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Apical sodium-sugar transport in pulmonary epithelium in situ

Guy Basset, Georges Saumon, Francine Bouchonnet and Christian Crone *

INSERM U82 and Département de Physiologie, Faculté Xavier Bichat, Paris (France)

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The presence of an apical sodium-coupled transport system for D-glucose in lung alveolar epithelial cells has been demonstrated in lungs instilled with Ringer's fluid and perfused with either blood or Ringer's fluid (Basset et al. (1987) J. Physiol. 384, 325–345). The direction of transport is from alveoli towards interstitium. The characteristics of the system were evaluated in similar preparations by use of sugar analogues such as α-methyl-glucopyranoside, 2-deoxyglucose, 3-*O*-methylglucose and L-glucose. The main finding was the presence of a transport system for α-methylglucopyranoside and 2-deoxyglucose in the apical cell membrane. This system was unaffected by phloretin. Both α-methylglucopyranoside and 2-deoxyglucose transports were inhibited by phloridzin and by the presence of glucose (10^{-2} mol · l^{-1}). Competition was demonstrated between D-glucose and α-methylglucopyranoside or 2-deoxyglucose, but not for 3-*O*-methylglucose or L-glucose. 3-*O*-Methylglucose was cleared as slowly as L-glucose. The results comply partly with those known from intestinal epithelium and kidney proximal tubular epithelium, but the handling of 3-*O*-methylglucose was different. The relative transport rates of Na^+ and glucose are compatible with a Na^+: glucose coupling ratio larger than one.

Introduction

Na^+-coupled glucose transport has mainly been described in two 'leaky' epithelia, jejunum and proximal kidney tubules. This transport system in combination with active sodium extrusion at the basolateral cell membrane, enables glucose transport to contribute to the isoosmotic reabsorption of water in these epithelia. Recently, we described a similar cotransport system in the luminal cell membrane of alveolar epithelium in rat lung [1]. Transepithelial glucose transport depending on the presence of Na^+ in the alveolar fluid was revealed in experiments where Na^+ was substituted by choline. Under these conditions glucose transport rate fell to one third of its value under control conditions. The effect of phloridzin in the instillate (10^{-3} mol · l^{-1}) confirmed the presence of a Na^+/glucose symport since the rate of D-glucose transport fell to almost zero. When phloretin (10^{-3} mol · l^{-1}) was present in the instillate the rate of removal of D-glucose was hardly affected [1].

Since only few details of this cotransport system in lung epithelium are known, we here report supplementary information on the specificity of the system using D-glucose analogues and inhibitors of sugar transport.

The majority of the experiments were carried out on rat lungs perfused from a donor rat ('circulation croisée'). Isoosmotic Ringer's fluid was instilled into the alveolar spaces via the trachea as

* Present address: Institute of Medical Physiology, Dept A, The Panum Institute, DK 2200 Copenhagen N, (Denmark).

Correspondence: G. Saumon, INSERM U82 and Département de Physiologie, Faculté Xavier Bichat, 75018 Paris, France.

earlier described in detail [1,2]. To measure transport in the reverse direction, from the perfusate into alveolar fluid, an isolated Ringer-perfused preparation was used. The glucose analogues used to assess the system were: α-methylglucopyranoside, 2-deoxyglucose, 3-*O*-methylglucose, and L-glucose.

The main result was that the Na^+/glucose cotransport system in the luminal membrane of alveolar epithelium shares characteristics with that in the two above-mentioned epithelia except that 3-*O*-methylglucose was not transported transcellularly.

Materials and Methods

The rat preparations used in the present experiments have been described in detail previously [1,2] and only the main points and special techniques are presented. Two preparations were used: cross circulation and Ringer perfusion.

