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# Transport of organic compounds in renal plasma membrane vesicles of cadmium intoxicated rats

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**Transport of organic compounds in renal plasma membrane vesicles of cadmium intoxicated rats.** Effects of cadmium intoxication on renal transport systems for various organic compounds were studied. Subcutaneous injections of $CdCl_2$ (2 mg Cd/kg · day) for two to three weeks induced marked polyuria, glycosuria, and proteinuria without altering glomerular filtration rate. In renal cortical brush border membrane vesicles (BBMV) isolated from cadmium treated rats, $Na^+$-dependent D-glucose uptake was markedly attenuated, and this was due to reduction in $V_{max}$ and not Km. Likewise, $Na^+$-driven L-glutamate transport and $H^+$-driven tetraethylammonium transport were significantly reduced. In renal cortical basolateral membrane vesicles (BLMV) of cadmium intoxicated rats, $Na^+$-dependent succinate transport was drastically reduced. These results indicate that cadmium intoxication impairs various transport systems for organic compounds in the brush border and basolateral membranes of proximal renal tubules.

Renal glycosuria has been documented in cadmium intoxicated humans and experimental animals. Axelson and Piscator [1] and Nomiyama, Sato and Yamamoto [2] observed that urinary excretion of glucose is markedly increased in rabbits exposed to chronic subcutaneous injections of $CdCl_2$. Nomiyama et al [3] also observed in rabbits and monkeys that oral exposures of cadmium in food pellets induced glycosuria. In clinical studies, glycosuria was commonly found among cadmium intoxicated patients [4, 5]. Despite these observations, few attempts have been made to elucidate the mechanism with which cadmium induces glycosuria.

Under normal conditions, filtered glucose is mostly reabsorbed in the proximal tubule via the $Na^+$-dependent transport process. We, therefore, questioned if cadmium impaired the $Na^+$-glucose cotransport system in the brush border membrane of the proximal tubule. The present study was undertaken to evaluate this possibility, using renal cortical plasma membrane vesicles isolated from cadmium intoxicated rats. For comparison, the effect of cadmium intoxication on the transport of several other organic compounds (L-glutamate, succinate, and tetraethylammonium) was also studied.

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## Methods

### *Animals*

Sprague-Dawley male rats (250 to 300 g) were maintained under standard laboratory conditions with *ad libitum* access to food and water, unless otherwise mandated by experimental protocol. Cadmium intoxication was induced by daily subcutaneous injections of $CdCl_2$ at a dose of 2 mg Cd/kg body wt · day for two to three weeks. Saline was injected as the control.

### *Urinalysis*

At one week intervals, animals were kept in metabolic cages and were denied food and water for 24 hours. Urine was collected under mineral oil, and was analyzed for creatinine (Wako Technical Bulletin No. 271-10509, Wako Pure Chemical Ind., Osaka, Japan), protein [6] and glucose (Sigma Diagnostics No. 315, Sigma Chemical Co., St. Louis, Missouri, USA). In some animals, blood samples were collected by heart puncture, and analyzed for creatinine.

### *Preparations of brush border and basolateral membrane vesicles from kidney cortex*

Renal plasma membrane vesicles were isolated by a procedure similar to those described by Kinsella et al [7] and Scalera et al [8]. Kidneys removed from animals were perfused through the renal artery with an ice-cold solution containing 140 mM NaCl, 10 mM KCl and 1.5 mM $CaCl_2$. The cortex was cut off, minced, and placed in 250 mM sucrose-10 mM triethanolamine hydrochloride, pH 7.6 at 4°C (sucrose buffer). Cortical tissues from five to six animals were pooled and then homogenized with 20 strokes in a motor-driven glass homogenizer with a tight-fitting Teflon pestle (clearance 0.15 mm) at 1,800 rpm. The tissue homogenate was centrifuged at 1,075 × g for 10 minutes in a Sorvall Refrigerated Centrifuge. The supernatant was saved, and the pellet was suspended again in half the original volume of sucrose buffer and homogenized with 10 strokes at 1,800 rpm. The homogenate was centrifuged as above for 10 minutes at 1,075 × g. The supernatant was decanted and combined with the previous supernatant (Fraction 1). The fraction 1 was centrifuged at 14,460 × g for 15 minutes, the resulting supernatant and the soft light portion of the pellet were taken, and pooled (Fraction 2). The fraction 2 was then centrifuged at 47,800 × g for 30 minutes, the supernatant and the lower dark pellet were discarded, and the upper fluffy layer of

the pellet was suspended in sucrose buffer to a total volume of 26.5 ml (Fraction 3). This constituted the microsomal fraction. Plasma membranes in this fraction were purified further by centrifugation on a gradient of Percoll (a suspension of colloidal silica particles which generates a self-orienting density gradient during centrifugation). To the fraction 3, 3.5 ml stock solution of Percoll (Pharmacia Fine Chemicals, Uppsala, Sweden) was added; the solution was mixed by inversion and centrifuged at 47,800 × g for 40 minutes. The spontaneously formed Percoll gradient was fractionated from the top by careful pipetting and was collected in 1 ml fractions. Initially, each fraction was assayed for $Na^+$-$K^+$-ATPase (a marker enzyme of basolateral membrane, BLM) and alkaline phosphatase (a marker enzyme of brush border membrane, BBM). Routinely thereafter, fractions were pooled according to the distribution of marker enzymes to obtain aliquots enriched in either BLM or BBM. Typically, the first 4 ml were discarded; 5 to 15 ml were pooled as BLM and 16 to 30 ml were pooled as crude BBM. Each pooled fraction was mixed with an equal volume of sucrose buffer, and the Percoll was removed by centrifuging at 100,000 × g for one hour in an ultracentrifuge (Sorvall OTD-75). The Percoll forms a glassy pellet, and membranes form a fluffy layer on top of the Percoll. In the case of BLM, they were resuspended in an appropriate vesicle buffer by passing the membrane pellet several times through a 25 gauge needle. The protein concentration of the vesicle fraction was adjusted to be 6 to 8 mg/ml. Unless stated otherwise, the composition of the vesicle buffer was 300 mM mannitol, 20 mM Hepes, pH 7.4 with Tris-base. BBM were further purified from a crude fraction by the magnesium precipitation method [9]. The crude BBM were suspended in 10 ml of vesicle buffer solution containing 10 mM $MgCl_2$, stirred on ice for 60 minutes, and then centrifuged at 1,075 × g for 10 minutes. The supernatant was saved, and the pellet was resuspended in the same buffer and centrifuged again at 1,075 × g for 10 minutes. The resulting supernatant was combined with the first supernatant. The BBM contained in the combined supernatants was packed by centrifugation at 100,000 × g for one hour. The resulting pellet was suspended in the vesicle buffer solution as described above. The BLM and BBM were incubated for 30 minutes at 37°C, and were stored on ice or at 4°C until used. If the fractions were not used within one hour, they were stored at −70°C and were used within two weeks.

