Biochimica et Biophysica Acta 815 (1985) 44–50
Elsevier

BBA 72531

Glucose transport across the basal plasma membrane of human placental syncytiotrophoblast

Larry W. Johnson * and Carl H. Smith

The Edward Mallinckrodt Department of Pediatrics and Department of Pathology, Washington University School of Medicine and St. Louis Children's Hospital, St. Louis, MO 63110 (U.S.A.)

(Received August 30th, 1984)
(Revised manuscript received January 4th, 1985)

Key words: Glucose transport; Placental membrane; Plasma membrane; (Human syncytiotrophoblast)

Transfer of glucose from maternal to fetal circulations requires transport across both the microvillous (maternal-facing) and basal (fetal-facing) plasma membranes of the placental syncytium. We have previously reported transport properties of the microvillous membrane and we now report those of the basal membrane. Basal plasma membrane vesicles were prepared by selective sonication and density gradient centrifugation. Glucose or glucose analogues were rapidly transported across these membranes by facilitated diffusion. Transport was inhibited by cytochalasin B, phloretin and phloridzin. L-Glucose at 1 mM was transferred at only 1/700 of the rate of D-glucose, which indicated an insignificant nonspecific diffusion component. Transport was independent of sodium gradients, and kinetic studies under equilibrium-exchange conditions demonstrated a K_m of 23 mM. Competition studies demonstrated that aldohexoses in the C-1 chair conformation were the preferred substrates. Placental steroids estriol and progesterone inhibited transport. In contrast to other polarized epithelia, the basal and microvillous membranes of the human placental syncytium possess transport systems with similar properties. Thus, the directionality and rate of transfer of glucose across the intact syncytium are likely to be direct functions of the materno-fetal concentration gradient and the total transport capacities of the two plasma membranes.

Introduction

Glucose is a major fetal nutrient and understanding its transport across placenta is fundamental to understanding fetal nutrition. Earlier work on the net transfer of glucose across placenta indicated a specific carrier system was present, since saturation kinetics and monosaccharide specificity were observed [1–4]. To understand this transport at the cellular level we must consider the structure of the placenta and the plasma membranes involved.

Term human placenta has a villous structure and the external surfaces of the villi are covered with a syncytial cell layer of about 16 m^2 area, through which all fetal nutrients must pass [5]. The microvillous plasma membrane of the maternal-facing side of this cell layer has been isolated [6] and shown to possess a highly efficient facilitated diffusion system for transport of glucose [7,8]. Its K_m of 31 mM, which is well above normal maternal blood glucose concentrations [9], and its capacity strongly suggest that the glucose concentration of the syncytial cytosol must be nearly the same as that of maternal blood [7]. Since the basal mem-

* To whom correspondence should be addressed at: Department of Pediatrics, Washington University, St. Louis Children's Hospital, P.O. Box 14871, St. Louis, MO 63178, U.S.A.

brane has a smaller surface area, limitation to the transfer of glucose to the fetus is more likely to be at the fetal side of the syncytium. This membrane may also be a potential site for physiological transport control mechanisms and its transport properties may reveal essential information about materno-fetal transfer of glucose.

Recent work in our laboratory established a procedure for purification of the basal plasma membrane [10]. Enzyme markers and electron microscopy indicate it contains little contamination from the microvillous membrane or other organelles and should be suitable for transport studies. This paper reports an investigation of glucose transport across this membrane and initial studies of its mechanism and control.

Materials and Methods

Substrates. Unlabeled monosaccharides were obtained from Sigma (St. Louis, MO). ^{3}H-labeled D- and L-glucose and 3-*O*-methyl-D-glucose were from Amersham/Searle (Arlington Heights, IL).

Isolation of membrane vesicles. Basal plasma membrane was isolated from human placental trophoblast [10]. Briefly, tissue was sonicated to remove the maternal-facing plasma membrane and syncytial cytoplasm. It was washed in hypotonic medium, soaked in isotonic 10 mM EDTA and then resonicated to free the basal plasma membrane from the basal lamina. Free plasma membrane fragments were isolated by differential and gradient centrifugations. Isolated basal membrane was usually resuspended in Krebs-Ringer/phosphate buffer, 130 mM NaCl, 10 mM Na_2HPO_4, 4.2 mM KCl, 1.2 mM $MgSO_4$ and 0.75 mM $CaCl_2$, pH 7.4 (buffer I) and stored at 0°C without freezing to preserve the vesicular structure. Vesicles were found to be stable with quantitative retention of glucose transport properties for at least 5 days. Protein content was measured by the method of Lowry et al [11].

