Permeability characteristics of complement-damaged membranes: Evaluation of the membrane leak generated by the complement proteins C5b-9

(immune lysis/membrane transport/erythrocytes)

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ABSTRACT Permeability characteristics of the membrane lesion generated by the terminal complement proteins are considered in light of recent observations that the measured diffusion of solute across complement-damaged membranes does not conform to the "doughnut hole" model of a discrete transmembrane pore formed by the inserted C5b-9 complex. By using the measured kinetics of steady-state tracer isotope diffusion of nonelectrolytes across resealed erythrocyte ghost membranes treated with C5b-9, a new transport model is developed. This model considers the apparent membrane lesion strictly in terms of the operational criteria of a functional conducting pathway for the observed diffusing solute, independent of *a priori* assumptions about the geometry or molecular properties of the membrane lesion. With this definition of the unit membrane lesion and the assumption that the exclusion size of the conducting pathway varies directly with the multiplicity of bound C5b-9 (as suggested by previous measurements under conditions of varying input of C5b-9), numerical estimates of the apparent permeability of the complement-damaged membrane to four diffusing nonelectrolytes are derived. These results suggest that the pathway for a particle diffusing across the complement lesion cannot be a pore and is functionally equivalent to an aqueous leak pathway, free of pore constraints. Implications of these results are discussed in terms of current molecular models for the mechanism of membrane damage by the complement proteins.

It is generally accepted that the terminal proteins of the complement system (C5b-9) insert through the target membrane, providing a diffusion pathway for the transmembrane equilibration of cell solute—consequently, the cell is lysed by the colloid osmotic expansion of intracellular water (1). Support for this model has derived from substantial evidence that the C5b-9 proteins form a highly lipophilic complex that embeds within the fatty acid matrix of biological membranes (2, 3). The mechanism by which membrane permeability is consequently altered has been assumed to be analogous to that of nystatin or gramicidin, the channel-like activity of the complement proteins being inferred from the cylindrical pore-like structure of the C5b-9 complex as viewed by negative-stain electron microscopy (2, 4, 5) and from electrical conductance fluctuations recorded across voltage-clamped C5b-9-treated planar lipid bilayers (6–8).

Nevertheless, attempts to quantitate the kinetic characteristics of solute flow across the membrane lesion generated by the C5b-9 complex (9–11) have led to the suggestion that the measured transport properties of complement-damaged cells are not consistent with the proposal of Mayer (2) that the membranes are altered by the insertion of discrete transmembrane channels. In those studies (9–11), it was directly demonstrated that solute flow across C5b-9 membranes occurred orders of

magnitude slower than predicted on the basis of the morphological appearance of the complement lesion. Furthermore, it was demonstrated that C5b-9-treated cells failed to exclude solute uniformly above a limiting molecular radius (contrary to the results anticipated for membranes embedded with fixed, uniform channels) and it was noted that the apparent size of the membrane lesion was related to the number of assembled C5b-9 complexes (11). It was therefore concluded that membrane-bound C5b-9 complexes do not behave as discrete pores but interact or coalesce to form lesions of increasing size.

Despite the conclusions of these studies, it was nevertheless noted that the uptake of radiolabeled solute by cells treated with the C5b-9 proteins and suspended under steady-state conditions appeared to conform to a first-order kinetic function that had been derived on the basis of the *a priori* assumption that each membrane lesion was discrete and uniform, and could therefore be defined by a characteristic permeability parameter (10, 11). It is necessary, therefore, to reconsider the data of these experiments in terms of a model derived on the basis of the observed heterogeneity of the C5b-9 membrane lesion.

Phenomenological model of the C5b-9 membrane leak

From measurements of solute diffusion across C5b-9-damaged erythrocyte ghost membranes, it was concluded that the membrane lesions detected in a population of C5b-9 cells increased in size as the total number of assembled C5b-9 complexes increased (11). It is assumed, therefore, that the surface area of the membrane lesion on a particular cell (A_n^*) is related to the number of C5b-9 complexes bound to that cell (n) and that the average lesion size under a given condition of C5b-9 input reflects the distribution of C5b-9 complexes among the cells:

$$\overline{A_n^*} = \sum_n A_n^* F(n). \quad [1]$$

Because of the experimental condition of random C5b-9 assembly from the fluid phase components (see methods in ref. 11) it is also assumed that the distribution of C5b-9 complexes among the cells obeys a Poisson function:

$$F(n) = \frac{z^n e^{-z}}{n!} \quad [2]$$

in which z is the mean number of bound complexes per cell.

