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Mechanisms for Cadmium Lumen-to-Cell Transport by the Luminal Membrane of the Rabbit Proximal Tubule

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MECHANISMS FOR CADMIUM LUMEN-TO-CELL TRANSPORT BY THE LUMINAL MEMBRANE OF THE RABBIT PROXIMAL TUBULE

by

YANHUA WANG

Under the Direction of Delon W. Barfuss

ABSTRACT

The lumen-to-cell transport, cellular accumulation, and toxicity of ionic cadmium ($^{109}Cd^{2+}$) and cadmium-cysteine conjugate (Cys-S-^{109}Cd-S-Cys) were studied in isolated perfused S_2 segments of the proximal tubule of the rabbit kidney. All perfusion solutions were HEPES buffered and contained ^{3}H-L-glucose which functioned as a volume and leak marker along with 250 nM FD & C Green dye as a vital dye. When ionic cadmium, 0.73µM Cd^{2+}, or 0.73µM cadmium-cysteine conjugate (Cys-S-^{109}Cd-S-Cys) containing solution was perfused through the lumen of the tubule there was no visual evidence of toxicity such as blebbing of the luminal membrane, cellular vital dye uptake, and cellular swelling. Ionic Cd^{2+} transport was temperature dependent (87% reduction at 22°C and 100% at 11°C) and inhibited by $FeCl_2$ (42% reduction at 10µM) and $ZnCl_2$ (48%

reduction at 20µM), and high Ca^{2+} concentrations (27% reduction at 1.95mM and 69% at 2.6mM). The ionic Cd^{2+} transport was not affected by verapamil and diltiazem. The cadmium conjugate (Cys-S-Cd-S-Cys) transport was also temperature dependent (76% reduction at 22°C and 100% at 11°C) and inhibited by the amino acids L-cystine and L-arginine (55% and 50% respectively), stimulated by L-methionine (56%), but not affected by L-aspartate, L-glutamate and Gly-Sar. 2, 3-Dimercaptopropane-1-Sulfonate (DMPS) co-perfused with Cd^{2+} decreased absorption of 20µM Cd^{2+} (39% reduction at 30 µM and 94.6% reduction at 200 µM), while DMPS added to the bathing solution has no effect on the luminal transport of Cd^{2+}. DMPS co-perfused with 20 µM Cys-S-Cd-S-Cys substantially reduced Cd^{2+} transport (62% reduction at 30 µM). We conclude that cadmium can be transported at the luminal membrane of the S_2 segment of the proximal tubule by multiple mechanisms, depending on the form which it is presented to membrane. Ionic cadmium appears to be transported by iron (DCT1), zinc (ZTL1) transporters and some kind of calcium-selective channel while cadmium conjugate of L-cysteine appears to be transported by L-cystine transporters (system b^{0+}). Dipeptide transporter is not involved in the transport of cadmium. DMPS appears to be a chelator for cadmium.

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TUBULE

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YANHUA WANG

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LIST OF ABBREVIATIONS

ABC	ATP binding cassette
$b^{0,+}$ AT	Sodium-independent neutral and basic amino acid transporter
Cd	Cadmium
CdO	Cadmium oxide
CdS	Cadmium sulfide
Cys	Cysteine
DCT1	Divalent cation transporter 1
DHP	1, 4-dihydropyridine
DMPS	2, 3-dimercaptopropane-1-Sulfonate
DMT1	Divalent metal transporter 1
DTPA	Diethylenetriamine pentaacetic acid
EDTA	Ethylenediaminetetraacetic acid
fmol	Femtamolar
Gly-Sar	Glysylsarcosine
GSH	Glutathione
Hcys	Homocysteine
LLC-PK1	Porcine kidney epithelial cell from proximal tubule
MDCK	Madin-Derby canine kidney
MRP2	Multidrug resistance protein 2
MT	Metallothionein

NAC	N-acetylcysteine
NRAMP2	Natural resistance-associated macrophage protein
OAT1	Organic anion transporter 1
PepT2	Peptide transporter 2
PT	Proximal tubule
ROS	Reactive oxygen species
rBAT	Rat broad-specificity amino acid transporter
rZip10	Rat Zrt/Irt-like protein 10
TETA	Triethylenetetramine
ZnT	Zinc transporter
ZTL1	ZnT like transporter 1

CHAPTER I: INTRODUCTION

Cadmium (Cd) is a highly toxic heavy metal, which is known as one of the commonest industrial and environmental metal poisons. After it is released into the environment, it can be absorbed by plants, seep into groundwater and enter the food chain. Humans are often exposed to cadmium by smoking cigarette, drinking tea or coffee, eating food with a relatively high level of cadmium such as shellfish, organ meat (liver, kidneys, etc), refined grain, cereal products, potatoes, leafy vegetables, and root vegetables and drinking tap water from cadmium-coated water pipes, etc. The kidney is one of the primary sites for cadmium toxicity, which is in line with the fact that the kidney is a main organ that collects, processes, and excretes a number of endogenous and exogenous toxic substances. Therefore, the current study will focus on the transport of cadmium by kidneys.

