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OSMOTIC PERMEABILITY OF NOVIKOFF HEPATOMA CELLS

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Summary

The osmotic permeability coefficient (P_f) for water movement across Novikoff hepatoma cells was found to be 82 ± 3 (S.E.) · 10^{-5} cm · s^{-1} at 20°C. The corresponding diffusional permeability coefficient for ^{3}HHO (P_d) was 97 ± 10 (S.E.) · 10^{-5} cm · s^{-1}, therefore the ratio P_f/P_d is close to unity. The apparent activation energy for water filtration was 10.4 ± 0.4 (S.E.) kcal · mol^{-1}. This value is significantly greater than the activation energy for the self diffusion of water. The product of the hydraulic permeability coefficient and the viscosity coefficient for water was temperature-dependent. However, the product of the hydraulic permeability coefficient and the viscosity coefficient for membrane lipid did not vary with temperature. These data are interpreted as evidence for water movement across a lipid membrane barrier rather than through aqueous channels.

Introduction

The paucity of published data on the permeability properties of isolated nucleated cells prompted the current investigation. The red cell has been the only isolated mammalian cell characterized sufficiently to permit comparison of the filtration permeability coefficient, P_f, with the diffusional permeability coefficient for water, P_d [1—3]. Although the osmotic permeability properties of several cell types have been studied [4—8] no quantitative data have been reported on the diffusional movement of water across these cells. This work describes the osmotic permeability properties of the Novikoff hepatoma cell.

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Since the diffusional permeability coefficient for water across the Novikoff hepatoma cells was reported in the preceding paper [9], it is now possible to calculate the P_f/P_d ratio for this isolated mammalian cell. Solomon and co-workers [1—3] have postulated that the red-cell membrane contains aqueous channels which facilitate the movement of water and small hydrophilic solutes [1—3]. Three lines of evidence have been presented in support of this hypothesis. (1) The filtration permeability coefficient is larger than the diffusional permeability coefficient for water ($P_f > P_d$); (2) the apparent activation energies for osmotic flux and diffusional flux of water across the plasma membranes are similar; (3) the product of the hydraulic permeability coefficient, L_p, and the bulk viscosity of water, η_w, is constant with respect to temperature. Evaluation of the permeability properties of the Novikoff hepatoma cell in terms of the above criteria rules out the existence of functional aqueous channels in the hepatoma cell plasma membrane.

Materials and Methods

Novikoff hepatoma ascites cells were maintained and isolated as described previously [9]. The cells were suspended in Na^+/K^+ Ringer's buffer [10] which contained: 1.165 mmol NaH_2PO_4, 9.35 mmol Na_2HPO_4, 6.15 mmol KCl and enough NaCl to yield 290 mosM/kg. Solution osmolality was measured by freezing-point depression (Precision Instruments). The response of the Novikoff cell to changes of external solute concentration was determined by suspending packed cells in Na^+/K^+ Ringer's buffer with osmolalities ranging from 260 to 670 mosM/kg. Cellular volume was determined from the cytocrit and corrected for the extracellular volume according to the procedure of Redwood et al. [11] with [^{14}C]sucrose as the marker molecule. Novikoff cell surface area was calculated morphometrically from transmission and scanning electronmicrographs [12].

Measurement of hydraulic permeability coefficient

The hydraulic permeability coefficient, L_p, was measured for exosmosis by a modification of the technique described by Blok et al. [13]. A 3 ml aliquot of cell suspension ($4.0 \cdot 10^6$ cells/ml) in 290 mosM/kg Ringer's buffer was transferred to a cuvette which was maintained at constant temperature (±0.1°C) by a circulating water bath. After temperature equilibration, a zero-time baseline was established. A solute concentration gradient was induced by rapidly injecting 25—100 µl of either 1.5 M NaCl or 2.7 M sucrose solution with a Hamilton syringe. Cell volume change, as a function of time, was monitored as a change in absorbance at 600 nm with an Aminco DW-2 spectrophotometer and ancillary recorder (American Instrument Co.). The final osmolality of the cell suspension, after osmotic shock, was determined by the freezing-point depression following the removal of cells by centrifugation (Beckman microfuge).