### *Marker enzyme assays*

$Na^+$-$K^+$-ATPase activity was measured according to Jørgensen and Skou [10]. Samples containing approximately 0.25 mg membrane protein were treated with deoxycholate by incubating in a solution containing 2 mM EDTA, 25 mM imidazole (pH 7.0) and 0.6 mg/ml deoxycholate (total volume 1 ml) for 30 minutes at 25°C. Aliquots of 100 μl were transferred to assay tubes containing 1 ml of an appropriate solution (NaCl-KCl-histidine solution for the total ATPase and NaCl-histidine-ouabain solution for the $Mg^{2+}$-ATPase). After preincubation for 10 minutes at 37°C, the enzyme reaction was initiated by adding ATP and $MgCl_2$ stock solutions. The concentration of $Na^+$, $K^+$, histidine, $Mg^{2+}$, ATP and ouabain in the incubation mixture was 130, 20, 30, 3, 3, and 1 mM, respectively, and the pH was adjusted to 7.5 at 37°C. After 10 minutes of incubation, the reaction was stopped by adding 0.2 ml of 6% $HClO_4$ and placing the test tubes on ice. The mixture was centrifuged at 3,500 × g for 15 minutes, and the inorganic phosphate (Pi) in the supernatant was determined by the method of Fiske and Subbarow [11]. The enzyme activity was expressed as μmol of Pi liberated per mg protein per hour.

Alkaline phosphatase activity was determined as described in the Wako Technical Bulletin No. 270-04609 for serum alkaline phosphatase. Membrane samples were incubated in 0.05 M carbonate buffer (pH 10.0) with 95 mg/dl phenyl phosphate as the substrate for 15 minutes at 37°C. The reaction was stopped by adding a color reagent (0.05 M phosphate buffer, pH 6.8, 1.2 g/dl potassium ferricyanide). The absorbance was determined at 500 nm, and the enzyme activity was expressed as King-Armstrong (K-A) units per mg protein per hour. Protein concentration in the membrane sample was determined by the method of Lowry et al [6].

### *Determination of substrate transport in membrane vesicles*

Transport of glucose and other substances in membrane vesicles was determined using a rapid filtration method [12]. For the glucose uptake, an aliquot of membrane vesicles was incubated in 8 volumes of incubation medium (100 mM NaCl, 100 mM mannitol, and 20 mM Hepes, pH 7.4 with Tris) containing 50 μM D-[$^{14}$C]-glucose at 25°C. At appropriate intervals, a 100 μl aliquot was removed and quickly filtered through Millipore filter (Type HA, pore size 0.45 μm), which was soaked overnight in distilled water prior to use. The filter was washed with 5 ml of ice-cold stop solution (200 mM NaCl-0.5 mM phlorizin). $^{14}$C-compound in the filter was dissolved in 1.0 ml of methoxyethanol. After the addition of 10 ml of scintillation fluid, the $^{14}$C-activity was counted on a liquid scintillation counter (Packard Tricarb 3000 C). Nonspecific binding of the radioactive material to the plasma membrane was determined by incubating vesicles in distilled water containing 0.1% deoxycholate and D-[$^{14}$C]-glucose for one hour. An aliquot of 100 μl was filtered through the millipore filter, and the filter was rinsed with the stop solution as above. The value of nonspecific binding was subtracted from the experimental value, and the vesicular uptake was expressed as pmol per mg protein for a given time.

When vesicular uptakes of other substances, such as $^{22}$Na, L-[$^{14}$C]-glutamate, $^{14}$C-succinate and $^{14}$C-tetraethylammonium were determined, compositions of the intra- and extravesicular media were appropriately adjusted. All the radioactive compounds used in this study were purchased from the Amersham International (Amersham, UK).

### *Statistical analysis*

Statistical evaluation of the data was done using the Student's *t*-test (unpaired comparison) and all results were presented as the mean ± SEM.

## Results

### *Renal functions*

Table 1 summarizes the effect of cadmium treatment on urine flow and urinary excretions of creatinine, glucose and protein. Subcutaneous injections of $CdCl_2$ at a dose of 2 mg Cd/kg · day for two weeks resulted in a marked polyuria. The creatinine excretion was not significantly changed as was the plasma