Cross circulation technique. The vascular systems between two rats were connected in the following way: in the donor rat a catheter was placed in the abdominal aorta. To establish the blood pressure necessary for the coronary perfusion a Starling resistor was placed in the leads to

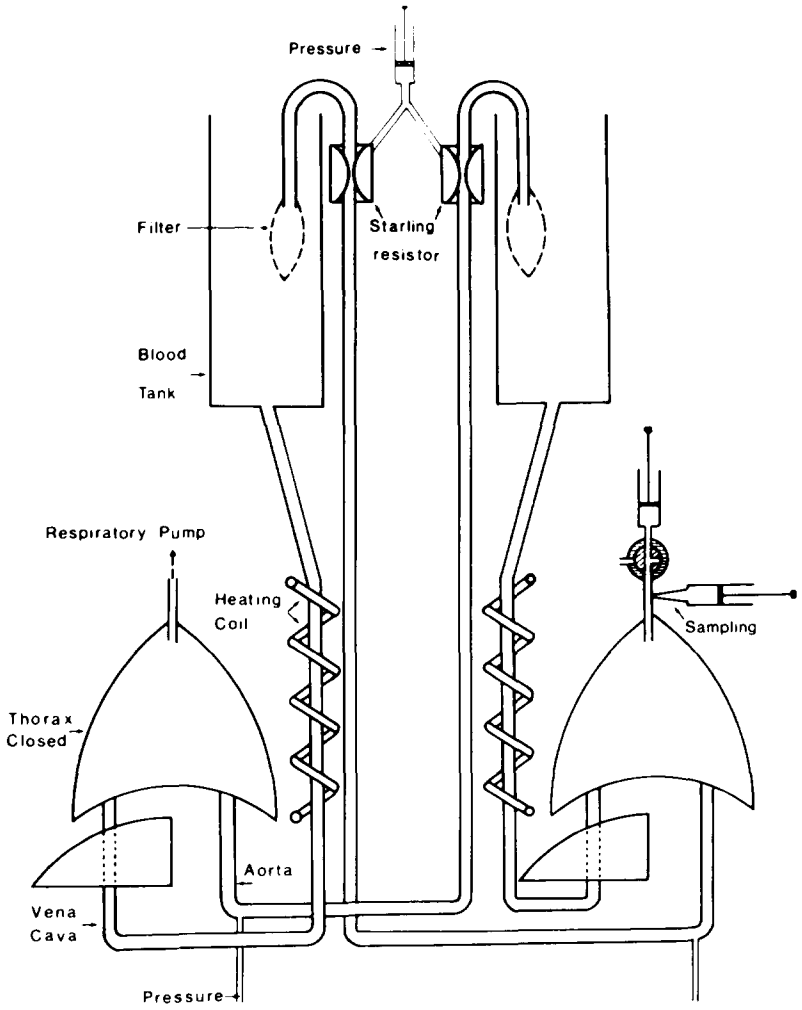

Fig. 1. Sketch of the preparation used in cross-circulation experiments. The donor rat was ventilated, thus assuring oxygenation of the blood. This was passed via a Starling resistor to the experimental preparation where it was perfusing the experimental lung, instilled with Ringer's fluid. The blood was returned to the inferior caval vein of the donor rat via a Starling resistor.

the recipient (Fig. 1). The catheter leads to the inferior caval vein of the recipient, thus allowing oxygenated blood to pass via the right heart to the lungs. These were instilled with Ringer's fluid from which samples were taken at intervals. Blood leaving the lungs was returned via a Starling resistor and connected to the inferior caval vein of the donor, thus establishing a complete circuit. The abdomen of the two rats was closed and by adjustment of the venous blood pressure it was assured that the cardiac outputs from the two hearts were the same. A concentration of about 10 mmol $\cdot l^{-1}$ of D-glucose in the blood was obtained by 0.5 ml of 50% glucose placed subcutaneously in the donor rat.

Isolated, Ringer-perfused lungs. The perfusion system contained a rotating disc oxygenator ensuring proper oxygen transport carrying capacity of the fluid that contained about 4% red cells (v/v). When the perfusion system was ready the lungs were dissected free from the thoracic cavity and ventilated. The perfusate was circulated by means of a roller pump. The temperature of the perfusate was kept at 37 °C by means of water jackets. The isolated lungs were placed in a thermostated glass chamber. The volume of the perfusate was about 25 ml. The rate of perfusion was about 8 ml $\cdot$ min^{-1}.

