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PERMEABILITY STUDIES ON ANIMAL MEMBRANE/CARBOHYDRATE SYSTEMS

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ABSTRACT

Hydrodynamic permeability measurements on water, and on aqueous solutions of D-glucose and sucrose of various concentrations, through frog-skin membrane were made. The conductance of the membrane equilibrated with the permeants was also measured. Some comments regarding the dependence of the permeability coefficient and the membrane conductance on the concentration were made. It was observed that the membrane behaves anisotropically in so far as the hydrodynamic permeability is concerned.

INTRODUCTION

Biological-membrane transport is a complex phenomenon[1] which plays a vital role in many areas of basic biology and medicine[2]. The membranes may be considered as constituted of several permeability barriers of lipid bilayers[3] that consist mostly of lipids and proteins[4]. In addition to lipoproteins, the membranes also contain some carbohydrate associated with the binding sites on the membrane surface[5]. Hence, a comprehensive study of a natural membrane equilibrated with aqueous solutions of carbohydrates is needed, in order to determine the directional characteristics of biomembranes. For this purpose, hydrodynamic permeability and conductance measurements have been conducted on frog-skin membrane/carbohydrate systems, and the data have been analyzed from the viewpoint of non-equilibrium thermodynamics.

EXPERIMENTAL

The membrane was obtained by dissecting the ventral side-skin of a frog (*Rana tigrina*). The membrane was thoroughly washed, and its fixation was performed in the membrane assembly. D-Glucose, sucrose (AR; BDH) and double-distilled water having a specific conductance of the order of 10^{-6} $ohm^{-1}.cm^{-1}$ were used.

The measurements of hydrodynamic flux were made by use of a technique already described[6,7]. A pressure difference was applied with the help of a pressure head. The volumetric flux induced by the pressure difference was measured by noting

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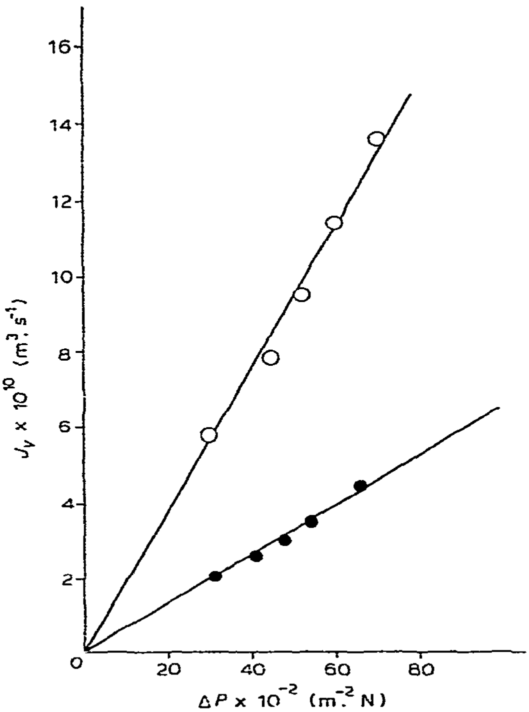
the change in the advance of fluid in a horizontal capillary-tube of known, cross-sectional area. Care was taken to avoid the formation of bubbles in the capillary tube by passing steam through it for ~2–3 h. The fluid whose permeability was to be measured was equilibrated with the permeant for ~8–10 h. The conductance of the membrane equilibrated with the fluid was measured with an AC conductivity bridge (Toshniwal, India) at 50 Hz. All measurements were conducted in an air thermostat maintained at 30 ±0.5°.

RESULTS AND DISCUSSION

The hydrodynamic-flux data were plotted against the pressure difference applied for the flow of permeants from the outer to the inner side of the membrane, and *vice versa* (see Figs. 1–5), the inner side being that portion which remained in contact with tissues.



