Glucose Transport through Cell Membranes of Modified Lipid Fluidity†

I. Yuli, W. Wilbrandt,‡ and M. Shinitzky*

ABSTRACT: Carrier-mediated transport of glucose in human erythrocytes and 3T3 mouse fibroblasts was examined at different lipid viscosities of the cell membrane. Rigidification of the membrane lipid layer was accomplished by incorporation of cholesterol or one of the hydrophilic esters, cholesteryl hemisuccinate or cholesteryl betainate, whereas fluidization was accomplished by cholesterol depletion. In both cells the dependence of the maximal rate of glucose transport at 37 °C, V_{max}, on the lipid microviscosity of the cell plasma membrane, $\bar{\eta}$, is of a similar pattern which does not obey simple diffusion considerations. When the $\bar{\eta}$ value of untreated cells is slightly increased (10–20%), V_{max} increases to a peak value, beyond which a further increase in $\bar{\eta}$ progressively reduces it. Decrease of the natural $\bar{\eta}$ is also accompanied by a progressive decrease of V_{max}. This general pattern was also observed for the transport of α-aminoisobutyric acid in 3T3 fibroblasts (unpublished results). A theoretical analysis of the dependence on $\bar{\eta}$ of the transport turnover number and of the accessibility of carrier sites was carried out in order to account for this behavior. On the basis of this analysis, a general expression for the dependence of V_{max} on $\bar{\eta}$, which fits reasonably well with the experimental data, was derived. This expression is also valid for the dependence on $\bar{\eta}$ of the overt activity of membrane-bound enzymes and receptors.

Facilitated transport through biological membranes is executed by operationally defined protein carriers (Wilbrandt, 1972; Lauger, 1972). The mode of action of these carriers involves at least one step of mechanical movement, which for most cases is rate limiting. Since the carrier presumably spans the membrane, its motion and therefore its rate of operation are determined to a large extent by the viscosity of the membrane lipid layer. Models offered for carrier-mediated transport therefore assume a rate of transport which is linearly dependent on diffusion rate constant or inversely proportional to the lipid viscosity, the latter acting as the retardation force of the transport process (Kotyk & Janacek, 1969).

Transport of glucose is characterized as carrier mediated (Stein, 1972; Olefsky, 1978; Jung et al., 1980). Verification of the relation between membrane lipid fluidity and the transport of glucose was studied in various cells after treatment with fluidity-modulating agents (Plagemann & Erbe, 1975; Read & McElhaney, 1976; Pilch et al., 1980). No clear and unequivocal dependence for transport rate and ligand affinity could be drawn from these studies. Furthermore, counter to the expected trend, lipid fluidization by cholesterol depletion was found to reduce glucose transport in human erythrocytes (Masiak & Le Fevre, 1974) or in LM mouse fibroblasts (Saito & Silbert, 1979).

As outlined above, carrier-mediated transport can be strongly affected by the membrane lipid viscosity, but in a complex manner which is markedly different from the presumed linear dependence on diffusion. In this study we have examined the effect of lipid viscosity on the carrier-mediated transport of glucose in human erythrocytes and mouse fibroblasts. A comprehensive analysis of the effect of lipid viscosity on the transport kinetic constants, which can account for the experimental data, is presented.

Materials and Methods

Chemicals. Cholesterol (Chol-OH) of high purity was purchased from Steraloids Inc. (Pawling, NY). Cholesteryl hemisuccinate, +99% (CHS, Chol-$OCOCH_2CH_2COO^-$), was purchased from Pfaltz and Bauer (Stanford, CT). Cholesteryl betainate [CB, Chol-$OCOCH_2N^+(CH_3)_3$] was prepared by bubbling trimethylamine gas (Fluka, Buchs, Switzerland) into a solution of cholesteryl chloroacetate (Sigma Chemical Co., St. Louis, MO) in chloroform, at 60 °C. The reaction was followed by monitoring inorganic chloride and stopped after 18 h. Crystallization was afforded from methanol, yielding 96% pure material. Poly(vinylpyrrolidone) (PVP), M_r 40000, pharmaceutical grade, and bovine serum albumin (BSA), RIA grade, were obtained from Sigma. Egg lecithin, grade I, was purchased from Lipid Products (South Nutfield, England).

[^{3}H]Cholesteryl hemisuccinate was prepared by reacting [^{3}H]cholesterol (Amersham, Buckinghampshire, England, 150 mCi/mg) with excess succinyl dichloride in dry pyridine for 1 h, followed by addition of water. The product was isolated by preparative thin-layer chromatography on silica gel G-60 (Merck, Darmstadt, West Germany), eluted with ethyl acetate–benzene, 1:5 v/v, and was kept in chloroform. 2-Deoxy[^{3}H]glucose (2dG, 18.2 Ci/mmol) was obtained from Amersham.

Cells. Human erythrocytes were isolated from freshly drawn blood and washed 3 times with phosphate-buffered saline, pH 7.2 (PBS). BALB/c-3T3 mouse fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM, Biolab, Jerusalem, Israel), containing 10% heat-inactivated fetal calf serum (Biolab) in 5% CO_2-humidified atmosphere at 37 °C. Cells were passaged every 3–4 days before reaching confluency.

Modulation of Membrane Lipid Viscosity. Washed erythrocytes were incubated at 37 °C for up to 48 h in cholesterol-enriched medium containing sonicated liposomes of 4 mg/mL cholesterol–egg lecithin (approximately 2:1 mol ratio) in PBS (Cooper et al., 1975). Cholesterol depletion was accomplished analogously by incubation with liposomes of egg lecithin, 2.5 mg/mL (Cooper et al., 1975). The ratio of cholesterol to phospholipid in the treated erythrocytes was determined with isolated membranes (Dodge et al., 1963) by chemical analysis of cholesterol (Brown et al., 1954) and organic phosphate (Bottcher et al., 1961). Cholesteryl hemisuccinate and cholesteryl betainate were incorporated into cell membranes (erythrocytes and fibroblasts) via dispersion in PVP (Shinitzky et al., 1979). A stock solution of 250 mg/mL in tetrahydrofuran of the cholesterol esters was diluted 500-fold with vigorous mixing into a medium consisting of 3.5% PVP

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‡ Pharmakologisches Institut, 3000 Berne, Switzerland; deceased.