**Table 1.** Effect of cadmium treatment on urine flow and urinary excretions of creatinine, glucose and protein in rats

| | Days of treatment | Control ($N$ = 4) | Cadmium ($N$ = 8) | $P$ |
|---|---|---|---|---|
| Urine flow | 0 | 22.4 ± 2.0 | 24.4 ± 2.0 | NS |
| *ml/kg · day* | 7 | 28.6 ± 2.0 | 23.8 ± 1.9 | NS |
| | 14 | 25.8 ± 2.9 | 50.1 ± 7.4 | <0.01 |
| Creatinine exc. | 0 | 33.6 ± 0.9 | 34.5 ± 3.1 | NS |
| *ml/kg · day* | 7 | 33.6 ± 0.9 | 31.5 ± 3.9 | NS |
| | 14 | 32.6 ± 8.7 | 42.9 ± 10.6 | NS |
| Glucose exc. | 0 | 2.8 ± 1.3 | 2.7 ± 0.2 | NS |
| *ml/kg · day* | 7 | 2.5 ± 0.5 | 2.1 ± 0.4 | NS |
| | 14 | 3.7 ± 0.4 | 13.2 ± 3.9 | <0.01 |
| Protein exc. | 0 | 233 ± 13 | 280 ± 21 | NS |
| *ml/kg day* | 7 | 274 ± 37 | 249 ± 21 | NS |
| | 14 | 276 ± 41 | 622 ± 150 | <0.01 |

Values represent mean ± SEM.

**Table 2.** Activities of $Na^+$-$K^+$-ATPase and alkaline phosphatase in renal cortical homogenate and membrane fractions of control and cadmium treated rats

| | Alkaline phosphatase *K-A units/mg protein · hr* | | $Na^+$-$K^+$-ATPase *μmol Pi/mg protein · hr* | |
|---|---|---|---|---|
| | Control | Cadmium | Control | Cadmium |
| Homogenate | 2.8 ± 0.29 | 0.85 ± 0.08 | 15.2 ± 2.99 | 14.2 ± 2.06 |
| BBMV | 21.3 ± 1.93 | 7.2 ± 0.08 | 29.6 ± 4.71 | 67.1 ± 1.63 |
| BLMV | 9.1 ± 1.51 | 3.5 ± 0.38 | 156.5 ± 4.11 | 159.7 ± 3.0 |
| BBMV/ homogenate | 7.6 | 8.4 | 1.9 | 4.7 |
| BLMV/ homogenate | 3.2 | 4.1 | 10.3 | 11.2 |

Values represent the mean ± SE of 5 determinations from 2 separate batches of membrane preparations in each group.

creatinine level (0.86 ± 0.08 mg/100 ml in the control and 0.91 ± 0.11 in the cadmium group); thus, the glomerular filtration rate remained unchanged. However, excretions of glucose and protein appeared to be significantly increased after two weeks of cadmium treatment, as observed in other studies [1, 13–15].

These results indicate that the cadmium treated rats in the present study developed renal functional changes typical of chronic cadmium intoxication.

### *Transport of organic compounds in renal cortical plasma membrane vesicles*

*Enzymatic characteristics of vesicle membranes.* To evaluate the purity of membrane vesicles, activities of $Na^+$-$K^+$-ATPase and alkaline phosphatase in each fraction were determined. $Na^+$-$K^+$-ATPase and alkaline phosphatase are well established marker enzymes for BLM and BBM, respectively [16, 17]. As described in **Methods**, fractions 5 to 15 ml from the top of the Percoll gradient were pooled to obtain BLM and fractions 16 to 30 ml for BBM.

Table 2 summarizes the results. In the control group, the specific activity of alkaline phosphatase in the purified BBM (after $Mg^{2+}$ precipitation) was 21.3 K-A units/mg protein · hr, which was 7.6-fold greater than that in the homogenate [2, 8]. $Na^+$-$K^+$-ATPase activity in this fraction (BBM) was 29.6 μmol Pi/mg protein · hr as compared with 156.5 in the BLM. Nevertheless, $Na^+$-$K^+$-ATPase activity was enriched 1.9-fold in the

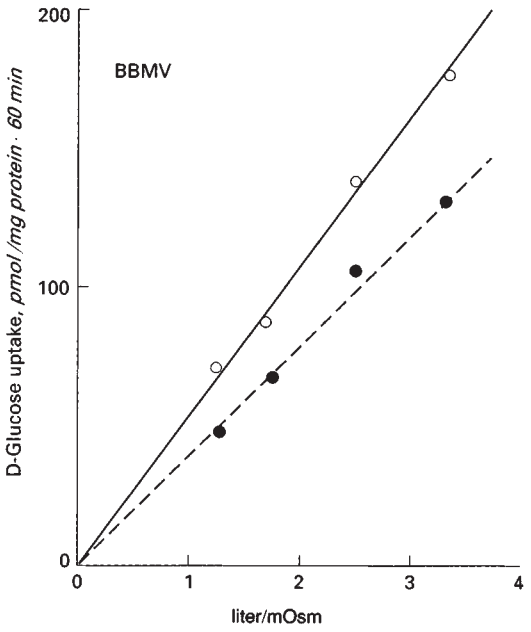


**Fig. 1.** *Steady state accumulation of D-glucose by renal cortical BBMV of control (○—○) and cadmium treated (●----●) rats as a function of the reciprocal of medium osmolarity.* The osmolarity of medium was changed by changing sucrose concentration. Each point represents the mean of 4 determinations from the same batch of vesicle preparations in each group.

BBM, indicating some contamination with BLM. $Na^+$-$K^+$-ATPase activity in the BLM (156.5 μmol Pi/mg protein · hr) was 10.3-fold greater than the homogenate value (15.2). Alkaline phosphatase activity in the BLM (9.1 K-A units/mg protein · hr) was much smaller than that in the BBM, but it was 3.2 times the activity in the homogenate; thus there was some contamination with the BBM. Similar results were obtained in the cadmium group, although the alkaline phosphatase activity in each fraction was significantly lower than the corresponding value in the control group.

