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# Endogenous D-Glucose Transport in Oocytes of *Xenopus laevis*

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**Summary.** Endogenous glucose uptake by the oocytes of *Xenopus laevis* consists of two distinct components: one that is independent of extracellular $Na^+$, and the other one that represents $Na^+$-glucose cotransport. The latter shows similar characteristics as 2 $Na^+$-1 glucose cotransport of epithelial cells: The similarities include the dependencies on external concentrations of $Na^+$, glucose, and phlorizin, and on pH. As in epithelial cells, the glucose uptake in oocytes can also be stimulated by lanthanides. Both the electrogenic cotransport and the inhibition by phlorizin are voltage-dependent; the data are compatible with the assumption that the membrane potential acts as a driving force for the reaction cycle of the transport process. In particular, hyperpolarization seems to stimulate transport by recruitment of substrate binding sites to the outer membrane surface. The results described pertain to oocytes arrested in the prophase of the first meiotic division; maturation of the oocytes leads to a downregulation of both the $Na^+$-independent and the $Na^+$-dependent transport systems. The effect on the $Na^+$-dependent cotransport is the consequence of a change of driving force due to membrane depolarization associated with the maturation process.

**Key Words** *Xenopus* oocyte · glucose · cotransport · flux · voltage clamp

## Introduction

The oocytes of *Xenopus laevis* have become a widely used expression system for mRNA encoding ion-selective channels (Barnard, Miledi & Sumikawa, 1982; Gundersen, Miledi & Parker, 1983) and carriers (Morgan et al., 1985) from other cells. However, the oocytes possess a variety of endogenous transport systems in their plasma membrane, the study of which is interesting in its own right. These transport systems include ion-selective channels (*see*, e.g., Robinson, 1979; Baud, Kado & Marcher, 1982; Kusano, Miledi & Stinnakre, 1982; Lotan, et al., 1982), as well as carriers (Wallace & Steinhardt, 1977; Richter, Jung & Passow, 1984; Eckard & Passow, 1987) with properties similar to those found in somatic cells of different sources. The large size (more than one millimeter in diameter) of the oocytes makes them particularly suited for the study of carrier-mediated transport. The oocytes are large enough to perform flux measurements, even with a single cell, and to apply simultaneously electrophysiological methods (Grygorczyk et al., 1989). In the present paper, we report on the uptake of $^{14}C$-labeled glucose. We demonstrate that glucose uptake by the oocytes consists of a $Na^+$-independent and a $Na^+$-dependent flux component. The $Na^+$-dependent component represents an electrogenic process; the kinetics of this component, notably the dependence on membrane potential of glucose uptake and inhibition of uptake by phlorizin, suggest a cotransport of $Na^+$ and glucose. In the past, such cotransport was thought to be confined to certain epithelia. The characteristics of the transport systems observed in the oocytes closely resemble those of $Na^+$-glucose cotransport described for the epithelial cells. A short report on part of the results has been presented previously (Weber, Schwarz & Passow, 1988).

## Materials and Methods

### Oocytes

Females of the clawed toad *Xenopus laevis* were anesthetized on ice, and parts of the ovary were removed. Full-grown oocytes arrested in the prophase of the first meiotic division (type V or VI after Dumont (1972)) were selected after removal of enveloping tissue by treatment of the ovarial pieces with collagenase (1.5 U/ml Barth's solution (*see below*)) and subsequent washing in $Ca^{2+}$-free Barth's solution. In vivo maturation to the metaphase of the second meiotic division was induced by injection of human chorionic gonadotropin (600 IU) into the lymph sack of the female. The maturated, shed oocytes were collected and the jelly coat was removed by washing in Barth's solution with mercaptoethanol (45 $\mu$M, pH 9.5 for 2 min).

### Uptake Experiments

To measure the uptake of glucose, oocytes were incubated at room temperature in oocyte Ringer's solution (*see below*) or

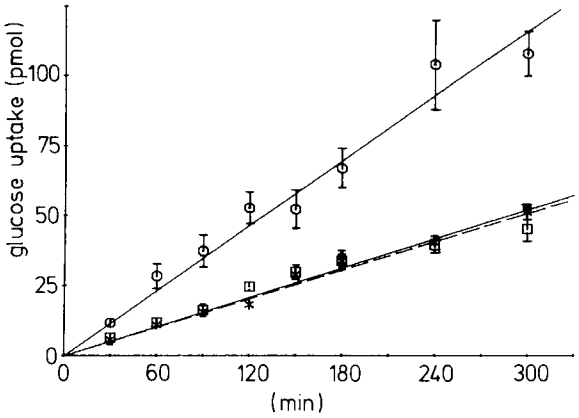


**Fig. 1.** Effect of $Na^+$ and phlorizin on the time course of glucose uptake. Circles represent measurements in control Ringer's solution, squares in $Na^+$-free solution, and asterisks in Ringer's solution containing 1 mM phlorizin. Each data point represents the average of 10 oocytes, the bars indicate SEM; for symbols without error bar SEM is smaller than the size of the symbol. The straight lines are fitted to the data using least-squares methods. The slopes (in pmol/(hr oocyte) ± SEM) are: for control solution, 23.2 ± 0.8; for $Na^+$-free solution, 10.4 ± 0.2; and for Ringer's solution containing 1 mM phlorizin 10.1 ± 0.5. The glucose concentration was the same for all curves and amounted to 2.01 mM

