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The use of potential-sensitive cyanine dye for studying ion-dependent electrogenic renal transport of organic solutes

Spectrophotometric measurements

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Renal transport of four different categories of organic solutes, namely sugars, neutral amino acids, monocarboxylic acids and dicarboxylic acids, was studied by using the potential-sensitive dye 3,3′-diethyloxadicarbocyanine iodide in purified luminal-membrane and basolateral-membrane vesicles isolated from rabbit kidney cortex. Valinomycin-induced K^+ diffusion potentials resulted in concomitant changes in dye–membrane-vesicle absorption spectra. Linear relationships were obtained between these changes and depolarization and hyperpolarization of the vesicles. Addition of D-glucose, L-phenylalanine, succinate or L-lactate to luminal-membrane vesicles, in the presence of an extravesicular > intravesicular Na^+ gradient, resulted in rapid transient depolarization. With basolateral-membrane vesicles no electrogenic transport of D-glucose or L-phenylalanine was observed. Spectrophotometric competition studies revealed that D-galactose is electrogenically taken up by the same transport system as that for D-glucose, whereas L-phenylalanine, succinate and L-lactate are transported by different systems in luminal-membrane vesicles. The absorbance changes associated with simultaneous addition of D-glucose and L-phenylalanine were additive. The uptake of these solutes was influenced by the presence of Na^+-salt anions of different permeabilities in the order: $Cl^- > SO_4^{2-} >$ gluconate. Addition of valinomycin to K^+-loaded vesicles enhanced uptake of D-glucose and L-phenylalanine in the presence of an extravesicular > intravesicular Na^+ gradient. Gramicidin or valinomycin plus nigericin diminished/abolished electrogenic solute uptake by Na^+- or $Na^+ + K^+$-loaded vesicles respectively. These results strongly support the presence of Na^+-dependent renal electrogenic transport of D-glucose, L-phenylalanine, succinate and L-lactate in luminal-membrane vesicles.

Renal transport of various organic solutes has been examined with a wide variety of physiological preparations, including the intact animal, tubular segments and cortical slices. This has led to many, and sometimes conflicting, observations on the stereospecificity, saturability, competition and Na^+-dependency of transport processes. In particular, the relative contributions of the luminal and basolateral membranes to transepithelial transport of solutes have been difficult to evaluate from these studies. With the advent of convenient methods to separate the luminal from the basolateral plasma membrane (Evers *et al.*, 1978; Kinsella *et al.*, 1979; Sheikh *et al.*, 1982), it is now possible to assess individually the contribution of both membranes to overall transepithelial movement of small molecules.

In recent years a number of reports of studies utilizing isolated renal luminal membrane vesicles have been published, which demonstrate Na^+-gradient-dependent uptake of sugars (Aronson & Sacktor, 1975; Beck & Sacktor, 1975, 1978*a*,*b*), amino acids (for reviews see Sacktor, 1977, 1978), monocarboxylic acids (Barac-Nieto *et al.*, 1980) and di- and tri-carboxylic acids (Kippen *et al.*, 1979; Wright *et al.*, 1980, 1981). It has been shown that the Na^+-dependent transport of organic substrates can produce changes in membrane potential of intact cells (Laris *et al.*, 1978; Pershadsingh *et al.*, 1978) and preparations of luminal-membrane vesicles (Beck & Sacktor, 1978*a*,*b*; Burckhardt *et al.*, 1980; Wright *et al.*, 1981). Electrical potentials have originally been measured by electrodes. However,

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membrane vesicles are too small to be studied with this technique. More recently extrinsic probes, especially cyanine dyes, have been used for monitoring changes in membrane electrical potential in a number of different biological systems, e.g. liposomes, erythrocytes and erythrocyte 'ghosts' (Guillet & Kimmich, 1981), mitochondria (Kinnally *et al.*, 1978; Smith *et al.*, 1980), sarcoplasmic-reticulum vesicles (Meissner & Young, 1980; Bennett & Dupont, 1981), giant axons from the squid (Gupta *et al.*, 1981) and renal luminal-membrane vesicles (Burckhardt *et al.*, 1980; Wright *et al.*, 1981).

The purpose of the present series of studies is mainly to investigate the ion-dependent mechanism of renal transport of a wide variety of organic solutes in purified luminal-membrane and basolateral-membrane vesicles by using the potential sensitive dye 3,3'-diethyloxadicarbocyanine iodide. The present paper deals with the question whether changes in the absorption spectrum of this dye, occurring during alterations in membrane potential, can be utilized to study the electrogenic renal transport of organic substances in vesicle preparations.

Experimental

Materials

Lactic acid and Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. L-Glucose, L-phenylalanine and D-phenylalanine were purchased from Fluka A.G., Buchs, Switzerland. D-Glucose monohydrate was bought from J. T. Baker Chemicals B.V., Deventer, The Netherlands. All other reagents were of A.R. grade.

Preparation of luminal-membrane and basolateral-membrane vesicles

Purified luminal-membrane and basolateral-membrane vesicles were prepared from rabbit kidneys as described in an accompanying paper (Sheikh *et al.*, 1982). Both types of membrane vesicle were suspended in 300mM-mannitol/25mM-Hepes adjusted to pH 7.0 with Tris and used within 24h after the preparation. The protein concentration was determined by the method of Lowry *et al.* (1951), as modified by Peterson (1977), with human serum albumin (Sigma Chemical Co.) as standard. Vesicles were preloaded by first removing the extravesicular mannitol/Hepes/Tris solution by centrifugation (25000*g* for 30min at 2°C) and afterwards resuspending the loose pellet in an iso-osmolar loading medium as described in the legends to the Figures. During the preloading process the suspensions were placed, under magnetic stirring, at 2°C for 1h. The quality of the membrane preparations was evaluated by measuring specific activities of various enzyme markers as reported in an accompanying paper (Sheikh *et al.*, 1982).