*Glucose transport.* To ascertain that the uptake of glucose by brush border membrane vesicles (BBMV) represents transport into the vesicles rather than binding to the membrane, the vesicular uptake of D-glucose (D-[$^{14}$C]-glucose) was determined first as a function of the medium osmolarity. The uptake of D-glucose was measured after 60 minutes of incubation in the absence of $Na^+$ and increased amounts of sucrose in the medium. Since the D-glucose uptake in the absence of $Na^+$ gradient reached equilibrium after 1.5 minutes of incubation, the amount of D-glucose taken up at 60 minutes should, if it were transported, be dependent upon the intravesicular volume. The volume of sealed vesicles will be decreased by increasing the medium osmolarity with sucrose, a relatively impermeable solute. The results depicted in Figure 1 indicate that, in both controls and cadmium treated rats, the D-glucose uptake was directly proportional to the reciprocal of the medium osmolarity, and the amount of membrane-bound glucose, estimated by extrapolation to the infinite osmolarity (that is, the intercept of the line with y axis), was negligible. However, at a given osmolarity, the D-glucose uptake per mg of membrane protein appeared to be considerably lower in the vesicles from

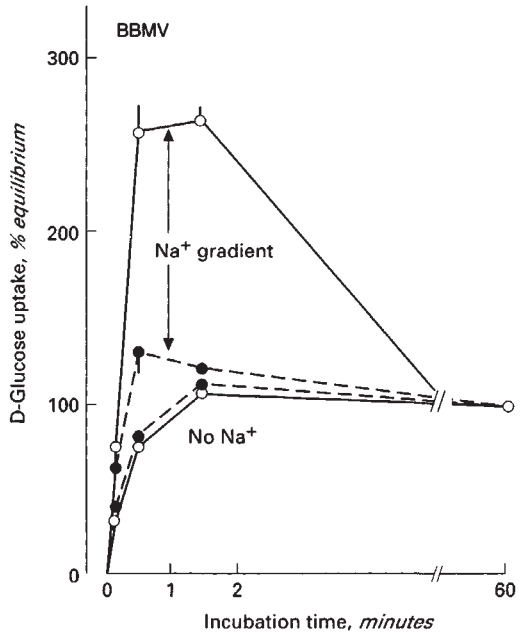


**Fig. 2.** *Time course of $Na^+$-dependent and $Na^+$-independent D-glucose uptakes by renal cortical BBMV of control (–) and cadmium (---) treated rats.* Vesicles containing 100 mM mannitol, 100 mM KCl and 20 mM Hepes-Tris (pH 7.4) were incubated in a medium containing 50 μM D-[$^{14}$C]-glucose, 100 mM mannitol, 100 mM NaCl (or KCl in the case of $Na^+$-independent uptake), 20 mM Hepes-Tris (pH 7.4) and 2 μM valinomycin at 25°C. The ionophore (valinomycin) was included to generate $K^+$ diffusion potential, thereby facilitating electrogenic $Na^+$ glucose cotransport [18–20]. Values are expressed as percentage of D-glucose taken up by vesicles after 60 min incubation (116 ± 10 pmol/mg protein in the control and 75 ± 6 in the cadmium group). Each datum represents the mean ± SEM of 7 determinations from 2 different batches of vesicle preparations in each group.

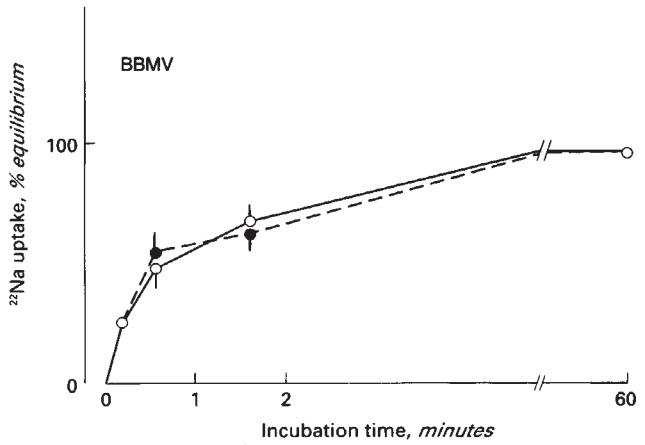


**Fig. 3.** *Time course of $Na^+$ uptake by renal cortical BBMV of control (○—○) and cadmium treated (●----●) rats.* Vesicles containing 100 mM mannitol, 100 mM KCl, 20 mM Hepes-Tris (pH 7.4) were incubated in a medium containing 100 μM $^{22}$Na, 100 mM NaCl, 100 mM mannitol and 20 mM Hepes-Tris (pH 7.4) at 25°C. Values are expressed as percentage of the $Na^+$ uptake after 60 min incubation (203 ± 11 pmol/mg protein in the control and 187 ± 11 in the cadmium group). Each datum represents the mean ± SEM of 4 determinations from the same batch of vesicles in each group.

cadmium treated rats, as compared with the controls. This indicates that the relative population of sealed vesicles was smaller in the cadmium group than in the control group. Thus, in all subsequent experiments, the vesicular uptake of substrate was expressed as a percentage of the equilibrium value in each situation.

Figure 2 illustrates the time course of the D-glucose uptake by the BBMV in the presence and absence of a 100 mM, inwardly-directed $Na^+$ gradient. In the control vesicle, the $Na^+$-dependent D-glucose uptake increased rapidly during the initial period, then declined to the equilibrium value, showing a characteristic "overshoot" phenomenon of a $Na^+$-gradient-dependent transport process [21–23]. The level of the D-glucose uptake at 30 seconds was approximately 2.5 times the equilibrium (60 min) level. In the vesicles of cadmium treated animals, however, the initial rate of the D-glucose uptake was markedly reduced and the overshoot phenomenon was much less apparent. The $Na^+$-independent uptake of D-glucose was not different between the vesicles of controls and cadmium treated animals. In both cases, the uptake increased gradually at a similar rate during the initial 1.5 minutes, and then it levelled off. The steady state uptake was similar, but the initial 30 second uptake was 50% (cadmium) to 70% (control) lower than that with the $Na^+$-gradient. These findings indicate that the $Na^+$-dependent transport system for glucose was seriously impaired by cadmium treatment.