Abbreviations: C5, C6, etc., individual components of the complement system; C5b6, stable complex between b fragment of C5 and C6; C5b-9, complex of C5b, C6, C7, C8, and C9 (see recommendations of the Committee of Complement Nomenclature of the World Health Organization [Bull. WHO (1968) 39, 939]).

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In the experiments described in ref. 11, a C5b-9 lesion was functionally defined by the cellular equilibration of radiolabeled solutes. It follows, therefore, that, to detect a lesion on a particular cell, A_n^* must equal or exceed the cross-sectional area of the particular solute whose cellular uptake is measured (given by πr_s^2 , in which r_s is the equivalent molecular radius of the radiolabeled solute). With this area (πr_s^2) defined operationally as the minimal detectable unit lesion—resulting from the insertion of n_s C5b-9 complexes in a single cell— A_n^* may be expressed as a multiple of functional lesion units ($n_s^* = n/n_s$), each with an area πr_s^2 :

$$A_n^* = n_s^* \pi r_s^2. \quad [3]$$

The average apparent lesion due to C5b-9 assembly may now be expressed in terms of the distribution of detectable units of membrane damage:

$$\begin{aligned} \sum_n A_n^* F(n) &= \pi r_s^2 \sum_{n_s^*} n_s^* F(n_s^*) \\ &= \pi r_s^2 \bar{n}_s^*. \end{aligned} \quad [4]$$

It should be noted that the above formulation expresses the apparent membrane lesion (phenomenologically defined by the transmembrane solute leak) as an aggregate of strictly hypothetical units, each representing the smallest lesion that can be detected by the measured flux (an area equivalent to the collisional cross section of the diffusing solute). Accordingly, it is necessary to caution that these units neither define the physical dimension of the C5b-9 membrane complex nor suggest the molecular arrangement or state of aggregation of the C5b-9 monomer. As will be shown below, by defining the apparent membrane lesion in terms of arbitrary domains of area πr_s^2 , it is possible to express the transport parameters of the C5b-9 membrane lesion independent of *a priori* assumptions as to the mechanism of membrane damage or the physical size of the C5b-9 membrane complex. The strictly operational significance of the defined unit membrane lesion is underscored by noting that, for a given distribution of C5b-9 complexes, the number of detectable membrane lesions per cell ($\bar{n}_s^*$) will appear to diminish as the radius of the diffusing solute (r_s) increases (cf. table 1 in ref. 11). This conclusion will be further considered below.

In their analysis of the experimental data, Sims and Lauf (10, 11) calculated z_s , the mean number of apparent functional lesions per cell, by assuming a Poisson distribution of functional lesions, and it was assumed that the end point of tracer uptake F_s^* (the fraction of the cell population permeable to s),

$$F_s^* = 1 - \sum_{n=0}^{n_s-1} F(n), \quad [5]$$

was related to z_s by

$$F_s^* = 1 - e^{-z_s}, \quad [6]$$

i.e., a Poisson function. It is necessary to consider, therefore, whether the distribution of functional lesions $F(n_s^*)$ may be assumed to obey a Poisson function, and whether z_s (calculated by Eq. 6) is equivalent to $\bar{n}_s^*$ (as defined by Eq. 4).

It has been assumed that the distribution of C5b-9 complexes obeys a Poisson function (Eq. 2). A single functional unit has been defined operationally as the number of complexes (n_s) required to generate a unit area of permeable membrane, πr_s^2 , equivalent to the cross-sectional area of the diffusing solute. The number of detectable functional units on a given cell (n_s^*) is therefore:

$$n_s^* = \frac{n}{n_s} \quad \text{for } \frac{n}{n_s} \geq 1 \quad [7]$$

$$n_s^* = 0 \quad \text{for } n < n_s. \quad [8]$$

Note that, although the function $F(n)$ is assumed to be binomially distributed, $F(n_s^*)$ is not (except for the case $n_s = 1$): the distribution is skewed for all cases in which cells bind $n < n_s$ complexes and therefore are not permeable to s ($n_s^* = 0$).