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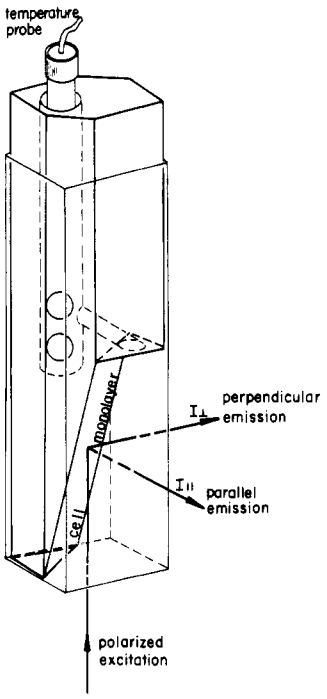

FIGURE 1: Optical arrangement for fluorescence depolarization measurement of cells in a monolayer. Cells, cultured on a microscope cover slip (24 × 10 mm), are placed outward on a black perspex plane mounted in a quartz cuvette containing 2 mL of PBS. The buffer fills the remaining free space which includes a temperature probe. The excitation-polarized beam enters the cuvette through the bottom wall, and the emitted fluorescence is collected through analyzers in two mutually perpendicular directions. Most of the reflection generated by the cover slip is absorbed by the black support.

and 2% BSA in PBS. The dispersion was briefly sonicated (30 s, 30 W), then diluted 10-fold with the cells, and allowed to incubate for up to 2 h at 37 °C.

Transport Assays. Glucose efflux from preloaded erythrocytes was measured by the method of Sen & Widdas (1962), monitoring the change in light scattering during glucose exit (Wilbrandt, 1978). Transport in fibroblasts was measured in quiescent monolayers depleted of intracellular glucose by incubation with DMEM without glucose, containing 1 mg/mL BSA for 60–90 min at 37 °C. Glucose transport was assayed by incubating the cell monolayer (~2 × 10^5 cells) with 0.225 mL of 50 mM [^{3}H]2dG in DMEM containing 1 mg/mL BSA, devoid of glucose for 5 min at 37 °C. These conditions were verified to provide the maximal rate of transport.

Fluorescence Polarization Measurements. Suspensions of erythrocyte membranes (Dodge et al., 1963) were labeled with 1,6-diphenyl-1,3,5-hexatriene (DPH), and the degree of fluorescence polarization was measured as previously described (Shinitzky & Inbar, 1976). Fibroblasts, grown as a monolayer on thin glass plates, were labeled with DPH without detachment as described (de Laat et al., 1977). For measurements with monolayer of cells in a fixed orientation, a special fluorescence polarization setup, similar to that described by de Laat et al. (1977), was constructed. Figure 1 illustrates the optical arrangement and the sample holder of the setup. The polarized excitation beam (2 mW He–Ca laser, 326 nm, Liconix Model 403) enters into the cuvette from the bottom, and the emission is detected at two mutual perpendicular directions after passing through analyzers of fixed orientation and appropriate cutoff filters. The cell layer faces the external medium buffer and is exposed to the excitation beam (see Figure 1). Excited-state lifetime, τ, of DPH-labeled membranes was measured by the pulse-sampling method, combined with deconvolution analysis (Grinvald & Steinberg, 1974).

Evaluation of Lipid Microviscosity. The apparent microviscosity ($\bar{\eta}$) of the lipid core in human erythrocyte membranes was derived from the fluorescence depolarization of DPH [for review, see Shinitzky & Barenholz (1978)]. Since DPH in fibroblasts can be incorporated into intracellular organelles (Berlin & Freg, 1977; Collard & de Wildt, 1978), we have applied a recent method (Grunberger & Shinitzky, 1981) for the resolution of the DPH fluorescence signal emitted from the plasma membrane. In this method the fluorescence signal from the plasma membrane is quenched by reacting the cells with the impermeable reagent 2,4,6-trinitrobenzenesulfonic acid (TNBS). Accordingly, the apparent fluorescence intensity (F) and anisotropy (r) of DPH-labeled monolayers were first measured, and then, without removal of the cell sample from the compartment, the monolayer was incubated with 2 mg/mL TNBS in PBS at 4 °C for 1 h. After two washes with PBS, the fluorescence intensity (F_{in}) and anisotropy (r_{in}) were measured. For all cell samples measured, the residual fluorescence after TNBS quenching, which was attributed to intracellular fluorescence, was about 70%. A simulation to a binary system comprised of the cell plasma membrane fluorescence, which is assumed to be fully quenched by TNBS (Grunberger & Shinitzky, 1981), and intracellular fluorescence will yield (Weber, 1952)

$$r_m = (Fr - F_{in}r_{in})/(F - F_{in}) \quad (1)$$

The evaluated fluorescence anisotropy relating to the plasma membrane, r_m, could then be used for derivation of the corresponding $\bar{\eta}$ (Shinitzky & Barenholz, 1978). The dependence of log $\bar{\eta}$ on the reciprocal of absolute temperature ($1/T$) was found to be linear with the corresponding flow activation energy of 7 kcal/mol for both the erythrocytes and fibroblasts (Shinitzky & Barenholz, 1978). Accordingly, $\bar{\eta}_{25°C}/\bar{\eta}_{37°C}$ = 1.6, which could be used for estimation of $\bar{\eta}_{37°C}$ from the $\bar{\eta}_{25°C}$ values presented throughout this study.