Instillation technique. 2 ml of alveolar air were withdrawn, followed by instillation of 5 ml of Ringer's fluid, containing various sugars as dictated by the nature of the experiment. When phloridzin or phloretin was added to the instillate the concentration was 10^{-3} mol $\cdot l^{-1}$. After instillation, 0.5 ml of air were pushed into the airways to clear the bronchial dead space from fluid.

Samples. After 10 min of equilibration between the perfusate and instillate, samples were taken every 30 min. Altogether the experiments lasted 100 min (three experimental periods). First, 0.5 ml of bronchial air was aspirated; then about 0.5 ml of the instillate was withdrawn into a syringe and weighed. Then fluid was returned to the alveoli, followed by 0.5 ml of air to clear the dead space.

Composition of Ringer's fluid (mmol $\cdot l^{-1}$). Na^+, 145; K^+, 5; Ca^{2+}, 1; Mg^{2+}, 1; Cl^-, 129; HCO_3^-, 25. This fluid was equilibrated with 95% O_2/5% CO_2 for half an hour before the experiment. D-Glucose (10 mmol $\cdot l^{-1}$) was added to the perfusate in the isolated lung preparations. The instillate was usually free from D-glucose, and D-mannitol was present at 10 mmol $\cdot l^{-1}$ to ensure isoosmolarity. In some experiments, D-glucose was placed in the instillate in order to study competition between D-glucose and the analogues.

Tracers. ^{125}I-albumin (CNTS, Orsay, France); L-[1-^{3}H]- and L-[1-^{14}C]glucose (New England Nuclear, Frankfurt, F.R.G.); 3-*O*-methyl[1-^{3}H]-glucose and methyl (α-D[U-^{14}C]gluco)pyranoside (Amersham, Buckingham, U.K.), and 2-deoxy-D-[1-^{14}C]glucose (CEA, Saclay, France). The unlabelled compound concentrations ranged from $0.2 \cdot 10^{-6}$ to $4 \cdot 10^{-6}$ mol $\cdot l^{-1}$.

Inhibitors. Phloridzin and phloretin were obtained from Sigma Inc., St. Louis, MO, U.S.A.

Determination. After centrifugation of the sample of instillate, determinations of solute concentrations in the supernatant were performed. D-Glucose concentration was measured with the glucose-oxidase method (Sigma); Na^+ and K^+ were determined by flame photometry (Eppendorf, Hamburg, F.R.G.), and Cl^- by coulometry (Radiometer, Copenhagen, Denmark); γ- and β-radiating isotopes were counted in a γ-counter and a liquid scintillation counter (Intertechnique, Plaisir, France). Proper corrections for spill-over between ^{3}H and ^{14}C channels were made.

Calculations. The activity of ^{125}I-albumin was assessed on every sample to indicate the movement of fluid, on the assumption that albumin stays within the instillate during the experiment. The movement of fluid out of the alveoli was calculated from the following expression.

$$V_{t_2} = V_{t_1} C_{t_1} / C_{t_2}$$

where V_{t_1} is the volume of instillate at time t_1, V_{t_2} the volume of instillate at time t_2, and C_{t_1} and C_{t_2} the activity ^{125}I-albumin at time t_1 and t_2.

The clearance (Cl) of the sugar from the instillate is calculated as

$$\text{Clearance} = (V_{t_1} C_{t_1} - V_{t_2} C_{t_2}) / (\bar{C}(t_2 - t_1))$$

where C refers to the sugar concentrations. $\bar{C}$ is the arithmetic mean concentration during the experimental period. The clearance is given as $\mu l \cdot s^{-1}$. Since the passive paracellular passage of the

sugar analogues was not determined, we use the term 'clearance' to indicate the efficiency of transalveolar transport without reference to the pathway followed by the substance. It is a reasonable assumption because molecular weights of tracers are close to that of mannitol; a good estimate of paracellular passage for all substances is given by mannitol clearance, reported in earlier studies to be about 0.05–0.1 $\mu l \cdot s^{-1}$ [1,2].

The results are given as means of the first, second and third period, respectively (in different experiments). The experimental variation is expressed as the standard error of mean and results were analyzed by one-way analysis of variance.