The kidneys are both absorptive and excretory organs. In addition to absorption of certain valuable endogenous compounds, primarily glucose and amino acids, the kidneys also excrete a number of end products of metabolism that are no longer needed by the body, xenobiotics and exogenous toxic substances. To better understand the function of the kidneys, it is necessary to be familiar with their anatomy. If a kidney were dissected coronally, two regions would be evident: an outer region called the cortex and an inner

region called the medulla (Figure 1). The cortex and medulla are composed of nephrons (Figure 2). Nephron is the functional unit of the kidney and all absorption and excretion occur in nephron. Each human kidney contains approximately 0.4×10^6 to 1.2×10^6 nephrons. The nephron consists of a renal corpuscle and tubules. The renal corpuscle consists of glomerular capillaries and Bowman's capsule. The tubules are composed of:
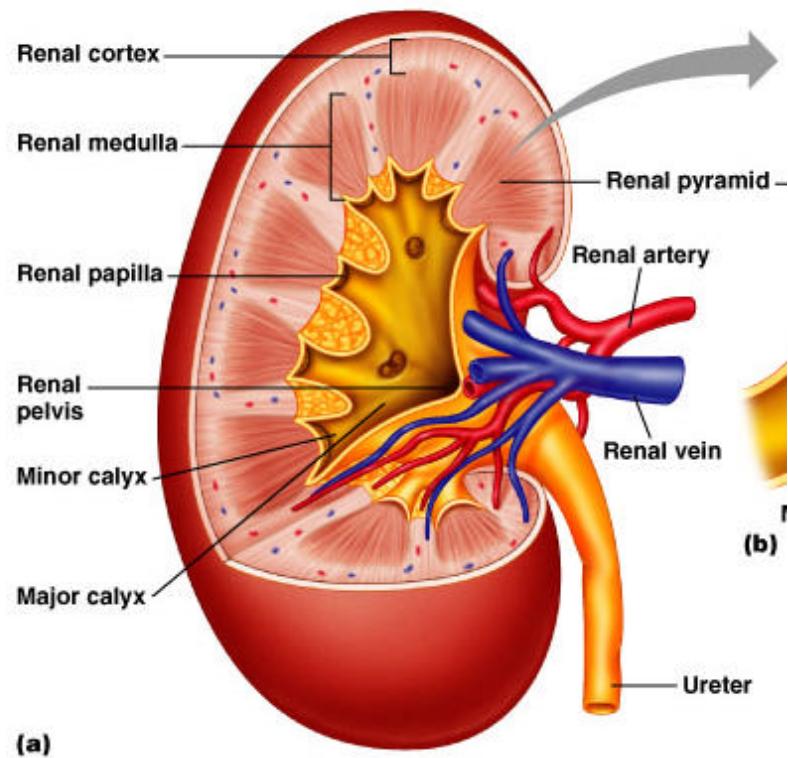
Figure 1: Macroscopic anatomy of the kidney (Principles of Human Physiology, Second Edition, Figure 19.2a, The Urinary System: Renal Function @2006 Pearson Education, Inc, publishing at Benjamin Cumming Co.)

(1) a proximal tubule, (2) a loop of Henle including a descending thin limb, an ascending thin limb, and a thick ascending limb, (3) a distal convoluted tubule, and (4) a collecting duct system (Figure 2). When blood passes through the glomerular capillaries, an

essentially protein-free fluid is filtered into Bowman's space. Then the fluid passes through the tubules, and finally formed into urine.