Computer Analysis. Least-squares fitting was performed with the aid of the weighted NLIN procedure of the Statistical Analysis System computation package (SAS Institute Inc., Cary, NC). Experimental data were weighed according to standard deviation.

Results

Glucose Efflux in Human Erythrocyte. In a large series of experiments human erythrocytes were either enriched with or depleted of cholesterol (see Materials and Methods), resulting in 0.6–3.0 mg of cholesterol/mL of packed cells, corresponding to a 0.5–2.5 cholesterol to phospholipid mole ratio (C/PL). The related range of the membrane lipid microviscosity ($\bar{\eta}$), as determined by DPH fluorescence polarization and excited-state lifetime, was 3–12 P at 25 °C. The average excited-state lifetime of DPH, τ, ranges between 9.5 ns in the cholesterol-depleted membranes and 10.8 ns in the cholesterol-enriched membranes. These values are close to the limiting value of 11.4 ns (Shinitzky & Barenholz, 1974). It should be noted that a minor component (5–10%) of a short τ of 2–3 ns could be resolved from the fluorescence decay curves. Such a component was also resolved by Klausner et al. (1980) in the lymphocyte plasma membrane labeled by DPH. However, the autofluorescence of the human erythrocyte membrane, excited and detected at the same wavelengths as DPH, was of the same lifetime as this minor component. This apparent heterogeneity was not treated here any further. At the determined range of $\bar{\eta}$ a linear correlation between C/PL and $\bar{\eta}_{25°C}$ was observed, which obeys the empirical function

$$\bar{\eta}_{25°C} = (1.84 \pm 0.03) + (3.55 \pm 0.03)(C/PL) \quad (2)$$

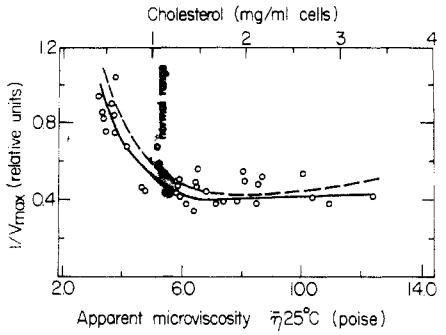

FIGURE 2: Dependence of the reciprocal maximal rate of glucose efflux from human erythrocytes at 37 °C ($1/V_{max}$) on apparent membrane microviscosity. Modulation of microviscosity was achieved by incubation with liposomes of various cholesterol to lecithin ratios for different time intervals. The final content of cholesterol in the treated erythrocytes is also presented. The solid curve represents the experimental pattern, whereas the dashed curve was obtained from data fitting according to eq 14.

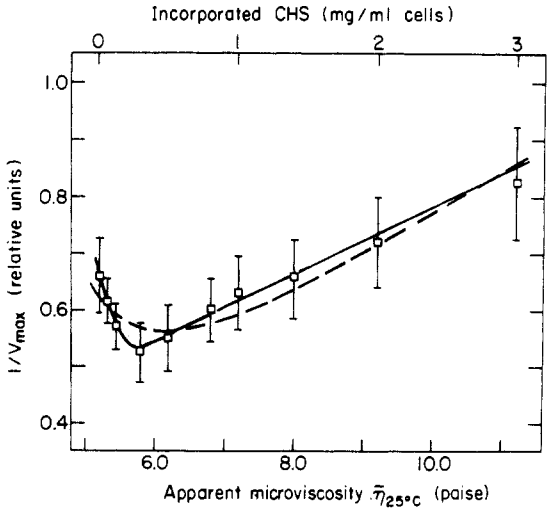

FIGURE 3: Effect of apparent microviscosity, modulated by incorporation of cholesteryl hemisuccinate (CHS), on the reciprocal maximal rate of glucose efflux ($1/V_{max}$) in human erythrocytes at 37 °C. The squares represent the mean of six to nine independent experiments, and the bars indicate their standard deviation. The experimental pattern is given by the solid line, whereas the dashed line was computed for data fitting according to eq 14.

Figure 2 summarizes the results of the reciprocal of maximal rate of glucose efflux ($1/V_{max}$), obtained with these cells as a function of their membrane cholesterol level or lipid microviscosity. For further verification of the effect of lipid fluidity on $1/V_{max}$ of glucose efflux, erythrocytes were enriched with the hydrophilic cholesterol esters, cholesteryl hemisuccinate (CHS), and cholesteryl betainate (CB), which can render an increase in lipid viscosity similar to the effect of cholesterol (Shinitzky et al., 1980). Both of these esters (CE) obey the linear relation when added to the untreated membrane

$$\bar{\eta}_{25°C} = (5.26 \pm 0.02) + (2.21 \pm 0.23)(CE/PL) \quad (3)$$

The dependence of $1/V_{max}$ on the lipid microviscosity, modulated by incorporation of CHS or CB, is shown in Figures 3 and 4.

Glucose Transport in Mouse 3T3 Fibroblasts. Attempts to alter the cholesterol content in 3T3 fibroblasts by the conventional liposome treatment (Cooper et al., 1975) resulted in a substantial decrease in cell viability. Therefore, increase in membrane microviscosity was approached by incorporation of CHS, administrated as a dispersion in PVP (see Materials and Methods). A profile of CHS incorporation, as traced with

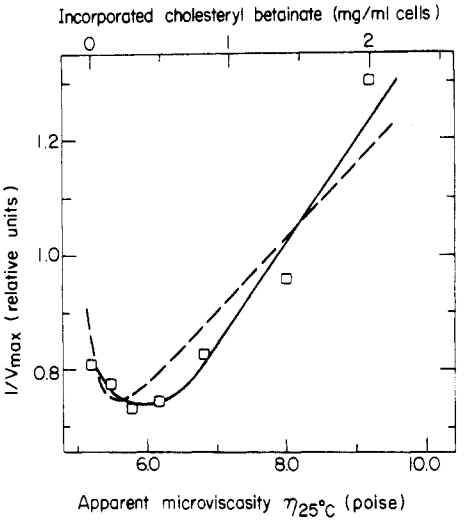

FIGURE 4: Effect of apparent microviscosity, modulated by incorporation of cholesteryl betainate (CB), on the reciprocal maximal rate of glucose efflux ($1/V_{max}$) in human erythrocytes at 37 °C. The squares and the solid lines represent a single set of experiments. The dashed line was computed for data fitting according to eq 14.

