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The cytoplasmic matrix: Its volume and surface area and the diffusion of molecules through it

(cytoplasm/protein diffusion/cytoskeleton)

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ABSTRACT **In this work we look into the problem of why proteins, unlike small molecules, diffuse in the cytoplasm much more slowly than in aqueous solutions. In order to examine whether the cytoplasmic matrix could, by simple obstruction, retard protein diffusion to such an extent, we developed a method to measure semiquantitatively the fractional volume occupied by the cytoplasmic matrix (which includes the microfilaments, intermediate filaments, microtubules, and the microtrabeculae of the cytoplasmic matrix). This method yielded values in the range of only 16–21%. Thus, a more elaborate model is suggested in which the diffusing proteins bind and dissociate constantly from the surfaces in the cytoplasmic matrix. From this model, the diffusion coefficients and the measured values of the fractional volumes, we calculated the corresponding binding constants. These values indicate that most of the diffusing proteins are bound to the matrix at any given time, in spite of the possibility that they may bind and dissociate very rapidly. In addition, from our measurements, we estimate the surface area of structures within the cytoplasmic matrix to be in the range of 69,000–91,000 μm^2 per cell.**

The concept of the cell as a tiny bag of enzymes containing a few membrane-limited structures is being laid to rest. In its place a much more complicated image is emerging, one in which filaments and a gel-like structure are prominent. This structure is known to comprise a dynamic three-dimensional network of skeletal elements that includes at least three distinct filamentous components disposed in a lattice of more slender and labile structures that has been called the microtrabecular lattice (MTL) (2,3,4). There are reasons to conclude that these components in some manner combine functionally to control cell shape and motility.

Using high-voltage electron microscopes, cell biologists are able to examine thick sections (up to 5 μm thick) and even whole, thinly spread cultured cells and, thus, to obtain useful high resolution images of substantial depth of the cytoplasmic matrix. Porter and co-workers (1–4) have discovered that, in addition to the discrete cytoskeletal structures listed above, the cytoplasm is composed of a three-dimensional lattice of slender strands coextensive with the cortex of the cytoplasm and the cytoplasmic surfaces of the endoplasmic reticulum (ER) membranes. In addition, the cytoskeletal filaments are found to be contained within this network. This system, known as the microtrabecular network (or lattice), has been observed in all eukaryotic cells examined. The strands are found to be 5–10 nm in diameter and 50–100 nm in length. Thus, the cytoplasm is a two-phase system, one a protein-rich phase and the other a water-rich phase. These phases are in most instances continuous throughout the cytoplasm.

It is known from experiments that small molecules such as metabolites, some proteins, and various ions diffuse through the cytoplasm. Recent diffusion studies have shown that, while small molecules [like spin labels (5–7)] move through the cytoplasm about half as rapidly as through water, proteins such as bovine serum albumin and IgG move much more slowly than in water (8–10). If the cytoplasm were basically a solution, it would be difficult to understand why proteins diffuse so slowly through it.

In order to find out why, we developed a new method for estimating quantitatively the fractional volume occupied by the cytoplasmic matrix (the microtrabecular lattice and the cytoskeleton) and to correlate the results of these measurements with the values of diffusion constants of protein in cells. We then examined how a cytoplasmic matrix, with the resulting values of the fractional volume, can act to slow down the diffusional motion of proteins. From the measurements of this fractional volume, we estimated values for the surface area associated with the cytomatrix and its weight ratio in the cytoplasm (assuming it is solely composed of proteins). In addition, by assuming a simple model of the interaction of the diffusing proteins with the surfaces in the cytoplasmic matrix, values of the "binding" constants and the free energy were calculated.

Method for Quantitatively Estimating the Fractional Volume of the Cytoplasmic Matrix, its Surface Area, and the Protein Fractional Weight in the Cell

The cytoplasm, as seen in high-voltage electron micrographs is shown in stereo in Fig. 1. It is clearly depicted in this example (as well as in all other instances) to comprise a three-dimensional network. We developed a method for estimating quantitatively the volume of the cytoplasm occupied by this network.