Based on the location of renal corpuscle, nephron is divided into two types: superficial and juxtamedullary. The renal corpuscle of each superficial nephron is located in the outer region of the cortex. Compared to the juxtamedullary nephron, its proximal straight tubule is long, its loop of Henle is short, and its efferent arteriole branches into peritubular capillaries that surround the nephron segments of its own and adjacent nephrons. The renal corpuscle of each juxtamedullary nephron is located in the region of
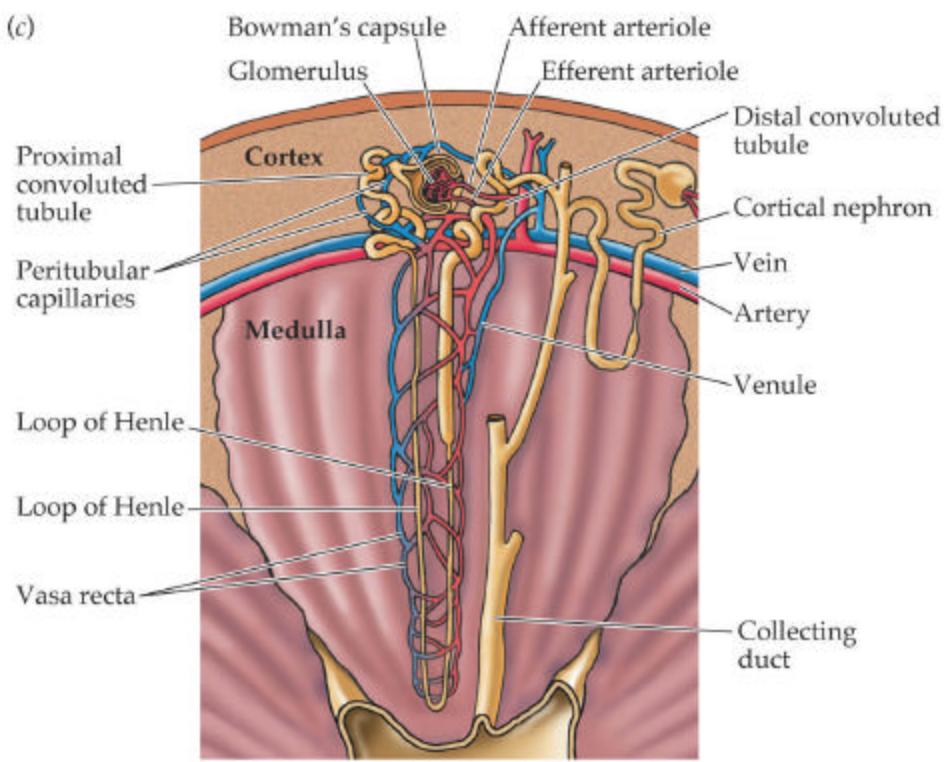
Figure 2: Microscopic anatomy of nephron (Life: The Science of Biology, Seventh Edition, Figure 51.9, The Human Excretory System (Part 2) @ 2004 Sinauer Associates, Inc. and W. H. Freeman & Co.)

the cortex next to the medulla; its loop of Henle is long and extends deeper into the medulla. Its efferent arteriole forms not only a network of peritubular capillaries, but also a series of vascular loops called the vasa recta (Figure 2). Among the total amount of nephrons, superficial nephrons account for 80-85%, and juxtamedullary nephrons account for 15-20%.

The proximal tubule is a direct continuation of the parietal epithelial cells of Bowman's capsule. The length of the proximal tubule is about 10 mm in the rabbit, 8 mm in the rat and 14 mm in the human. The outside diameter of the proximal tubule is approximately 37.5-50 µm while the inside diameter of the proximal tubule is approximately 20-25 µm for the rabbit. The proximal tubule is composed of proximal convoluted tubule (pars convoluta) and proximal straight tubule (pars recta). In humans and several animals, including the rat, the rabbit, and the mouse, three morphologically distinct segments, S_1, S_2, and S_3, can be histologically distinguished in the proximal tubule. The S_1 segment includes the initial portion of the proximal tubule; it begins at the glomerulus and constituents approximately two thirds of pars convoluta. The S_2 segment comprises the remainder of the pars convoluta and most of the pars recta to the cortical-medullary junction. The transition from S_1 to S_2 is gradual. The S_3 segment represents the remaining portion of the pars recta; it is located from the cortical-medullary junction to the beginning of thin descending limb. The transition from S_2 to S_3 is either abrupt as found in rat or gradual as seen in rabbit.