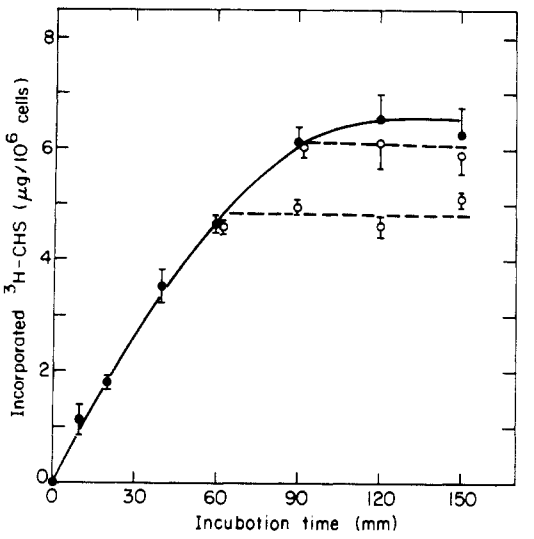

FIGURE 5: Time-dependent incorporation of [^{3}H]CHS into 3T3 mouse fibroblasts. The solid circles represent six independent experiments (bars represent the standard deviation). Open circles represent combined results of successive incubation with dispersions of lecithin–PVP (four experiments) or PVP (four experiments) after 60 or 90 min of [^{3}H]CHS incorporation (bars represent standard deviation of the combined results).

[^{3}H]CHS, is shown in Figure 5, indicating a linear rate of incorporation during the first 50 min of incubation. The incorporated CHS seems to be well integrated into the membrane lipids, since any successive incubation of the CHS-treated cells with a PVP solution or even with a 100 μg/mL lecithin–PVP dispersion did not delete it out (see Figure 5). At various levels of incorporated CHS, the DPH fluorescence anisotropies from the plasma membrane and from the intracellular organelles were resolved (see Materials and Methods) and are given in Figure 6. As clearly shown, CHS exclusively effects the TNBS-quenched compartment, which suggests that the CHS does not partition into intracellular membranes during the course of the experiment.

Uptake of [^{3}H]2dG by 3T3 fibroblasts was carried out at various levels of CHS incorporation. Cells were first incubated for up to 70 min in a CHS dispersion (see Materials and Methods), and the apparent $\bar{\eta}$ was evaluated at 25 °C. For each incubation time six to nine separate 2dG uptake exper-

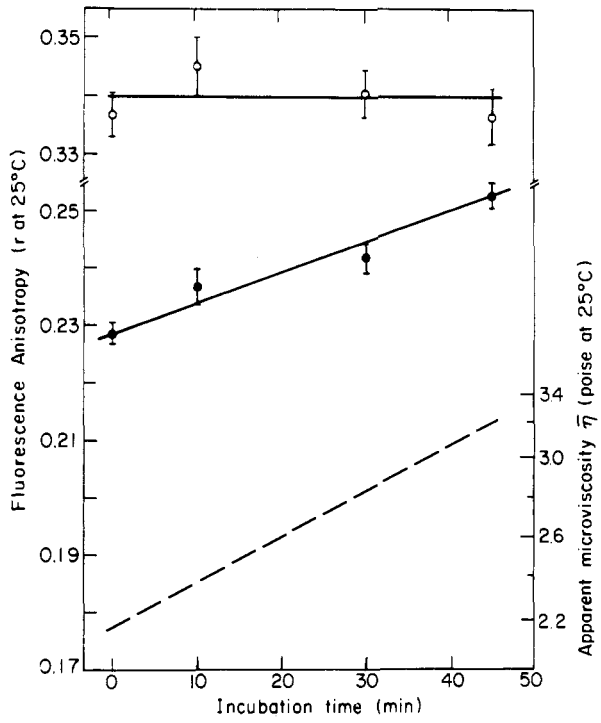

FIGURE 6: Effect of incorporated CHS into 3T3 mouse fibroblasts on the DPH fluorescence anisotropy measured in cell monolayers before (●) and after (○) treatment with TNBS. The dashed line is the calculated residual DPH fluorescence anisotropy emitted from the quenched plasma membrane and its translation to apparent microviscosity (right ordinate).

iments were carried out. The results are summarized in Figure 7.

Discussion

Passive transport of glucose (Morgan & Neely, 1972), across a biological membrane, is characterized as carrier mediated. Despite the kinetic complexity of this process, it can formally be simulated by the Michaelis–Menten scheme for enzymatic activity (Janacek & Kotyk, 1969) and is assumed to be linearly dependent on the rate of diffusion of the carrier (Janacek & Kotyk, 1969), irrespective of the actual microscopic steps of the process.

Passive transport of glucose in human erythrocytes and mouse 3T3 fibroblasts was demonstrated in this study to be affected markedly by the membrane cholesterol level. Upon mild cholesterol enrichment the transport is significantly enhanced, until it reaches a maximal rate which, upon further enrichment, is decreased progressively (Figures 2–4 and 7). Furthermore, depletion of cholesterol from erythrocytes decreases the rate of the glucose transport markedly (Figure 2), similarly to that reported by Masiak & Le Fevre (1974). Such a trend was also observed in LM mouse fibroblasts where cholesterol depletion was found to reduce the rate of 3-*O*-methylglucose uptake (Saito & Silbert, 1979).