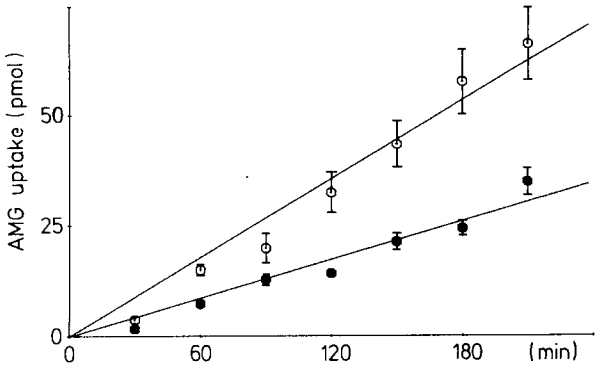


**Fig. 2.** Time course of AMG (α-methyl D-glucopyranoside) uptake. AMG is transported only by the $Na^+$-dependent transport system. Each data point represents the average of 20 oocytes; the bars indicate SEM; for symbols without error bar SEM is smaller than the size of the symbol. The slopes of the fitted lines (in pmol/(hr oocyte ± SEM) and are 8.4 ± 0.4 and 18.0 ± 0.6 for 1.04 mM nd 3.04 mM AMG, respectively

Barth's solution containing either D-glucose or AMG (α-methyl D-glucopyranoside) labeled with $^{14}C$ (370 and 148 kBq in 500 μl incubation medium, respectively). After suitable lengths of time, 8 to 15 oocytes were removed from the medium. The cells were washed, placed individually into counting vials, and dissolved in 0.1 ml sodium dodecyl sulfonate solution. The radioactivity taken up by the oocytes was then determined by liquid scintillation counting. All data presented in the Results refer to average values obtained from single oocytes.

The dependence of uptake rates ($k$) on external glucose or $Na^+$ concentrations ($[X_o]$) could be described by the expression

$$k = k_{max} \frac{[X_o]^n}{(K_{1/2})^n + [X_o]^n} . \quad (1)$$

The parameter values were calculated using a curve-fitting procedure based on least-squares methods. The dependence on phlorizin concentration ($[P]$) of the degree of inhibition ($I$) could be described correspondingly by

$$I = \frac{[P]}{K_I + [P]} . \quad (2)$$

## Electrophysiological Measurements

Measurements of membrane potential and voltage clamp were performed by conventional microelectrode techniques (*see* Lafaire & Schwarz, 1986). For uptake measurements under voltage clamp, oocytes were exposed to Ringer's solution containing $^{14}C$-glucose and kept under constant voltage-clamp conditions for 0.5 or 1 hr. To determine voltage dependence of inhibition by phlorizin, this procedure turned out to be unsuitable; measurements at the very low rates of glucose uptake, observed in the presence of phlorizin, are distorted by unspecific $^{14}C$-glucose influx due to the impalement with microelectrodes. The membrane potential was, therefore, established by suitable $K^+$ gradients across the cell membrane. Two sets of experiments were performed either in 60 mM $Na^+$ Ringer's solution (*see below*, average potential: −50 mV) or in 60 mM $Na^+$ solution, where the 50 mM TMACl (tetramethylammoniumchloride) was replaced by 50 mM KCl (average potential: −10 mV).

## Solutions

The composition of the Barth's solution was (in mM): 90 NaCl, 2.4 $NaHCO_3$, 1 $K_2SO_4$, 0.8 $MgSO_4$, 0.3 $Ca(NO_3)_2$, 0.4 $CaCl_2$, 5 HEPES (N-2-hydroxylethylpiperazine-N′-ethansulphonic acid (pH 7.6)), 0.08 penicillin, and 0.03 streptomycin. The oocyte Ringer's solution was composed of (in mM): 110 NaCl, 3 KCl, 2 $CaCl_2$, and 5 HEPES (pH 7.6). If lower $Na^+$ concentrations were used, NaCl was replaced by equimolar concentrations of TMACl.

# Results

During exposure of prophase-arrested oocytes to glucose-containing bath solution, the uptake of D-glucose follows a linear time course for several hours (*see* Fig. 1). A large fraction of uptake is inhibited by 1 mM phlorizin. The same degree of inhibition is obtained in the absence of phlorizin when $Na^+$ is omitted from the incubation medium. The rate of uptake for the $Na^+$-dependent and/or phlorizin-sensitive component can reach values of up to 20 pmol/hr at a concentration of 2 mM glucose in the bath medium.

From epithelial cells of kidney and intestine it is known that $Na^+$-glucose cotransport systems accept as substrate the nonmetabolizable glucose de-