According to the model, n_s increases as the radius of the diffusing solute increases (i.e., the minimal number of C5b-9 complexes required to form a detectable lesion of area πr_s^2 increases as r_s increases). Accordingly, it is expected that, as the size of the radiolabeled solute used to detect a functional lesion increases, the further z_s will deviate from the true population mean $\bar{n}_s^*$:

$$\bar{n}_s^* = \sum_{n_s^*} n_s^* F(n_s^*) \quad [9]$$

$$[\text{cf.}, z_s = -\ln(1 - F_s^*)].$$

In the analysis of the data of refs. 10 and 11, the calculation of an apparent single lesion permeability (P_s^*) was based upon the assumption that the distribution of lesions obeyed a Poisson function and that $\bar{n}_s^* = z_s$. For sucrose (table 2 of ref. 10), it was noted that P_s^* was relatively constant over a wide range of z_s , suggesting that the distribution of lesions obeyed a Poisson function conforming to the one-hit condition ($n_s = 1$). The data of figures 2–4 of ref. 11, however, reveal that, under the condition of low C5b-9 input, a sucrose-impermeant, inositol-permeant lesion is detected, suggesting that the minimal lesion formed by C5b-9 complexes must be smaller than sucrose ($r_s = 4.4 \text{ \AA}$) and therefore, that n_s (for sucrose) cannot be unity. From these observations, it would be expected that, under conditions of low C5b-9 input (i.e., cells with $n < n_s$ are prevalent), a nonlinear relationship between z_s and sucrose net flux should be observed. Unfortunately, accurate measurements under these conditions (low C5b-9 input) are difficult because the net flux is not substantially higher than background levels.

In hemolytic titrations with the complement components, it has been observed that cell hemolysis conforms to a one-hit model, assuming lesion distribution according to a Poisson function (12). In these titrations, the measured event (hemolysis) presumably follows the equilibration of Na^+ and K^+ [atomic radii, 0.95 and 1.3 $\text{\AA}$, respectively (13)] across the primary membrane lesion. As in the case for sucrose, if it is assumed that the effective membrane lesion at low C5b-9 multiplicity permits equilibration of these solutes, the apparent one-hit behavior of immune hemolysis can be reconciled with the current model.

Reevaluation of the kinetics of solute flow

In their analysis of the kinetics of solute flux across C5b-9 membranes, Sims and Lauf (10, 11) implicitly assumed that all cells permeable to the labeled solute were equally permeable and could be treated as a single compartment. In the case of a model that envisions lesions of various sizes, it is necessary to consider how the apparent flux (J_s) may be related to the distribution of functional lesions $F(n_s^*)$.

It is assumed that solute flow occurs by a diffusional process that is proportional to the effective area of the membrane lesion (A_n^*). For any cell, the unidirectional flux is given by:

$$j = [s] P^* A_n^* \quad [10]$$

in which P^* is the lesion permeability coefficient (in cm sec^{-1})

for solute s .[‡] The single cell flux (j) is therefore a function of the number of membrane bound complexes (n). The net apparent flux depends upon the number of cells in population (N_c) and the distribution of complexes,

$$J_s = \sum_n N_c j F(n) \quad [11]$$

$$= N_c [s] P^* \sum_n A_n^* F(n). \quad [12]$$

From Eq. 4, A_n^* may be expressed in terms of the radius of the diffusing molecule (r_s) and the distribution of functionally detectable lesion units:

$$J_s = N_c P^* [s] \pi r_s^2 \sum_{n_s^*} n_s^* F(n_s^*) \quad [13]$$

$$J_s = N_c P^* [s] \pi r_s^2 \bar{n}_s^*. \quad [14]$$

In their analysis of solute flow, Sims and Lauf (10, 11) defined an apparent single-lesion permeability (P_s^*) (in $\text{cm}^3 \text{sec}^{-1}$) in terms of the net flux (J_s) and the estimated mean number of lesions