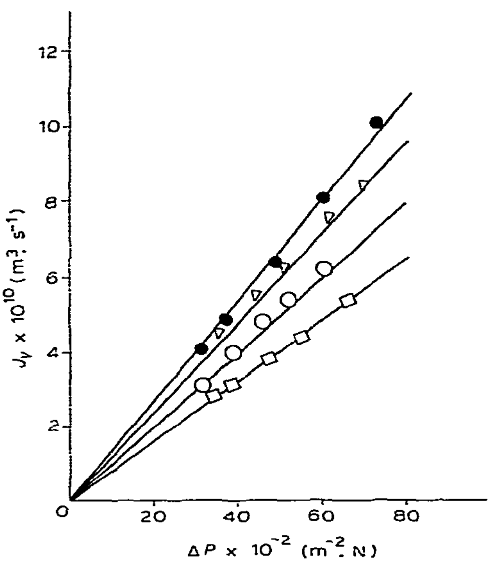
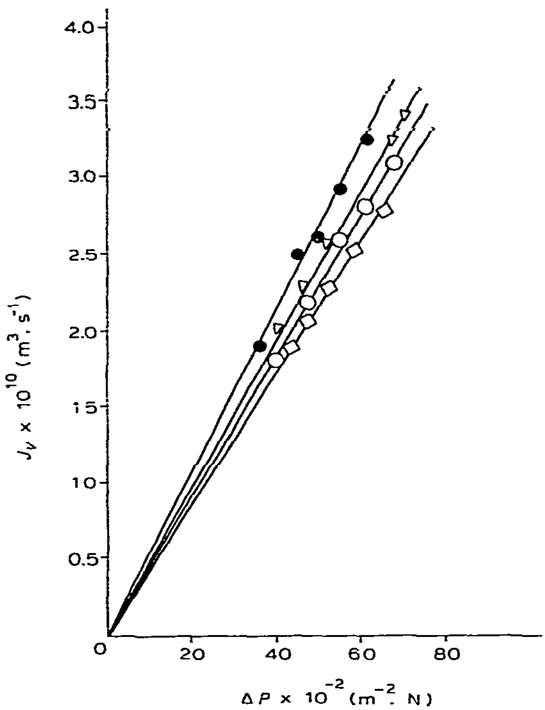


Fig. 1. Dependence of hydrodynamic permeability (J_v) on applied pressure difference (ΔP) for frog-skin membrane/water system. [Key: (●) flow from outer to inner side, and (○) flow from inner to outer side.]

Fig. 2. Dependence of hydrodynamic permeability (J_v) on applied pressure difference (ΔP) for the flow of outer to inner side of frog-skin membrane/aqueous solutions of D-glucose system. [Key: (●) 0.1M, (△) 0.2M, (○) 0.3M, and (□) 0.4M.]



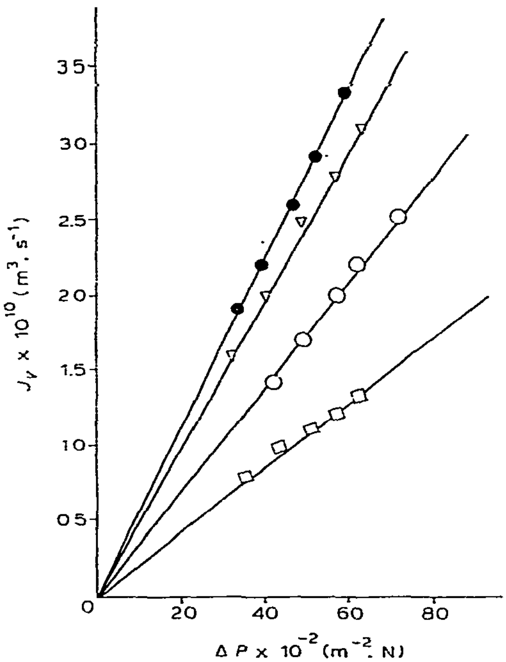


Fig. 3. Dependence of hydrodynamic permeability (J_v) on applied pressure difference (ΔP) for the flow of inner to outer side of frog-skin membrane/aqueous solutions of D-glucose system. [Key: (●) 0.1M, (△) 0.2M, (○) 0.3M, and (□) 0.4M.]

Fig. 4. Dependence of hydrodynamic permeability (J_v) on applied pressure difference (ΔP) for the flow of outer to inner side of frog-skin membrane/aqueous solutions of sucrose system. [Key: (●) 0.05M, (△) 0.10M, (○) 0.15M, and (□) 0.20M.]

The flux is directly proportional to the pressure difference, ΔP, for all of the cases studied, and the following, linear, phenomenological equation is adequate for description of the results[8,9].

$$(J_v)_{\Delta\phi=0} = L_{11}(\Delta P/T), \qquad (1)$$

where $(J_v)_{\Delta\phi=0}$ is the volumetric flow when the potential difference is zero, L_{11} is a phenomenological coefficient, and T is the absolute temperature. The values of the phenomenological coefficients obtained from the slopes in Figs. 1–5 are given in Tables I and II.