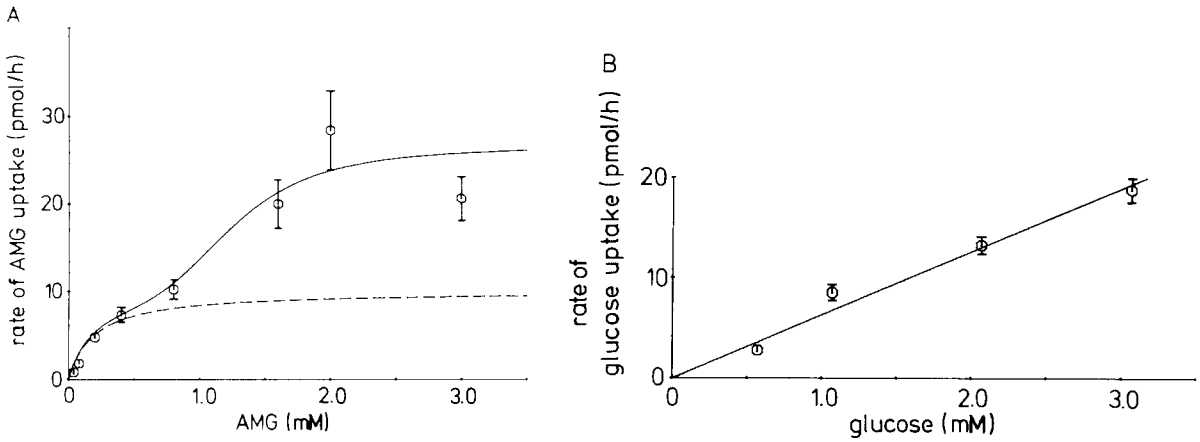


**Fig. 3.** (*A*) Dependence of rate of AMG uptake on AMG concentration in the Ringer's solution. The data serve to characterize the concentration dependence of the $Na^+$-dependent transport component. The dotted line represents a fit of Eq. (1) to the data up to 0.8 mM AMG ($K_{1/2} = 0.19 \pm 0.06$ mM; $k_{max} = 10$ pmol/hr; $n = 1$). The solid line is drawn by eye. (*B*) Dependence of rate of D-glucose uptake on D-glucose concentration in $Na^+$-free Ringer's solution. The data characterize the concentration dependence of the $Na^+$-independent transport component. The fitted straight line has a slope of $6.30 \pm 0.25$ pmol/(hr mM). All data are averages of measurements in 20 oocytes; bars indicate SEM; for the symbol without error bar SEM is smaller than the size of the symbol. For all uptake measurements oocytes were kept for 3 hr in the incubation medium

rivative AMG; AMG, however, is not transported by $Na^+$-independent glucose transporters (Kimmich & Randles, 1981; Brot-Laroche et al., 1987). In the oocytes, AMG is also specifically transported by the $Na^+$-dependent system; in $Na^+$-free solution or in presence of 1 mM phlorizin, the residual rate of AMG uptake is about 10% of the rate in normal, $Na^+$-containing Ringer's solution (*not shown*). Figure 2 demonstrates that, with AMG as substrate, uptake also follows a linear time course for several hours. At similar substrate concentrations the rate of AMG uptake had about the same magnitude as the rate of the $Na^+$-dependent D-glucose transport (*compare* Figs. 1 and 2).

The linearity of substrate uptake with time is not surprising because of the large volume of an oocyte (about 1 $\mu$l). Even after four hours, the increase in intracellular glucose or AMG concentration would be far below 1 $\mu$M.

## Concentration Dependence of Glucose and AMG Uptake

Figure 3*A* shows the dependence of the rate of AMG uptake on AMG concentration. The concentration dependence of this $Na^+$-dependent transport can be approximated by the sum of two saturating components with $K_{1/2}$ values of about 0.2 and 1.5 mM. The glucose dependence of the $Na^+$-independent transport does not show any sign of saturation. In $Na^+$-free solution, the rate of glucose uptake increases nearly linearly up to 3 mM glucose (Fig. 3*B*). This transport is insensitive to cytochalasin B (0.1 mM) but is inhibited by 1 mM PCMBS (*p*-chloromercurybenzol sulfonic acid) or 1 mM phloretin (*not documented*). In this respect, the transport resembles glucose transport in human red blood cells where cytochalasin B is effective only from the inside, but PCMBS and phloretin inhibit the glucose transport (for reviews, *see* Widdas, 1988; Baly & Horuk, 1988).

## Dependence of Glucose Uptake on $Na^+$ Concentration

For $Na^+$-dependent glucose uptake in epithelial cells, $Na^+$ dependencies with different Hill coefficients have been reported (*see*, e.g., Kaunitz & Wright, 1984). In the *Xenopus* oocytes, a Hill coefficient of at least $n = 2$ is necessary to describe the $Na^+$ dependence at 0.24 mM glucose in the bath solution (Fig. 4). This suggests that the $Na^+$-dependent glucose uptake in the *Xenopus* oocytes is mediated at this glucose concentration by a transport system that requires binding of at least two $Na^+$ ions for transporting one glucose molecule across the cell membrane.

## Dependence of Glucose Uptake on Phlorizin Concentration

Phlorizin is a specific inhibitor of $Na^+$-glucose cotransport in epithelial cells. Figure 1 has demonstrated that the $Na^+$-dependent component of glu-