Since cholesterol and its hydrophilic esters, CHS and CB, which are considerably different in their hydrophilic head groups, similarly affected the glucose transport, it may be assumed that the effect is primarily mediated by the membrane lipid fluidity. Yet, expressing the cholesterol level in lipid microviscosity terms is inconsistent with the carrier diffusion model, which predicts a monotonous decrease in transport rate upon an increase in the ambient viscosity (Janacek & Kotyk, 1969). This inconsistency could, however, be accounted for by assuming that the number of carriers, in which the substrate recognition sites are accessible to binding, can vary with lipid viscosity by a "passive modulation" mechanism (Shinitzky,

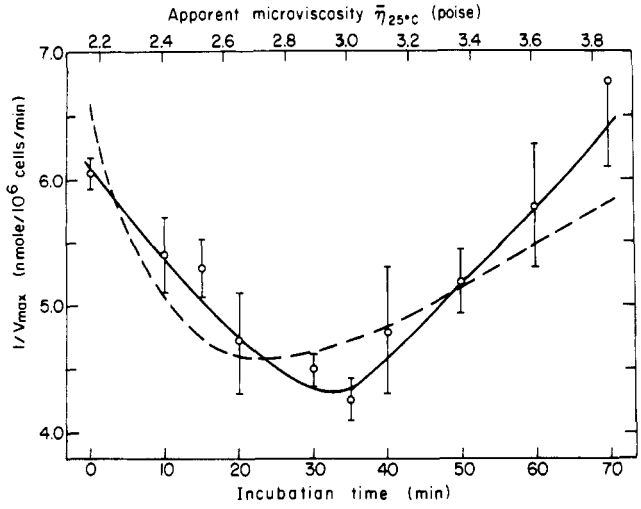

FIGURE 7: Effect of incorporated CHS (presented in a scale of incubation time) on the uptake of 2-deoxy-*d*-[^{3}H]glucose in monolayers of 3T3 mouse fibroblasts. The experimental points represent the mean and the standard deviation of 6–9 independent measurements. The solid line represents the experimental pattern, and the dashed line was computed for data fitting according to eq 14.

1979). Carriers with masked binding sites can, therefore, be assumed to remain dormant. Thus, while the rate of diffusion and, therefore, the rate of transport of each carrier site decrease upon an increase in lipid viscosity, the number of operating sites can increase concomitantly. The overt transport rate can, therefore, increase or decrease according to the relative weight of each of these two counteracting trends along a scale which can be represented by the lipid fluidity. It should be stressed, however, that a fully quantitative description of membrane lipid viscosity is, in principle, of formidable complexity, due to its many anisotropic features (Saffman & Delburck, 1975; Evans & Hochmuth, 1978; Shinitzky & Henkart, 1979). Diffusion considerations in membrane transport are, therefore, still limited to isotropic systems, where the average viscosity parameters are the operational units. The average lipid microviscosity, as derived from fluorescence depolarization with DPH and described in macroscopic units (i.e., poise), was found to present well the dynamics of membrane lipid layers, especially when correlated with the cholesterol level (Shinitzky & Inbar, 1976; Shinitzky & Barenholz, 1978). The approach presented in the following is based on the averages of both the transport activity and the apparent microviscosity. Obviously, the statistical heterogeneity of carrier activity between cells themselves and within each individual cell membrane is inherent in such systems. Furthermore, from the teleological point of view, the in vivo restoration of impaired membrane viscosity, due to external and environmental conditions, is accomplished by alternative and interchangeable (Baldassare et al., 1979; Sinensky, 1980) pathways of alteration in either the cholesterol level or the phospholipid acyl chains. In these processes of homeoviscous adaptation (Hazel & Prosser, 1974; Cossins, 1977) the microviscosity, as derived from the DPH fluorescence anisotropy, was found to be restored to its original level, which indicates a direct relevance of the membrane functions to the average physical state of the lipid region rather than to the nature of the microscopic domains.

The use of viscosity parameters which, by definition, are kinetic terms poses another fundamental problem while discussing membrane thermodynamic disciplines. This, however, can be overcome by the use of the empirical relation for the nonassociated liquids (Batschinski, 1913)

$$\eta = C/[\bar{V} - w] \quad (4)$$

where η is the liquid viscosity, $\bar{V}$ is the specific volume (i.e., the reciprocal of the density), w is the limiting volume (at infinite viscosity), and C is a proportionality factor. The denominator $[\bar{V} - w]$ expresses the "free volume" of the fluid. Equation 4 holds exceptionally well for a nonassociated series of liquids like hydrocarbons and weakly associated liquids at temperatures far from the solidification point (Batschinski, 1913; Bingham & Kinney, 1940) and reveals a linear correlation between the free volume and the reciprocal of viscosity (i.e., the fluidity). In the case of membranes eq 4 can be used for delineation of microviscosity in terms of the free volume of the lipid core. On the basis of the above, a quantitative evaluation of the dependence of carrier-mediated transport on the membrane lipid viscosity is presented in the following.