To measure L_p for endosmosis, cells were pre-equilibrated for 30 min, in 360 mosM/kg Ringer's buffer. Volume flow was initiated by injecting 100 µl distilled water into the cuvette containing the cell suspension. This resulted in a decrease in the osmolality of the bathing solution of 10.3 mosM/kg. The subse-

quent change in cell volume and buffer osmolality was monitored as described above.

The density of the Novikoff cell was determined according to the procedure described by Arnold and Lacy [14].

Calculation of the hydraulic permeability coefficient

Introduction of cells into nonisosmolar conditions of nonpermeating solute (reflection coefficient equal to unity) initiates a volume flow either into or out of the cells. The rate of change of volume approximated by the expression:

$$\frac{d(Vol)}{dt} = L_p \cdot S \cdot RT\Delta C \tag{1}$$

where $d(Vol)/dt$ is the cell volume change with time, S the cell surface area, ΔC the difference between internal and external solute concentration, R the gas constant, and T the absolute temperature. Since the initial relative change in absorbance is directly proportional to the initial volume change, the following relationship was used:

$$\frac{d(Vol)}{dt} = K \cdot \frac{d\left(\frac{1}{A}\right)}{dt} \tag{2}$$

where K is the proportionality constant obtained from a plot of absorbance (A) against the reciprocal of cell volume. The relative change in A is calculated from the following equation:

$$A = \frac{(A_0 - A_{max})}{A_0} \tag{3}$$

where A_0 and A_{max} are the absorbance at $t = 0$ and $t = 1$ min, respectively. The initial shrinkage velocity $d(1/A)/dt$ is obtained from the slope of the initial ascending portion of the shrinkage curve (cf. Fig. 3).

Results and Discussion

Osmotic permeability properties of the Novikoff hepatoma cell at 20° C

The dependence of the cell volume on the buffer osmolality is illustrated in Fig. 1. Over the NaCl concentration range from 290 mosM/kg (isosmolal) to 588 mosM/kg the cell volume decreased as a linear function of reciprocal osmolality (1/osM). Above 588 mosM/kg the cell shrinkage ceased. The minimum cellular volume attained was $1.37 \cdot 10^{-15}$ cm^3 which was 67.8% of the isosmolar cellular volume. The density of the Novikoff cell was measured in isosmolar medium and found to be 1.079 ± 0.19 (S.E.) g/cm^3, therefore the osmotically available cellular water in Novikoff hepatoma cells in isosmolar medium was approximately 34.7% on a weight/weight basis. In comparison, the total cellular water content measured by a dry weight analysis was 85.1 ± 2.1%. It is probable that a large fraction of the cellular water is associated with the relatively large nucleus of the Novikoff cell [15]. The minimum cellular volume attainable may therefore correspond to the limitations on cell shrinkage imposed by

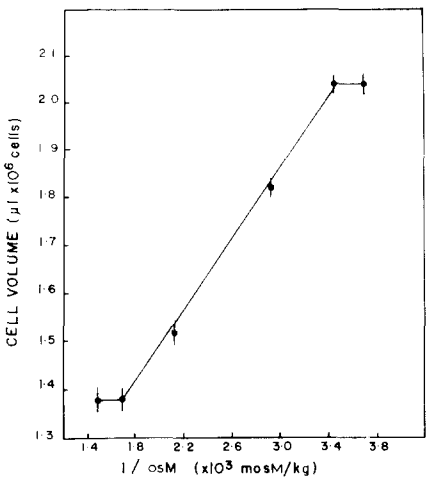

Fig. 1. Novikoff cell volume as a function of the reciprocal of osmolality. Cell volume measured by cytocrit and corrected for extracellular space.

the relatively large nucleus if the nucleus is osmotically unresponsive.

Incremental swelling of the cells did not occur on decreasing the osmolality to below 290 mosM/kg. Two factors may be involved. A cytoskeleton could reinforce the plasma membrane and thereby permit a higher hydrostatic pressure to develop and oppose the osmotic pressure. Alternatively, the efflux of mobile solutes could reduce the intracellular osmolality. Both phenomena could occur.