Electron micrographs displaying the structure of the cytoplasmic matrix (especially the microtrabeculae) were digitized by using a Perkin–Elmer microdensitometer. The magnification of the transparencies used was usually ×44,000. The microdensitometer with an aperture of 2500 μm^2 scanned an area of about 6×10^8 μm^2, creating a matrix of optical densities for further computer processing. Subareas consisting of 512 × 480 pixels were processed at a time. A pixel is a single matrix element (area) measuring 2500 μm^2, the optical density of which may be measured. A representation of the pixel analysis of one of the cytoplasmic images is shown in Fig. 2 *Lower*. The computer is schematically described in Fig. 3.

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Abbreviation: FRAP, fluorescence recovery after photobleaching.

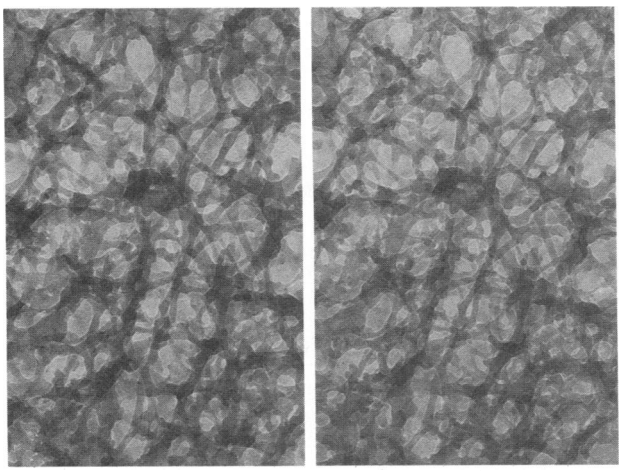

FIG. 1. A sample of one of the micrographs in which the fractional volume was measured. It shows a stereo pair of micrographs taken with a high-voltage (1 Mev) electron microscope. The images represent a small area of the cytoplasm of a cultured PTK cell. In preparation for microscopy, the cell was frozen rapidly by immersion in a bath of propane cooled to −185°C and thereafter dried in a vacuum while kept at −90°C. This took approximately 48 hr. The cells were not chemically fixed or stained at any point. Other details of the preparation procedure are described by Porter and Anderson (4).

Processing was performed on a PDP-11/70 computer with an attached Evans and Sutherland Picture System II, video frame buffer, and camera station.

In order to measure how much of a micrograph is occupied by the cytoplasmic matrix, a threshold value for the optical density of the palest area (representing a structural element) in the image of this structure was required. To select the threshold, the micrograph was first displayed on the video frame buffer (a device that contains the digital image, allowing its manipulation, fast storage, and retrieval and a simultaneous display on a video television screen). A tablet and a graph pen were used to select 10 or more pale regions, for which a threshold value could be determined. Then, given the density of the faintest structure on the image, the computer could calculate how much cytoplasmic structure was represented within a given area. To do this we developed the following method.

All of the pixels in the image that are as dark as or darker than the threshold value are considered to represent a structure. The computer calculates the amount and fraction of the image that is occupied by dark pixels or, equivalently, by structure. We denote the fraction of the picture area covered with structure by f_b. To determine how much of the volume is occupied by the structure, we must take into account the thickness of the sample represented in the micrograph. We made thickness measurements by using a Ladd stereoscope (model SB180/183). The average diameter of the different structures in the picture, d, was calculated from measurements done on a Summagraphics ID digitizer. By assuming that the cross sections of the structures are circular, the average thickness of the structure is $2d/\pi$. If the same weight is given to all of the dark pixels, the fraction of the volume occupied by the structure is then:

$$\text{structure volume fraction} = \frac{\text{fraction covered by structure} \times \text{average structure thickness}}{\text{cell thickness}}$$