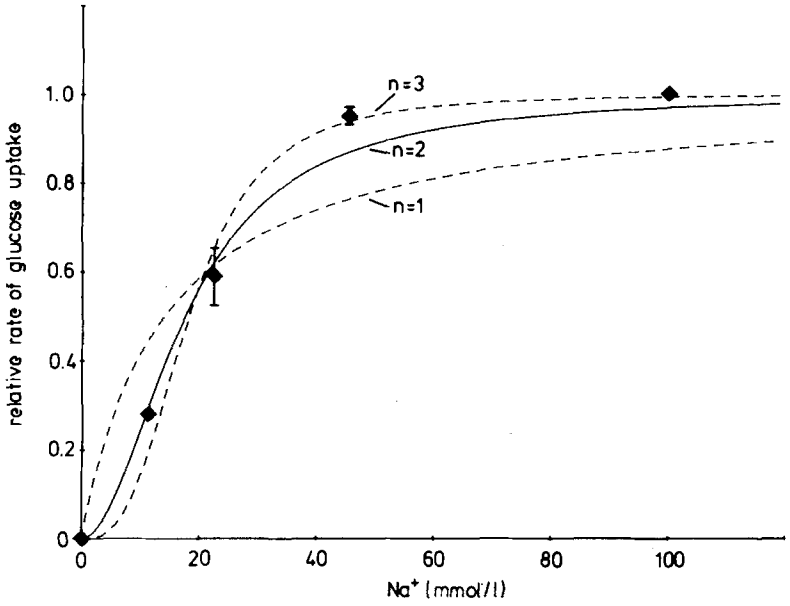


**Fig. 4.** Dependence of rate of glucose uptake on external $Na^+$. $Na^+$-independent transport has been subtracted. Data represent averages from oocytes of three different females; bars indicate SEM; for symbols without error bar SEM is smaller than the size of the symbol. The lines are fits of Eq. (1) to the normalized data points. The fitted parameters are: for $n = 1$, $K_{1/2} = (14 \pm 5)$ mM; for $n = 2$, $K_{1/2} = (17.6 \pm 2.4)$ mM; and for $n = 3$, $K_{1/2} = (18.3 \pm 3.6)$ mM. For the measurements, the oocytes were incubated for 3 hr in solutions containing different concentrations of $Na^+$ and 0.24 mM glucose

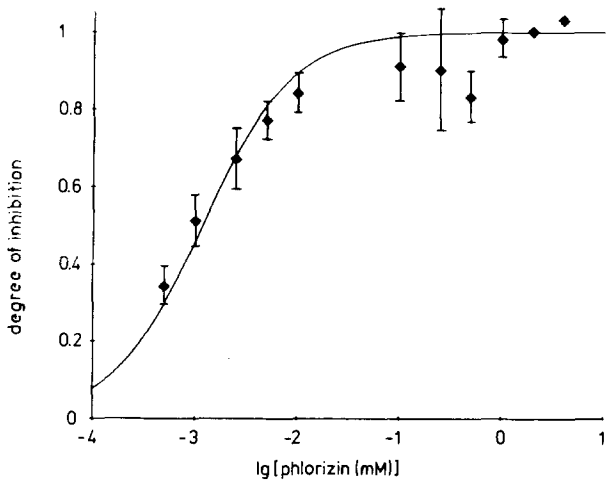


**Fig. 5.** Inhibition by phlorizin of the $Na^+$-dependent glucose uptake. Data represent averages from 2 to 6 experiments; bars indicate SEM; symbols without error bar are from single measurements. The solid line represents a fit of Eq. (2) to the data points ($K_{1/2} = (1.19 \pm 0.35)$ $\mu$M). For the measurements, the oocytes were incubated for 3 hr in the Barth's solution containing 0.1 mM glucose

cose uptake in *Xenopus* oocytes can also be inhibited specifically by 1 mM phlorizin. The degree of inhibition of the phlorizin-dependent transport on phlorizin concentration is plotted in Fig. 5. The experiments were performed at saturating $Na^+$ concentration and 0.1 mM glucose. The inhibition can be described by a $K_{1/2}$ value of about 1.2 $\mu$M.

## Electrogenicity of Glucose Uptake

In order to explore whether or not the $Na^+$-dependent glucose transport is electrogenic, we investigated the effect of transport stimulation on membrane potential. The stimulation of the transport system was achieved by addition of glucose to the bath solution. Figure 6 shows that such a depolarization in membrane potential in fact occurs, demonstrating the electrogenicity. This effect is specific for D-glucose. Mannose cannot substitute. Typically, the depolarization amounts to no more than a few millivolts (as shown in Fig. 6*A*, similar results are obtained with AMG). Occasionally, however, glucose-induced depolarizations may be as large as 20 or 30 mV (Fig. 6*B*); but this was seen only during a brief summer period. Nevertheless, the results clearly demonstrate that charges are transported together with the glucose. Consequently, the rate of transport should in turn depend on membrane potential.

## Voltage-Dependence of Glucose Uptake

Figure 7 summarizes the result of experiments in which total $^{14}C$-glucose uptake was measured at two different membrane potentials under two-microelectrode voltage-clamp conditions. When the potential is made less negative, a tendency for a reduction of the rate of glucose uptake becomes apparent.

Similar observations were made in experiments in which the membrane potential was adjusted by a

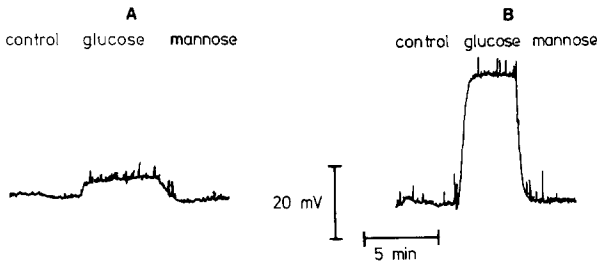


**Fig. 6.** Changes of resting potential in response to replacement of glucose-free Ringer's solution by Ringer's solution containing 10 mM glucose or 10 mM mannose. Polarizations of the magnitude as shown in *B* were observed in the oocytes of only three females (May–June, 1987). Usually polarizations as small as those shown in *A* can be detected. The resting potential before and after addition of glucose is about −70 mV in *A* and *B*; upward deflections represent depolarizations

