# Intracellular Water and the Cytomatrix: Some Methods of Study and Current Views

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ABSTRACT The extent to which the properties of water in cells are like those of water in dilute aqueous solutions is a question of broad significance to cell biology. A detailed answer is not available at present, although evidence is accumulating that the properties of at least a large fraction of intracellular water are altered by interactions with cell ultrastructure, notably the cytomatrix. That and related evidence also suggests that the properties, composition, and activities of the "aqueous cytoplasm" of intact cells bear little resemblance to those of the "cytosol" obtained by cell fractionation. This paper will consider some of the evidence for these possibilities and some of their potential consequences with regard to cellular structure and function.



In spite of the well-known and often-stated fact that most of the volume and mass of living cells consists of water, we know very little about the structure and properties of intracellular water and its participation in cellular structure and function. Moreover, what information has been acquired has been subjected to a variety of interpretations, and it is fair to say that the topic is controversial. This brief paper cannot hope to present the details of all views, nor can details of the methodology applied to the problem be given. My major objective will be to provide a general account of the current status of the question for those not familiar with the area and to indicate how water-cytomatrix interactions may be of significance.

One reason for our poor understanding of cell water is that it is difficult to study, and, compared with macromolecules, for example, relatively little effort has been devoted to it. At the same time it is clear that the importance of this remarkable liquid is widely appreciated. Indeed, the "literature" goes back at least 3,000 years to when the Upanishad thinker said (see reference 32):

"It is water that assumes the form of this earth, mid-region, this heaven, these mountains, these gods and men, cattle and birds, herbs and trees, and animals together with worms, flies and ants. Water indeed is all these forms. Meditate on water."

Accepting that advice, I begin this meditation, suspecting that the cytomatrix may be an important addition to the forms recognized, so long ago, to depend on water.

## Pure Liquid Water

The concensus seems to be that liquid water is made up of an essentially random network of water molecules connected by hydrogen bonds, many of which are "strained" or broken at any given time. Such networks continually undergo change on a time scale of about $10^{-11}$ to $10^{-12}$ s. The specific molecular arrangements of water molecules and intermolecular forces operating between them are difficult to investigate and poorly understood but remain active areas of research. Recent review articles (10, 36) and a book series (11) provide ready access to the enormous literature.

The properties of liquid water are obviously consequences of its structure, and those properties have been described in some detail (10, 11). A question of importance to us is the extent to which the structure and properties of water in cells are altered by interactions with surfaces, be they macromolecular or ultrastructural. At present, there is no clear-cut answer, but the following exercise provides what might be a first approximation.

## Intracellular Surfaces and Water

I adopt the picture described by Porter et al. (31) for the cytoplasmic matrix of animal cells, notably the microtrabecular lattice. In that view an extraordinary network of structures ramifies throughout the aqueous cytoplasm (Fig. 1), providing enormous surface area. Estimates of this surface area for a spherical cell 16 $\mu$m in diameter and having a nucleus 10 $\mu$m in diameter range between 50 and $100 \times 10^3$ $\mu m^2$ (15). It has been calculated that a monolayer of water placed on all this surface would "involve" 2–4% of the total cytoplasmic water (7). It is well established that water adjacent to surfaces has properties that differ from pure water, but