In keeping with its role in transport of many molecules, the morphology of the epithelial cells of the proximal tubule exhibits polarity of structure and function. In the

epithelial cells, as shown in figure 3, the basolateral (the blood side of the cell) and luminal (apical) (the urine side of the cell) domains of the plasma membrane begin and end at the tight junctions, structures that segregate the transport proteins of the basolateral and luminal membranes and restrict paracelluar transport movement. But in contrast to the tight junctions in distal tubules and collecting ducts, the tight junctions in proximal tubules are relatively leaky. For example, they are permeable to water and most ions. The basolateral membrane forms many deep invaginations that both increase surface area and place sites of active transport in close proximity to mitochondria. The luminal membrane of proximal tubule epithelial cells is greatly amplified as a brush border containing a thick carpet of microvilli, which is unique to the proximal tubule of the nephron and also increases surface area. The brush border and the basolateral invaginations equally increase the surface area of the luminal and basolateral membranes by forty fold. Functional polarity of the proximal tubular cells is also evidenced by the asymmetric distribution of enzymes, receptors and membrane-bound transporters between the luminal membrane and basolateral membrane such as the exclusive expression of system b^{0+} in the luminal membrane and the exclusive expression of Na^+-K^+-ATPase in the basolateral membrane of the proximal tubule.
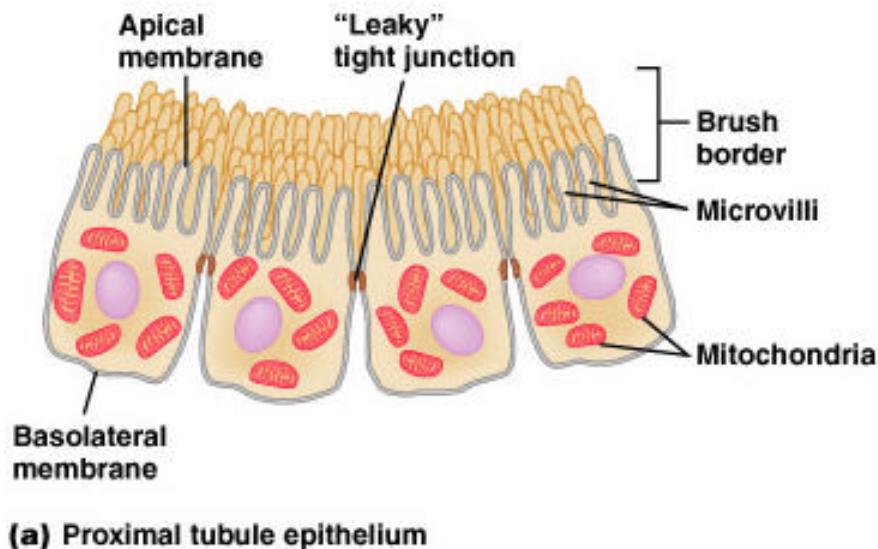
Figure 3: Proximal tubule epithelium (Principles of Human Physiology, Second Edition, Figure 19.17a, The Urinary System: Renal Function @2006 Pearson Education, Inc, publishing at Benjamin Cumming Co.)

The three segments of the proximal tubule have differences in morphology based on cellular histology. The S_1 segment has a tall brush border, a well-developed vacuolar-lysosomal system, and extensive invaginations of the basolateral plasma membrane. Compared to the S_1 segment, the S_2 segment has a shorter brush border, less prominent basolateral invaginations, fewer and smaller mitochondria. The epithelium of the S_3 segment is simpler than that of the S_1 and S_2 segments. Basolateral invaginations of the plasma membrane are essentially absent, mitochondria are small and randomly distributed throughout the cytoplasm, and the intracellular spaces are smaller and less complex. These morphological characteristics suggested that the S_3 segment may be less involved in the absorption of solute and water, which is evidenced by the findings that fluid absorption in the S_3 segment is significantly less than in the S_1 and S_2 segments

(Clapp, Park et al. 1988), and Na^+-K^+-ATPase activity in the S_3 segment is significantly lower than in the S_1 and S_2 segments (Katz, Doucet et al. 1979). The length of the brush border in the S_3 segment varies among species. It is tall in the rat, fairly short in the rabbit, and intermediate in length in the human kidney.

In the kidneys, the proximal tubule (PT) is a portion of the nephron that is often damaged by nephrotoxic compounds, including various drugs and heavy metals. Previous research experiments have shown that the proximal tubule is the major site where cadmium is absorbed and expresses its toxic effect (Felley-Bosco and Diezi 1987; Robinson, Barfuss et al. 1993; Brzoska, Kaminski et al. 2003; Barbier, Jacquillet et al. 2004). However, very little data have been obtained concerning the transport systems involved in the uptake of Cd^{2+} across the basolateral membrane of the proximal tubule. Although a considerable amount of information is known about uptake of Cd^{2+} across the luminal membrane of the proximal tubule, most of these findings were obtained from cultured epithelial cells and other *in vitro* techniques, in which bipolarity of epithelial cells is lost and dedifferentiation occurs. Thus, those techniques are not able to provide the conditions in which cadmium is presented to the target epithelial cells *in vivo*.