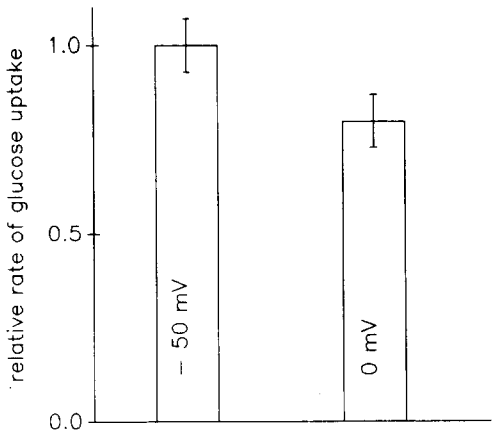


**Fig. 7.** Dependence of rate of glucose uptake on membrane potential. Oocytes were incubated in Ringer's solution containing 148 $\mu$M $^{14}$C-labeled glucose and kept under two-microelectrode voltage clamp for half an hour. The data represent the average of 50 oocytes ± SEM. The holding potentials were as indicated

$K^+$ gradient rather than by voltage clamp with microelectrodes (Fig. 8*A*). The data shown in Figs. 7 and 8*A* represent total glucose uptake. To determine the effect of the $Na^+$-dependent component, the difference of uptake rates without and with a saturating concentration of phlorizin was determined. For the phlorizin-sensitive flux component (Fig. 8*B*), the voltage dependence is more obvious than for the total flux. The phlorizin-insensitive glucose uptake shows little, if any, voltage dependence (*compare* Fig. 8*A* and *B*).

## Voltage Dependence of Inhibition by Phlorizin

Phlorizin is a specific inhibitor of $Na^+$-glucose cotransport. It competes with glucose for binding to the transport protein and has been used as a probe for the localization of the binding step in the reaction cycle that accomplishes $Na^+$-glucose cotransport (Toggenburger, Kessler & Semenza, 1982). The study of the voltage dependence of phlorizin action or binding plays an important role in understanding the voltage dependence of the mechanism of the cotransport process. For this reason, fluxes were measured in solutions containing $Na^+$ at a concentration of 60 mM that produces nearly maximum stimulation of transport. Either 3 or 50 mM KCl was used to establish different membrane potentials. To confine the measurements to $Na^+$-dependent transport, AMG rather than glucose was used as substrate. In each oocyte, the AMG uptake and the membrane potentials were determined at the end of a fixed incubation period. Figure 9 shows the voltage dependence of rate of AMG uptake and its decrease with depolarization. If phlorizin is added to the medium, the degree of inhibition decreases with depolarization. At 0 mV, nearly no inhibition of glucose uptake by phlorizin can be detected. As a consequence, the steepness of the voltage dependence of the transport rate is reduced in the presence of phlorizin.

## pH Dependence of Glucose Uptake

It has been speculated that a change of protonation of carboxyl groups plays a role in the conformational changes associated with the $Na^+$-glucose cotransport. Changes in pH may, therefore, influence the rate of transport (Fuchs, Graf & Peterlik, 1985). Figure 10 shows that glucose uptake in fact is reduced by acidification and increased by alkalization. However, over the pH range of 5.5 to 8.5 the titration curve shows no clear inflection point that could be related to the dissociation of a specific amino acid residue.

## $Me^{3+}$ Dependence of Glucose Uptake

In epithelial cells, lanthanides have been demonstrated to be capable of substituting for $Na^+$ in the activation of glucose transport (Stevens & Kneer, 1988). This can also be demonstrated for glucose uptake in the oocytes. Figure 11 shows that $Tb^{3+}$ is slightly less effective than $Na^+$, while $Eu^{3+}$ is about two times more effective. It should be pointed out that these experiments were performed with a glucose concentration of 74 $\mu$M, a concentration where $Na^+$-independent glucose transport is small compared to the cotransport.

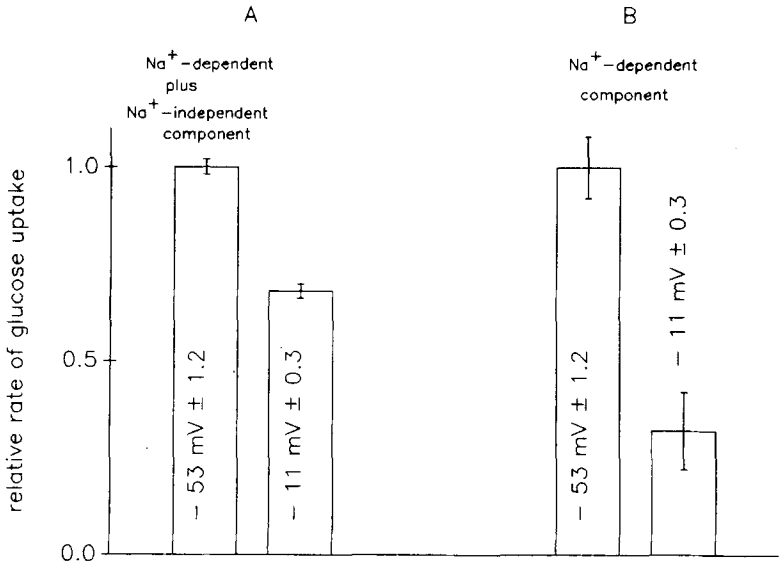


**Fig. 8.** Dependence of the rate of glucose uptake on membrane potential. 50 oocytes were incubated in solutions containing 74 $\mu$M $^{14}$C-labeled D-glucose for 3 hr. The incubation medium contained either 60 mM NaCl and 50 mM TMACl (left columns in *A* and *B*) or instead of the TMACl 50 mM KCl (right columns in *A* and *B*). Average membrane potentials are indicated. The $Na^+$-dependent component (*B*) was determined as the difference of transport rate with and without 0.5 mM phlorizin in the bath solution