Transport measurements. Measurements of transport from the medium into the vesicles were performed under isotonic conditions [7]. Membrane vesicles suspended in buffer I (40 μl) were mixed with substrate, either D-[2^3H]glucose or 3-*O*-[1-^{3}H]methyl-D-glucose, (20 μl), with a sudden vortexing. They were incubated at 22°C for transport periods as short as 1 s (timed with a metronome) and the transport was stopped by diluting the medium 20-fold with ice-cold phloretin (0.5 mM, in buffer I plus 2% ethanol). The vesicles were separated from the substrate medium by filtration with mixed ester filters (0.45 μm pore size, Millipore Corp., Bedford, MA) and washed extensively with buffer I containing 0.1 mM phloretin and 1% ethanol. Filters were dissolved, and radioactivity was determined by scintillation spectrometry.

Measurement of the transport of the same substrates in an outward direction was performed by rapid dilution of preloaded vesicles (60 μl) with a large volume (1.1 ml) of buffer I at 22°C. Dilution of the external substrate to a low concentration initiated net outward transport of the internal substrate. The flux was stopped after measured intervals with 4 ml of ice-cold phloretin stopping solution, and the remaining internal substrate was determined as before.

For experiments involving equilibrium exchange of substrate, the vesicles were preequilibrated with substrate for at least 90 min. Uptake was then initiated by the addition to the medium of radioactive substrate at the same concentration as that used for preequilibration. Internal radioactivity was measured as before.

The effectiveness of the phloretin stopping solution was investigated in preliminary experiments. Losses of trapped glucose from vesicles during isolation were less than 3% and considered acceptable.

Results

Time course of uptake and egress

Transport of 3-*O*-[^{3}H]methylglucose into as well as out of the vesicles was measured in separate experiments. In either direction transport was very rapid for the initial 2 s and then slowed markedly to give biphasic curves (Fig. 1). The half-time for equilibration was less than 1 s for the rapid phase and approx. 1000 s for the slow phase. It would appear that at least two populations of membrane vesicles were present, one with a very efficient and rapid transport system, and one with very little or no transporter. This conclusion was supported by other observations. (1) The egress curve was bi-