It is evident from the results in these Tables that the hydrodynamic permeability coefficients have different values for flow in the two directions, *i.e.*, they are direction-dependent. The directional dependence arises on account of (*i*) complexity in the texture of the membrane, and (*ii*) differences in the nature of their flow across the membrane. Biological membranes may be regarded as composed of a number of

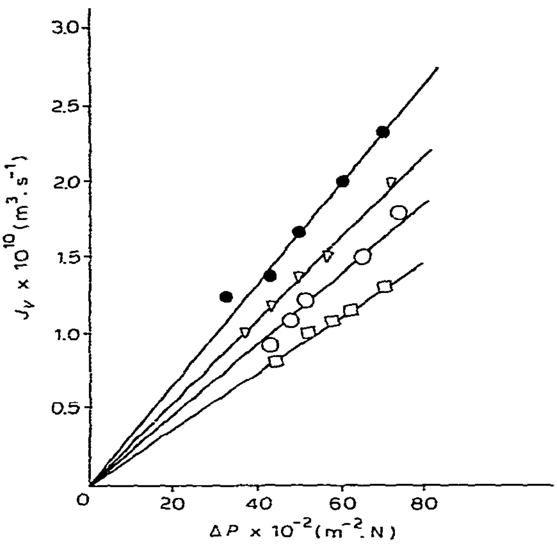


Fig. 5. Dependence of hydrodynamic permeability (J_v) on applied pressure difference (ΔP) for the flow of inner to outer side of frog-skin membrane/aqueous solutions of sucrose system. [Key: (●) 0.05M, (△) 0.10M, (○) 0.15M, and (□) 0.20M.]

thin layers having different pore-diameters, and thus it may be expected that extra- and intra-cellular surfaces have different characteristics[10].

There are two types of flow mechanism across the membranes, depending on their pore size[11]: (*i*) diffusion, and (*ii*) viscous. When the pore size ≫ mean free-path of the permeating species, viscous flow takes place, and the volume flow may be given as follows

$$J_v = n\pi r^4/(8\eta l)\Delta P, \qquad (2)$$

TABLE I

DIRECTIONAL DEPENDENCE OF PERMEABILITY COEFFICIENT FOR MEMBRANE/D-GLUCOSE SYSTEM

Concentration (M)	*Direction of flow*	$[L_{11}/T] \times 10^{14}$ ($m^5N^{-1}.s^{-1}$)
0.00	→	18.75
	←	6.66
0.10	→	13.30
	←	5.38
0.20	→	12.00
	←	4.80
0.30	→	10.00
	←	4.60
0.40	→	8.20
	←	4.28

TABLE II

DIRECTIONAL DEPENDENCE OF PERMEABILITY COEFFICIENT FOR MEMBRANE/SUCROSE SYSTEM

Concentration (M)	*Direction of flow*	$[L_{11}/T] \times 10^{14}$ ($m^5N^{-1}.s^{-1}$)
0.00	→	18.75
	←	6.66
0.05	→	5.10
	←	3.50
0.10	→	4.90
	←	3.00
0.15	→	3.27
	←	2.30
0.20	→	2.09
	←	1.80

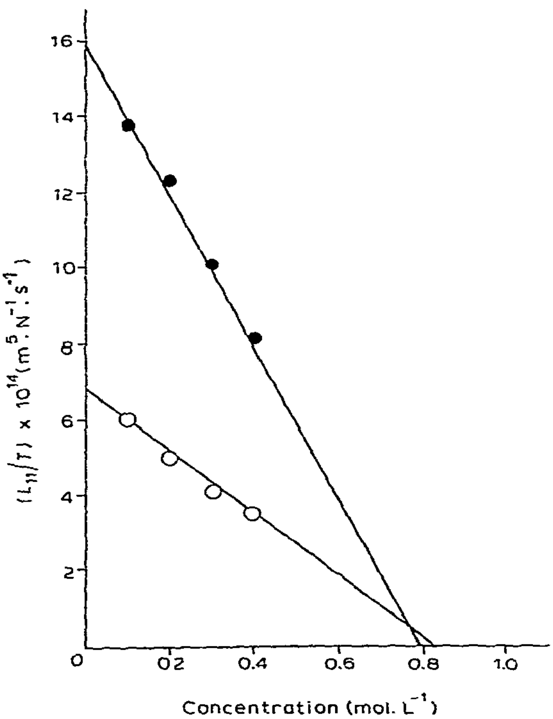


Fig. 6. Dependence of permeability coefficient (L_{11}/T) on concentration of the permeants for frog-skin membrane/aqueous solutions of D-glucose system. [Key: (●), flow from outer to inner side, and (○), flow from inner to outer side.]