Cell volume and osmolality products were constant (Fig. 1) over the concentration range from 290—588 mosM/kg, an indication that the Novikoff cell

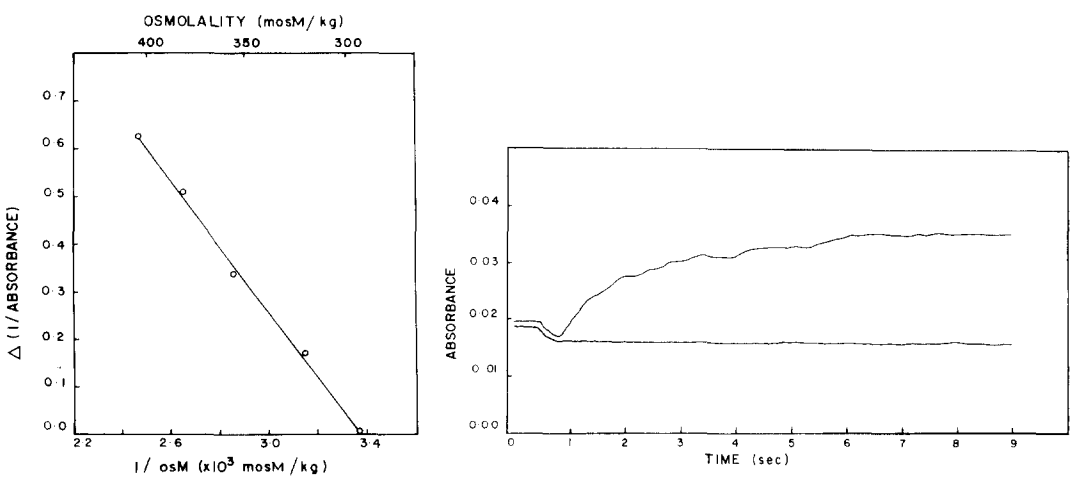

Fig. 2.Relationship between the relative absorbance change of Novikoff cell suspension with the reciprocal of buffer osmolality. Absorbance was measured at 600 nm and at a cell density of $4.0 \cdot 10^6$ cells/ml.

Fig. 3. Kinetics of Novikoff cell volume change. Recorder tracing showing the change in absorbance of Novikoff cell suspension following injection of 100 µl of 1.5 M saline (upper curve) or 100 µl of isotonic buffer (lower curve). Conditions identical to Fig. 2.

behaves as an ideal osmometer with respect to NaCl. Measurement of the hydraulic permeability coefficient (L_p) requires monitoring of rapid changes in cell volume. The time course of volume changes may be conveniently followed from changes in the absorbance of a cell suspension. A plot of $\Delta(1/A)$ against the reciprocal osmolality (1/osM) (Fig. 2) shows that there is a linear dependence over the range of osmolality in which the cell volume also varies linearly with 1/osM. Combination of these relationships permits the use of absorbance measurements as an index of cell volume changes in the above range of osmolality.

Data from a representative shrinking experiment and isotonic control run are presented in Fig. 3. The lower curve results from injecting isotonic buffer into a cuvette containing the cell suspension. The initial decrease in absorbance was a dilution effect which stabilized in 0.5 s. Detectable variations of optical density were not observed for more than 0.6 s after injection of the same volume of trypan blue. Cell settling produced a change of $8.3 \cdot 10^{-5}$ A per s. This did not interfere with volume measurements since the reaction was complete in 6—7 s. Injection of a hypertonic saline solution produced a movement of water out of the cells, which was reflected as an increase in absorbance (upper curve) due to cell shrinkage. When 100 μl 1.5 M NaCl was mixed with the cell suspension, the buffer osmolality was increased by approximately 117 mosM/kg and a volume change of 0.020 A was measured.

Hydraulic permeability coefficients (L_p) were calculated according to Eqn. 1. The rate of volume change was taken from the slope of the initial ascending portion of the shrinkage curve (Fig. 3). This procedure was adopted since mixing was maximal during the early portion of the experiment. At later time periods 'unstirred layer' effects tended to attenuate shrinkage velocity by approximately 20%. This was estimated by comparing the reaction half-time for the initial portion of the curve (1.2 s) to the half-time for the entire curve (1.5 s). The cell surface area required for the calculation of L_p was found by a morphometric analysis of transmission and scanning electron micrographs to be $1.9 \cdot 10^{-5}$ cm^2 [12]. Listed in Table I are the osmotic permeability coefficients calculated for cells which were transferred from isosmolar medium to different hyperosmolar media. The permeability coefficients did not vary significantly ($P > 0.05$) with osmolality, and the mean hydraulic permeability coefficient was calculated to be $5.9 \pm 0.2 \cdot 10^{-10}$ (S.E.) cm $\cdot$ (cm $H_2O)^{-1} \cdot s^{-1}$. Relative to the human red-cell membrane, water movement across the hepatoma cell membrane is an order of magnitude slower [16]. However, the hydraulic permeability coefficient found for the Novikoff cell is similar to the values determined by Hempling and associates [6,8] for human lymphocytes, human leukocytes and leukemic cells.