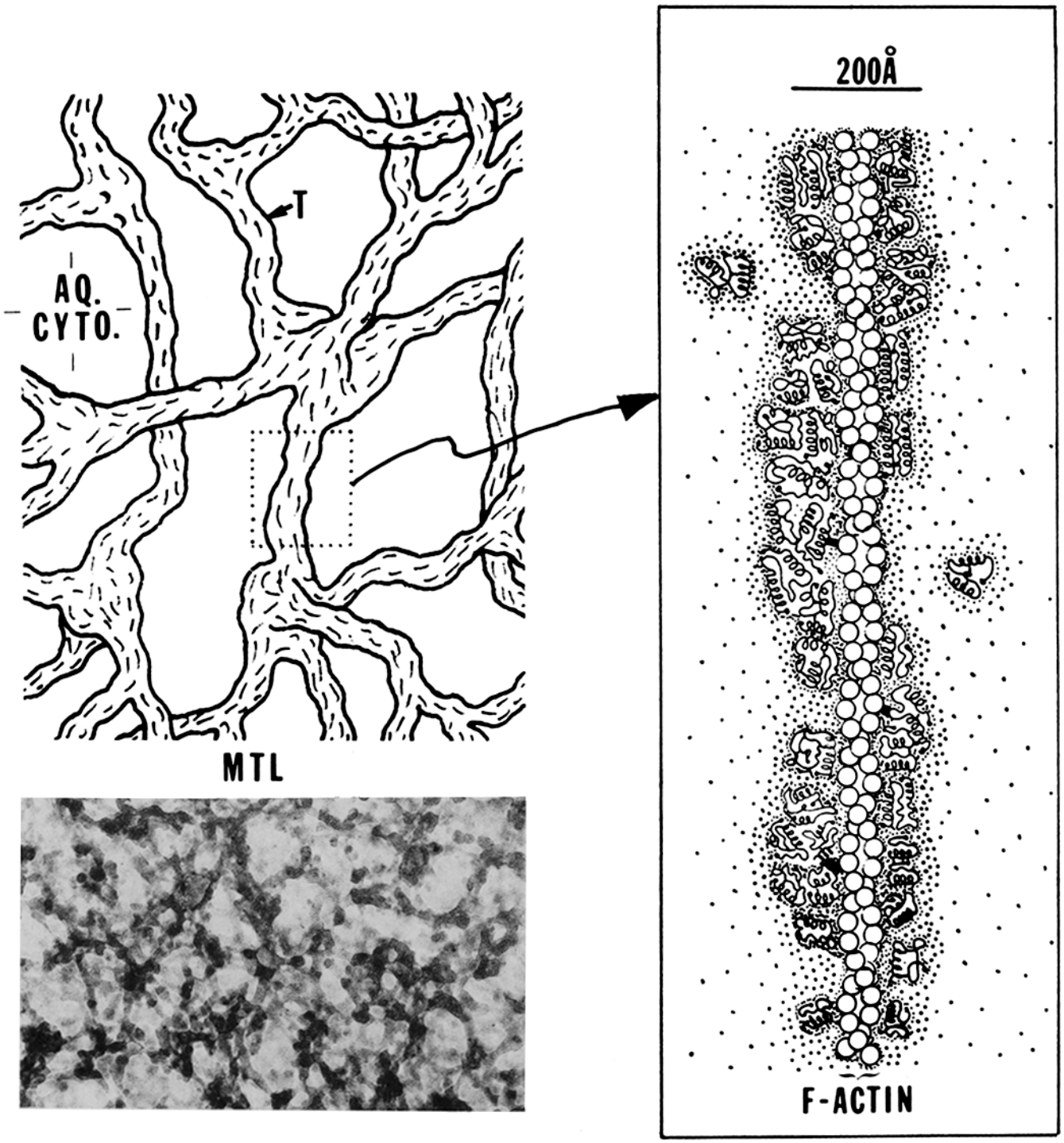


FIGURE 1 Diagrammatic description of the microtrabecular lattice (*MTL*) and surrounding aqueous cytoplasm (*AQ. CYTO.*). The photomicrograph of the MTL at the lower left was kindly supplied by K. R. Porter. The drawing at the top left is an enlarged simplified version of the MTL. One possible organization of a trabecula (*T*) is shown on the right: a central helical F-actin filament is associated with various enzymes and other proteins, some tightly (bars), others loosely; metabolites are not shown in the aqueous cytoplasm. Stippling represents water molecules. Redrawn from reference 7.

there is disagreement about the distance from the surface over which the structure and properties of water are changed. Classic surface and colloid chemistry allows for one or two such layers of water (about 6 Å maximum). The latter would involve 4–8% of cytoplasmic water. However, other workers (18, 24, 29) propose that the distance of influence might be as great as 50 Å. In that case, 33–66% of cytoplasmic water would be involved. Drost-Hansen (9) believes that water as far as 500 Å from surfaces has altered properties and he coined the term "vicinal" to refer to that water. That seems hard to imagine, but if his view is correct none of the cytoplasmic water could possibly escape the effects of some surface. What seems clear is that the cytomatrix must play a crucial role in determining the structure and properties of cell water.