Results

1. Accumulation of α-methylglucopyranoside in the alveolar epithelium

It is agreed that the apical entry of D-glucose in absorbing epithelia depends on the electrochemical gradient for Na^+ [3]. The 'force' represented by this gradient enables D-glucose to be accumulated against a considerable concentration difference, depending on the coupling ratio of Na^+/glucose and passive permeabilities [4]. To avoid problems due to cellular consumption of D-glucose we used α-methylglucopyranoside that traces D-glucose in the Na^+/glucose symport [5]. A further advantage of this substance relates to its extremely small passive leakage from cells [6]. α-Methylglucopyranoside was present in tracer doses. Due to the low specific activity of the tracer the unlabelled concentration was about 5–10 nmol $\cdot l^{-1}$.

To estimate the intracellular accumulation an indirect method was used since it is impossible to specifically isolate the epithelium from lung tissue. The difference between the amount of α-methylglucopyranoside disappearing from the instillate and the amount appearing in the perfusate is assumed to represent the net accumulation in the epithelium. The results depicted in Fig. 2 were obtained. It is seen that the accumulation of α-methylglucopyranoside progresses rapidly and reaches a plateau after 70 min, a rate of accumula-

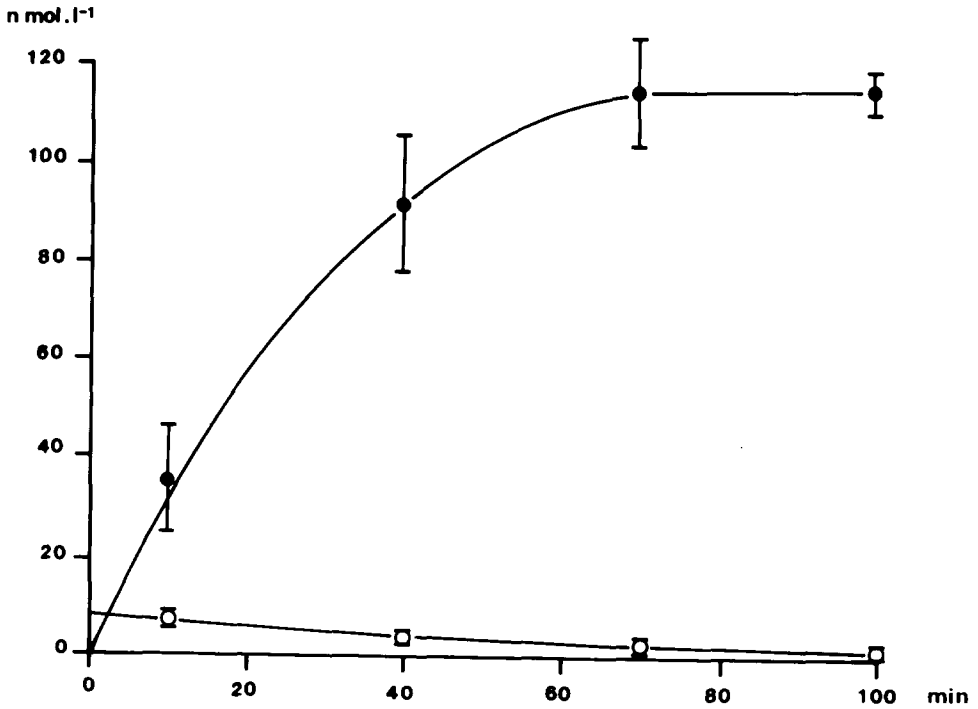

Fig. 2. Accumulation of α-methylpyranoside in lung alveolar epithelium when presented to the epithelium from the alveolar instillate (●). The simultaneous reduction of the concentration of this substance in the instillate is shown on the lower curve (○). The estimate of the accumulation ratio was calculated as explained in the result section. D-Glucose was not present in the instillate.

tion that corresponds closely to that found by Kimmich and Randles [6] in intestinal epithelium. Morphometric studies have shown that a 300 g rat has a total epithelial cell volume of 0.12 ml [7]. Disregarding the fact that the cell only has a free water space of about 75%, and although this estimate, evidently, is relatively uncertain, it suggests that the intracellular concentration is at least 100 times that in the alveolar fluid. Fig. 2 also shows the corresponding decline in concentration of α-methylglucopyranoside in the instillate.