Preparation of dye solutions

Because of the moderate instability of 3,3'-diethyloxadicarbocyanine iodide caused by photodynamic damage and dye bleaching (Sims *et al.*, 1974), the dye was dissolved in degassed buffer (15mM-Tris/HCl buffer, pH 7.0). The dye solution was bubbled with N_2 and stored in a dark bottle covered with aluminium foil at 2°C. The glass wall of the bottle was saturated with dye before use. Spectrophotometric measurements at 577nm showed that the stock solutions prepared in this way were stable for 3–4 days.

Spectrophotometric measurements

The spectrophotometric recordings were performed on an Aminco DW-2a UV/VIS spectrophotometer with a constant temperature in the sample compartment of 20°C. The experiments were conducted in a room with minimal light. Until and unless described, a cuvette was placed in the sample compartment and the following solutions were added: 1.5ml of buffered salt solution, 1.5ml of buffered 3,3'-diethyloxadicarbocyanine iodide solution (final dye concentration 15μM) and 0.1ml of vesicle suspension (final protein concentration 0.25mg/ml). The sample compartment was closed and the solution was mixed for about 40s by magnetic stirring with a small Teflon-coated magnet placed inside the cuvette. The solutions were stirred throughout the spectrophotometric recordings, and no whirls influenced the spectra to a measurable extent. After about 40s a small volume of a stock solution of ionophore or organic solute or buffer was added through a small opening in the top of the sample compartment.

Results and discussion

Absorption spectra

Fig. 1 shows the absorption spectra of 3,3'-diethyloxadicarbocyanine iodide in the absence and presence of luminal-membrane vesicles with increasing protein concentrations (0.06–0.96mg/ml). It is seen that addition of vesicles to dye solution results in a shift of $\lambda_{max.}$ from 577nm to 592nm. An isosbestic point at wavelength 588nm is observed at relatively low protein concentrations (0.06–0.36mg/ml). The results obtained with basolateral-membrane vesicles (results not shown) were similar to those of the luminal-membrane vesicles.

The difference spectra depicted in Fig. 2 show that introduction of changes in the potential of the membrane vesicles result in alterations of the dye–membrane-vesicle spectra. The potential changes were initiated by establishing K^+ gradients over the membranes and adding valinomycin. Fig.

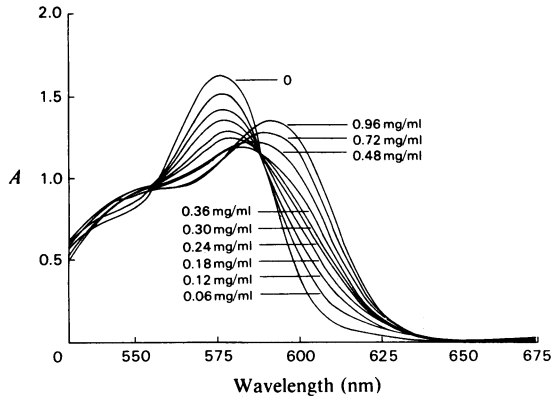

Fig. 1. *Effect of luminal-membrane vesicles on the absorption spectrum of 3,3'-diethyloxadicarbocyanine iodide*

Luminal-membrane vesicles were prepared and loaded with 300mM-mannitol/25mM-Hepes/Tris buffer, pH7.0. The external medium contained 7μg of dye/ml and various concentrations of membrane protein dissolved in 300mM-mannitol/25mM-Hepes/Tris buffer, pH7.0. 0 denotes the dye spectrum in the absence of membrane vesicles. The spectrophotometer was operated in the split-beam mode with 650nm as a reference wavelength. The curves are corrected for absorption of medium alone and light-scatter caused by the membrane vesicles.

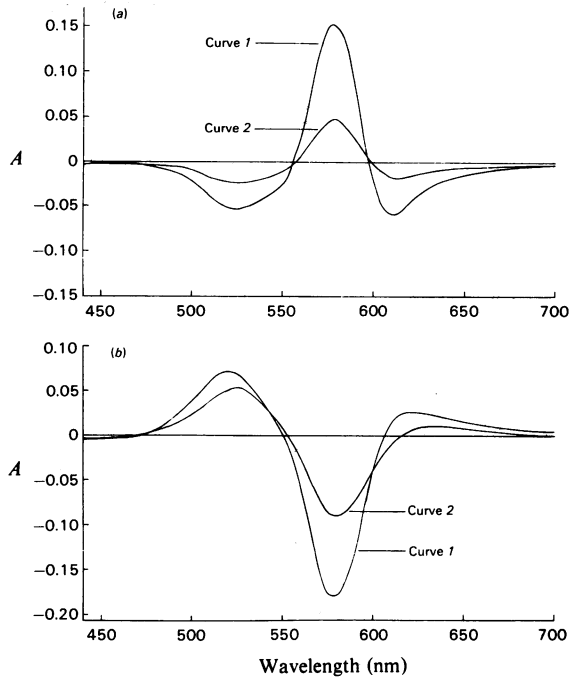

Fig. 2. *Effect of valinomycin-induced potential changes on dye–membrane-vesicle difference spectra*

(*a*) Membrane vesicles were preloaded with 5mM-KCl/290mM-mannitol/25mM-Hepes/Tris buffer and suspended in 155mM-KCl/15mM-Tris/HCl buffer. (*b*) Membrane vesicles preloaded in 155mM-KCl/15mM-Tris/HCl buffer and suspended in 5mM-KCl/290mM-mannitol/25mM-Hepes/Tris buffer. Curves *1* and curves *2* represent the results obtained with luminal-membrane vesicles and basolateral-membrane vesicles respectively. Baselines were recorded with two cuvettes of identical composition without valinomycin. Common experimental conditions: protein, 0.25mg/ml; dye, 7μg/ml; valinomycin, 5μg/mg of protein; pH7.0. The spectrophotometer was operated in the split-beam mode with 650nm as a reference wavelength.