In the current research project, using the isolated perfused tubule technique (Figure 4), the mechanisms for transport of cadmium across the apical membrane of isolated perfused segments of the proximal tubule of rabbit were investigated. In this technique, a proximal tubule segment is manually dissected and transferred into a special temperature-controlled bathing chamber. Both ends of the tubule were held in glass tube, and the tubule is perfused through a micropipette with its tip centered in the tubule lumen

while being viewed through a microscope. This technique provided a dynamic *in vitro* system in which intact segments of the nephron were perfused through the lumen under conditions similar to those found *in vivo*. In contrast to some other *in vitro* renal systems (e.g. cell culture), in which bipolar orientation is lost and some dedifferentiation of the tubular epithelial cells occurs, the *in vivo* bipolarity of the epithelial cells and the electrochemical gradient across the apical membrane and basolateral membrane are maintained. This technique allows us to measure the rate at which a given solute is transported across the proximal tubule into the bathing solution from the luminal fluid (urine) (absorption) or transported across the proximal tubule into the luminal fluid from the bathing solution (secretion). This technique makes it possible to manipulate and control both the luminal environment (luminal fluid) and the basolateral environment (bathing solution) surrounding the perfused tubular segment. For example, ionic and osmotic composition, presence or absence of toxicants, presence or absence of amino acids, proteins, hormones and/or other plasma solutes, temperature, transepithelial hydrostatic pressure, and other factors can all be controlled. In addition, using this technique, the transport in all three segments of the proximal tubule (S_1, S_2, and S_3 segments) can be studied, while S_2 and S_3 segments are not accessible to study by other techniques. The current experiments were done primarily using isolated S_2 segments of rabbit renal proximal tubules to explore the mechanisms for cadmium lumen-to-cell transport and accumulation. The S_2 segment was mostly used for the study because the epithelial cells in the S_2 segment avidly absorb Cd^{2+} (Robinson, Barfuss et al. 1993), and

the S_2 segment is straight, easier to dissect and perfuse than the S_1 and S_3 segments. The following hypotheses were developed for the study of luminal transport of cadmium.

First Hypothesis: luminal transport of ionic cadmium (Cd^{2+}) is mediated by a Fe^{2+} transporter (DCT1), a Zn^{2+} transporter (ZTL1), and a calcium channel. The rationale for testing this hypothesis is that Cd^{2+}, being a divalent cation, could possibly share specific transport mechanism with Fe^{2+}, Zn^{2+}, and Ca^{2+}. Thus, these latter three cations are expected to inhibit luminal Cd^{2+} transport when co-perfused.

Second Hypothesis: cadmium conjugated to sulfhydryl-containing amino acids is transported across the luminal membrane into the epithelial cells via an amino acid transporter (system b^{0+}) and a dipeptide transporter (PepT2). The rationale for this hypothesis is that the molecular structure of the cadmium-sulfhydryl conjugate of L-cysteine (Cys-S-Cd-S-Cys) is very similar to that of the amino acid L-cystine (Cys-S-S-Cys) and the dipeptide Gly-Sar, thus being homologues in structure. Therefore, it would be expected L-cystine and Gly-Sar will inhibit luminal transport of Cys-S-Cd-S-Cys when co-perfused through the renal tubule.