Solomon and associates [17,18] have reported that there are two mechanisms for water transport across lumen red-cell membranes, diffusion and convection or bulk flow. This hypothesis is based upon the assumption that the difference between the filtration coefficient and the diffusional permeability coefficients reflect qualitative differences in water movement. Accordingly, a P_f/P_d ratio significantly greater than unity is taken as evidence for viscous flow through aqueous channels [1]. The diffusional permeability coefficient for ^{3}HHO through the Novikoff cell membrane has been reported in the preceding

TABLE I

OSMOTIC PERMEABILITY COEFFICIENTS AT THREE OSMOLALITIES

Values are mean ± S.E. with number of determination in parentheses. $P_f = L_p(RT/\bar{V}_w)$, where $\bar{V}_w$ is the partial molar volume of water.

Osmolality (final osmolality, 290 mosM/kg)	$L_p \times 10^{-10}$ (cm · (cm H_2O)$^{-1}$ · s^{-1})	P_f ($\times 10^{-5}$) (cm · s^{-1})
60	6.29 ± 0.33 (12)	87 ± 5 (12)
88	5.54 ± 0.23 (9)	77 ± 3 (9)
117	5.70 ± 0.30 (9)	79 ± 5 (9)

paper to be 97 ± 10 · 10^{-5} (S.E.) cm · s^{-1} [9]. The mean value for the filtration coefficient calculated from Table I is 82 ± 3 · 10^{-5} (S.E.) cm · s^{-1}. These coefficients are not significantly different ($P > 0.05$), consequently there is no evidence for bulk flow of water through aqueous channels in the Novikoff cell membrane.

In order to confirm that the values for L_p calculated in Table I were not underestimates due to the solute reflection coefficient for NaCl being actually less than the assumed value of unity, the experiments were repeated with sucrose as the impermeant solute. It has been shown in ancillary experiments with radioactively labeled sucrose that the Novikoff cell membrane is impermeable to this solute [12]. For these studies the hydraulic permeability coefficient was determined with either NaCl or sucrose as solute, using the same cell population for both of the measurements. This prevented variations between sets due to use of different isolated cell preparations. Table II lists the L_p values determined in four independent studies of the comparison between NaCl and sucrose as solutes. It can be seen that there is no significant difference between the data sets at $P > 0.05$. These results support the assumption that the reflection coefficient for NaCl is unity and that the L_p values listed in Table I are valid estimates of the hydraulic permeability coefficients.

Rich et al. [19] and Farmer and Macey [20] have shown a directional preference for water movement across the red-cell membrane. These studies indicated that water movement into red cells under hypotonic conditions is more rapid than water movement out of these cells under hypertonic conditions. In order to study the question of possible directionality to osmotic flow in the

TABLE II

EFFECTS OF SOLUTES AND DIRECTION OF WATER FLOW ON HYDRAULIC PERMEABILITY COEFFICIENT

The values are mean ± S.E. with number of determinations in parentheses.

Directionality	Solute	$L_p(\times 10^{-10})$ (cm · (cm H_2O)$^{-1}$ · s^{-1})
Exosmosis	NaCl	5.80 ± 0.26 (4)
Exosmosis	Sucrose	6.00 ± 0.24 (4)
Exosmosis	NaCl	6.94 ± 0.17 (4)
Endosmosis	NaCl	7.28 ± 0.27 (4)

hepatoma cell, it was necessary to pre-equilibrate the cells in hypertonic medium prior to the measurement of cell swelling caused by the introduction of distilled water into the medium. This maneuver was necessary since it was known from Fig. 1 that the Novikoff cell would not swell beyond its isotonic volume. Table II lists the results of four separate paired studies which compare the rates for cell swelling and shrinking from a preequilibrated medium osmolality of 360 mosM/kg NaCl. The hydraulic permeability coefficient for endosmosis was $7.3 \pm 0.3 \cdot 10^{-10}$ (S.E.) cm · (cm H_2O)$^{-1}$ · s^{-1}, a value not significantly different ($P < 0.05$) from $6.9 \pm 0.2 \cdot 10^{-10}$ (S.E.) cm · (cm H_2O)$^{-1}$ · s^{-1} which was determined for exosmosis using the paired cell preparations in the initial buffer medium of 360 mosM/kg. In the calculation of L_p, the assumption was made that the surface areas on either side of the membrane are identical. Based on this assumption these data indicate that the Novikoff cell membrane is a symmetrical barrier for water movement.