2(*a*) shows the difference spectra for luminal-membrane and basolateral-membrane vesicles with a concentration of intravesicular K^+ ($[K^+]_i$) of 5mM and an extravesicular K^+ concentration of 155mM ($[K^+]_o$). It is seen that the depolarization results in spectra with negative maxima at 525nm and 610nm and in a positive maximum at 580nm for both types of membrane vesicles. Fig. 2(*b*) shows the spectra measured when the vesicles are hyperpolarized ($[K^+]_i = 155$mM and $[K^+]_o = 5$mM). In this case also three maxima are observed, but the direction of the maxima are reversed as compared with those caused by depolarization.

Effect of potential on absorbance

The results presented in Fig. 3(*a*) show that there is a linear relationship between the difference in absorbance at 580nm and 610nm (reference wavelength) and $\log([K^+]_o/[K^+]_i)$ when luminal-membrane and basolateral-membrane vesicles are depolarized by valinomycin. Fig. 3(*b*) shows that linear relationships also exist when the membrane vesicles are hyperpolarized.

The optical response accompanying alterations of the membrane potential probably is based on changes in the vesicle–dye binding (see, e.g., Guillet & Kimmich, 1981). In a series of experiments we have determined, by ultracentrifugation, the binding of dye before and 1h after addition of valinomycin. We found that the percentage dye binding in the suspensions to which valinomycin was added and in the solutions without valinomycin was as follows (means ± S.D., $n = 10$): luminal-membrane vesicles in the absence of valinomycin, 30.5 ± 2.3%, after addition of valinomycin resulting in depolarization, 30.0 ± 2.6%, and after addition of valinomycin resulting in hyperpolarization, 28.1 ± 3.3%; basolateral-membrane vesicles in the absence of valinomycin, 45.6 ± 1.9%, after depolarization, 48.8 ± 2.5%, and after hyperpolarization, 44.6 ± 2.9%. Thus in our systems the process by which the dye monitors changes in membrane potential seems to be reversible. This was also found to be the case in the other studies reported in the present paper.

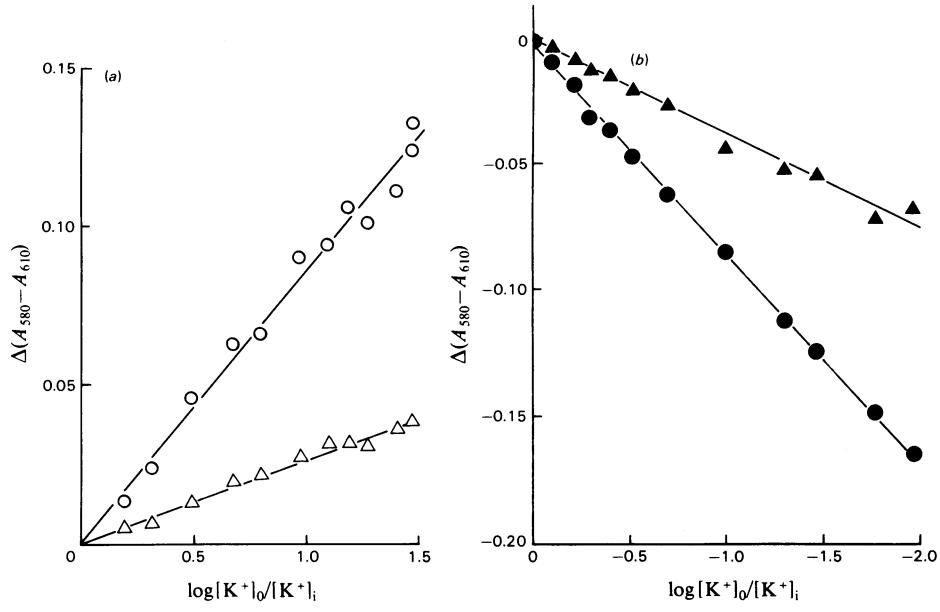

Fig. 3. *Relationship between absorbance changes of 3,3'-diethyloxadicarbocyanine iodide and K^+ diffusion potentials in renal membrane vesicles*

(*a*) Membrane vesicles were preloaded with 5 mM-KCl/290 mM-mannitol/25 mM-Hepes/Tris buffer and suspended in 5–155 mM-KCl/15 mM-Tris/HCl buffer. (*b*) Membrane vesicles were preloaded with 155 mM-KCl/15 mM-Tris/HCl buffer and suspended in 1.55–155 mM-KCl/15 mM-Tris/HCl buffer. Iso-osmolarity was maintained with mannitol. ○ and ●, Luminal-membrane vesicles; △ and ▲, basolateral-membrane vesicles. The spectrophotometer was operated in the dual-wavelength mode with 580 nm and 610 nm (reference wavelength). Other experimental conditions were the same as those mentioned in Fig. 2 legend.