Since the $Na^+$-glucose cotransport depends on the electrochemical potential gradient for $Na^+$ [21, 22, 24], it would decrease if the $Na^+$ gradient was more rapidly dissipated in the vesicles of cadmium treated animals. To evaluate this possibility, $^{22}$Na uptake by BBMV was measured in the presence of 100 mM $Na^+$ and absence of glucose in the medium. The results, however, indicated that cadmium intoxication had no apparent effect on the dissipation of the $Na^+$ gradient (Fig. 3).

Figure 4 shows kinetics of D-glucose uptake by the BBMV. Since the D-glucose uptake was found to change linearly up to five seconds in a preliminary experiment, the five second rates of uptake were measured at sugar concentrations of 0.1 to 10 mM in the presence of a 100 mM, inwardly-directed $Na^+$ gradient. In the vesicles of both controls and cadmium treated animals, the uptake rate increased curvilinearly with the substrate concentration, providing an evidence for saturability. In order to analyze the carrier mediated component, the uptake rate was corrected for the nonsaturable component. The latter was estimated by employing the straight-line equation generated at higher substrate concentrations.

Figure 5 presents the carrier mediated component of the D-glucose uptake in the form of a Hofstee plot. The relationship between the uptake (V) and the uptake/substrate concentration (V/[S]) appeared to be non-linear (Fig. 5A), suggesting that more than one transport system may be involved. This could be due to heterogeneity of the membrane source. Isolated renal tubular perfusion studies of Barfuss and Schafer [25] indicated that the nature of the glucose transport system varies along the proximal tubular segments, that is, a low affinity-high capacity system in the early proximal segment, and a high affinity-low capacity system in the late proximal tubule. In a study on rabbit renal BBMV, Turner and Moran [26] found that the $Na^+$-dependent glucose transport system in the outer cortical (early

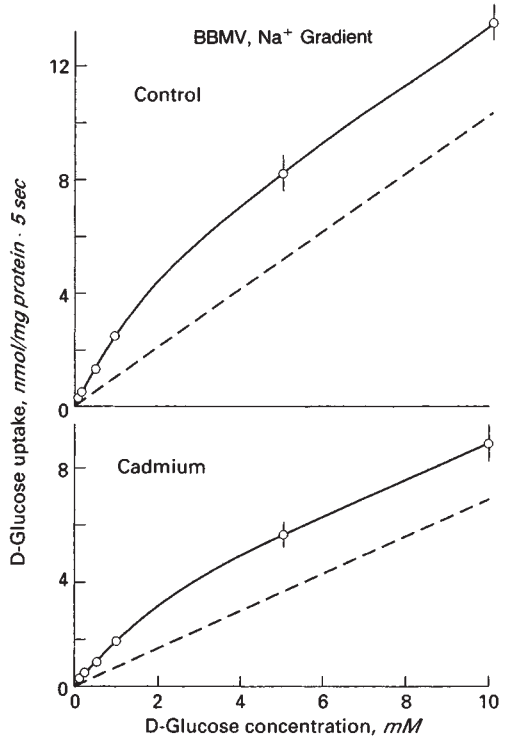


**Fig. 4.** *Initial (5 sec) rate of $Na^+$-dependent D-glucose uptake by renal cortical BBMV of control and cadmium-treated rats as a function of D-glucose concentration in the medium.* Compositions of intra- and extravesicular media, other than substrate concentration, are the same as described in Fig. 2. Incubation temperature was 25°C. The dashed line represents the nonsaturable component. Values are mean ± SEM of 4 determinations from the same batch of vesicles in each group.

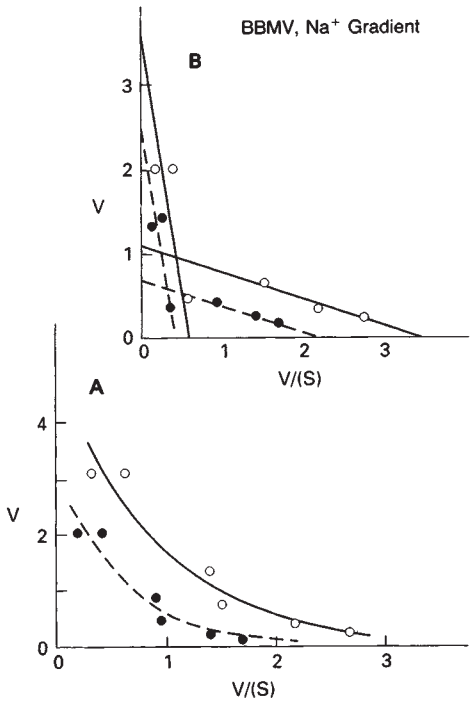


**Fig. 5. A.** *Hofstee plot of the carrier-mediated component of D-glucose uptake.* **B.** Resolution of carrier-mediated component of D-glucose uptake into high affinity-low capacity and low affinity-high capacity systems. The way of resolution is described in the text. Data are based on Fig. 4. Symbols are (○—○) control; (●----●) cadmium.

proximal tubule) BBMV has low affinity and high capacity, while that in the outer medullary (late proximal tubule) vesicle has high affinity and low capacity. Since in the present study, vesicles were prepared from the whole renal cortex, membranes of both the early and late proximal tubules might be involved. We therefore divided the D-glucose uptake into two different components:

$$V = V_1 + V_2 = \frac{V_{max_1}[S]}{Km_1 + [S]} + \frac{V_{max_2} + [S]}{Km_2 + [S]}$$

where $V_1$ and $V_2$ are the uptake by high affinity and low affinity system, respectively. From the straight line equation generated at high V/[S] values, $V_{max_1}$ and $Km_1$ were calculated, and using these values $V_1$ at each substrate concentration was calculated. $V_2$ was then estimated by subtracting $V_1$ from V at each substrate concentration. The Hofstee plot of the data (Fig. 5B) shows that cadmium intoxication reduced $V_{max}$ but not Km for both high affinity-low capacity and low affinity-high capacity systems. These results may suggest that capacity for glucose transport was decreased in both early and late segments of proximal tubules after cadmium intoxication.