$$= \frac{f_b(2d/\pi)}{\text{cell thickness}}$$

$$= f_b f, \qquad [1]$$

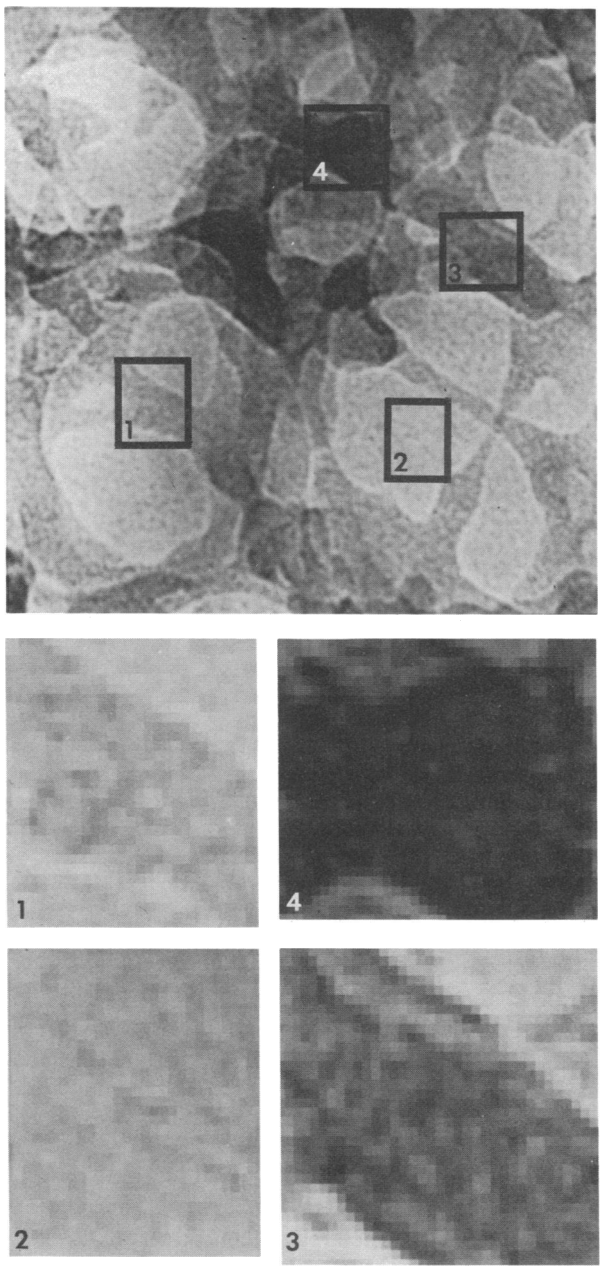

FIG. 2. The digital representation of the data used by the computer system. (*Upper*) A segment of a typical micrograph analyzed in this work. (*Lower*) Four areas marked correspondingly in *Upper*, with high magnification showing the individual pixels.

where f is the average structure thickness divided by the section thickness.

This simple approach does not differentiate between pixels containing one strand and those that represent two or more overlapping structures and is based on a binary representation of the image. As a result, this estimate may be lower than the real value of the fraction of the image that contains structure.

It is obvious that some pixels are darker than others. This is the result of overlapping structures, different thicknesses of elements or different chemical compositions. In order to take these variations into account, we developed an additional, more-sophisticated method. Pixels that are less dense or lighter than the threshold (the optical density of the faintest structure, determined on the screen, as defined above) are taken as not containing any structure as far as the lattice is concerned. On the other hand, pixels that are denser or darker than the threshold are weighted according to how much they exceed the threshold value. If b is the value of the

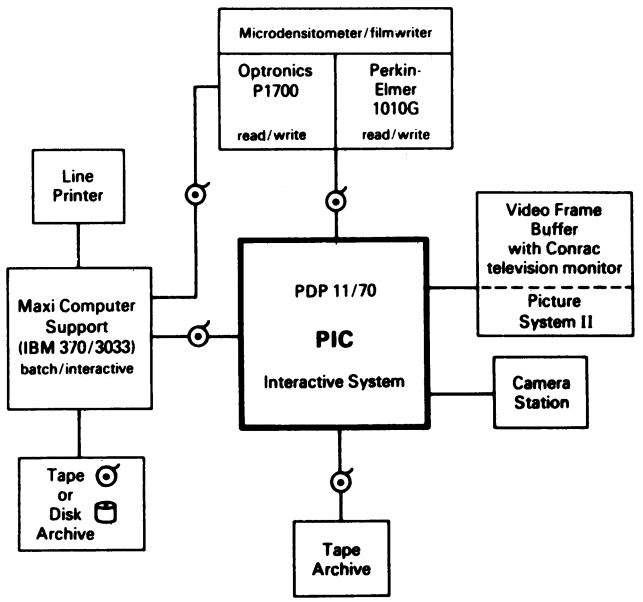

FIG. 3. The computer system used to quantitate the area in the micrographs occupied by cytoplasmic structure.