The main experimental characteristic of membrane transport is the maximal rate, V_{max}, which according to the Michaelis–Menten scheme is the product of the transport rate constant, k_{tr}, and the apparent concentration of the operating sites, C_+

$$V_{max} = k_{tr}C_+ = k_{tr}\alpha C_0 \qquad (5)$$

where α is the accessibility factor ($0 < \alpha < 1$). As stated above, the number of operating carriers may vary as a result of the distribution of the total number of existing carriers, C_0, between the operating (C_+) and the dormant (C_-) forms. If one assumes that each of the sites can thermally fluctuate between these two forms, a simulation of distribution can be ascribed to a quasi-equilibrium

$$C_- \underset{k_2}{\overset{k_1}{\rightleftharpoons}} C_+ \qquad k_1/k_2 = C_+/C_- \qquad (6)$$

The fraction of operating sites, α, which represents a statistical integration over time and area, is

$$\alpha = C_+/C_0 = 1/[1 + (k_2/k_1)] \qquad (7)$$

The kinetic constants, k_1 and k_2, determine the shuttling between the operative and the dormant forms of the carriers and, therefore, depend on the lipid free volume which is the main determinant of the solubilizing capacity of the lipid layer. This is supported by the observation that changes in lipid viscosity were found to change the degree of accessibility of various membrane proteins (Borochov & Shinitzky, 1976; Borochov et al., 1979; Shinitzky & Souroujon, 1979). Qualitatively, while k_1 increases with $\bar{\eta}$, probably due to the decrease in the average free volume of the lipid core, thus increasing the relative exposure of the carrier on the surface of the membrane, k_2 will decrease with $\bar{\eta}$ in the same manner. In a simple hypothetical case, the ratio of k_1/k_2 should be a second power of $\bar{\eta}$. However, in the real membrane, it is reasonable to assume that $k_1/k_2 \propto \bar{\eta}^m$, where m is a constant specific to the carrier system under discussion. One can further normalize $\bar{\eta}$ to units of $\bar{\eta}_{1/2}$, the specific viscosity at which half of the sites are in the operating form, yielding

$$\alpha = 1/[1 + (\bar{\eta}/\bar{\eta}_{1/2})^{-m}] = 1/[1 + \tilde{\eta}^{-m}] \qquad (8)$$

A family of computed curves of $\alpha = f(\tilde{\eta})$, presented according to eq 8, is shown in Figure 8. The power m can be regarded as an "expansion factor", which characterizes the degree of cooperativity between the lipid viscosity and the statistical accessibility of the sites. Positive m represents an increase in the site accessibility with $\tilde{\eta}$, while negative m represents the converse trend. In either direction, when $0 < |m| < 1$, the modulation of site accessibility is of a negative cooperativity form, whereas for $|m| > 1$ the modulation is positively cooperative (see Figure 8).

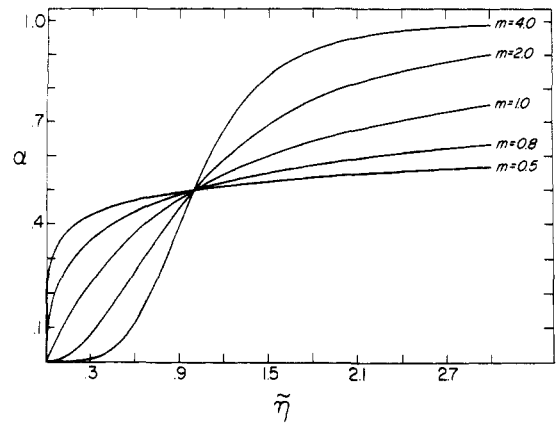

FIGURE 8: Computed curves describing the accessibility factor as a function of membrane viscosity according to eq 8.

In the general case of a heterogeneous population, modulation of accessibility can be simulated by a combination of fully affected sites (eq 8) and a constant fraction, f, in the operating form, which extends the expression for α to the general form

$$\alpha = [1 + f\tilde{\eta}^{-m}]/[1 + \tilde{\eta}^{-m}] \qquad (9)$$

where $0 < f < 1$ and therefore $f < \alpha < 1$.

The dependence of k_{tr}, which determines V_{max} (eq 5), on lipid viscosity can be derived from the analogy between membrane transport and enzymic activity. Accordingly, k_{tr} is inversely proportional to the ambient viscosity, as was shown by Gavish & Werber (1979)

$$k_{tr} = \eta^{-1} \exp(-\Delta E^*/RT) \qquad (10)$$

The presumed reciprocal relation, between k_{tr} and η, is also implied when local changes in volume, due to the activation process of the transport, are considered (Low & Somero, 1975). The energy barrier of the change in volume in the active state of the occupied site (ΔV^*) at a pressure P is $P\Delta V^*$, and, therefore

$$k_{tr} = k_{tr}^0 \exp(-P\Delta V^*/RT) \qquad (11)$$

where k_{tr}^0 corresponds to the rate constant at zero pressure. The dependence of viscosity on pressure can be expressed analogously by the flow activation volume ΔV^+ (Hirai & Eyring, 1958):

$$\eta = \eta_0 \exp(P\Delta V^+/RT) \qquad (12)$$

Since the activation of the transport is actually the initiation of some motion through the membrane, it could be assumed to be strongly coupled to the lipid activation of flow, and, therefore, $\Delta V^* \approx \Delta V^+$. This again indicates that k_{tr} is approximately inversely proportional to η (see eq 11 and 12).