Third Hypothesis: DMPS is an effective chelator for cadmium as an ion (Cd^{2+}) and as an organic form (Cys-S-Cd-S-Cys). The rationale for this hypothesis is that DMPS has been reported to be a heavy metal chelator, but its efficacy for chelating cadmium in the kidney has not been determined. Therefore, DMPS is expected to inhibit the luminal transport of Cd^{2+} and Cys-S-Cd-S-Cys when they are co-perfused with DMPS.
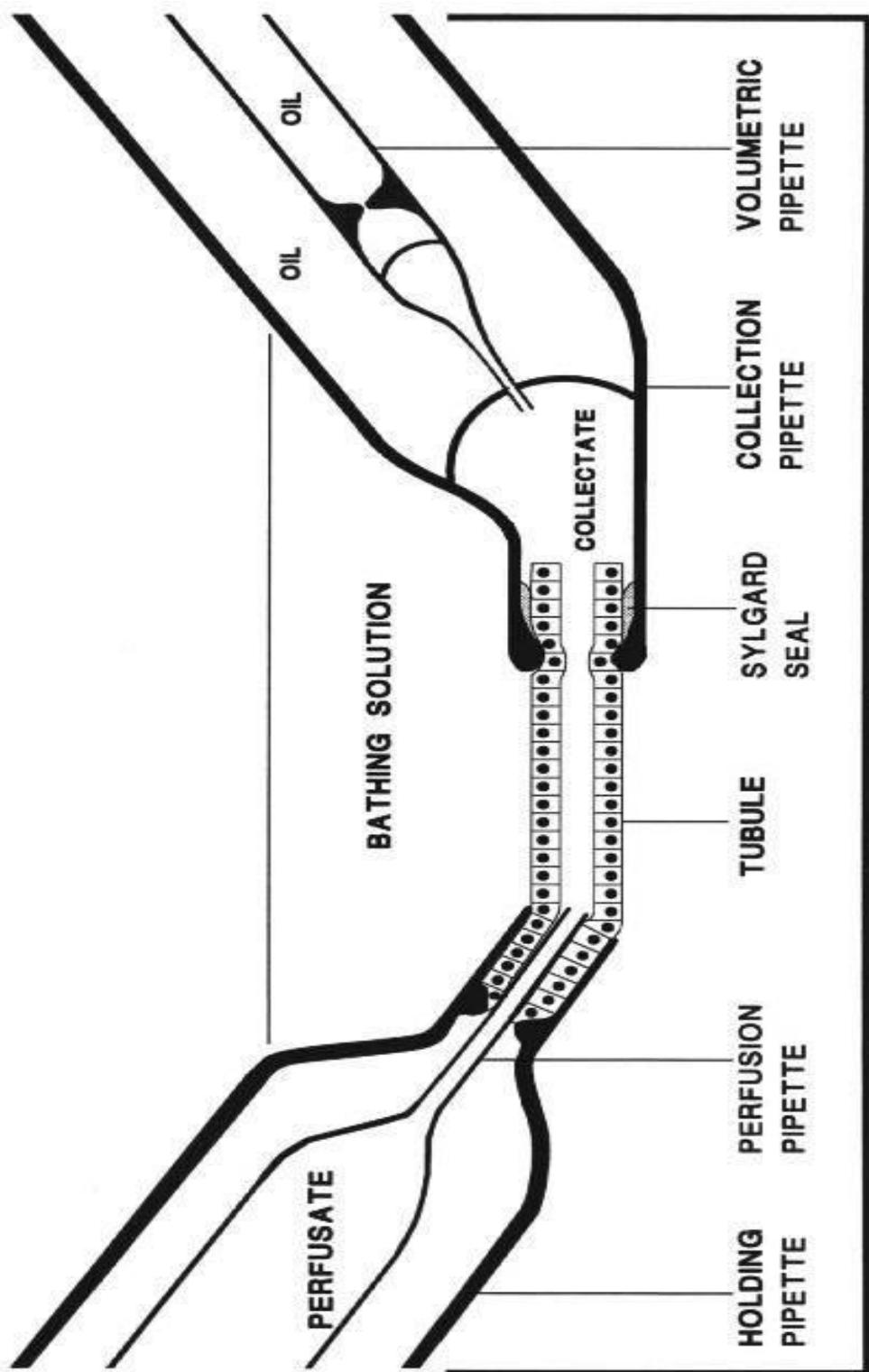
Figure 4: Isolated perfused tubule

CHAPTER II: REVIEW OF THE LITERATURE

Cadmium's history and toxicity

Cadmium (Cd) is a nonessential, soft, silver-white heavy metal, which was discovered in 1817 by the German chemist Friedrich Stromeyer (1776-1835). Cadmia is the ancient name for zinc oxide. This name reflects a close connection between cadmium and zinc. At that time, Stromeyer was studying a pharmaceutical project involving zinc carbonate ($ZnCO_3$). But, the zinc carbonate supplied by manufacturers turned yellow under some circumstances. The pharmacists refused to accept the shipment of this product because they thought it was not pure. Finally, Stromeyer discovered that this impurity was an as-yet-undentified element, cadmium. Actually, cadmium commonly occurs in zinc ores and is produced commercially as a by-product during the production of zinc from its ores. Cadmium is relatively rare in the earth's crust with an estimated abundance of about 0.1-0.2 parts per million. The most sought-after ore of cadmium is greenockite [cadmium sulfide (CdS)].

The close relationship between cadmium and zinc is also reflected by the locations of cadmium and zinc in the periodic chart of elements. In the periodic chart of elements, cadmium is found in Group IIB and located in the area of transition metals. Both cadmium and zinc belong to Group IIB. Cadmium has an atomic number of 48 and an atomic mass of 112.41. Its melting point is 610^0 F (321^0 C) and its boiling point is 1410^0 F (765^0 C). When cadmium is used in alloys, it tends to lower the melting point of the alloy. Cadmium reacts slowly with oxygen in moist air at room temperature and

forms cadmium oxide (CdO). It does not react with water, but it does react with most acids.