threshold and p is the intensity or the optical density of a pixel, then its weight in determining how much black there is in the picture is given by

$$\text{Weight} = 1 + (p-b)/b = p/b. \quad [2]$$

For pixels that are greater than or equal to the threshold, the weight is ≥ 1. The volume fraction of the structure is then

$$\text{structure volume fraction} = \frac{\Sigma p/b}{\text{no. of pixels in picture plane}} \times \frac{\text{average structure thickness}}{\text{pixel dimension}} \Big/ \frac{\text{section thickness}}{\text{pixel dimension}} = \frac{\Sigma p/b}{\text{no. of pixels in picture plane}} \times f. \quad [3]$$

This method was found to yield higher values for the volume fraction of black or structured elements than did the simple-minded approach expressed by Eq. 1, since overlapping structures are counted more than once.

From the results of the fractional volume of the structure in the cytoplasmic matrix, we were able to estimate two quantities: the amount of surface area associated with the structure and the weight ratio of the proteins in the cell (assuming that the structure is solely composed of protein). The description of these procedures is given as follows.

Surface Area Estimates. If the average diameter of the structure, d, is known, then by taking a sample cell (e.g., with a diameter of 16 μm and a nucleus of 10 μm in diameter) we can calculate the amount of the surface area associated with the structure. We denote the fractional volume of the structure by α and the volume of the cytoplasm by V_c and obtain the following relation for L, the estimated total length of the structure in the cytoplasm of the given cell:

$$L = \frac{V_c\alpha}{\pi(d/2)^2}, \quad [4]$$

and its estimated surface area, S, is

$$S = 2\pi(d/2)L = 2V_c\alpha/(d/2). \quad [5]$$

Fractional Weight of Proteins in the Cell. From the results of the fractional volume of the structural elements of the cytoplasmic matrix, one can further calculate the estimated fractional weight of the proteins in the cell (given that the structure is made solely of protein). The volume of the cytoplasm is denoted by V_c and that of the nucleus by V_n. If the specific weight of the protein is ρ, then the fractional weight of the protein in the cell, pw, is

$$pw = \frac{\text{cytoplasmic protein weight + nuclear protein weight}}{\text{cell total weight}} = \frac{V_c\alpha\rho + nV_c\alpha\rho}{(1-\alpha)V_c + (V_n - nV_c\alpha\rho) + V_c\alpha\rho + nV_c\alpha\rho} = \frac{\alpha\rho + n\alpha\rho}{(1-\alpha) + [(V_n/V_c) - n\alpha\rho] + \alpha\rho + n\alpha\rho}. \quad [6]$$

The total cell weight is composed of the water weight in the cytoplasm, the water weight in the nucleus, the protein weight in the cytoplasm and in the nucleus. Their fractional weights are given by $1-\alpha$, $(V_n - nV_c\alpha\rho)$, $\alpha\rho$ and $n\alpha\rho$ respectively. Here the factor n has a different meaning from n of the next sections. In this approximate calculation the relatively small amounts of nucleic acids were not included.

Results of Quantitative Estimates of Fractional Volume of the Cytoplasmic Matrix and the Diffusion of Proteins Through it

The methods outlined in the previous section were used to estimate quantitatively how much of the volume of the cytoplasmic matrix is occupied by electron scattering elements of the lattice.