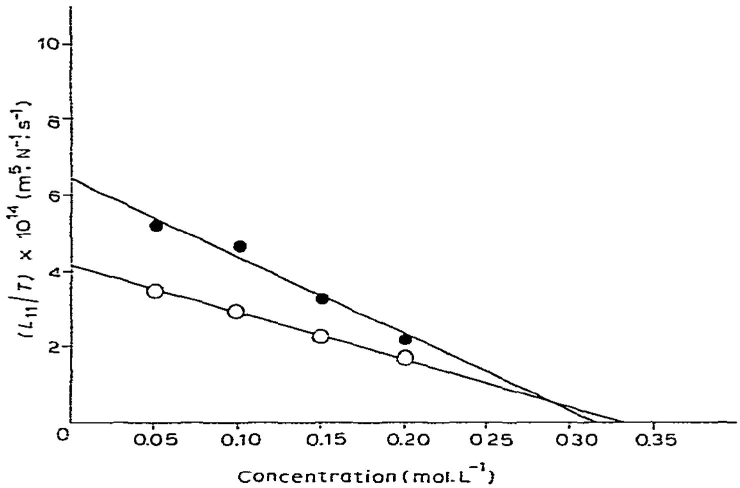


Fig. 7. Dependence of permeability coefficient (L_{11}/T) on concentration of the permeants for frog-skin membrane/aqueous solutions of sucrose system. [Key: (●), flow from outer to inner side, and (○), flow from inner to outer side.]

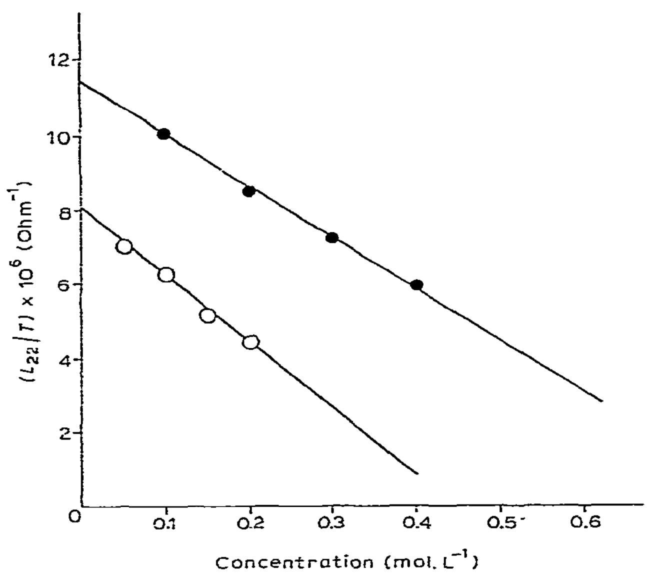


Fig. 8. Dependence of conductance (L_{22}/T) on concentration of the permeants for frog-skin membrane/aqueous solutions of carbohydrate systems. [Key: (●) D-glucose, and (○) sucrose.]

where η is the coefficient of viscosity, r is the pore radius, l is the thickness of the membrane, and n represents the number of capillaries across the membrane. On the other hand, when the pore size ≪ mean free-path of the permeative species, the flow is diffusive, and the volumetric flux is given as follows

$$J_v = D\varepsilon V/(RTl)\Delta P, \tag{3}$$

where D is the diffusion coefficient, ε is the fractional void-volume, and R is the gas constant.

The plots of the permeability coefficient, L_{11}/T, against the concentration, C, of the permeants yield straight lines, as shown in Figs. 6 and 7 for aqueous solutions of D-glucose and sucrose, respectively. The following, empirical relation was found to hold good.

$$L_{11}/T = a + bC, \qquad (4)$$

where a and b are constants, and are related to (*i*) solute–solvent, (*ii*) solvent–membrane, and (*iii*) solute–membrane interactions.

Biological membranes are very complex systems, and asymmetrical behavior in hydrodynamic flow can be understood by visualizing the membrane as composed of two membranes having different pore-dimensions[12]. Thus, the inner parts have pore dimensions different from those of the outer parts. The difference in L_{11}/T is a measure of the structural complexity. In the case of free diffusion, the solvent and solute migrate only with respect to each other. Hence, the hydrodynamic resistance to diffusional flow is due to the friction between the solute and the solvent alone, so that diffusion in a solution of a single solute is determined by a single diffusion-coefficient. The passage through a membrane involves, however, two additional factors, *viz.*, the friction between the solute and the membrane, and that between the solvent and the membrane. Thus, a full description of three coefficients whose values will depend on the nature of the three processes involved has to be taken into account. For coarse membranes having large pore-dimensions, the solute–solvent friction will contribute more than the other factors, and the permeability will approach the behavior of free diffusion. On the other hand, in dense membranes, in which part of the solute penetration takes place (*i.e.*, through dissolution in the membrane), the contribution of the friction between solute and membrane becomes predominant.

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