Substitution of α (eq 9) and $k_{tr} \propto \bar{\eta}^{-1}$ (eq 10) in eq 5 yields the general dependence of V_{max} on $\bar{\eta}$

$$V_{max} \propto \bar{\eta}^{-1}[1 + f\tilde{\eta}^{-m}]/[1 + \tilde{\eta}^{-m}] \qquad (13)$$

For data fitting, eq 13 can be presented in the convenient form

$$1/V_{max} = A\bar{\eta}[1 + \tilde{\eta}^{-m}]/[1 + f\tilde{\eta}^{-m}] \qquad (14)$$

A is a constant which is

$$A = A_0C_0^{-1} \exp(\Delta E^*/RT) \qquad (15)$$

where C_0 is the total number of transport carriers, ΔE^* is the activation energy of the transport, and A_0 is a proportionality factor. According to eq 14, when all sites are affected by the lipid viscosity (i.e., $f = 0$), the dependence of $1/V_{max}$ on $\bar{\eta}$ has

Table I: Fitted Parameters (±SE) for Dependence of the Maximal Rate of Glucose Transport (V_{max} at 37 °C) on Membrane Microviscosity

cell	viscosity modulator	fitted parameter $\bar{\eta}_{1/2}$ (P) 25 °C	$\bar{\eta}_{1/2}$ (P) 37 °C	m	asymptotic correlation between $\bar{\eta}_{1/2}$ and m
human erythrocyte	cholesterol	6.1 (±0.5)	3.8 (±0.3)	3.8 (±1.0)	0.73
	CHS	4.7 (±0.2)	3.0 (±0.1)	6.1 (±1.8)	0.76
	CB	4.9 (±0.5)	3.1 (±0.4)	>10	0.96
BALB/c-3T3	CHS	2.12 (±0.05)	1.33 (±0.03)	8.4 (±2.8)	0.23

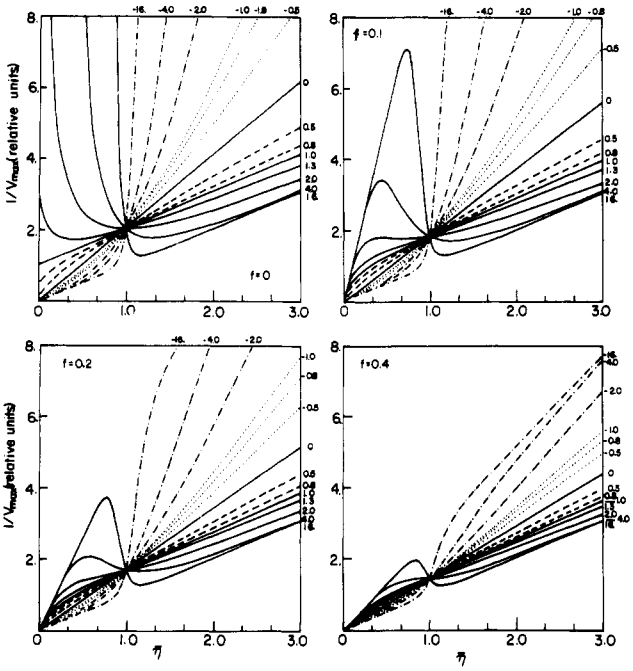

FIGURE 9: Computed curves describing dependence of $1/V_{max}$ on $\bar{\eta}$ according to eq 14 at f values of 0, 0.1, 0.2, and 0.4; m values are indicated at the margin.

the important feature that when $m > 1$ it reaches a peak activity at $\bar{\eta}_{max} < 4/3\bar{\eta}_{1/2}$ (see Figure 9). For all other m values (at any f value) $1/V_{max}$ changes monotonously with $\bar{\eta}$. The general boundary conditions for the function are $1/V_{max} = \bar{\eta}$ and $1/V_{max} = \bar{\eta}/f$. For the specific case of $f = 1$, namely, when site accessibility is unaffected by changes in lipid fluidity, these boundaries overlap and assume the classical form $1/V_{max} \propto \bar{\eta}$. For the general case of $f > 0$, the function $1/V_{max}$ vs. $\bar{\eta}$ can assume various shapes within these boundaries (see Figure 9).

All transport experiments presented in this study were fitted to eq 14. The fitted curves are shown together with the experimental data in Figures 2–4 and 7, and the relevant parameters are summarized in Table I. The fitting for the cholesterol-modulated glucose efflux in human erythrocytes (Figure 2) reveals a homogeneous population ($f \rightarrow 0$) of fluidity-sensitive glucose carriers. All other fittings were, therefore, carried out by assuming $f = 0$. A close examination of the optimized parameters (see Table I) indicates that the deviations of the fitted values arise mainly from the inaccuracy in m, while $\bar{\eta}_{1/2}$ could be estimated much more accurately. In both erythrocytes and fibroblasts, $\bar{\eta}_{1/2}$ for glucose transport is very close to the natural membrane lipid viscosity (see Figures 2–4 and 7). Thus, it may be assumed that the fraction of the operating glucose carriers in untreated cells (erythrocytes and fibroblasts) is nearly half of the total, resulting in submaximal transport activity. It is of interest to note that a similar observation was reported for the stoichiometry of binding of cytochalasin B to reconstituted glucose transporters from human erythrocytes (Hinkle et al., 1979). On the other hand, the power m appears to depend on the type of the lipid-rigidifying agent used (see Table I). Since cholesterol, CHS, and CB mediate a similar effect on the fluidity of the lipid hydrocarbon region (see eq 2 and 3), it is likely that their different effects on m originate from the lipid "head-group" region. It seems, therefore, that the charge distribution of the lipid head groups is a major determinant of the degree of cooperativity between the lipid matrix and the carrier accessibility.

Membrane lipid fluidity is now accepted as one of the major function regulators of cellular activities (Shinitzky & Henkart, 1979). For the carrier-mediated transports which were investigated in this study, the natural membrane microviscosity appears in a region very close to $\bar{\eta}_{1/2}$. Around this viscosity the carrier accessibility is very sensitive to subtle changes of the lipid fluidity which, under physiological conditions, can be utilized for fine regulation of transport activity.

The approach presented in this study can provide a potent substitute for the commonly employed temperature dependence of membrane function. While changing temperature affects simultaneously the energetics of all cellular compartments, many of which can indirectly affect the analyzed system, changes in viscosity can be directly correlated with changes in the thermodynamic free volume, as well as with diffusion. With the aid of methods for selective information on the cell plasma membrane this approach is now experimentally feasible.