Uptake of glucose by renal membrane vesicles

After having established that 3,3'-diethyloxadicarbocyanine iodide can be used to record changes in membrane potential of renal membrane vesicles, we investigated whether the dye also can be used to monitor electrogenic transport of organic solutes across the renal membranes. The optical changes of the dye associated with uptake of a number of different organic compounds were measured. Fig. 4 depicts the spectral changes observed in the presence of L- and D-glucose under various experimental conditions. Addition of D-glucose to luminal-membrane vesicles, in the presence of an extravesicular > intravesicular Na^+ gradient, results in a rapid and pronounced increment of absorbance (curve *1*). The maximal change is observed at approx. 1 min after addition of D-glucose to the solution. This is followed by a slower decrease in the absorbance change as time proceeds, approaching the basal value (i.e. the value recorded before addition of D-glucose) after 75 min. The transient nature ('overshoot') of the spectral curve suggests that the absorbance changes of the dye in the presence of D-glucose are the result of a transport process and not, e.g., binding of the sugar to the membrane vesicles. The uptake pattern of D-[^{14}C]glucose was studied by a Millipore-filtration technique, under experimental conditions similar to those originally described by Aronson & Sacktor (1975). For the sake of comparison the results obtained with this established method by us (·····) as well as by Aronson & Sacktor (1975) (– – – –) are plotted in the Figure. It is seen that the forms of the three curves are comparable. However, it should be noted that the Millipore-filtration technique measures the total uptake of glucose at any given time, whereas the spectrophotometric technique monitors the changes in membrane potential caused by the rate of change in glucose uptake. Since the membrane potential is governed by the direction and magnitude of the net flow of substances with Na^+, the extent of depolarization due to glucose is therefore greatest at the start of the run, when glucose is introduced, least at the peak of glucose accumulation, and reversed as glucose leaves the vesicles with Na^+. This interpretation is consistent with Beck & Sacktor (1978*b*).

Curve *2* shows the effect of adding D-glucose to luminal-membrane vesicles with the same intra-

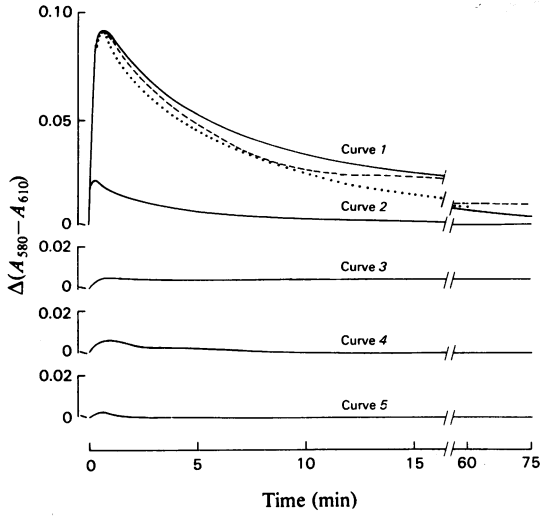

Fig. 4. *Absorbance changes of 3,3'-diethyloxadicarbocyanine iodide induced by glucose*

Unless otherwise noted the intravesicular medium was 300mM-mannitol/25mM-Hepes/Tris buffer. The external media were: curve *1*, 5mM-D-glucose/155mM-NaCl/15mM-Tris/HCl buffer; curve *2*, the same, but the internal medium was 155mM-NaCl/15mM-Tris/HCl buffer; curve *3*, 5mM-D-glucose/155mM-KCl/15mM-Tris/HCl buffer, and the internal medium was 155mM-KCl/15mM-Tris/HCl buffer; curve *4*, 5mM-L-glucose/155mM-NaCl/15mM-Tris/HCl buffer; curve *5*, 5mM-D-glucose/155mM-NaCl/15mM-Tris/HCl buffer. Curves *1–4* represent results obtained with luminal-membrane vesicles, and curve *5* shows results with basolateral-membrane vesicles. All curves in this and the following Figures were corrected for the effect of adding a small volume of 15mM-Tris/HCl buffer (the medium of the solute's stock solutions). Common experimental conditions: protein, 0.25mg/ml; dye, 7μg/ml; pH 7.0. The spectrophotometer was operated in the dual-wavelength mode with 580nm and 610nm (reference wavelength). The discontinuous curves represent D-glucose uptake as measured by the Millipore-filtration technique: ——, Aronson & Sacktor (1975); ·····, the present study.

vesicular and extravesicular Na^+ concentration. The form of curve *2* is the same as that of curve *1*. However, the maximal response is obtained faster than in curve *1*, and is only about 20% of that in the presence of an Na^+ gradient. Curves *3* and *4* show, in accordance with Aronson & Sacktor (1975), that the uptake of glucose by luminal membrane vesicles is Na^+-dependent and stereospecific respectively.

The absorbance change of the dye associated with addition of D-glucose to purified basolateral-membrane vesicles is shown by curve *5*. It is seen that even in the presence of an extravesicular > intravesicular Na^+ gradient only a very small optical response is detected, indicating that the sugar is not taken up by an electrogenic transport system in basolateral-membrane vesicles.