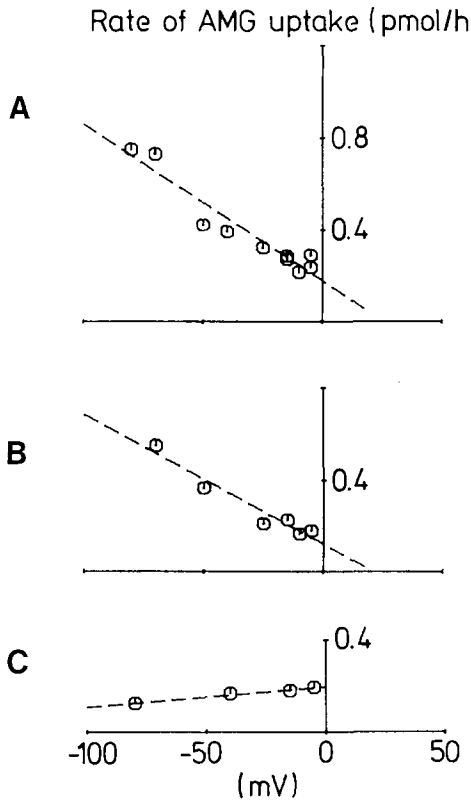


**Fig. 9.** Voltage dependence of the rate of AMG uptake in Ringer's solution with no (*A*), 1.0 (*B*) or 1.5 (*C*) $\mu$M phlorizin. Oocytes were incubated 3 hr in solution containing either 3 or 50 mM KCl. At the end of the incubation period the oocytes were washed and the membrane potential for each was determined. The AMG concentration was 40 $\mu$M. Dotted lines are drawn by eye

### Regulation of Glucose Uptake During Meiotic Maturation

The experiments described above were performed with oocytes arrested in the prophase of the first meiotic division. During meiotic maturation of the oocytes, several transport proteins are known to be downregulated in their transport activity (*see* Jung, Lafaire & Schwarz, 1984*a*, Richter et al., 1984). We, therefore, tried to measure glucose uptake also in maturated oocytes. In vivo maturation and shedding were induced by injection of gonadotropin into the female. Compared to the prophase-arrested cells, maturated oocytes exhibit glucose uptake at reduced rate. Both the phlorizin-sensitive and insensitive components are downregulated during the maturation (Fig. 12). Partial recovery can be obtained by membrane hyperpolarization (*not shown*).

## Discussion

In a previous study, Carvallo et al. (1981) demonstrated with follicles (oocytes covered with follicular epithelium) that the oocytes of *Xenopus laevis* are able to take up D-glucose from bath medium. This uptake was shown to be downregulated during meiotic maturation. In our present paper we confirm these observations, and we show that the plasma membrane of the oocytes is the site of transport mediation and regulation. In addition, we demonstrate that the glucose transport is composed of

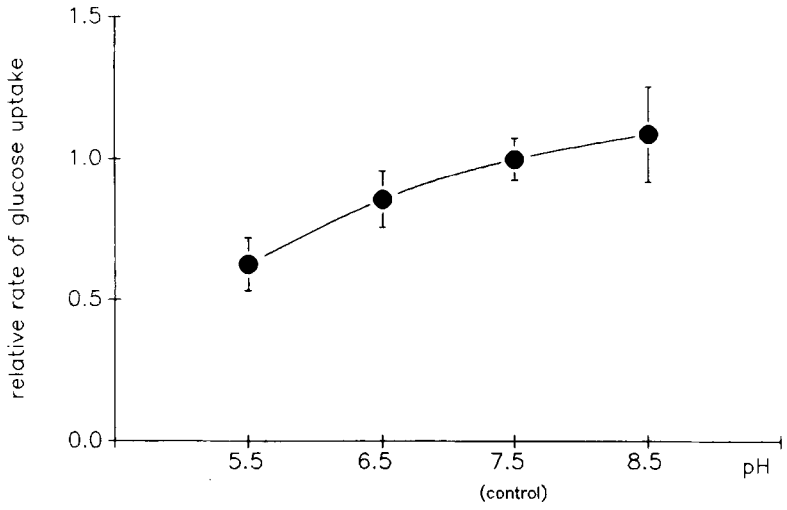


**Fig. 10.** pH dependence of D-glucose uptake. Oocytes were incubated in Ringer's solution adjusted to different pH values. Data represent average values of about 40 oocytes (bars indicate SEM). For the measurements, the oocytes were incubated for 3 hr in solution containing 74 $\mu$M glucose

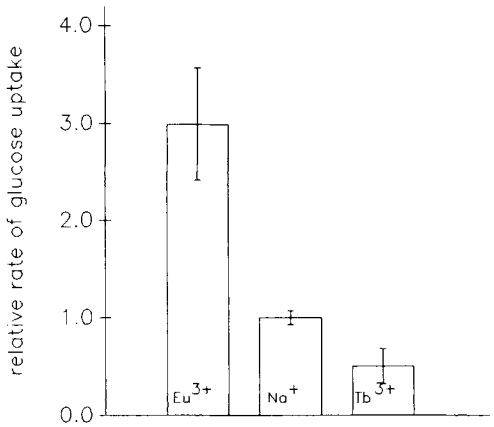


**Fig. 11.** Stimulation of glucose uptake by lanthanides. Oocytes were incubated in solution containing, instead of 110 mM NaCl, 70 mN NaCl and 40 mM choline chloride, or either 70 mM $EuCl_3$ or 70 mM $TbCl_3$. For the measurements, 40 oocytes were incubated for 3 hr in solution containing 74 $\mu$M glucose