At one time, cadmium was widely used for the electroplating of steel. After it was found that cadmium is very toxic to humans and other animals, the use of cadmium for this purpose was reduced significantly. Today, the vast majority of cadmium (75% in the United States) is used in the production of nickel-cadmium batteries that can be recharged and reused many times. Such batteries are used in a large variety of appliances, including compact disc players, cellular telephones, pocket recorders, handheld power tools, cordless telephones, laptop computers, camcorders, and scanner radios. It is also a component of certain specialty alloys used in semiconductors (such as cadmium selenide and telluride), in dyes and pigments, as a stabilizer in plastics such as polyvinyl chloride, and as a neutron absorber in nuclear reactor control rods and shields. The United States is among the top ten largest producers including Canada, Japan, Belgium, China, Kazakhstan, and Germany. Every year, over 1000 metric tons of cadmium is refined in the United States.

Currently, there is widespread environmental contamination with cadmium. The major air pollution of cadmium comes from zinc mining and smelting, oil and coal combustion, household waste incineration and the use of high phosphate and sewage sludge fertilizers. Approximately, 4,000 to 13,000 tons of cadmium is released into the environment from human activities annually. Cadmium particles in air can travel a long distance before falling to the ground or water. After cadmium is released into the environment, cadmium may contaminate water and enter the food chain through plants

that readily absorb cadmium. When people eat food and drink water contaminated by cadmium, the ingested cadmium passes through the gastrointestinal tract into the systemic circulation. In the US, the average person consumes approximately 30µg of cadmium per day in food and absorbs 1-3µg from the gastrointestinal tract. In addition, because tobacco plants concentrate cadmium from soil, cigarette smoking is the largest source of cadmium exposure for human beings. Each cigarette may contain from 1 to 2 µg of cadmium. Consequently, smokers take in an additional 1-3µg per day from the respiratory tract because 40-60% of the inhaled cadmium enters the systemic circulation. The remaining cadmium in the cigarette smoke enters the atmosphere to be inhaled by others or to contaminate the environment.

When cadmium is deposited in the various target organs via the systemic circulation, unlike organic toxicants, which can be degraded metabolically to less toxic derivatives in these organs, cadmium remains stored intact. Cadmium is stored in the body for a very long time (10-30 years) (Jarup, Berglund et al. 1998), and exerts an adverse effect on a number of organs and tissues such as the kidneys, liver, lung, pancreas, testis, placenta, and bone (Kamiyama, Miyakawa et al. 1995; Diamond and Zalups 1998; Habeebu, Liu et al. 1998; Jarup, Berglund et al. 1998; Sarkar, Yadav et al. 1998; Oteiza, Adonaylo et al. 1999; Liu, Liu et al. 2000; Liu, Umino et al. 2000; Kanter, Yoruk et al. 2003). Excretion of cadmium into urine or feces is extremely limited. Therefore, cadmium builds up over time and causes toxicity in the target organs.

Acute exposure to cadmium produces hepatic, pulmonary, stomach, and testicular injury, whereas chronic exposure results in renal and bone injury and cancer, as well as

toxicity to other organs such as cardiovascular system (Tzotzes, Tzilalis et al. 2007). Among the target organs of cadmium, the major portion of the body burden of cadmium is located in the kidneys and liver. Liver and kidney cadmium concentrations are comparable after short-term exposure, but the kidney concentration exceeds the liver concentration following chronic exposure (Vogiatzis and Loumbourdis 1998). The symptoms for acute cadmium toxicity have been reported. For instance, in low levels, eating food or drinking water with high levels of cadmium can severely irritate the stomach and cause vomiting, diarrhea and abdominal cramps; breathing high doses of cadmium can damage the lungs and cause accumulation of fluid in the lungs, dryness of the throat, coughing, and shortness of breath, headache, and pneumonia-like symptoms; The long-term effects of cadmium are not fully understood, but are thought to include heart and kidney problems, high blood pressure, calcium loss in bones and cancer. One of the most famous environmental diseases, itai-itai (Japanese for "ouch-ouch") resulted from cadmium poisoning. The syndromes for this disease were bone and joint aches and pains, deformities of the spine, and more easily broken bones. Interestingly, cadmium does not cross the placenta-fetal barrier nor the blood-brain barrier as lead and mercury do, so it is not toxic to fetuses, nor does it cause the mental symptoms similar to lead and mercury. Also, cadmium does not go to the breast milk with lactation. Therefore, cadmium is not transmitted to newborns. Cadmium levels in humans tend to increase with age due to chronic subtle exposure, usually peaking at around age 50 and then leveling off. However, what level of cadmium causes toxicity is not clear. Currently, below 2ppm in hair and 0.015ppm in whole blood are considered acceptable ranges for

body cadmium levels. Now, cadmium has been ranked as high as seventh on the Top *20 Hazardous Substances Priority List* by the Agency for Toxic Substances and Disease Registry and the US Environmental Protection Agency. Taken together, due to the wide distribution and high toxicity of cadmium, it is critical to elucidate the mechanisms by which cadmium is transported *in vivo.*