Uptake of phenylalanine by renal membrane vesicles

Addition of L-phenylalanine to luminal-membrane vesicles, in the presence of Na^+, results in 'overshoot' phenomena (Fig. 5, curves *1* and *2*). In the case of L-phenylalanine also, the presence of an extravesicular > intravesicular Na^+ gradient increases the maximal optical response severalfold. No 'overshoot' phenomenon was observed when Na^+ was replaced by K^+ (curve *3*), nor when L-phenylalanine was replaced by the D-isomer (curve *4*). These results indicate that L-phenylalanine is taken up by a stereospecific Na^+-dependent electrogenic process. Similar results were obtained by Evers *et al.* (1976) with the Millipore-filtration technique. In contrast with the luminal-membrane vesicles, addition of L-phenylalanine to basolateral-membrane vesicles, in the presence of an extravesicular > intravesicular Na^+ gradient, results in only a very small absorbance change of the dye (curve *5*), indicating that L-phenylalanine is not transported by an electro-

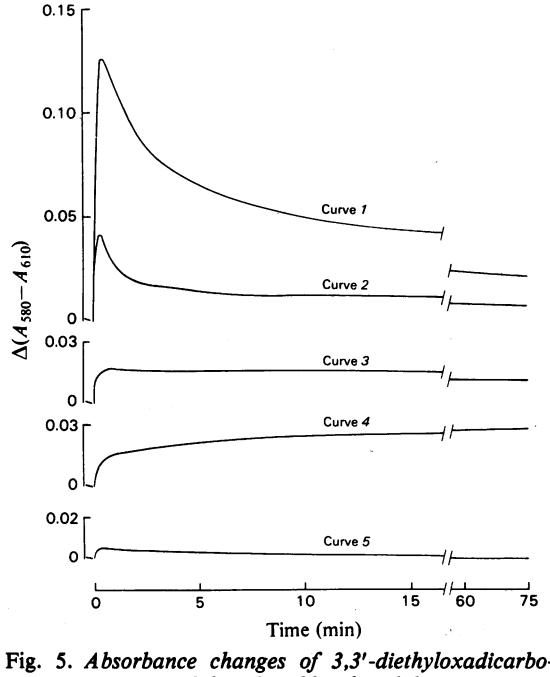

Fig. 5. *Absorbance changes of 3,3'-diethyloxadicarbocyanine iodide induced by phenylalanine*

The experiments were performed as described in Fig. 4 legend, except that L-phenylalanine (curves *1–3* and *5*) or D-phenylalanine (curve *4*) was added instead of glucose.

genic transport system in basolateral-membrane vesicles.

Uptake of carboxylic acids by renal membrane vesicles

The effect on the optical properties of the dye by addition of monocarboxylic acid or dicarboxylic acid to luminal-membrane vesicles is illustrated in Fig. 6. It is seen that addition of succinate, in the presence of Na^+, results in an 'overshoot' (Fig. 6*a*) of almost the same size as that obtained with D-glucose (Fig. 4, curve *1*). The finding that uptake of succinate is associated with depolarization of the membrane vesicles is in accordance with fluorescence studies by Wright *et al.* (1981). However, these observations are in contradiction with the Millipore-filtration studies, which showed that Na^+-dependent transport of tricarboxylic acid-cycle intermediates is an electroneutral process (Kippen *et al.*, 1979, 1980; Wright *et al.*, 1980). Fig. 6(*b*) reveals that addition of L-lactate, in the presence of Na^+, also resulted in a transient depolarization indicative of an electrogenic transport system for monocarboxylic acids. Similar results were obtained by Barac-Nieto *et al.* (1980) with rat luminal-membrane vesicles with Millipore-filtration technique.

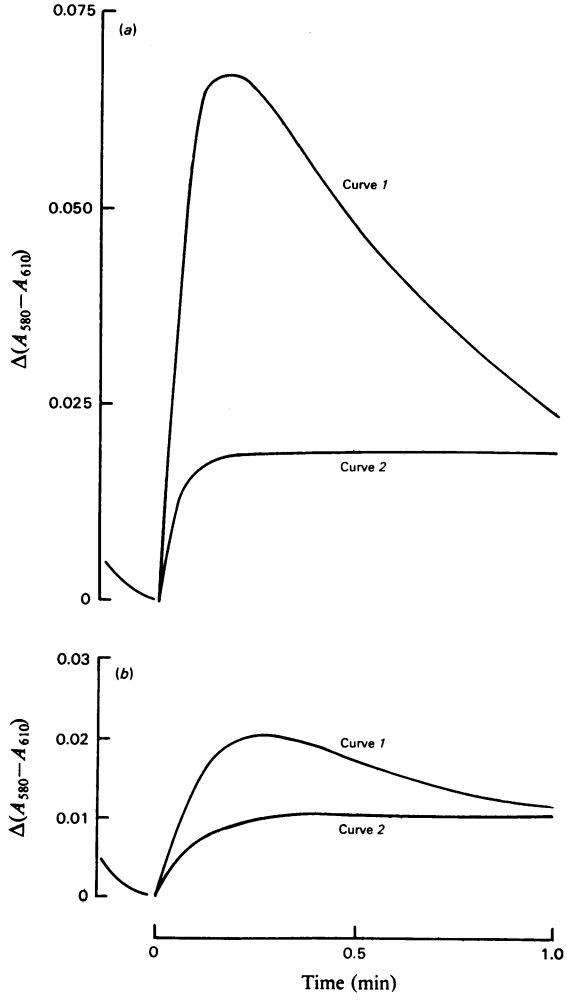

Fig. 6. *Absorbance changes of 3,3'-diethyloxadicarbocyanine iodide induced by succinate or lactate*
The intravesicular medium was 155 mM-KCl/15 mM-Tris/HCl buffer. The external media contained 155 mM-NaCl (curves *1*) or 155 mM-KCl (curves *2*), 15 mM-Tris/HCl buffer, and 5 mM-succinate (*a*) or 5 mM-lactate (*b*). For further details see Fig. 4 legend.