$$P_s^* = \frac{J_s}{N_c z_s [s]}. \quad [15]$$

Substitution for J_s from Eq. 14 gives

$$P_s^* = \frac{N_c \bar{n}_s^* [s] P^* \pi r_s^2}{N_c z_s [s]}. \quad [16]$$

Because $z_s \approx n_s^*$ (see above),

$$P_s^* = P^* \pi r_s^2. \quad [17]$$

According to the model, therefore, the experimentally defined apparent permeability (P_s^* , in $\text{cm}^3 \text{sec}^{-1}$) is a constant related to the true permeability of the solute across the membrane lesion (P^* in $\text{cm} \text{sec}^{-1}$) by a factor equivalent to the cross-sectional area of the diffusing solute. As discussed previously (see ref. 10), P_s^* was specifically defined as an "apparent permeability" (in $\text{cm}^3 \text{sec}^{-1}$) to avoid assumptions about the cross-sectional area of the C5b-9 pore. In the context of the present model, however, the detectable unit area of membrane damage is defined as the cross-sectional area of the diffusing solute, and therefore a conventional permeability coefficient (representing the apparent velocity of membrane diffusion across a $1 - \text{cm}^2$ patch of the lesion) can be defined:

$$P^* = \frac{P_s^*}{\pi r_s^2} \text{ (in cm sec}^{-1}\text{)}. \quad [18]$$

From this coefficient, a lesion diffusivity (D_s^*) analogous to the conventional diffusion coefficient may be defined for each solute by assuming that the permeating molecule must diffuse across a patch of C5b-9 damaged membrane 75 Å thick:

$$D_s^* = P^* \times 75 \times 10^{-8} \text{ (in cm}^2 \text{sec}^{-1}\text{)}. \quad [19]$$

In Table 1 are compiled the calculated values of the lesion permeability (P^*) and diffusivity (D_s^*) of each solute as derived within the context of the present model. Note that, with the exception of inulin, the apparent diffusivity of each solute—derived from the C5b-9-specific solute flux by considering a volume element of the membrane equal to the collisional path

Table 1. Rate characteristics for solute diffusion across C5b-9 membranes

Cmpd.	r_s , Å	D , $(\text{cm}^2 \text{sec}^{-1}) \times 10^5$	P_s^* , $(\text{cm}^3 \text{sec}^{-1}) \times 10^{14}$	P^* , cm sec^{-1}	D_s^* , $(\text{cm}^2 \text{sec}^{-1}) \times 10^5$
M-Inositol	3.6	0.90	6.68	16.37	1.23
Sucrose	4.4	0.69	4.83	7.94	0.59
Raffinose	5.6	0.58	4.25	4.31	0.32
Inulin	14.8	0.21	4.46	0.65	0.05

The computed values of the apparent lesion permeability (P_s^*), the lesion permeability coefficient (P^*), and the apparent lesion diffusivity (D_s^*) are derived by using Eqs. 15–19 above and data of table 1 of ref. 11. Unit area of the membrane lesion was assumed to be πr_s^2 . Molecular radii (r_s) and aqueous diffusion coefficients (D , at 37°C) were obtained from ref. 11.

of the diffusing molecule—closely approximates the free diffusivity of each solute in water (defined by the Fick diffusion coefficient). Note that, by pore theory (14, 15), unimpeded diffusion cannot occur across a channel with an equivalent pore radius equal to the radius of the diffusing molecule itself, confirming the conclusion reached previously (11) that the unit C5b-9 lesion cannot be a fixed membrane channel. Biochemical evidence suggesting an alternative physical model of the membrane lesion—one which conforms to the theoretical model developed in the present study—will be considered.

Unstirred layers

Under certain conditions, the observed rate of solute permeation through a biological membrane may be significantly limited by the rate of diffusion across the unstirred layer of solvent at the membrane boundary (16, 17). It therefore is necessary to consider whether the apparent permeability of the C5b-9 membrane (as estimated from the measured isotopic diffusional flux) actually may represent the rate of diffusion of solute across this unstirred layer and not across the membrane lesion *per se*. The suggestion that the diffusivities of the measured solutes within a unit element of the C5b-9 damaged membrane are similar to their aqueous diffusivities (Table 1) makes consideration of unstirred layer effects especially critical.

In the case of diffusion through a limited element of the membrane, it is necessary to consider the access resistance experienced by the solute as it approaches the membrane.