Porter's (31) image of the cytomatrix requires that the

surrounding aqueous phase, referred to here as the aqueous cytoplasm, be very dilute with respect to macromolecules, and recent studies on the microviscosity of the aqueous cytoplasm using electron-spin resonance support his contention (19, 20, 26, 27, 33), as do older data obtained from cells stratified by centrifugation (5, 21, 22) and by other techniques (7, 13, 25). Such a description, diagrammed in Fig. 1, arises from the use of a variety of independent experimental techniques and is markedly different from the very crowded cytosol obtained from cell fractionation studies in which the total concentration of macromolecules is very high. In my opinion the in vitro cytosol bears little resemblance to the aqueous cytoplasm of intact cells and provides a misleading portrayal of this major cell compartment (7). These matters bear directly on current conceptions of cytoplasmic organization.

The preceding discussion suggests that appreciable amounts of cell water can be expected to exhibit physical properties that differ from those of the pure liquid, but that is not at all evident from the literature (for a review, see reference 6): views range across the extremes that none (23, for example) or practically all (37, for example) of the water in cells has ordinary bulk properties. In the following sections I describe, very briefly, some studies on the motion of cell water that illustrate one aspect of this controversy.

## Nuclear Magnetic Resonance Spectroscopy (NMR)[1]

NMR can be used to probe the motion of water protons by a "pulse" method, the details of which have been described (1–3, 16). It has been applied to dozens of cells and tissue during the last 15 years without much resolution, chiefly because it is necessary to interpret the data within the confines of a model, and several have been constructed. Three parameters can be obtained from pulse NMR experiments that provide information about the motion of cell water: two "relaxation times," $T_1$ and $T_2$, and the self-diffusion coefficient, D. Simply put, the extent to which these parameters deviate from pure water will reflect altered motion of cell water. But things are not so simple. Measurements of $T_1$ and $T_2$ for pure water are ~3,000 ms, but for cell and tissue water the $T_1$s are ~150–1,000 ms and the $T_2$s are 20–250 ms. These reductions have been interpreted to mean that all of the cell water exhibits reduced motion or that almost all cell water (~95%) has the same motion as pure water. In the latter models, reductions in $T_1$ and $T_2$ are commonly explained by the rapid exchange of "bulk" water molecules with a small "tightly bound" fraction (5% of the total) whose existence greatly influences the relaxation times measured. Variations on this theme have also been proposed.

These various models carry different predictions about D (16), which, unlike relaxation times, is a simple average of the total cell water. For example, models that interpret $T_1$ and $T_2$ reductions as the result of "fast exchange" also predict that D for cell water should be nearly the same as that for pure water. NMR measurements of D in cells reveal twofold to sevenfold reductions. Fast-exchange models explain these reductions by obstruction and compartmentation effects, which indeed are plausible because NMR diffusion coefficients are ordinarily measured over distances on the order of the cell diameter. Therefore, the critical test of these models is to measure D over very short distances (and times), thereby not allowing the water molecules to encounter barriers. That, however, is very difficult to do with current NMR technology.

## Quasielastic Neutron Scattering (QNS)

QNS seems capable of resolving the problem because it gives information on the diffusive motion of water over periods of about $10^{-12}$ s and distances of 1 or 2 Å. Although interpretation is not free of difficulty, the limitations are less than those of NMR. A major reason why this technique has not been used to resolve the controversy is that the sample must remain closely packed and sealed in the measuring cell for at least several days, and usually about a week, if sufficient data for analysis is to be obtained. Most living systems cannot tolerate such treatments. One of my reasons for choosing the *Artemia* cyst as a model system for studying cell water is its extraordinary resistance to environmental insults: it tolerates QNS conditions with no trouble at all (4, 5). This system, which consists of a group of 4,000 closely packed cells surrounded by a complex shell, has been described in considerable detail (30).

In a recent interdisciplinary study, the diffusive motion of water in *Artemia* cysts was measured by QNS, and the results have been published in preliminary (38) and complete (39) form. Table I shows some of these findings, along with NMR parameters (34, 35) for cysts at the same water content, at which the cells are hydrated to a little less than the water content of rat liver cells. As commonly observed, $T_1$ and $T_2$ are greatly reduced compared with pure water, and D determined by NMR is reduced about sevenfold. However, QNS yields only a threefold reduction in D.