Quantitative aspects of solute uptake

The relations between the Na^+-dependent transport systems of luminal-membrane vesicles for D-glucose and other organic solutes were also studied. First, the maximal absorbance changes induced by addition of various concentrations of D-glucose to suspensions of luminal-membrane vesicles were determined. As shown in Fig. 7(*a*), the results follow a saturation curve. This curve resembles the saturation curve published by Beck & Sacktor (1978*b*). They measured the changes in the fluorescence of 3,3'-dipropylthiodicarbocyanine iodide accompanying uptake of D-glucose. Afterwards, experiments were performed by first adding D-glucose to luminal-membrane vesicles and about 2 min later adding different organic solutes to the same suspension (Fig. 7*b*). The concentration of D-glucose was 12.5 mM, a concentration that nearly saturates the D-glucose-transport system (cf. Fig. 7*a*). Therefore, if the compound added to the D-glucose/membrane-vesicle suspension is taken up by the same transport system as that for D-glucose, no, or only a small, additional absorbance change is expected to take place. By contrast, if the secondary added compound is transported by a separate system, further absorbance changes should be observed. Curve *1* illustrates that no secondary 'overshoot' phenomenon is seen when 5 mM-D-galactose is added to the D-glucose/membrane-vesicle suspension. Curve *5* is a control curve showing that addition of 5 mM-D-galactose, in the absence of D-glucose, results in an 'overshoot'. Thus the results show that D-glucose and D-galactose are probably taken up by the same transport system. Addition of 5 mM-L-lactate, -succinate or -L-phenylalanine introduced a second 'overshoot', indicating that these solutes are not taken up by the same transport system as D-glucose.

The question whether the optical responses generated by D-glucose and another solute, transported by a system different from that of the sugar,

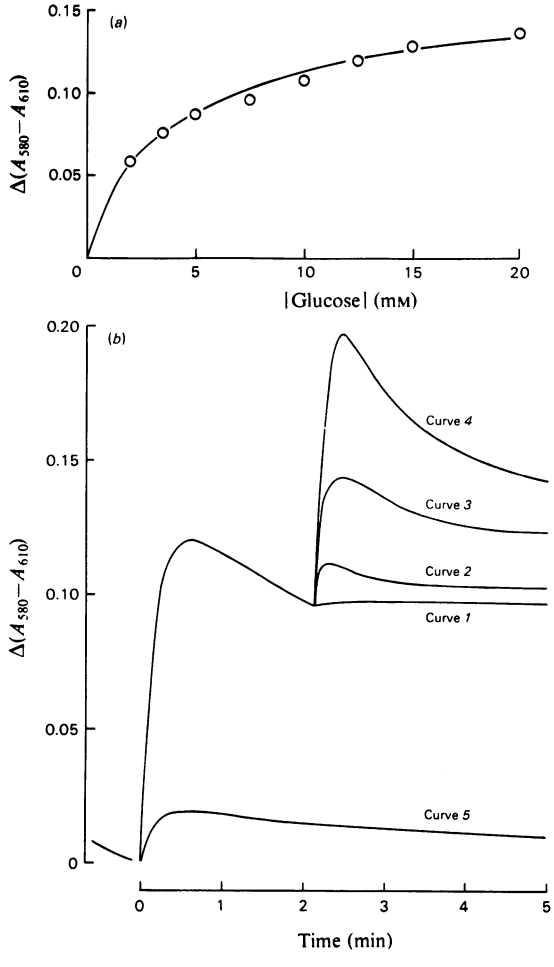

Fig. 7. *Absorbance changes of 3,3'-diethyloxadicarbocyanine iodide induced by various solutes*

(*a*) Maximal absorbance changes caused by addition of increasing concentration of D-glucose to luminal-membrane vesicles. (*b*) Absorbance changes associated with first adding 12.5 mM-D-glucose and later adding 5 mM-D-galactose (curve *1*), 5 mM-L-lactate (curve *2*), 5 mM-succinate (curve *3*) or 5 mM-L-phenylalanine (curve *4*). Curve *5* shows the effect of 5 mM-D-galactose on the absorbance of the cyanine dye in the absence of D-glucose. All results in the Figure were obtained with an intravesicular medium composed of 300 mM-mannitol/25 mM-Hepes/Tris buffer and an external medium of 155 mM-NaCl/15 mM-Tris/HCl buffer. For further details see Fig. 4 legend.

were additive was investigated by simultaneous administration of D-glucose and L-phenylalanine. Addition of 1 mM-D-glucose resulted in a maximal absorbance change of 0.038. The absorbance change caused by 1 mM-L-phenylalanine read at the same time after addition of the solute was 0.064. When 1 mM-D-glucose and 1 mM-L-phenylalanine were added simultaneously, the maximal absorbance change was 0.099, which is very close to the sum of the two individual responses. Thus the present dye technique seems to be useful in examining whether two organic solutes are transported by identical or different transport systems of the luminal-membrane vesicles.