As mentioned in the introduction, diffusion of proteins in the cytoplasm is much slower than in aqueous solutions (8–10). Table 1 contains the values of the diffusion constants of various proteins in cells as measured by fluorescence recovery after photobleaching (FRAP) (11–14). These results give rise to the question: why do proteins diffuse so slowly in the cytoplasm? In order to answer this question, we measured the fractional volume occupied by the cytoplasmic matrix by the method outlined in the previous section. The results are given in Table 2. From these results we also were able to estimate the amount of surface area associated with the cytoplasmic matrix as described above. The values for the surface area are presented in Table 3. To examine the possibility that the interaction of the diffusing proteins with the cytoplasmic matrix is responsible for the slow diffusion rate, we analyzed a simple case of such interaction. In our model, the diffusing proteins bind to the matrix instantaneously. This means that the binding times are much shorter than the time it takes for the concentrations in the aqueous phase to change, which should be comparable to translational

Table 1. Diffusion of proteins in the cytoplasm as measured by FRAP

Protein	Ratio D_w/D_c
Bovine serum albumin	68–113
Ovalbumin	27
Actin	341

These values are taken from refs. 8–10. Unlike these proteins, small molecules such as spin labels diffuse in the cytoplasm only about half as fast as in aqueous solutions (5–7). D_w and D_c, diffusion coefficient in water and cytoplasm, respectively.

Table 2. Fractional volume of the cytoplasmic matrix

Cell	Fixation procedures	Network volume fraction, %
PTK	Freeze-dry	20
	Freeze-substitution* Osmium tetroxide/Methyl Cellosolve	16
	Freeze-dry Osmium tetroxide/glutaraldehyde	19
NRK	Freeze-dry	21
	Freeze-substitution* Osmium tetroxide/acetone	16

*Freeze-substitution is a procedure by which water in the frozen specimen is replaced by acetone containing O_5O_4 at very low temperature.

diffusion times in this length scale. Under this condition, the concentration of the proteins "adsorbed" on the cytoplasmic matrix, n, is proportional to its concentration in the aqueous phase, c. Thus,

$$n = kc, \quad [7]$$

where k is the "equilibrium" constant. Both c and n are concentrations expressed per unit volume of the total cytoplasmic system—i.e., both the cytoplasmic matrix and the aqueous phase. The diffusion equation in this system is

$$\partial c/\partial t = D\partial^2 c/\partial x^2 - \partial n/\partial t, \quad [8]$$

where the diffusion coefficient of the protein, D, is the same as in a purely aqueous phase (to a first approximation). This equation can be transformed into a simple diffusion equation by substituting Eq. **7** into Eq. **8**, resulting in the following equation:

$$\partial c/\partial t = D_{\text{eff}}\, \partial^2 c/\partial x^2, \quad [9]$$

where

$$D_{\text{eff}} = [1/(1 + k)]D \quad [10]$$

or

$$D/D_{\text{eff}} = 1 + k \quad [11]$$

From this relation and from the diffusion coefficients measured by FRAP, we could calculate values for the "binding" constant. Also, "binding" energy could be calculated from the following relation:

$$k = \exp[-\Delta G/RT], \quad [12]$$

where R is the gas constant and T is the absolute temperature. Values for k and ΔG are given in Table 4.

Table 3. Protein weight fraction in the cell and the cytoplasmic matrix surface area*

Volume fraction	Protein weight in the cell,† %	Cytoplasmic matrix surface area,‡ $\mu m^2 \times 10^{-4}$
0.1	12	4.3
0.2	23	8.6
0.3	33	13.0

*This is for a cell 16 μm in diameter and with a 10-μm nucleus.
†The density of proteins is taken as 1/0.75 g/cm^3.
‡For comparison, the surface area of the outer membrane of the cell mentioned above is 804.25 μm^2.

Table 4. "Binding" constants and free energies for proteins diffusing in the cytoplasm with different values of D/D_{eff}

D/D_{eff}	Binding constant*	Binding free energy,† kcal/mol
20	19	1.74
70	69	2.50
115	114	2.79
340	339	3.44

*Calculated by using Eq. **11**.
†Calculated by using Eq. **12**.