The access resistance to a small circular pore has been calculated by Hall (18) and includes the convergence resistance to a hemisphere of solvent about the pore as well as the resistance contributed by the hemisphere itself:

$$R_{ul} = \frac{\rho}{4r} \quad [20]$$

in which R_{ul} is the total access resistance posed by the unstirred layer, ρ is aqueous resistivity, and r is the pore radius. Since ρ is the reciprocal of conductivity (g), the conductance (G_{ul}) through the unstirred layer is $G_{ul} = 1/R_{ul} = 4rg$, and the analogous diffusive permeability (in $\text{cm}^3 \text{sec}^{-1}$) is $\phi_{ul} = 4rD$, D being the aqueous diffusivity. The unstirred layer represents an impedance in series with the pore, and the apparent membrane permeability (P_s^* , see Eq. 15) is related to the true permeability through the membrane *per se* (P^{**}) by:

$$\frac{1}{P_s^*} = \frac{1}{P^{**}} + \frac{1}{\phi_{ul}}. \quad [21]$$

Division by pore area (in cm^2) to convert to terms of corresponding pore permeability coefficients (in cm sec^{-1} , see Eq. 17) gives

[‡] It should be noted that P^* is defined in terms of lesion area, not cell membrane area.

$$\frac{1}{P^*} = \frac{1}{P^{**}} + \frac{\pi r_s^2}{\phi_{ul}} \quad [22]$$

Rearranging and substituting for ϕ_{ul} :

$$\frac{1}{P^{**}} = \frac{1}{P^*} - \frac{\pi r_s}{4D} \quad [23]$$

In the analysis of solute diffusion across C5b-9 membranes, the lesion permeability (P^* , Table 1) was calculated from the experimental data by assuming a unit lesion area equivalent to the cross-sectional area of the diffusing molecule. Thus, the equivalent pore radius (r) of this unit of lesion area is the radius of the molecule (r_s , from Table 1). Eq. 23 therefore may be rewritten:

$$\frac{1}{P^{**}} = \frac{1}{P^*} - \frac{\pi r_s}{4D} \quad [24]$$

In Table 2 are compiled the computed values of the unit lesion permeability and diffusivity to each solute, after correction for the effects of the unstirred layer according to Eq. 24. Comparison of these values with those of Table 1 suggests that unstirred layer effects may have led to slight underestimation of the unit lesion permeabilities. Nevertheless, the error due to these effects would not have significantly altered the interpretation of the data.

Discussion

Limitations to the Model. In order to derive meaningful transport values for the C5b-9 specific conductance pathway directly from the observed diffusion of solute across complement damaged membranes, an attempt has been made to formulate the membrane lesion in terms of a strictly operational transport model, independent of *a priori* assumptions about the molecular mechanism of membrane damage. Nevertheless, because solute flow across erythrocyte membranes can only be observed for large cell populations, this analysis required that an estimate of the average cellular leak be derived from the total leak flux and an assumed distribution of contributing leaks from individual cells. To estimate the cellular distribution of membrane leak fluxes, assumptions about both the cellular distribution of C5b-9 complexes [assumed to obey a Poisson function (above and ref. 10)] and the functional dependence of the membrane leak on the multiplicity of bound C5b-9 were required. Thus, in the derivation of Eq. 3 it was implicitly assumed that the membrane lesion expands in a direct linear fashion with increasing multiplicity of C5b-9 monomer. Although this assumption is in qualitative agreement with the data of ref. 11, it is necessary to caution that there is no experimental basis for a strictly linear dependence of A^* on C5b-9 monomer. It therefore would be inappropriate to derive a quantitative estimate of either the physical dimension of the unit C5b-9 membrane complex or the state of aggregation of the C5b-9 monomer.

Table 2. Correction for access resistance due to unstirred layers

Cmpd.	r_s , Å	P^{**} , cm sec ⁻¹	D_s^{**} , (cm ² sec ⁻¹) × 10 ⁵
m-Inositol	3.6	17.3	1.30
Sucrose	4.4	8.27	0.62
Raffinose	5.6	4.46	0.33
Inulin	14.8	0.67	0.05

Values of the C5b-9 lesion permeability coefficient (P^{**}) and apparent lesion diffusivity (D_s^{**}) from Table 1 computed after correction for the effect of unstirred layers according to Eq. 24.

Evidence has been presented (11) on, and the present model assumes, cell-to-cell variability of the functional size of the membrane lesion, corresponding to the multiplicity of membrane-bound C5b-9. Therefore, one might expect that the rate constant for solute influx varies from cell to cell according to the distribution of C5b-9 and that, for measurements made on the total population, non-first-order kinetics would be observed. Nevertheless, as previously discussed (10, 11), within the resolution of the methods used first-order kinetics have been observed for the steady-state flux of all nonelectrolytes tested (semilogarithmic regressions, >0.98). In considering why apparent first-order kinetics are observed under circumstances suggesting lesion heterogeneity, it should be noted that under the circumstances of the experiments for which rate data were obtained, calculated values of z_s (from the end point of exchange) did not exceed unity (10, 11), suggesting that cellular lesions among the population contributing to the observed flux were narrowly distributed.