Figure 6 illustrates the D-glucose uptake by the BLMV in the presence of a 100 mM $Na^+$ gradient (medium > vesicles). In both control and cadmium treated animals the uptake increased gradually and reached equilibrium after 1.5 minutes. The initial rate of uptake was not significantly different between the two groups.

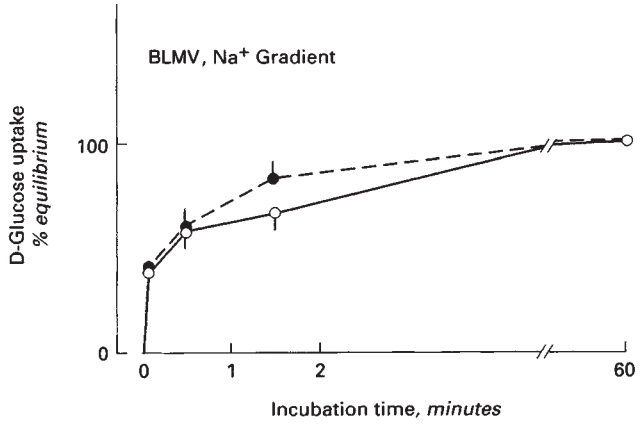


**Fig. 6.** *Time course of D-glucose uptake by renal cortical BLMV of control (○—○) and cadmium treated (●----●) rats.* The condition of incubation was identical to that described in Fig. 2. Values are expressed as percentage of equilibrium (60 min) level (121 ± 6 pmol/mg protein in the control and 94 ± 4 in the cadmium group). Values are mean ± SEM of 6 determinations from the same batch of vesicles in each group.

Since in the kidney, glucose is also transported from cell to blood across BLM, we measured the efflux of D-glucose from the preloaded BLMV. Vesicles were first permitted to accumulate D-[$^{14}$C]-glucose by incubating them in a medium containing D-[$^{14}$C]-glucose (1 μCi/ml) for about 60 minutes. The incubation

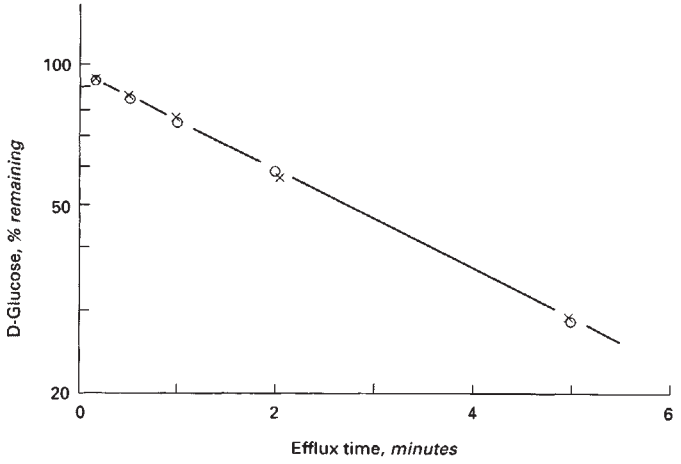


**Fig. 7.** *Efflux of D-glucose by renal cortical BLMV of control (○) and cadmium treated (×) rats.* Vesicles were loaded with D-[$^{14}$C]-glucose, by incubating them in a medium containing 1 $\mu$Ci D-[$^{14}$C]-glucose/ml, 0.1 mM D-glucose, 100 mM mannitol, 100 mM KCl and 20 mM Hepes-Tris (pH 7.4) for 60 min at 25°C. The mixture was then diluted (1/50) with a $^{14}$C-free medium and incubated for various period. The percentage of radioactivity remaining in the vesicle at the end of each efflux period was plotted as a function of time using a semi-logarithmic plot. The slope of the line represents the efflux rate constant. Values are mean of 4 determinations from the same batch of vesicles in each group.

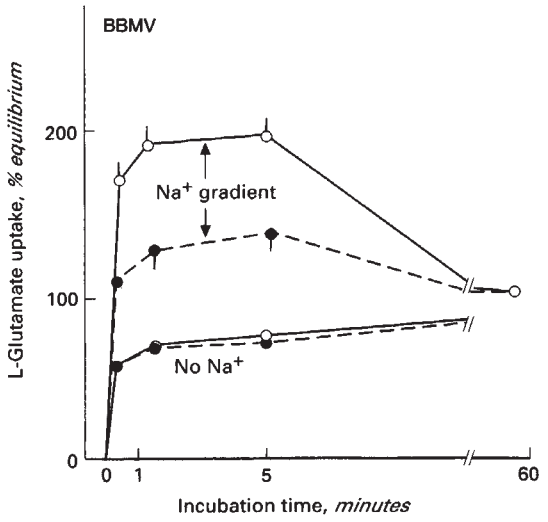


**Fig. 8.** *Time course of $Na^+$-dependent and $Na^+$-independent L-glutamate uptakes by renal cortical BBMV of control (○—○) and cadmium treated (●----●) rats.* Vesicles containing 100 mM mannitol, 100 mM KCl and 20 mM Hepes-Tris (pH 7.4) were incubated in a medium containing 50 $\mu$M L-[$^{14}$C]-glutamate, 100 mM mannitol, 100 mM NaCl (or KCl in the case of $Na^+$-independent uptake), 20 mM Hepes-Tris (pH 7.4) and 2 $\mu$M valinomycin at 25°C. Values are expressed as percentage of L-glutamate taken up after 60 min incubation (107 ± 10 pmol/mg protein in the control and 102 ± 7 in the cadmium group). Each datum represents the mean ± SEM of 6 determinations from the same batch of vesicles in each group.

mixture was then diluted (1/50) with a D-[$^{14}$C]-glucose-free medium and incubated again. At appropriate intervals, incubations were terminated and the D-[$^{14}$C]-glucose retained by the vesicles were determined. As shown in Figure 7, the rate of efflux (slope of the line) was not different between the control and cadmium treated groups. In a separate series of experiments, we have observed that approximately 50% of the efflux was phloretin (0.1 mM) sensitive in both the control and cadmium groups. These indicate that the glucose transport system in the BLM was not altered in cadmium treated animals.