The temperature dependence of the water permeability coefficient

Temperature coefficients for water movement across membranes have been employed to differentiate permeation pathways. According to Stein [21], permeation of water through unrestrictive pores would be characterized by an activation energy (E_a) similar to the 4.5 kcal · mol^{-1} activation energy for the self-diffusion of water [22]. Table III lists the temperature dependence of the hydraulic permeability coefficients for the Novikoff cell. An Arrhenius plot (Fig. 4) yields an apparent activation energy (E_a) of 10.4 ± 0.4 (S.E.) kcal · mol^{-1}. This value is greater than the E_a for the self-diffusion of water and also the 3.3 kcal · mol^{-1} E_a reported for the osmotic permeability of red cells [2]. Blok et al. [13] have reported an E_a of 10.4 kcal · mol^{-1} for lipid model membranes which lack aqueous pores. This similarity of activation energies would be consistent with a lipid pathway for osmotic movement of water through the Novikoff cell membrane. However, caution should be exercised in interpreting the apparent activities energies for water transport across such complex barriers as biological membranes where many factors may influence the measured temperature coefficient. In the relatively simple planar bilayer system it has been shown that compositional changes in microenvironments in the membrane occur with temperature changes which contribute to the apparent activation energy for osmosis [23].

The apparent activation energies for osmotic flux across the hepatoma cell is

TABLE III

HYDRAULIC PERMEABILITY COEFFICIENTS AT VARIOUS TEMPERATURES

The values are mean ± S.E. with number of determinations in parentheses.

Temperature (°C)	L_p ($\times 10^{-10}$) (cm · (cm H_2O)$^{-1}$ · s^{-1})
20	7.04 ± 0.32(5)
27	9.04 ± 0.23(5)
31	11.83 ± 0.29(5)
40	18.69 ± 0.41(5)

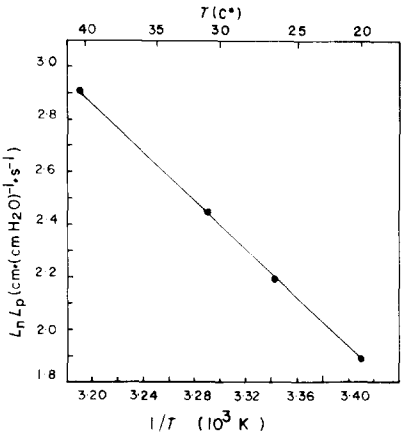

Fig. 4. Arrhenius plot of the logarithm of the hydraulic permeability coefficient as a function of the reciprocal of the absolute temperature. Data from Table III. E_a, 10.4 kcal · mol^{-1}.

similar to the values reported by Hempling and associates for water transport across Erhlich ascites cells (9.9 kcal · mol^{-1}, Ref. 4) and human lymphocytes (14.1 ± 1.6 kcal · mol^{-1}, Ref. 8).

In the preceding paper [9], an apparent activation energy of 6.7 ± 1.9 (S.E.) kcal · mol^{-1} was measured for the diffusional movement of ^{3}HHO across the Novikoff cell membrane. This value is intermediate between the apparent activation energy for osmotic movement of water across these membranes and the self diffusion of water. However, due to the large error associated with this E_a value further statistical analysis is not warranted. The evidence presented here and in the preceding paper [9] favor water movement across a lipid matrix. Vieira et al. [3] demonstrated that the product of L_p and the bulk viscosity of water, η_w, remains constant with temperature. This result was taken as evidence for viscous flow through pores in the red-cell membrane. An equivalent analysis for Novikoff cell (Table IV) shows that $L_p \cdot \eta_w$ is not independent of temperature. We interpret this result to suggest that water flow is not occurring through aqueous channels. Esko et al. [24] have measured the viscosity of mouse LM cell membranes by fluorescence polarization. Based on the assumption that the viscosity of the Novikoff cell membrane is not significantly different from the LM cell membrane, then the product ($L_p \cdot \eta_m$) is a constant