Two significant conclusions appear justified. First, as fast-exchange models have predicted, some of the reduction in the diffusive motion of cyst water evaluated by NMR does indeed appear to be due to obstruction and similar effects, which account for about one-half of the sevenfold reduction. Second, even over distances of ~1 Å, there is still a threefold reduction in D. Because the latter cannot be due to anything but the motions of the water molecules themselves, these data provide good evidence that the diffusion of at least a large fraction of the water in these cells, possibly all of it, differs markedly from that of water in dilute aqueous solutions. That being the

TABLE I

*NMR, QNS, and Microwave Dielectric (MD) Measurements on Artemia Cyst Water**

| | Parameter | *Artemia* water | Pure water |
|---|---|---|---|
| NMR: | $T_1$ (ms) | 275 | 3,000 |
| | $T_2$ (ms) | 53 | 1,750 |
| | D ($10^{-5}$ cm$^2$/s) | 0.38 | 2.4 |
| QNS: | D ($10^{-5}$ cm$^2$/s) | 0.75 | 2.4 |
| | $\tau$ ($10^{-12}$ s) | 4 | 1 |
| MD: | $\epsilon'$ at 2 GHz | 40 | 78 |
| | $\epsilon'$ at 35 GHz | 16 | 23 |
| | $\tau$ ($10^{-12}$ s) | 10–25 | 8 |
| | $\alpha$ (0.8–70 GHz) | 0.46 | 0.02 |

Cysts at 1 g/g were used in MD work. $\tau$ is the correlation time (which does not have precisely the same meaning for QNS and MD), $\epsilon'$ is the real part of the complex dielectric constant and $\alpha$ is the spread parameter (a measure of deviation from Debye relaxation).

* Cysts at water contents of 1.2 g/g were used in NMR and QNS studies.

[1] *Abbreviations used in this paper:* D, self-diffusion coefficient; MD, microwave dielectric; NMR, nuclear magnetic resonance spectroscopy; QNS, quasielectric neutron scattering.

case, $T_1$ and $T_2$ reductions cannot easily be accounted for by fast-exchange models.

Do results obtained with *Artemia* have general applicability? *Artemia* cysts are unusual in many ways, but I have given reasons to believe that the *Artemia* data will, more likely than not, apply to animal cells in general (4–8). If that is accepted, the *Artemia* studies provide firm evidence that the traditional view of cell water requires reevaluation. What remains to be determined is the relative amount of cell water that exhibits altered properties. Very likely that will differ in various cells and tissues, depending on their physiological state and other factors.

Of direct significance to the proposed relationship between the cytomatrix and cell water is the important work of Beall (1), who carried out NMR studies on HeLa and Chinese hamster ovary cells during the cell cycle. The results in both cases indicate that the water in mitotic cells is "more mobile" than other stages of the cell cycle, and the proposal is offered that this results from a decrease in ultrastructural organization of the cytoplasm of cells in S phase. She finds evidence for this in NMR studies on isolated HeLa nuclei at various stages of chromatin condensation, in cells treated with colcemid and cytochalasin B, and in a variety of in vitro studies on microtubules and microfilaments. The general conclusion drawn from these studies is that the assembly-disassembly of such cytoplasmic architecture has predictable effects on the properties of cell water, based on changes in surface area in cells. Beall (1) suggests that the microtrabecular lattice could play a major role in the NMR changes she observes, and that view is certainly supported by the evidence presented in the present paper. Further work on the relationships between water mobility and in vitro preparations of the various cytoskeletal components should prove to be of value in testing these hypotheses.

## Dielectric Measurements

Fewer studies have been carried out on cell water using dielectric measurements. Most have come from the laboratories of Schwan, Foster, and colleagues (see references 6 and 37), who believe that almost all of the water in cells is dielectrically like pure water, and Grant and colleagues (see references 6 and 14), who concluded otherwise. As is the NMR work, these studies are difficult to interpret. Also, to obtain unambiguous data it is necessary to make measurements over the frequency range of water relaxation (~2–100 GHz), which poses some serious technical problems. Almost all of the published data have been obtained at frequencies below ~10 GHz, requiring considerable extrapolation for interpretation. Thus, like those obtained by NMR, the data do not provide a direct and unambiguous description of the behavior of cell water.