Effect of permeant and impermeant anions on solute uptake

If the transport of solutes into luminal-membrane vesicles is electrogenic, then the solute uptake should be influenced by changes in the membrane potential. Two approaches are usually taken to modify the membrane potential, namely (1) use of salt anions of different permeability and (2) addition of specific ionophores. First, we studied the effect of changing the salt anions. The results obtained with D-glucose and L-phenylalanine in the presence of different extravesicular > intravesicular Na^+-salt gradients are shown in Figs. 8(*a*) and 8(*b*) respectively. In all cases 'overshoots' are seen. However, the magnitude of the 'overshoots' is dependent on the kind of salt anion. The uptake of both glucose and phenylalanine in the presence of various anions is decreased in the following order: $Cl^- > SO_4^{2-}$ > gluconate. The effect of the ions on the solute uptake can be related to their ability to penetrate biological membranes. Since in the proximal tubule the permeability coefficient for Cl^- was found to be 3 times that for Na^+ (Schafer *et al.*, 1974), it is reasonable to assume that Cl^- enters the intravesicular space of the membrane vesicles more rapidly than does Na^+, and therefore can render the interior of the vesicles more negative. The anion SO_4^{2-} is less permeant than is Cl^- (Frömter *et al.*, 1971). Gluconate is not able to permeate luminal-membrane vesicles (Schneider & Sacktor, 1980).

Effect of ionophores on solute uptake

The above-mentioned theory was further tested by examining the effects of various ionophores on the uptake of D-glucose and L-phenylalanine. The effects of valinomycin, which mediates electrogenic K^+ movements, on the uptake of the two organic compounds by luminal-membrane vesicles is illustrated in Figs. 9(*a*) and 9(*b*). The experiments were performed with both an intravesicular > extravesicular K^+ gradient and an extravesicular > intravesicular Na^+ gradient. It is seen that pretreatment of the membrane vesicles by valinomycin increases the uptake of the solutes, especially of L-phenylalanine (Fig. 9*b*). The increased solute uptake following administration of valinomycin can be explained as follows: in the presence of ionophore, K^+ diffuses out of the vesicles, rendering the interior of the vesicles more negative. The transient hyper-

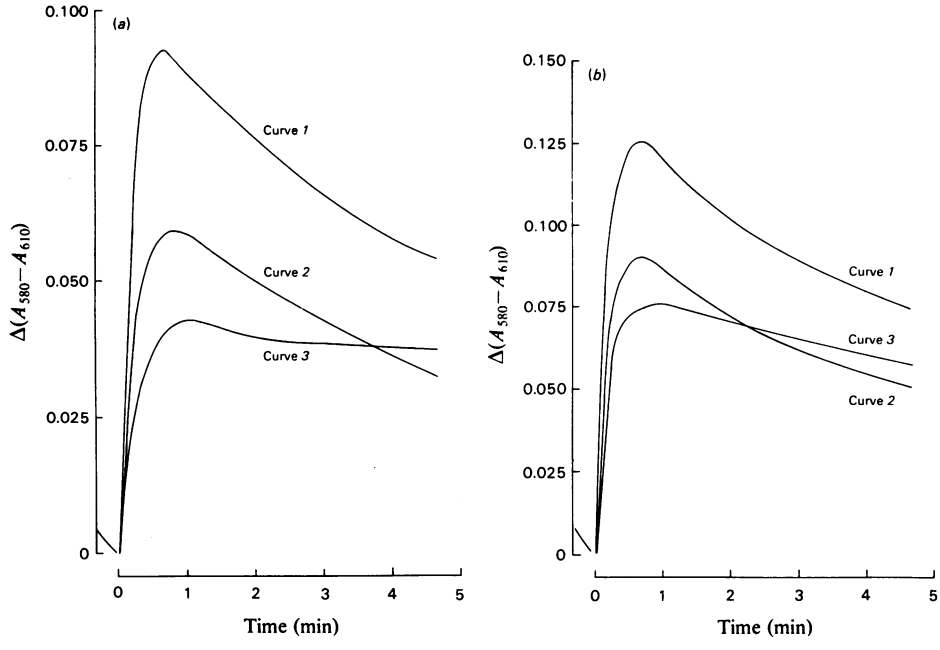

Fig. 8. *Effect of permeant and impermeant anions on the absorbance changes induced by* D-*glucose or* L-*phenylalanine*
(*a*) Absorbance changes caused by addition of 5 mM-D-glucose to luminal-membrane vesicles. (*b*) Absorbance changes caused by addition of 5 mM-L-phenylalanine. All curves were obtained with an intravesicular medium of 300 mM-mannitol/25 mM-Hepes/Tris buffer. The external media were 15 mM-Tris/HCl buffer and 155 mM-NaCl (curves *1*), 103 mM-Na_2SO_4 (curves *2*) or 155 mM-sodium gluconate (curves *3*). For further details see Fig. 4 legend.