2. Relative transport of the D-glucose analogues

The clearance of three analogues of D-glucose were studied besides that of α-methylglucopyranoside, 2-deoxyglucose, 3-*O*-methylglucose and L-glucose. As shown in our earlier studies the epithelium has a minor paracellular leak for small hydrophilic solutes. The paracellular clearance of mannitol is about 0.05 $\mu l \cdot s^{-1}$ [1]. In the present experiments L-glucose was supposed to move essentially via the paracellular route, an expectation that was confirmed inasmuch as the clearance of L-glucose was in the same range as that for mannitol, cf. Table I. Unexpectedly, the same was true for 3-*O*-methylglucose in the present experimental conditions (Table I).

We made a tentative evaluation of the kinetics of the transport systems. Since the sugar analogues were present in low concentrations their cellular uptake would be linearly dependent on the concentration in the instillate. Under such conditions the Michaelis-Menten expression takes the following form:

$$J = J_{max}C/K_m$$

where J is the observed efflux from the alveolar fluid; J_{max} is the maximal transport rate, and C is the concentration of the substance in the alveolar instillate. According to this version of the Michaelis-Menten expression the uptake is proportional to the value of J_{max}/K_m, that depends both on affinity and the maximal transport rate. According to Table I these 'permeabilities' varied in the following order: α-methylglucopyranoside > 2-deoxyglucose > 3-*O*-methylglucose > L-glucose.

Besides this evaluation of relative transport rates the specificity of the system was evaluated using phloridzin that mainly interferes with the cotransport system [8] and phloretin interfering with facilitated transport system [9].

With reference to Table I it is seen that the effects of the two inhibitors were very different. As regards α-methylglucopyranoside, phloridzin produced a major reduction of clearance from the alveoli. Phloridzin also exerted a clear effect on 2-deoxyglucose, a finding corresponding to that of Fricke and Longmore [10], who however used a preparation with 2-deoxyglucose added to the perfusate, 3-*O*-methylglucose and L-glucose were unaffected by either inhibitor.

As shown in Table I the presence of 10 $mmol \cdot l^{-1}$ D-glucose in the instillate clearly reduced the

TABLE I

TRANSPORT RATES OF ANALOGUES OF D-GLUCOSE ACROSS ALVEOLAR EPITHELIUM EXPRESSED AS CLEARANCES FROM INSTILLATE TO PERFUSATE

The clearance is the ratio of transport rate and mean sugar concentration. It is expressed as $\mu l \cdot s^{-1}$. The figures represent average values and standard deviation. The number of experimental periods is given in parenthesis. The inhibitors were placed in the alveolar fluid. 10 $mmol \cdot l^{-1}$ D-glucose was present in the perfusate. α-Methylglucopyranoside was studied simultaneously with 3-*O*-methylglucopyranoside.

Instillate with	α-Methylglucopyranoside	2-Deoxyglucose	3-*O*-Methylglucose	L-Glucose
Mannitol (10^{-2} $mol \cdot l^{-1}$)				
Control	1.36 ± 0.194 (15)	0.59 ± 0.035 (12)	0.09 ± 0.0200 (21)	0.01 ± 0.014 (26)
Phloridzin	0.11 ± 0.039 (10)	0.14 ± 0.030 (9)	0.10 ± 0.0418 (9)	0.02 ± 0.021 (12)
Phloretin	1.42 ± 0.295 (9)	0.48 ± 0.038 (9)	0.11 ± 0.020 (9)	0.02 ± 0.021 (9)
Glucose (10^{-2} $mol \cdot l^{-1}$)				
Control	0.23 ± 0.026 (12)	0.11 ± 0.021 (15)	0.05 ± 0.022 (9)	0.04 ± 0.012 (12)

removal of α-methylglucopyranoside and 2-deoxyglucose, whilst 3-*O*-methylglucose and L-glucose were unaffected.