Discussion

If the cytoplasm were a mere solution it would be unlikely that proteins would diffuse so slowly through it as compared with aqueous solutions. According to what has been reported from studies using high-voltage electron microscopy (1–4), the cytoplasm comprises a three-dimensional lattice of fine strands in addition to the cytoskeleton and the cell organelles. It has basically the structure of a gel. There is then the possibility that the diffusion of proteins in the cytoplasm is retarded because part of the cytoplasmic volume is occupied by these structures, which obstruct the diffusion of at least the proteins. Thus, any molecule will take more time to diffuse from one location to another. To examine this possibility, we developed the method described above to estimate quantitatively the fractional volume occupied by the structured matrix in the cytoplasm, using electron micrographs. The results of these quantitative estimates are given in Table 2.

It is clear from Table 2 that the volume occupied by the cytoplasmic matrix is not very high. By "not very high," we mean as compared with values obtained using a mere visual inspection. In addition, the dimension of the pores in the aqueous phase is about 100 nm, which is much larger than the size of the proteins examined. Based on the values of the fractional volumes and the pore dimensions, it is obvious that the diffusion of proteins cannot be slowed down to such an extent by a noninteractive cytoplasmic matrix occupying such a low fractional volume through an excluded volume effect.

Thus, we are forced to consider a more reasonable possibility—that the diffusing proteins interact with the surfaces of the cytoplasmic matrix. The interaction could have the form of an entanglement or chemical attraction. To test this possibility, we examined the following simple model. The diffusing protein molecules are instantaneously adsorbed on the cytoplasmic matrix. By "instantaneously," we mean that the reaction resulting in adsorption takes place in a much shorter time than it takes the protein molecules to diffuse through the aqueous phase. The mathematical description of this diffusion–adsorption model is given in the previous section. The resulting "binding" constants, k, and the calculated "binding" energies, ΔG, are given in Table 4. These numerical values for k and ΔG seem to be reasonable. Thus, it is likely that the interaction of the proteins with the large surfaces of the cytoplasmic matrix is responsible for the slow rate of diffusion in the cytoplasm of the proteins measured by Wojcieszyn *et al.* (8), Kreis *et al.* (9), and Wang *et al.* (10). More experimental work is needed in which different kinds of proteins are injected—e.g., those that are considered to be adherent compared with nonadherent proteins. By "adherent," we exclude proteins such as actin that can be incorporated into the existing filaments. Also the model used in this work is the simplest one. Once there are more detailed experimental results, it will be possible to construct and examine more elaborate models. In contrast to proteins, small molecules like spin labels diffuse more slowly in the

cytoplasm than in water by a factor of 2–3, as shown by Mastro and Keith (5, 6). They showed that over the dimensions of 50–100 Å and for small molecules, solvent viscous forces seem to be the major cause for slowing down the diffusional motion.

One important corollary of our measurements is the estimate of the amount of surface associated with the cytoplasmic matrix. The results, which are presented in Table 3, show that the estimated surface is in the range of 69,000–91,000 μm^2, depending on the value of the fractional volume of the cytoplasmic matrix in the cytoplasm. These values are estimated for a cell of 16 μm in diameter with a 10-μm nucleus. For comparison, the external surface area of such a round cell is only 804 μm^2. These results should be valuable in problems where the surface of the cytoplasmic structures participates in various reactions or when it is necessary to estimate how much water there is in the vicinity of these surfaces (15).

The values of the binding constant point up an important possibility. From Eq. 7 it follows that the percentage of molecules bound to the matrix at any given time is $100k/(1+k)$, which is 100 times the concentration of bound protein over the total concentration of protein present in the volume examined. If we take, for example, the value of $k=69$, then the percentage of the bound protein is 99%. Similar high values are obtained for the rest of the k values in Table 4. This value of the amount of bound protein in the cytoplasm is quite high. Thus, according to this model, at any given time, most of the diffusing proteins [which have slow diffusion times as measured by FRAP, as outlined before (8–10)] are bound to the cytoplasmic matrix, although the rates of association–dissociation can be quite high. This conclusion also points out that most of the native proteins present in the cell might be bound to the matrix, at any given time. Such a situation was observed by Kempner and Miller a few years ago (16). When in their studies Euglena cells were centrifuged in the ultracentrifuge, the cell contents became stratified. The cytoplasmic proteins that were examined moved in the direction of the gravitational field faster than would have been expected if they were merely soluble in the liquid compartment of the cytoplasm. This finding was interpreted to mean that these proteins were associated with large particulates or a structural system that sedimented faster than free macromolecules. A supernatant, water-rich stratum was essentially free of demonstrable proteins. Gershon *et al.* (17), Jacobson and Wojcieszyn (18), and Mastro and Keith (6) have considered the possibility that the diffusing proteins used as a probe are bound to cytoplasmic structures. Jacobson and Wojcieszyn (18) also pointed out that one can show [based on Elson and Reidler (19)] that, in order to account for the diffusion results, ≈98% of the diffusing species used as a probe will be bound.