TABLE IV

VARIATION OF $L_p \cdot \eta$ WITH TEMPERATURE

Temperature (°C)	L_p ($\times 10^{-10}$) (cm · dyn^{-1} · s^{-1})	η_w * (cP)	η_m ** (cP)	$L_p \cdot \eta_w$ ($\times 10^{-10}$) (cm)	$L_p \cdot \eta_m$ ($\times 10^{-10}$) (cm)
20	7.18	1.00	4.70	7.20	33.8
27	9.22	0.85	3.65	7.85	33.6
31	12.10	0.78	2.85	9.44	34.5
40	19.07	0.65	1.80	12.43	34.3

* Ref. 25.

** Fig. 4 of Ref. 24.

with temperature (Table IV). This result suggests that filtration is a function of membrane lipid viscosity (fluidity) and supports the concept of water movement across the lipid matrix.

In interpreting our data we call attention to the following items: (1) the ratio of the filtration coefficient to the diffusional permeability coefficient, P_f/P_d, is close to unity, (2) the apparent activation energy for osmotic water movement is greater than the activation energy for the self-diffusion of water, and (3) the hydraulic permeability coefficient does not show a reciprocal relationship to the bulk viscosity of water but does show a relationship to the viscosity of the membrane and thus membrane fluidity. We take these items as evidence that, in contrast to the red-cell membrane, the Novikoff cell membrane lacks aqueous channels.

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References

1 Solomon, A.K. (1968) J. Gen. Physiol. 51, 335s—364s
2 Rich, G.T., Sha'afi, R.I., Barton, T.C. and Solomon, A.K. (1967) J. Gen. Physiol. 50, 2391—2405
3 Vieira. F.L., Sha'afi, R.I. and Solomon, A.K. (1970) J. Gen. Physiol. 55, 451—466
4 Hempling, H.G. (1960) J. Gen. Physiol. 44, 365—379
5 Hempling, H.G. (1972) J. Cell Physiol. 81, 1—9
6 Hempling, H.G. (1974) Scand. Med. J. 67, 951—958
7 Hempling, H.G. and Wise, W.C. (1975) J. Cell Physiol. 85, 195—207
8 Hempling, H.G., Thompson, S. and Dupre, A. (1977) J. Cell Physiol. 93, 293—302
9 Polefka, T.G., Redwood, W.R., Garrick, R.A. and Chinard, F.P. (1981) Biochim. Biophys. Acta 641, 67—78
10 Hempling, H.G. (1967) J. Cell Physiol. 70, 237—256
11 Redwood, W.R., Rall, E. and Perl, W. (1974) J. Gen. Physiol. 64, 706—729
12 Polefka, T.G. (1979) Thesis Dissertation, Ann Arbor, MI; Dissertation Abstracts International
13 Blok, M.C., van Deenen, L.L.M. and DeGier, J. (1976) Biochim. Biophys. Acta 433, 1—13
14 Arnold, W.N. and Lacy, J.S. (1977) J. Bacteriol. 131, 564—571
15 Karasaki, S. (1970) Cancer Res. 30, 1736—1742
16 Paganelli, C.V. and Solomon, A.K. (1957) J. Gen. Physiol. 41, 259—276
17 Sidel, V.W. and Solomon, A.K. (1957) H. Gen. Physiol. 41, 243—259
18 Sha'afi, R.I. and Gary-Bobo, C.M. (1973) Prog. Biophys. Mol. Biol. 26, 105—146
19 Rich, G.T., Sha'afi, R.I., Romualdez, A. and Solomon, A.K. (1968) J. Gen. Physiol. 52, 941—954
20 Farmer, R. and Macey, R.I. (1970) Biochim. Biophys. Acta 196, 53—65
21 Stein, W.D. (1967) The Movement of Molecules Across Cell Membranes, Academic Press, New York
22 Wang, J.H. (1965) J. Phys. Chem. 58, 686—691
23 Redwood, W.R. and Haydon, D.A. (1969) J. Theoret. Biol. 22, 1—8
24 Esko, J.D., Gilmore, R. and Glaser, M. (1979) Biochem. 16, 1881—1890
25 Weast, R.C. (1970) Handbook of Chemistry and Physics. Cleveland The Chemical Rubber Co., Cleveland, OH