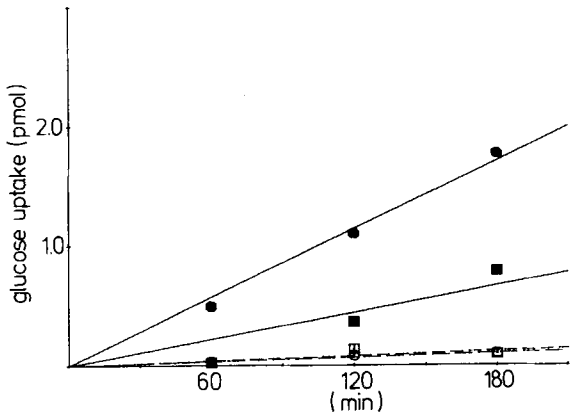


**Fig. 12.** Time course of glucose uptake by prophase-arrested (filled symbols) and metaphase-arrested (open symbols) oocytes in Barth's solution containing 0.1 mM glucose. Squares represent data from experiments where 0.5 mM phlorizin was present in the bath solution. The slopes of the fitted straight lines (in pmol/(hr oocyte) ± SEM) are for prophase-arrested oocytes 0.57 ± 0.02 and 0.22 ± 0.05, and for metaphase-arrested oocytes 0.035 ± 0.002 and 0.041 ± 0.012, without and with 0.5 mM phlorizin, respectively. Data are obtained from experiments with prophase-arrested oocytes from four different females and with metaphase-arrested oocytes from three different females

at least two components: (i) One component is independent of extracellular $Na^+$ and shows no saturation for glucose concentrations up to 3 mM; the transport is insensitive to external cytochalasin B, but can be inhibited by PCMBS or phloretin. In this respect, $Na^+$-independent transport in the oocytes resembles the facilitated diffusion of glucose in red cells (Widdas 1988, Baly & Horuk 1988). (ii) The other component is mediated by a transport system that requires extracellular $Na^+$, and is inhibited by phlorizin. The activity of both transport systems is downregulated during meiotic maturation of the prophase-arrested oocytes. At glucose concentrations below 0.5 mM, transport is primarily mediated by the $Na^+$-dependent system, and hence can be characterized separately. In this contribution we wanted to characterize primarily the $Na^+$-dependent transport. Therefore, the majority of experiments described in the Results were performed in the presence of less than 0.5 mM glucose, or AMG was used as substrate.

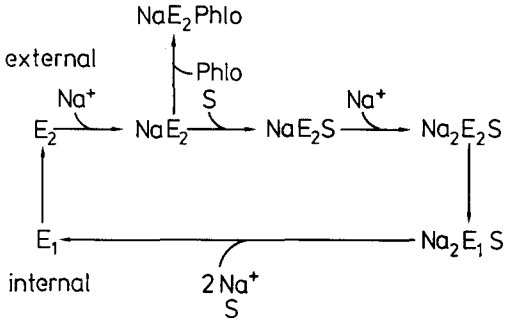


**Fig. 13.** Reaction diagram for $Na^+$-glucose cotransport and inhibition by phlorizin. It is based on generally accepted ideas of the $Na^+$-glucose-$Na^+$ binding sequence and that phlorizin binds competitively to the glucose-binding site (*see*, e.g., Semenza et al., 1984)

## The $Na^+$-Dependent Transport is 2Na-1Glucose Cotransport

$Na^+$-glucose cotransport has most extensively been investigated in brush-border membranes (*see*, e.g., Semenza et al., 1984). Two transport systems or two modes of transport have been described (Crane & Dorando, 1979; Kaunitz & Wright, 1984; Koepsell et al.[1]), one with low affinity and the other with high affinity for glucose. For the renal $Na^+$-glucose cotransporter, values of 5.8 and 0.15 mM, respectively, have been reported (*see* Koepsell et al.[1]). In the *Xenopus* oocytes, the dependence of glucose uptake on glucose concentration can also be described by superimposition of two transport components, one with $K_{1/2}$ value of about 1.5 mM and the other with a $K_{1/2}$ value of 0.2 mM. Inhibition of the glucose uptake in the oocytes by phlorizin also shows similarities to the cotransport of epithelial cells. The $K_I$ value of about 1.2 $\mu$M is of the same order of magnitude as reported for kidney (*see* Alavi, Spanger & Jung, 1987). Furthermore, the dependence of glucose uptake in the oocytes on external pH and transport stimulation by lanthanides is also similar to the corresponding effects on $Na^+$-glucose cotransport in epithelial cells (Lever, 1984; Stevens & Kneer, 1988). This suggests that the $Na^+$-dependent glucose uptake in the oocytes is mediated by $Na^+$-glucose cotransport. This is strongly supported by electrogenic effects of the glucose uptake on membrane potential, which demonstrates that charges are translocated across the cell membrane when glucose transport is stimulated. For the $Na^+$-alanine cotransporter of the *Xenopus* oocytes, alanine-dependent current and $^{22}Na^+$ uptake could be determined (Jung, Schwarz & Passow, 1984*b*); comparison of these parameters with the rate of alanine uptake yielded a $Na^+$-alanine stoichiometry of 2:1. For the $Na^+$-glucose cotransporter, the transport rates are two small to determine the currents and $^{22}Na^+$ fluxes quantitatively. However, an estimate of the upper limit of the glucose-induced current is possible. The glucose-induced depolarization usually amounts up to 5 mV (*see*, e.g., Fig. 6), and the resting membrane resistance to about 5 M$\Omega$ (*see*, e.g., Schweigert, Lafaire & Schwarz, 1988). From these values a glucose-induced membrane current can be estimated to 1 nA or less, which would indeed be hardly detectable. This current corresponds to an upper limit of charge uptake of about 40 pmol/hr. At saturating substrate concentrations, the rate of AMG uptake amounts to about 25 pmol/hr. This would be compatible with a 2 Na-1 glucose stoichiometry.