In addition to the volume fraction of the cytoplasmic matrix and the amount of surface associated with it, we also could calculate an estimate of the percent of protein weight in the cell (see method above and Table 3). These values range from 12% to 33% depending, of course, on the value of the fractional volume of the cytoplasmic matrix in the cytoplasm. These numbers are in the expected range.

This work attempts to measure the fractional volume of the cytoplasmic matrix. The values obtained by this method point out that a simple noninteractive cytoplasmic matrix cannot slow down the diffusional motion of proteins to the measured extent. Thus, we are led to believe that there is a more complicated mechanism responsible for this amount of retardation. We analyzed a simple association–dissociation model that yielded high values of the binding constant. According to this model, most of the diffusing proteins are bound to the cytoplasmic matrix at any given time. This means that most of the native proteins in the cytoplasm may be bound to the cytoplasmic matrix, rather than being freely dissolved in the aqueous phase. This possibility must have important biochemical implications.

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1. Buckley, I. K. (1975) *Tissue Cell* **7**, 51–72.
2. Wolosewick, J. J. & Porter, K. R. (1979) *J. Cell Biol.* **82**, 114–139.
3. Porter, K. R. & Tucker, J. B. (1981) *Sci. Am.* **244**, 56–67.
4. Porter, K. R. & Anderson, K. L. (1982) *Eur. J. Cell Biol.* **29**, 83–96.
5. Mastro, A. M. & Keith, A. D. (1981) in *The Transformed Cell*, eds. Cameron, I. L. & Pool, T. B. (Academic, New York), pp. 327–345.
6. Mastro, A. M. & Keith, A. D. (1984) *J. Cell Biol.* **99**, 180s–187s.
7. Mastro, A. M., Babich, M. A., Taylor, W. D. & Keith, A. D. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 3414–3418.
8. Wojcieszyn, J., Schlegel, R., Wu, E.-S. & Jacobson, K. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 4407–4410.
9. Kreis, T., Geiger, B. & Schlessinger, J. (1982) *Cell* **29**, 835–845.
10. Wang, Y.-L., Lanni, F., McNeil, P., Ware, B. & Taylor, L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4660–4664.
11. Poo, M.-M. & Cone, R. A. (1974) *Nature (London)* **247**, 438–441.
12. Peters, R., Peters, J., Tews, K. H. & Bahr, W. (1974) *Biochim. Biophys. Acta* **367**, 282–294.
13. Edidin, M., Zagyansky, Y. & Lardner, T. J. (1976) *Science* **191**, 466–468.
14. Schlessinger, J., Koppel, D., Axelrod, D., Jacobson, K., Webb, W. W. & Elson, E. L. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 2409–2413.
15. Clegg, J. (1984) *J. Cell Biol.* **99**, 167s–171s.
16. Kempner, E. S. & Miller, J. H. (1968) *Exp. Cell Res.* **51**, 150–156.
17. Gershon, N. D., Porter, K. R. & Trus, B. L. (1983) in *Biological Structures and Coupled Flows*, eds. Oplatka, A. & Balaban, M. (Academic, New York), pp. 377–380.
18. Jacobson, K. & Wojcieszyn, J. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6747–6751.
19. Elson, E. & Reidler, J. (1979) *J. Supramol. Struct.* **12**, 481–489.