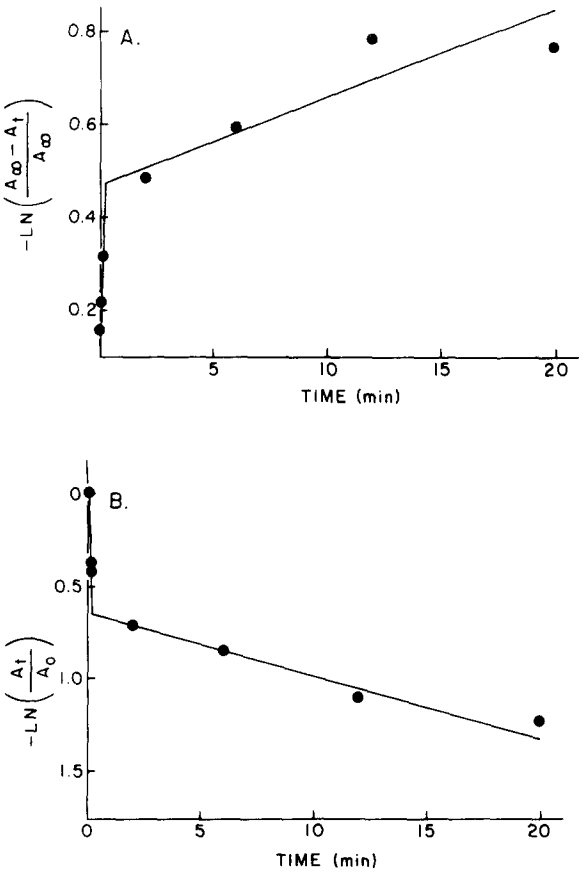

Fig. 1. (A) Time course of D-[^{3}H]glucose uptake. Membrane vesicles and D-[^{3}H]glucose solution (1 mM) were mixed, and glucose entry was stopped at the times indicated by dilution with ice-cold phloretin solution. The internal substrate was measured after isolation of the vesicles. The data are plotted with semi-logarithmic coordinates to demonstrate the clearly different rates of uptake at early versus late times. A_0 is initial internal substrate concentration, A_∞ is internal concentration at equilibrium and A_t is concentration at time t. (B) Time course of egress of D-[^{3}H]glucose. Vesicles preloaded with D-[^{3}H]glucose (1 mM) in buffer I were rapidly diluted with buffer I (20 vol. at 22°C) to reduce external D-[^{3}H]glucose concentrations. At the times indicated, vesicles were flooded with ice-cold phloretin solution and the internal D-[^{3}H]glucose concentration was determined. Rates of egress are markedly different in the first few seconds than in the next 20 min.

phasic only after a lengthy loading. A short loading period apparently filled predominently the rapidly filling vesicles, since subsequent egress was monophasic. (2) The slow uptake mechanism was apparently not saturable by increasing concentrations of substrate and thus not mediated. (3) Uptake during the slow phase was not inhibited by phloretin, although the rapid phase was very sensitive to this inhibitor. It is apparent that the slowly filling vesicles do not contain a typical mediated transport system.

Since we were concerned with the membrane which provides transport between syncytium and the fetal compartment, we report data hereafter using short uptake measurements. These measure only uptake into the population of vesicles with efficient transport, which may be expected to transport most of the glucose in vivo.

Nonspecific contributions to rapid transport

Nonmediated diffusion of monosaccharide into the rapidly transporting vesicles was estimated by comparing the uptake of L-glucose and of D-glucose. With 1 mM glucose and zero-*trans* uptake, the rates were approx. 4400 pmol/s per mg protein for D-glucose and 6.2 pmol/s per mg protein for L-glucose. Partially sealed vesicles which leak L-glucose at appreciable rates were not detected. This 700-fold difference in rates indicates that virtually all D-glucose uptake in short periods occurs by mediated transport and that mediated uptake can be measured directly without correction for a nonspecific contribution.

Kinetics

The kinetic properties of the transport system were measured under conditions of equilibrium exchange [12], since these conditions most nearly reflect the condition in vivo [7]. Vesicles were preequilibrated with 3-*O*-methylglucose and then uptake of the same substrate in the form of labeled tracer was measured. The rates of transport of labeled monosaccharide were strongly concentration dependent (Fig. 2).

Since the very rapid uptake process made measurements of initial velocities impossible, an integrated equation was used (Eqn. 1) [12,13]:

$$\frac{N_\infty - N}{N_\infty} = \Sigma w_i e^{-k_i rt} \qquad (1)$$

N is specific activity in the vesicles at time t, N_∞ is specific activity at equilibrium, w_i is a fractional weighting for the contribution of a given vesicle to