*Glutamate transport.* To investigate if cadmium treatment affected the $Na^+$-dependent amino acid transport system, the L-glutamate uptake by the renal BBMV was measured. Figure 8 shows the time course of the L-glutamate uptake in the presence and absence of a 100 mM, inwardly-directed $Na^+$ gradient. In the presence of the $Na^+$ gradient, the glutamate uptake increased rapidly during the initial 1.5 minutes to a value of 30% (cadmium) to 90% (control) above the equilibrium level, showing an overshoot phenomenon. However, the rate of initial (30 sec) uptake appeared to be significantly ($P < 0.01$) lower in the cadmium group (106 ± 5% equilibrium) than in the control group (169 ± 13% equilibrium). In the absence of the $Na^+$ gradient, the uptake was not different between the control and the cadmium group. These indicates that the mechanism of the $Na^+$-dependent acidic amino acid transport in the renal BBM was impaired in cadmium intoxicated animals.

*Tetraethylammonium (TEA) transport.* To evaluate the effect of cadmium intoxication on the $H^+$-organic cation antiport system, the TEA uptake by the renal BBMV was studied. Vesicles prepared in 300 mM mannitol-20 mM Hepes-Tris (pH 6.0) were incubated in a medium containing 50 $\mu$M $^{14}$C-TEA, 300 mM mannitol and 20 mM Hepes-Tris (pH 7.4), thus the outwardly directed $H^+$ gradient was 1/25 (medium/vesicle). As depicted in Figure 9, initial (30 sec) uptake of TEA was

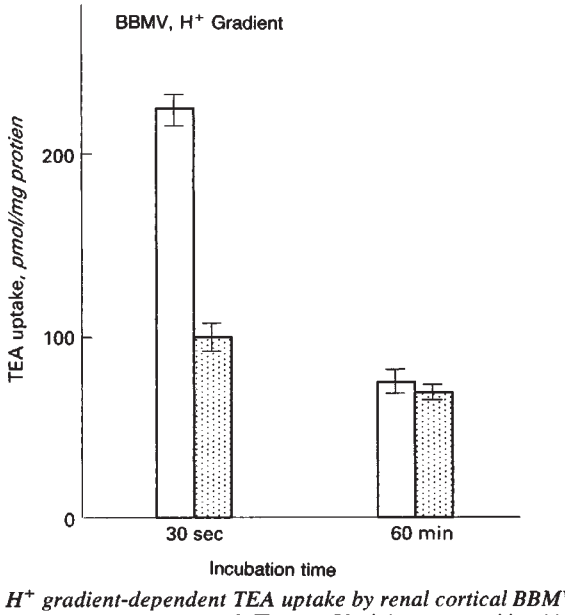


**Fig. 9.** *$H^+$ gradient-dependent TEA uptake by renal cortical BBMV of control (□) and cadmium treated (▨) rats.* Vesicles prepared in 300 mM mannitol-20 mM Hepes-Tris (pH 6.0) were incubated in the medium containing 50 $\mu$M $^{14}$C-TEA, 300 mM mannitol and 20 mM Hepes-Tris (pH 7.4) at 25°C. Each datum represents the mean ± SEM of 6 determinations from the same batch of vesicles in each group.

drastically reduced in the cadmium group, as compared with the control group. This indicates that the $H^+$-TEA antiport system in the renal BBM was severely damaged by cadmium intoxication. The membrane potential-dependent TEA transport in the

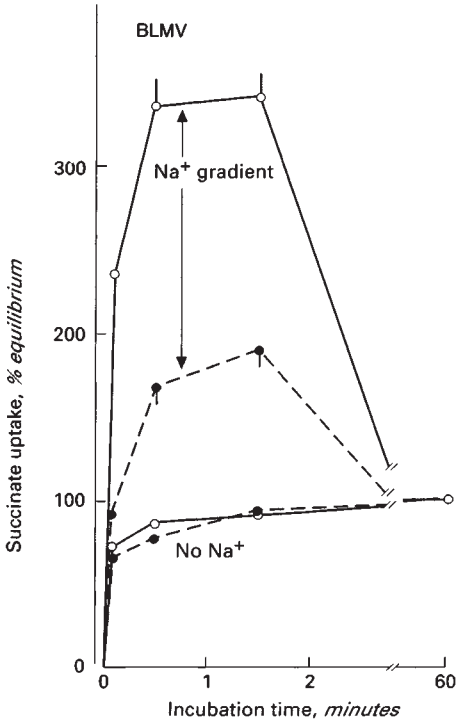


**Fig. 10.** *Time course of $Na^+$-dependent and $Na^+$-independent succinate uptakes by renal cortical BLMV of control (○—○) and cadmium treated (●----●) rats.* Vesicles prepared in 100 mM mannitol, 100 mM KCl and 20 mM Hepes-Tris (pH 7.4) were incubated in the medium containing 50 μM $^{14}C$-succinate, 100 mM NaCl (or KCl in the case of $Na^+$-independent uptake), 100 mM mannitol, 20 mM Hepes-Tris (pH 7.4), and 2 μM valinomycin at 25°C. Values are expressed as percentage of the equilibrium (60 min) level (321 ± 13 pmol/mg protein in the control and 318 ± 17 in the cadmium group). Each datum represents the mean ± SEM of 4 determinations from the same batch of vesicles in each group.