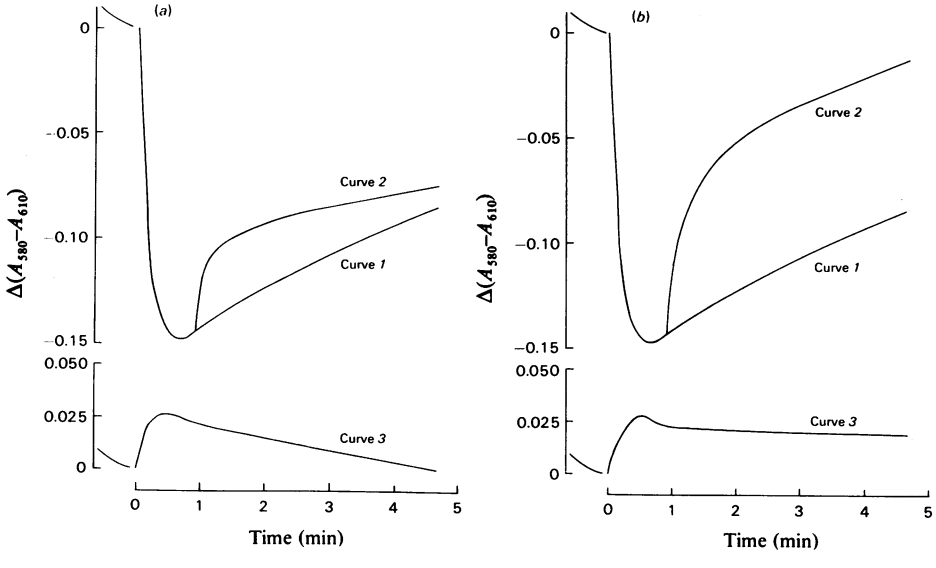

Fig. 9. *Effect of valinomycin on the absorbance changes induced by* D-*glucose or* L-*phenylalanine*
(*a*) Absorbance changes caused by addition of 5 mM-D-glucose to luminal-membrane vesicles. (*b*) Absorbance changes caused by addition of 5 mM-L-phenylalanine. Luminal-membrane vesicles were preloaded with 155 mM-KCl/15 mM-Tris/HCl buffer and suspended in 155 mM-NaCl/15 mM-Tris/HCl buffer. Curves *1*, addition of 5 μg of valinomycin/mg of protein; curves *2*, addition of valinomycin and, about 1 min later, D-glucose or L-phenylalanine; curves *3*, addition of solute in the absence of valinomycin. For further details see Fig. 4 legend.

polarization favours the electrically positive Na^+ co-transport of D-glucose and L-phenylalanine into the vesicles. The increased D-glucose uptake after the addition of valinomycin was also found by Beck & Sacktor (1975, 1978*a*). A K^+-diffusion-potential-sensitive uptake of L-phenylalanine has previously been reported by Evers *et al.* (1976), who used the Millipore-filtration technique.

The effect of gramicidin on the Na^+-dependent transport of glucose and phenylalanine was also investigated (results not shown). The experiments were performed in the presence and in the absence of an Na^+ gradient, $[Na^+]_i = [Na^+]_o$. It was found that pretreatment of the membrane vesicles by gramicidin drastically diminished the optical responses with both the sugar and the amino acid. The Na^+ co-transport of glucose and phenylalanine will tend to depolarize the interior of the membrane vesicles and would create an Na^+ gradient (intravesicular > extravesicular). However, in the presence of gramicidin Na^+ will rapidly migrate out of the vesicles and thereby diminish/abolish the depolarization, which in turn decreases the absorbance change of the dye.

The effect of simultaneous addition of valinomycin and nigericin, the latter ionophore mediating electroneutral exchange of Na^+ for K^+ (Douglas & Cockrell, 1974), on the optical response associated with addition of D-glucose or L-phenylalanine was also studied (results not shown). The experiments were performed in the absence of Na^+ and K^+ gradients, $[Na^+]_i = [Na^+]_o$ and $[K^+]_i = [K^+]_o$. It was observed that pretreatment of the membrane vesicles by the ionophores greatly diminished the optical response with both D-glucose and L-phenylalanine. The explanation of the small optical changes after administration of valinomycin and nigericin probably is analogous to that given for the gramicidin experiments: under these conditions Na^+ and K^+ are rapidly diffusible and thereby diminish the tendency to build up a depolarization of the interior of the membrane vesicles

Concluding remarks

The results presented in this paper confirmed and extended the previous findings that uptake of glucose, phenylalanine, succinate and lactate is Na^+-dependent and results in depolarization of renal membrane vesicles. They also showed that these transport systems are specifically situated at the luminal membrane of the rabbit kidney proximal tubules. In the past, uptake of organic solutes was studied classically by a rapid-filtration technique (Kaback, 1974). As pointed out by Murer & Kinne (1980), despite the many advantages, this method has the following drawbacks. (1) During separation of the vesicles from the extravesicular medium, solutes may leak out from the vesicles. Depending on the permeability of the membrane vesicles and on the time needed for the separation procedure, the intravesicular content of the solute under examination may therefore be altered to a certain degree. (2) During rapid suction filtration the vesicle's membrane may in part be damaged, resulting in leakage of solutes from the intravesicular space. (3) The existence of a specific transport system in membrane vesicles is usually inferred from observations such as different transport rates of structurally related compounds (for an example see Wright *et al.*, 1980). Such demonstrations require that the substrates are radioactively labelled, if the Millipore-filtration technique has to be used, and this is an obvious problem when such forms are unavailable.

An alternative method for the measurement of electrogenic transport of solute is described in the present paper. Although the principle of the spectrophotometric method is simple, some precautionary measures should be taken when the method is used in practice. (1) It is desirable to determine the effect of the selected dye on the transport of organic compound under study. In a series of control experiments, with luminal-membrane and basolateral-membrane vesicle preparations, we found that 3,3′-diethyloxadicarbocyanine iodide is non-destructive and did not influence the renal uptake of a number of organic solutes (results not shown). Therefore this cyanine dye was chosen for the present series of studies. (2) Care should be taken in analysing the spectrophotometric results. The effect of the selected organic compound on the binding of dye to membrane vesicles should be examined before performance of the transport studies. In this connection it is important to note that the renal transport of lipophilic compounds, which unspecifically bind to membrane vesicles, cannot be studied by this method. (3) It is apparent that organic compounds that are electroneutrally transported by membrane vesicles cannot be examined by this technique.

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