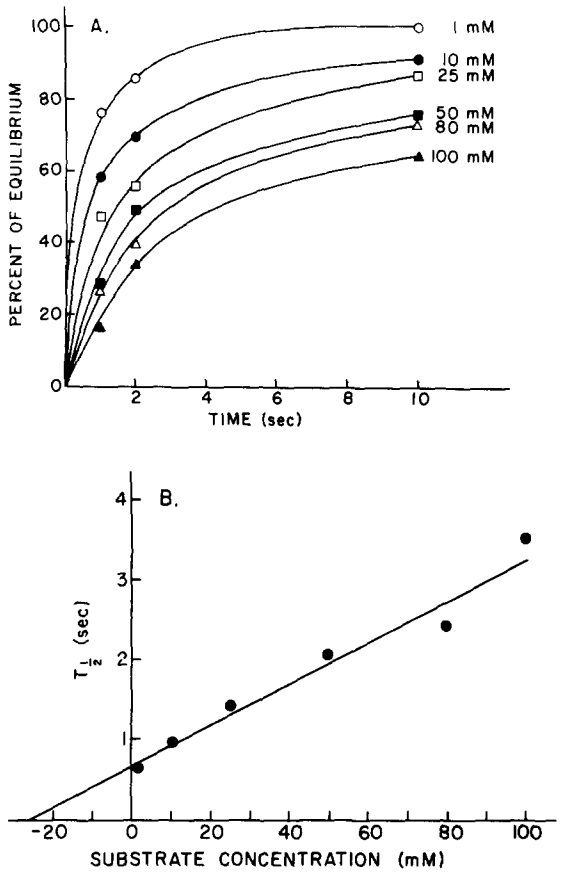

Fig. 2. Rates of transport as a function of substrate concentration. Membrane vesicles were first equilibrated with unlabeled 3-*O*-methylglucose, and then 3-*O*-[^{3}H]methylglucose, at the same concentration, was added. (A) Uptake of label as a function of time is shown for each substrate concentration. (B) Replot of data from (A). Half-times for equilibration are plotted against concentration. The *x*-intercept gives a K_m of 27 mM in this representative experiment.

the total label exchange of all vesicles, h_i is a heterogeniety factor depending on vesicle size and variations in transporter density, and r is a rate constant. This equation has been shown to take on Michaelis-Menten form at the half-time for exchange [12]:

$$\frac{1}{t_{1/2}} = \frac{1}{K_m + [S]} \cdot \frac{V_{max\ app.}}{\ln 2} \qquad (2)$$

The time $t_{1/2}$ when 50% of equilibration was achieved was derived from the time course at each concentration. These times were plotted against concentrations of substrate, and the K_m was taken from the *x*-intercept [14] as shown in Fig. 2B. The K_m thus derived was 23 ± 5 mM (mean ± S.D.) for five placentas.

Substrate specificity

To determine the structural requirements for substrate specificity, a variety of mono- and disaccharides present in a 9-fold excess were tested as competitors for 3-*O*-methylglucose transport (Table I). D-Glucose, 2-deoxy-D-glucose, 3-*O*-methyl-D-glucose, D-galactose, D-mannose, D-xylose and maltose all competed strongly, α- and β-methyl glucosides and fructose weakly, and L-glucose and L-xylose essentially not at all. Thus, the D-monosaccharides were all good competitors regardless of hydroxyl orientation. Hydroxyls number two and three were not essential and substitution at the anomeric carbon strongly hindered the recognition of substrate. L-Configuration monosaccharides were virtually unrecognized by the site.

TABLE I

COMPETION OF VARIOUS MONOSACCHARIDES WITH LABELED 3-*O*-METHYL-D-GLUCOSE

Uptake of labeled substrate (20 mM) was measured in the presence of various unlabeled competitors (180 mM) in media made isotonic with salts of buffer I. To avoid osmotic changes of internal volume caused by transport of sugars, times of uptake were kept short. Volume changes amounted to less than 10%.

Competitor	% inhibition of 3-*O*-methyl-D-glucose transport	Structural differences from glucose
L-Glucose	3	mirror image
L-Xylose	8	L-configuration, pentose
Fructose	11	ketose
α-Methyl-D-glucose	12	methylated at No. 1
β-Methyl-D-glucose	20	methylated at No. 1
Maltose	45	disaccharide
D-Xylose	49	pentose
D-Mannose	61	epimer at No. 2
3-*O*-Methyl-D-glucose	63	methylated at No. 3
D-Glucose	64	none
D-Galactose	68	epimer at No. 4
3-Deoxy-D-glucose	69	missing hydroxyl No. 2

Sodium gradients and monosaccharide transport

Since sodium is cotransported with glucose in other epithelia [15,16] and with amino acids in placental microvillous membrane [17], the effects of sodium concentration gradients on 3-*O*-[^{3}H]methylglucose transport were determined. Membrane vesicles were initially prepared in isotonic solutions of choline chloride buffered with potassium phosphate. Sodium and labeled monosaccharide were added simultaneously to the medium, and uptake of labeled monosaccharide was monitored over a short time course. Neither increased velocity of transport nor overshoot was observed. Sodium gradients do not affect glucose transport in basal plasma membrane.