Nevertheless, the NMR and QNS results obtained with *Artemia* cysts predict that the dielectric properties of their water should differ from those of pure water and water in dilute aqueous solutions. That result was obtained over the frequency range of 0.8–70 GHz (8). Table I also summarizes some of these data: the dielectric relaxation time ($\tau$) of cyst water is slightly longer, most of it being much longer (8), and the average permittivity $\epsilon'$ (dielectric constant) is considerably lower (about one-half) than that of pure water. The latter result, incidentally, is fully consistent with the finding that cell water seems to have altered solvent properties compared with pure water (6, 17, 23).

In summary, it appears that most of the water in the cells of *Artemia* cysts exhibits rotational and translational motions that differ appreciably from those of the pure liquid. These findings are in general agreement with those obtained by other workers, who have used these and other methods to study a variety of other cell and tissue types (1–3, 6, 14, 16, 19, 23 and the references therein). Nevertheless, it appears that the prevailing opinion still is that almost all of the water in cells is virtually the same as that in ordinary dilute aqueous solutions; I believe that view requires extensive revision.

## Some Implications and Concluding Comments

It is fair to ask what difference it makes if cell water has properties unlike those of an ordinary solution? Several answers come to mind.

1. Much current thought about macromolecular function in cells is based on data obtained in vitro, almost always in dilute aqueous solution. If intracellular water differs from that in test tubes, as some of us believe, then information obtained in vitro may not allow us to construct (or better, "reconstruct") an accurate description of these molecules and their activities within cells, including those concerned with the cytomatrix.

2. Interactions between macromolecules and their aqueous environment appear to be even more important than has commonly been believed. Welch et al. (40) have recently reviewed the abundant evidence for this, and their analysis makes it very likely that water plays subtle but important roles in metabolism: to understand these roles we must know the details of the aqueous microenvironment in which most of this activity occurs.

3. Available evidence suggests that the solvent properties of at least a large fraction of the total cell water, notably that in cytoplasm, differ from those of ordinary aqueous solutions. At least some contribution to the uneven distribution of certain solutes across the plasma membrane as well across membranes within cells (organelles) could arise from such "solvent" differences. Thus, small metabolites might "partition" between various intracellular aqueous phases, a possibility made likely by work on the solvent properties of cell water and notably the work of Garlid on mitochondria (see references 4 and 6). Even protein distribution within cells may be influenced in a similar fashion (see references 4–7 and 28). A speculative "model" on the organization of enzymes in the aqueous cytoplasm includes the possibility that a loose association of enzymes with the cytomatrix (Fig. 1) may occur by water interactions involving their surfaces (4, 6).

4. Assembly-disassembly processes are clearly influenced by the properties of the aqueous phase within which they occur. Such mechanisms likely are important to the dynamic turnover of the cytomatrix and possibly other cell structures.

5. A great many of the molecular interactions in cells involve ionic interactions, which should be quite sensitive to the dielectric properties of the surroundings. Thus, the possibility that the permittivity of cell water is considerably lower than that of dilute solutions may be of some importance. Indeed, Frohlich (12) has developed a theory of cell function based on coherent oscillations of cellular macromolecules that involves not only the properties of cell water but probably the cytomatrix as well. Although his proposals have direct bearing on cytomatrix function, they have not been given much attention by cell biologists.

I conclude by emphasizing that the cytomatrix can be expected to play a major role in determining the properties of intracellular water (in spite of our current lack of understanding of the details). Likewise, water may very well play an important role in mechanisms that regulate the cytomatrix, and both seem linked to most cellular activities. Evidently, it is this entire system that must be studied if we are to understand the participation of water and the cytomatrix in cell structure and function. I believe they should be thought of as a continuum and not as two separate and somewhat independent entities in contact with one another. The potential importance of these and other relationships has been discussed elsewhere (7). One example may serve as a final point for meditation. A reasonably good correlation exists between modifications in the cytomatrix and changes in the amount and properties of water, both of which commonly, although not always, accompany cell transformation. Although this may be fortuitous, it is notable that the usual chain of events involves a reduction in cytomatrix surface area and an increase in the amount of cell water that has "bulklike" properties (1–3). That is at least consistent with the proposed relationship between the cytomatrix and its surrounding aqueous environment. It has also not escaped our attention that many of the metabolic changes accompanying the transformation process are associated with "soluble" enzymes, which, perhaps, are not really soluble but instead part of the water-cytomatrix system (7).

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This paper is dedicated to Keith Porter whose image of the cell has been so valuable in our attempts to understand the properties and functions of the aqueous intracellular environment.

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