For example, under the conditions of C5b-9 input for which rate data are reported in ref. 11, even for the smallest solute (*m*-inositol), one can estimate from the end point of exchange ($F_s^* = 0.41$) that cells with $n_s^* > 1$ functional lesions accounted for only 10% of the cells (assuming distribution according to a Poisson function). Furthermore, if one assumes that the rate constant of the leak for cells with $n_s^* \geq 2$ is at least twice that of the first-order rate constant observed for the net flux [3.79 hr⁻¹ (11)], then at least 0.63 time constants have elapsed before the first data point was obtained (5 min), and at least 1.9 time constants have elapsed by the second (15 min). Accordingly, one might expect that significant deviation from apparent first-order kinetics would be observed only under conditions for which a high multiplicity of functional lesions is generated ($z_s \gg 1$) and very rapid sampling during the initial period of tracer solute diffusion is experimentally obtained.

Speculation About the Molecular Mechanism of Lesion Formation. Consideration of the permeability characteristics of the complement-damaged membrane has led to the proposal of a transport model that operationally defines the membrane lesion strictly in terms of a C5b-9-specific leak pathway. Analysis of the data in terms of this model suggests that solute flow (at least for nonelectrolytes with molecular radii of 3–6 Å) is unimpeded, when diffusion is considered within a minimal lesion unit, arbitrarily defined as the collisional volume element traversed by the diffusing solute.

Because this model suggests a C5b-9-specific disruption of the permeability barrier intrinsic to the ordered lamellar structure of membrane lipid, it is necessary to consider the molecular basis for a possible detergent-like action of the complement proteins.

As has been previously reviewed, substantial evidence has accumulated to suggest that the C5b-9 complex is a highly lipophilic structure that intimately binds large quantities of membrane lipid (3). The data of these studies suggest that, as a consequence of the activation of complement against both biologic and liposomal (lipid bilayer) membranes, not only are extensive structural domains of the C5b-9 proteins intercalated into the membrane interior but also it would appear that the structural integrity of the membrane is compromised, as evidenced by the release of significant amounts of intrinsic phospholipid in the form of soluble micellar aggregates.

Recently, Müller-Eberhard and collaborators (19–22) suggested that the functional unit for the membranolytic action of the complement proteins is not the C5b-9 monomer as previously assumed but rather at least a dimeric aggregate (C5b-9)₂ that can be extracted from the membrane as a mixed micellar structure binding phospholipid at a molar ratio of about 1460:1.

Based upon evidence for the micellar organization of lipid bound to the (C5b-9)₂ dimer in the fluid phase and for the reorientation of membrane phospholipid about the C5b-9 proteins *in situ*, they proposed a molecular model for the membrane lesion which envisions a localized lamellar-to-micellar phase transition of membrane lipid. It is proposed that this membrane complex results in loss of cellular permeability control due to the instability of the lipid structure at the boundary regions between lamellar and micellar phases (20, 21).

The implication of this model is that the functional nature of the lesion can change as larger domains of membrane lipid are disrupted by the insertion of additional C5b-9 complexes. This model, therefore, represents one physical analogue of the theoretical analysis developed in the present study and *prima facie* accounts for the apparent heterogeneity in lesion size (related to C5b-9 multiplicity) noted in the experiments previously discussed (11). One might also speculate that, according to such a model, the organization of membrane lipid may undergo very rapid C5b-9 induced lamellar-micellar fluctuations within a molecular time frame, thus accounting for the comparatively slow rate of permeation by small ions and nonelectrolytes (as measured against a time scale of minutes) compared to the significant degree of membrane permeation by rather large molecules (of the size of inulin). Production of transient high dielectric "gaps" for unimpeded solute diffusion by such fluctuations might also account for the rather surprising conclusion of the present study, that molecules permeating C5b-9 membranes appear to diffuse freely—on consideration of the volume element equivalent to their respective collisional paths—and do not appear to be subject to the relative diffusional restraints characteristic of passage through fixed pores.

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