Effects of inhibitors

Several inhibitors of glucose transport have been shown to have different effects depending on cell types and sodium dependence [16,18,19]. In placental basal membrane cytochalasin B at $1 \cdot 10^{-4}$ M proved to be a very effective inhibitor (81% inhibition) followed by phloretin (76%) and phloridzin (51%). Cytochalasin E, in spite of its structural similarity to cytochalasin B, was without effect (Fig. 3). This order of effectiveness resembles that seen in the red cell [18] and contrasts that of intestinal brush-border [19] or kidney apical membrane [16].

Steroids as inhibitors

Phloretin, a potent inhibitor of glucose transport in the basal membrane vesicles, is also weakly estrogenic [20]. Therefore, estrogens and progesterone, which were found to be inhibitory to glucose transport in apical plasma membrane of placenta [7], were tested for effects on glucose transport in basal membrane. Basal vesicles were preincubated with steroids or steroid analogues for 10 min and then subjected to zero-*trans* transport of ^{3}H-3-*O*-methyl glucose (Fig. 4). Diethylstilbestrol proved to be a potent inhibitor, with progresterone also showing good inhibition. Smaller inhibition was seen with estradiol and estriol. Cholesterol had little effect. The 5% ethanol present as solvent for the steroids had no effect. Moreover, addition of steroid after uptake of sugar did not destroy vesicles nor render them leaky. Transport inhibitory effects required large concentrations of steroid, ranging upwards from $1 \cdot 10^{-6}$ M.

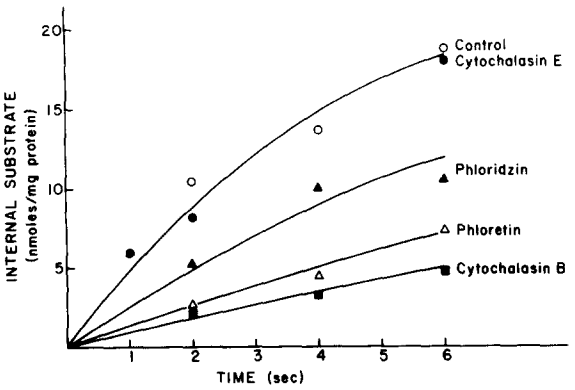

Fig. 3. Effects of various inhibitors on transport of 3-*O*-methylglucose. Uptake of substrate (50 mM) was measured in the presence of $1 \cdot 10^{-4}$ M inhibitor.

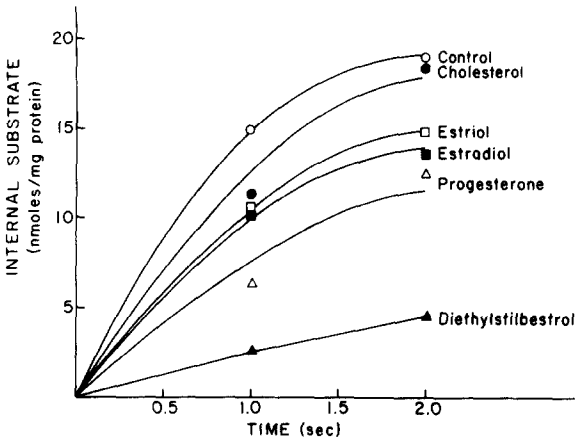

Fig. 4. The effects of steroids and steroid analogues on 3-*O*-methylglucose transport. Steroids or analogues were added to membrane suspensions to give 750 μM solution in 5% ethanol plus buffer I. During transport assay, the concentrations were 250 μM steroid and 50 mM 3-*O*-methylglucose.

Discussion

In order to understand transport across placental syncytium we must know whether there are differences in the characteristics of the transporter of the maternal- and fetal-facing plasma membranes. The highly efficient facilitated diffusion system of the basal membrane is substrate specific,

is inhibited by cytochalasin B, phloretin and phloridzin and has a high K_m. Sodium cotransport appears to play no part in the mechanism, since neither increased rates nor overshoot were evident in the presence of sodium gradients. In all of these characteristics this transport system seems similar to that in the opposite (microvillous) plasma membrane [7]. In many respects, however, the two membranes are quite different. The microvillous membrane contains large amounts of actin, alkaline phosphatase, and 5′-nucleotidase, while the basal membrane is rich in ouabain binding (($Na^+ + K^+$)-ATPase), adenylate cyclase and β-adrenergic receptors [10]. Thus, the two limiting membranes of the synctium clearly differ in their protein composition, yet their glucose transport proteins are functionally identical. In this regard, placental syncytium is quite different from other polarized epithelia such as those of gut and kidney, in which the two plasma membranes have glucose transport systems differing in sodium sensitivity, substrate preference, phloretin and phloridzin inhibition and kinetic properties [15,19,21–23].