It has been assumed for epithelial cells that $Na^+$-glucose cotransport generally operates at a 2:1 stoichiometry (*see* Kimmich & Randles, 1984). On the other hand, it has been suggested that the glucose transporter may operate at either high or low affinity. In the high-affinity mode, glucose cotransport operates at 2:1 stoichiometry, in the low-affinity mode at 1:1 stoichiometry (Turner & Moran, 1982; *see also* Koepsell et al.[1]). In the *Xenopus* oocytes the glucose uptake clearly shows sigmoidal dependence on extracellular $Na^+$ at 0.24 mM glucose, a concentration where transport presumably is mediated primarily by the high-affinity mode. The description with a Hill coefficient of about 2 would be compatible with a 2:1 stoichiometry and hence would correspond to the high-affinity cotransport of epithelia.

## Potential Dependence of the Glucose Cotransporter

$Na^+$-glucose cotransport is usually discussed on the basis of the kinetic scheme presented in Fig. 13. The electrogenicity of the transport process should lead to voltage dependence of the transport rate provided the charge translocations determine rate-limiting steps. For epithelial transport, a negative slope in the transport-voltage relation has been demonstrated (Murer & Hopfer, 1974; Kaunitz & Wright, 1984). This would be compatible with positive charges moving inwardly during $Na^+$-glucose

[1] Koepsell, H., Fritzsch, G., Korn, K., Madrala, A. 1989. Two substrate sites in the renal $Na^+$-D-glucose cotransporter studied by model analysis of phlorizin binding and D-glucose transport measurements (*unpublished*).

translocation, and/or negative charges moving outwardly during the reorientation of the unloaded carrier. Also for the oocytes, a negative slope is demonstrated (Figs. 7–9).

Experiments with the intestinal cotransport system yielded the result that negative membrane potentials increase phlorizin binding (Aronson, 1978; Toggenburger et al., 1982). In contrast, unbinding of phlorizin has been demonstrated to be voltage-independent (Aronson, 1978; Lever, 1984; Semenza et al., 1984). These observations were interpreted by suggesting that the unloaded transport protein carries one negative charge during the translocation step from inside to outside. As a consequence, the negative membrane potential may recruit the glucose or phlorizin binding sites from inward to outward orientation, and hence increase phlorizin binding. As an alternative explanation for voltage-dependent phlorizin binding voltage-dependent $Na^+$ binding prior to phlorizin binding has been discussed by Aronson (1978) and Lever (1984). Though these authors rejected the possibility of potential-dependent $Na^+$ binding, Kimmich and Randles (1988) demonstrated dependence of $K_m$ and $V_{max}$ for $Na^+$ on membrane potential. In any case, in the presence of phlorizin, membrane potential has two opposing effects of glucose uptake. The observed electrogenicity of the cotransport should lead to stimulation of transport when the inside is made more negative (stimulating $Na^+$ inward movement). On the other hand, such hyperpolarization recruits more binding sites for glucose or phlorizin to the external membrane surface and hence enhances inhibition by phlorizin. The voltage dependence of the inhibitory effects would be responsible for the reduced (or even inverted) voltage dependence of the rate of glucose uptake in the presence of phlorizin (Fig. 9).

The results presented here demonstrate that the $Na^+$-glucose cotransport in the oocytes is essentially similar to the cotransport in renal and intestinal cells and in *Xenopus* oocytes after injection of mRNA from intestine tissue (Hediger et al., 1987*a*,*b*).

### Regulation of Glucose Uptake During Meiosis

During completion of the first meiotic division most transport systems are drastically reduced in their activity (Richter et al., 1984), and the membrane potential depolarizes nearly completely (Bellé, Ozon & Stinnakre, 1977; Wallace & Steinhardt 1977, Grygorczyk, Schwarz & Passow, 1987). These potential changes play an essential role in downregulation of $Na^+$ cotransport systems during maturation. Downregulation by membrane depolarization has been demonstrated for endogenous $Na^+$-alanine cotransport (Jung et al., 1984*a*), and for erythroid band 3-mediated anion exchange expressed in the oocytes (Grygorczyk et al., 1987). The decrease of $Na^+$-glucose cotransport during the maturation process can also be explained as a consequence of the negative slope in the voltage dependence. If maturated oocytes are hyperpolarized by two-microelectrode techniques to the resting potential of prophase-arrested oocytes, the $Na^+$-glucose cotransport is indeed restored to its original value before maturation (*not shown*).

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