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References

Baldassare, J. J., Saito, Y., & Silbert, D. F. (1979) *J. Biol. Chem. 254*, 1108–1113.

Batschinski, A. J. (1913) *Z. Phys. Chem., Stoechiom. Verwandtschaftsl. 84*, 643–706.

Berlin, R. D., & Freg, J. P. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 1072–1076.

Bingham, E. C., & Kinney, P. W. (1940) *J. Appl. Phys. 11*, 192–202.

Borochov, H., & Shinitzky, M. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 4526–4530.

Borochov, H., Abbott, R. E., Schachter, D., & Shinitzky, M. (1979) *Biochemistry 18*, 251–255.

Bottcher, G. J. F., van Gent, C. M., & Pries, C. (1961) *Anal. Chim. Acta 24*, 203–204.

Brown, H. H., Zlatkis, A., Zak, B., & Boyle, A. J. (1954) *Anal. Chem. 26*, 397–399.

Collard, J. G., & de Wildt, A. (1978) *Exp. Cell Res. 116*, 447–450.

Cooper, R. A., Arner, E. C., Wiley, J., & Shattil, S. J. (1975) *J. Clin. Invest. 55*, 115–126.

Cossins, A. R. (1977) *Biochim. Biophys. Acta 470*, 341–395.
de Laat, S. W., van der Saag, P. T., & Shinitzky, M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 4458–4461.
Dodge, J. T., Mittchel, C. M., & Hanahan, D. J. (1963) *Arch. Biochem. Biophys. 100*, 119–130.
Evans, E. A., & Hochmuth, R. M. (1978) *Curr. Top. Membr. Transp. 10*, 1–64.
Gavish, B., & Werber, M. M. (1979) *Biochemistry 18*, 1269–1275.
Grinvald, A., & Steinberg, I. Z. (1974) *Anal. Biochem. 59*, 583–598.
Grunberger, D., & Shinitzky, M. (1981) *Biochim. Biophys. Acta* (in press).
Hazel, J. R., & Prosser, C. L. (1974) *Physiol. Rev. 54*, 620–677.
Hinkle, P. C., Sogin, D. C., Wheeler, T. J., & Telford, J. N. (1979) in *Function and Molecular Aspects of Biomembrane Transport* (Quagliariello, E., et al., Eds.) pp 487–494, Elsevier/North-Holland Biomedical Press.
Hirai, H., & Eyring, H. (1958) *J. Appl. Phys. 29*, 810–816.
Janacek, K., & Kotyk, A. (1969) in *Cell Membrane Transport: Principles and Techniques* (Kotyk, C., & Janacek, K., Eds.) pp 57–98, Plenum Press, New York.
Jung, C. Y., Hsu, T. L., Hah, J. S., Cha, C., & Haas, M. N. (1980) *J. Biol. Chem. 255*, 361–364.
Klausner, R. D., Kleinfield, A. M., Hoover, R. L., & Karnovsky, M. J. (1980) *J. Biol. Chem. 255*, 1286–1295.
Kotyk, A., & Janacek, K. (1969) *Cell Membrane Transport: Principles and Techniques* (Kotyk, C., & Janacek, K., Eds.) Plenum Press, New York.
Lauger, P. (1962) *Science (Washington, D.C.) 178*, 24–30.
Low, P. S., & Somero, G. N. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3014–3018.
Masiak, St. J., & Le Fevre, P. G. (1974) *Arch. Biochem. Biophys. 162*, 442–447.
Morgan, H. E., & Neely, J. R. (1972) in *Handbook of Physiology* (Field, J., Ed.) Sect. 7, Vol. 1, p 323, Williams & Wilkins, Baltimore, MD.
Olefsky, J. M. (1978) *Biochem. J. 172*, 137–145.
Pilch, P. F., Thompson, P. A., & Czech, M. P. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 915–918.
Plagemann, P. G. W., & Erbe, J. (1975) *J. Mol. Biol. 25*, 381–396.
Read, B. D., & McElhaney, R. N. (1976) *Biochim. Biophys. Acta 419*, 331–341.
Saffman, P. G., & Delbruck, M. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 3111–3113.
Saito, Y., & Silbert, D. F. (1979) *J. Biol. Chem. 254*, 1102–1107.
Sen, A. K., & Widdas, W. F. (1962) *J. Physiol. (London) 160*, 392–403.
Shinitzky, M. (1979) *Dev. Cell Biol. 4*, 173–181.
Shinitzky, M., & Barenholz, Y. (1974) *J. Biol. Chem. 249*, 2652–2657.
Shinitzky, M., & Inbar, M. (1976) *Biochim. Biophys. Acta 433*, 133–149.
Shinitzky, M., & Barenholz, Y. (1978) *Biochim. Biophys. Acta 515*, 367–394.
Shinitzky, M., & Henkart, P. (1979) *Int. Rev. Cytol. 60*, 121–147.
Shinitzky, M., & Souroujon, M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4438–4440.
Shinitzky, M., Skornick, Y., & Haran-Ghera, N. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5313–5316.
Shinitzky, M., Borochov, H., & Wilbrandt, W. (1980) in *Membrane Transport in Erythrocytes* (Lassen, U. V., Ussing, H. H., & Weith, J. O., Eds.) pp 91–102, Munksgraad, Copenhagen.
Sinensky, M. (1980) *J. Cell Biol. 85*, 166–169.
Stein, W. D. (1972) *Ann. N.Y. Acad. Sci. 195*, 412–428.
Weber, G. (1952) *Biochem. J. 51*, 145–155.
Wilbrandt, W. (1972) *J. Mol. Biol. 10*, 357–366.
Wilbrandt, W. (1978) in *Cell Membrane Receptors for Drug and Hormones: A Multidisciplinary Approach* (Straub, R. W., & Bolis, L., Eds.) pp 243–249, Raven Press, New York.