It should be noted that contamination of the basal membrane preparation with microvilli is not responsible for the glucose transport reported here. The microvillous membrane marker alkaline phosphatase, indicates that at most 5–10% of the basal membrane preparation could be contaminating microvilli [10]. Glucose transport specific activities are nearly equal in the two membranes as prepared.

The transport properties reported here seem to classify this transport system as being a member of a particular group including that of the red blood cell [24], hepatocyte [25] and baso-lateral membrane of intestinal cells [26], in which the K_m is well above the normal blood concentration of about 5 mM [9]. In contrast, there is another group including that in the adipocyte [27] and kidney brush border [16] in which the K_m is much lower. The additional properties that phloretin inhibits more readily than phloridzin, and that transport is independent of sodium gradients, also indicate that the glucose transport system under study here is similar to the group typified in red blood cells.

The high K_m suggests an important physiological consequence. Our earlier work demonstrated that syncytial cytosol probably contains nearly the same glucose concentrations as does maternal blood [7]. Since we now find the K_m for basal membrane transport to be well above those concentrations, it follows that the transfer of glucose to fetal circulation is never saturated and must be a direct function of maternal blood glucose concentration.

A logical extension of the present work could be the use of its in vitro data to estimate total transport capacity of this membrane in vivo. However, one must be cautious in making such a calculation because of the heterogeniety in size of the isolated vesicles, as demonstrated by electron microscopy [10]. This heterogeniety in size implies that the exchange rates measured experimentally are really weighted averages derived from the heterogeneous vesicles (Eqn. 1). Eqn. 2, which is used to calculate kinetic parameters, therefore contains the term $1/t_{1/2}$ in place of velocity, and the apparent V_{max} is actually a constant proportional to the true V_{max} [13]. Nevertheless, the apparent V_{max} (166 nmol/s per mg protein) seems to imply that the transport capacity per unit of membrane area is similar to that in the microvillous membrane [7]. If the capacities per unit area are similar, then the basal membrane in vivo has less total capacity, since its surface area is only one-fifth that of the microvillous surface area [5]. This conclusion requires confirmation by independent methods and this work is in progress. It should be noted that the placenta itself may metabolize a major fraction of the entering glucose [28] and the microvillous membrane may need additional capacity to accommodate syncytial as well as fetal needs.

The preferred substrates for the transport site in basal membrane are all conformational analogues of D-glucose. The equatorial hydroxyl configuration in D-glucose tends to stabilize the C-1 chair conformation [29] and other sugars with this conformation are also recognized at the transport site. In the opposite extreme, the L-sugars tested tend to be in the 1-C conformation and were not recognized. Direct interactions of equatorial hydroxyls number two or three with the transporter was not necessary, since these hydroxyls could be absent (2-deoxy-D-glucose) or methylated (3-*O*-methyl-D-glucose) with no loss of activity. We found little

recognition of α- or β-substituted glucosides, which indicates that a free hydroxyl on the anomeric carbon is an essential recognition feature. The substrate specificity found in the present work for placental basal membrane appears to be identical to that found earlier for microvillous membrane of the syncytium [7].

The basal membrane glucose transport system is inhibited by large concentrations of placental steroids, much as is that of the microvillous membrane. These steroids are synthesized by the placenta and their local concentration in vivo, while not known precisely, may be presumed to be quite high. Smith and Brush [30] have reported a value of 1–2 μg of progesterone per g of isolated microvilli from placenta. This corresponds to about $5 \cdot 10^{-6}$ M, which is within the range where we observed inhibition of glucose transport. If steroids in vivo do inhibit transport they would appear to have effects on transport through both maternal-facing and fetal-facing membrane, since both sites are inhibited at the same steroid concentrations. This form of control over glucose flow would be unique in terms of mechanism and autonomy.