3. Transport of sugar analogues from perfusate to alveoli

In experiments using isolated ventilated rat lungs, the presence of a Na^+-dependent α-methylglucopyranoside accumulation in lung tissue has been reported as the sugar was placed in the perfusate [11]. This observation leaves open the possibility that a cotransport system is also present at the plasma side. To evaluate this possibility, we followed on isolated, liquid-filled lungs, the fate of α-methylglucopyranoside and 3-*O*-methylglucose placed in the perfusate. Were a cotransport for α-methylglucopyranoside present, the activity ratio α-methylglucopyranoside/3-*O*-methylglucose would decrease. After 100 min of experiment, this ratio did not vary from its initial value (Fig. 3). In contrast, conditions in the instillate were such that this ratio was low, reflecting the activity of the apical cotransporter (Fig. 3). It is likely that the observed uptake of α-methylglucopyranoside [11] was a consequence of the apical cotransport: the sugar leaking into alveoli via the paracellular pathway, and then being accumulated in epithelial cells. The presence of phloridzin in the instillate did not affect the course of concentration of the two sugars in the perfusate. As expected, their ratio did not decrease in the instillate (Fig. 3). This lack of effect of inhibition of the apical cotransport on the perfusate α-methylglucopyranoside activity is probably due to the very low fraction of the tracer that leaked into the alveoli, as a result of the low paracellular permeability of the epithelium to hydrophilic solutes. It is however possible that the presence of 10 mmol · l^{-1} D-glucose might have interfered. However, in supplementary experiments with low concentrations of D-glucose the same pattern was observed. Since alveolar epithelium needs a supply of D-glucose from the perfusate, there are experimental limits to how much the concentration of D-glucose can be lowered. We used 1 mmol · l^{-1} glucose and the preparation tolerated this low concentration well.

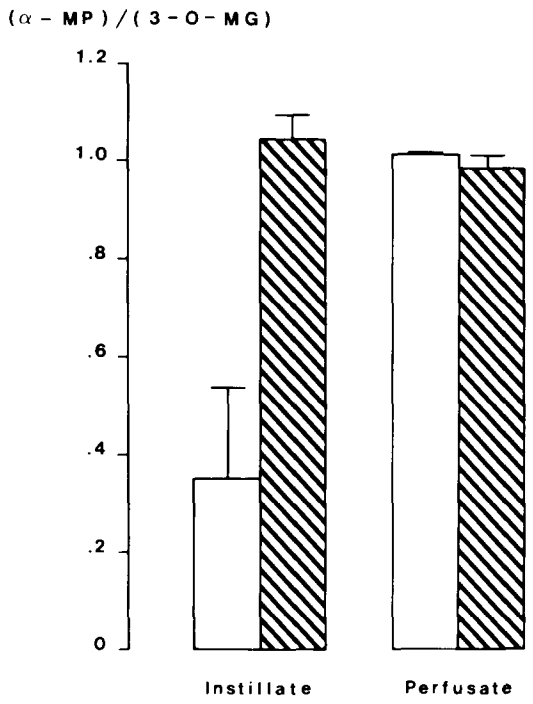

Fig. 3. α-Methylglucopyranoside/3-*O*-methylglucose activity ratios (expressed relative to the ratio at the beginning of the experiment) at the end of 100 min experiments (three in each situation). White bars: control conditions; dashed bars: phloridzin (10^{-3} mol·l^{-1}) added in the instillate. Were a Na^+/α-methylglucopyranoside co-transport present in the apical membranes, this ratio would decrease, as observed for the instillate. When phloridzin was placed in the instillate, the α-methylglucopyranoside/3-*O*-methylglucose ratio stayed at the perfusate level, indicating the inhibition of the cotranport. Since the α-methylglucopyranoside/3-*O*-methylglucose ratio did not decrease in the perfusate, this signifies absence of Na^+/sugar cotransport at the interstitial side of alveolar cells.

Discussion

The main interest in this paper relates to similarities of D-glucose transport in pulmonary epithelium and in intestinal or proximal kidney epithelium. Since the alveolar epithelium is a newly discovered fluid-absorbing epithelium [1,2] it is of interest to know possible similarities with other fluid-absorbing epithelia. Results obtained so far suggest that the alveolar epithelium compares in many ways with 'leaky' epithelia such as the above-mentioned.