BLMV [27] was also measured, but there was no difference in the transport between the control and cadmium group (data not shown).

*Succinate transport.* In another series of experiments the $Na^+$-driven transport system for succinate in the BLM was studied. Figure 10 shows that the succinate uptake by BLMV in the presence of a 100 mM, inwardly-directed $Na^+$ gradient revealed an overshoot phenomenon and that the rate of initial uptake was markedly reduced by cadmium intoxication. The $Na^+$-independent uptake of succinate was not affected by cadmium intoxication. These results indicate that the $Na^+$-dependent transport system for succinate in the renal BLM was also changed in cadmium intoxicated animals.

## Discussion

### *Glucose transport*

Glycosuria is one of the typical renal functional changes in cadmium intoxication [3–5]. Our previous study in rats [28] demonstrated that cadmium intoxication caused glycosuria without changing GFR, indicating that renal tubular reabsorption of filtered glucose was reduced. The site and mechanism responsible for this alteration, however, remained to be determined.

The results of the present study indicate that the $Na^+$-glucose cotransport system in the kidney is impaired by cadmium intoxication. In the renal cortical BBMV prepared from cadmium treated rats, the $Na^+$-dependent glucose transport was markedly attenuated (Fig. 2), whereas the $Na^+$-independent component of glucose transport (Fig. 2) and the permeability of the BBM to $Na^+$ (Fig. 3) did not appear to be significantly altered. Furthermore, glucose transport in the BLMV was little affected (Figs. 6 and 7). It is therefore apparent that the process of glucose transport coupled with $Na^+$ transport in the BBM was specifically altered by cadmium treatment.

Kinetic analysis (Fig. 5) suggested that reduction of $Na^+$-dependent glucose transport in the BBMV was due to alteration in $V_{max}$ and not Km. We therefore presume that the substrate affinity of the carrier was not changed, but the number of effective carrier was decreased by cadmium intoxication. The mechanism with which cadmium altered glucose carrier is not certain. Loss of carrier units within the membrane or changes in lipid composition of the membrane could be responsible. With respect to the latter possibility, it is known that the renal BBM has a high content of sphingomyelin and phosphatidylserine and a high cholesterol-to-phospholipid ratio [29], and alterations in these lipid compositions affect $V_{max}$ of the $Na^+$-dependent glucose transport without changing Km [30], as observed in the present study. Direct analysis of the carrier number, using a specific binder to the $Na^+$-dependent glucose carrier, such as phlorizin [31, 32], and determinations of membrane lipids would resolve this problem.

### *Glutamate transport*

Although many previous studies [3, 4] have shown that amino acid excretion into the urine is increased after cadmium intoxication, the underlying mechanism has not been identified. In the present study we have observed that the $Na^+$-dependent transport of L-glutamate in the renal cortical BBMV was significantly reduced after the animal was exposed to cadmium (Fig. 8). Since, in the kidney, amino acids including L-glutamate are mainly reabsorbed in the proximal tubule via a cotransport system with $Na^+$ [33], the present data indicate that one mechanism of cadmium induced aminoaciduria is a defect in the $Na^+$-amino acid cotransport system in the renal brush border membrane.

### *Tetraethylammonium (TEA) transport*

Transport of organic cation, such as TEA, in the renal BBM is known to be mediated by a $H^+$-organic cation antiport system [27]. The present results (Fig. 9) provide strong evidence that this system is severely impaired by cadmium intoxication. In the presence of an initial outwardly-directed $H^+$ gradient of 1/25 (medium/vesicle), the renal cortical BBMV of normal rats accumulated TEA over three times the equilibrium (60 min) level during the initial 30 second period, confirming the existence of a $H^+$-TEA antiport system. In the vesicle of cadmium treated rats, however, the 30 second uptake of TEA was only slightly (20%) higher than the equilibrium value. It is therefore apparent that the uphill transport of TEA driven by $H^+$ gradient was markedly attenuated by cadmium intoxication. The $H^+$-organic cation antiport system is reported to be responsible for the active secretion of cationic drug in the kidney [34]. Although the TEA uptake in the BBMV, as measured in the

present study, simulates reabsorption rather than secretion because of the vesicle orientation, it has been demonstrated that the organic cation carrier catalizes substrate transport in either direction [34, 35]. Thus, in the light of present results, it is possible that tubular secretion of organic cation is inhibited by cadmium intoxication.

### *Succinate transport*

Krebs cycle intermediates filtered through glomeruli are reabsorbed in the proximal tubules of the kidney [30–39]. Their transport across the BBM of tubular cells occurs by cotransport with $Na^+$. Studies involving the renal membrane vesicles indicated that transport of dicarboxylic acids, such as succinate is driven by $Na^+$ gradient not only in the BBM [40–42] but also in the BLM [43]. In the present study, the succinate uptake by the BLMV revealed $Na^+$-dependent "overshoot" (Fig. 10) in agreement with the above notion. The degree of overshoot, however, was significantly lower in the vesicles of cadmium treated animals than in the normal vesicles. We therefore suspect that the $Na^+$-dicarboxylate cotransport system in the renal tubular BLM was impaired in cadmium treated rats. Effects of cadmium on the cotransport system in the BBM is not known. If the system was also changed by cadmium, as in the $Na^+$-glucose and $Na^+$-amino acid cotransport systems (Figs. 2 and 8), then one would argue that reduction of the $Na^+$-dependent succinate transport in the BLMV of cadmium group was due to a contamination with the BBMV (Table 2). However, the fact that the D-glucose uptake in the BLMV was not stimulated by $Na^+$ (Fig. 6) suggest that the degree of contamination, if anything, was rather small.

In conclusion, the present study shows that cadmium intoxication in rats impairs various $Na^+$-dependent and $H^+$-dependent secondary active transport systems in renal tubular plasma membranes. Unraveling the mechanism by which these changes are induced needs further investigation.

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