In summary, glucose transport across the basal membrane of placental syncytium occurs by a facilitated diffusion system. The characteristics of this transport system are very similar to those of the membrane on the opposite (maternal-facing) side of the syncytium. The high capacity of that microvillous membrane almost certainly causes the syncytial cytoplasm to be equilibrated with maternal blood. The flow of glucose across the syncytium and the resultant maternal-fetal gradient apparently depend on the relation between fetal glucose utilization and the transport capacity of the basal membrane.

References

1 Huggett, A.S.G., Warren, F.L. and Warren, N.V. (1951) J. Physiol. 113, 258–275
2 Widdas, W.F. (1952) J. Physiol. 118, 23–39
3 Davies, J. (1955) Am. J. Physiol. 181, 532–538
4 Rice, P.A., Rourke, J.E. and Nesbitt, E.L.,Jr. (1976) Gynecol. Invest. 7, 213–221
5 Laga, E.M., Driscoll, S.G. and Munro, H.N. (1973) Biol. Neonate 23, 231–259
6 Smith, N.C., Brush, M.G. and Luckett, S. (1974) Nature 252, 302–303
7 Johnson, L.W. and Smith, C.H. (1980) Am. J. Physiol. 238, C160–C168
8 Bissonnette, J.M., Black, J.A., Wickham, W.K. and Acott, K.M. (1981) J. Membrane Biol. 58, 75–80
9 Caraway, R.T. (1976) in Fundamentals of Clinical Chemistry (Tietz, N.W., ed.), p. 242, W.B. Saunders Co., Philadelphia
10 Kelley, L.K., Smith, C.H. and King, B.F. (1983) Biochim. Biophys. Acta 734, 91–98
11 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
12 Eilam, Y. and Stein, W.D. (1974) Methods Membrane Biol. 2, 304
13 Hopfer, U. (1981) Fed. Proc. 40, 2480–2485
14 Kotyk, A. and Janacek, K. (1975) Cell Membrane Transport, Principles and Techniques, p. 76, Plenum Press, New York
15 Goldner, A.M., Schultz, S.G. and Curran, P.F. (1969) J. Gen. Physiol. 53, 362–383
16 Turner, R.J. and Silverman, M. (1977) Proc. Natl. Acad. Sci. USA 74, 2825–2829
17 Boyd, C.A.R. and Lund, E.K. (1981) J. Physiol. 315, 9–19
18 LeFevre, P.G. (1961) Pharmacol. Rev. 13, 39–70
19 Hopfer, U., Sigrist-Nelson, K., Ammann, E. and Murer, H. (1976) J. Cell. Physiol. 89, 805–810
20 Dodds, E.C. and Lawson, W. (1938) Proc. R. Soc. Lond., Ser. B 125, 222–232
21 Bihler, I. and Cybulsky, R. (1973) Biochim. Biophys. Acta 298, 429–437
22 Kimmich, G.A. and Randles, J. (1976) J. Membrane Biol. 27, 363–379
23 Stevens, B.R., Kaunitz, J.D. and Wright, E.M. (1984) Annu. Rev. Physiol. 46, 417–433
24 Naftalin, R.J. and Holman, G.D. (1977) in Membrane Transport in Red Cells (Ellory, C.J. and Lew, V.L., ed.), pp. 257–299, Academic Press, New York
25 Elliott, K.R.F. and Craik, J.D. (1982) Biochemical Society Transactions 10, 12–13
26 Wright, E.M., Van Os, C.H. and Mircheff, A.K. (1980) Biochim. Biophys. Acta 597, 112–124
27 Gliemann, J. and Rees, W.D. (1983) Current Topics in Membranes and Transport 18, 339–379
28 Meschia, G., Battaglia, F.C., Hay, W.W. and Sparks, J.W. (1980) Fed. Proc. 39, 245–249
29 Reeves, R.E. (1950) J. Am. Chem. Soc. 72, 1499–1506
30 Smith, N.C. and Brush, M.G. (1978) Med. Biol. 56, 272–276