Although the treatment of glucose by lung tissue has been studied with different techniques, only few of these investigations allow comparison with the present results. The work that most closely

resembles the present one was carried out by Wangensteen and Bartlett [12] who compared the fate of D- and L-glucose instilled into the alveoli of perfused rat lungs. They found that D-glucose appeared faster in the perfusate than L-glucose. At variance with the present findings, they reported that α-methylglucopyranoside left the alveoli as slowly as did L-glucose. Since they calculated transport rate from the appearance of tracers in the perfusate, their results may reflect the trapping of α-methylglucopyranoside in the epithelial cells, that would occur in the absence of a basolateral leakage of this tracer. Thus their results comply with the present results as regards the absence of a cotransport system for α-methylglucopyranoside in the baso-lateral membrane of alveolar cells (Fig. 3). They described that phloridzin reduced the transcellular movement of that D-glucose by about 60%, but did not draw the conclusion that glucose is cotransported with sodium. We have earlier provided evidence in favour of such a system in the apical cell membrane of alveolar epithelium [1] and the present results fully support this conclusion.

Our studies show that a coupled Na^+/α-methylglucopyranoside system is present in the apical membrane. Kerr et al. [11] studied α-methylglucopyranoside transport out of the perfusate in the presence and absence of Na^+ or ouabain and showed that α-methylglucopyranoside accumulated in the lung suggesting that a cotransport transport system was present in the alveolar epithelium basolateral membrane. If, as we propose, α-methylglucopyranoside did leak into the alveolar film via paracellular pathways to be recaptured by the apical membrane (similarly to what was observed in the small intestine [13]) their results would agree with the present findings.

The presence of an apical cotransport system for α-methylglucopyranoside is strongly supported by the differential effect of phloridzin and phloretin on the clearance of α-methylglucopyranoside. As shown in Table I phloretin does not exert any effect, whilst phloridzin dramatically reduces α-methylglucopyranoside transport rate.

The clearance of 2-deoxyglucose (Table I) was rather large. It is commonly assumed that 2-deoxyglucose passes into cells via facilitated transport to be trapped intracellularly and phosphorylated. The macrophages contain a facilitated transport system for D-glucose and 2-deoxyglucose [14] as do other cells. Since the influx of 2-deoxyglucose into the alveolar macrophages continue because of the intracellular trapping of a phosphorylated 2-deoxyglucose molecule, even a small population of cells might be responsible for a part of the removal of 2-deoxyglucose which was present only in tracer doses in these experiments.

Surprisingly the clearance of 2-deoxyglucose was inhibited by phloridzin and not by phloretin. Silverman et al. [15] describe a similar finding in kidney epithelium and proposes the presence of both an apical Na^+-dependent system and a baso-lateral facilitated carrier system for this analogue.

3-*O*-Methylglucose had a very low clearance (Table I) and was unaffected by the two inhibitors. As mentioned earlier this possibly signifies that this analogue is not taken up by the alveolar epithelium, at variance which other cell types [6,16].

Although the stoichiometry of the cotransport cannot be precisely assessed with the present technique, a semi-quantitative estimation favours a coupling Na^+/glucose ratio larger than one. First, when the amiloride dependent pathway of Na^+ transport in the apical membrane was blocked [1], leaving only Na^+ absorption via the Na^+/glucose cotransport, the net flux of glucose was 3.8 nmol · s^{-1} from alveoli simultaneously with a net Na^+ flux of 8.5 nmol · s^{-1} [1]. Second, the calculation of a cellular concentration of α-methylglucopyranoside of 100-fold that in the instillate is a too large value to be explained by a 1 : 1 coupling ratio [17], since there is a low transalveolar negative potential in the alveoli [18] and no special reason to expect an electrochemical gradient of Na^+ across the apical membrane of alveolar cells different from that in other epithelial cells.

A consequence of the presence of a D-glucose/Na^+ cotransport in alveolar apical cell membranes is that the glucose concentration in the alveolar lining layer must be low. A gross estimation of glucose concentration in alveolar lining layer based on recent observations in this laboratory from broncho-alveolar lavage in rats using the urea dilution method [19] support this contention.

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