Cadmium-induced nephrotoxicity

Two forms have been suggested for *in vivo* cadmium: ionic cadmium (Cd^{2+}) and cadmium-sulfhydryl conjugates. It is believed that cadmium has a propensity of bonding with sulfhydryl groups. Therefore, it is likely that the majority of cadmium in plasma is in amino acid and/or protein-bound forms because the plasma is rich in sulfhydryl-containing amino acids such as L-cysteine (L-Cys), L- homocysteine (L-Hcys), and N-acetyl-cysteine (NAC), and low-molecular weight peptides and proteins such as glutathione (GSH), albumin and metallothionein (MT). But since there is a small fraction of cadmium that will escape from bonding with amino acids and/or proteins, free ionic form of cadmium (Cd^{2+}) is considered as possible *in vivo* form of cadmium. Thus, luminal fluid of the proximal tubule could contain both the ionic form and protein-bound complex of cadmium.

The toxic form of cadmium is ionic cadmium (Cd^{2+}) (Erfurt, Roussa et al. 2003). The direct cytoplasmic effect of cadmium is to cause formation of reactive oxygen species (ROS) indirectly. ROS include oxygen ions, free radicals and peroxide, which are generally very small molecules. ROS are highly reactive because they have unpaired valence shell electron. ROS can damage cell membranes and break DNA by causing

oxidative stress, and finally lead to apoptosis (programmed cell death) (Erfurt, Roussa et al. 2003; Pulido and Parrish 2003). When free Cd^{2+} enters the cytosol of renal proximal tubular cells, ROS are generated (Thevenod and Friedmann 1999). Cd^{2+} has been shown to produce hydroxyl radicals in the presence of metallothioneins (MTs) containing Fenton metals, such as Fe^{2+} and Cu^{2+}. This suggests that Cd^{2+}-mediated production of ROS is a consequence of Cd^{2+}-induced displacement of endogenous redox active metals (Fe^{2+}, Cu^{2+}, Zn^{2+}). The released endogenous redox active metals from metallothioneins produced ROS directly. For example, cadmium replaces Fe^{2+} from metallothioneins. The released Fe^{2+} produced ROS in the Fenton reaction:

$$H_2O_2 + Fe^{2+} \text{ ----- } HO^{\cdot} + OH^- + Fe^{3+}$$

The $HO^{\cdot}$ is able to oxidize all organic materials at close to diffusion-controlled rates. Cadmium may also compete to bind with GSH and protein sulfhydryls, endogenous intracellular radical scavengers, and deplete these scavengers, resulting in the formation of ROS. ROS causes DNA strand breaks, lipid and protein peroxidation (The oxidative process whereby free radicals "steal" electrons from the lipids or proteins in cell membranes, resulting in cell damage and increased production of free radicals.). This oxidative stress caused by cadmium-induced ROS formation can induce structural changes or misfolding of cellular proteins including vital membrane transporters, such as the Na^+/K^+-ATPase or Na^+-dependent nutrient transporters, which is subsequently degraded by both the ubiquitin-proteasome complex and endo-lysosomal proteases (Thevenod and Friedmann 1999). The cadmium-induced ROS formation also can cause

an outer membrane rupture and an uncoupling of mitochondrial respiration, which inhibits electron transfer and oxidative phosphorylation, resulting in the release of numerous death signals like ROS and cytochrome *c*. If the repair processes do not balance the ROS-mediated stress events, the affected cells will undergo cell death via apoptosis or necrosis. In addition to the direct cytotoxic effect, cadmium may also activate proto-oncogens in target cells. Acute administration of the free ionic form of cadmium will lead to acute toxicity. In the rat, an acute perfusion of Cd^{2+} caused hypercalciuria, hyperphosphaturia and hypokaliuria without modification of glomerular filtration rate.

Protein-bound conjugates of cadmium are not toxic by themselves but the ionic form released from the complexes is responsible for the cellular toxicity, which results in chronic toxicity. The proximal tubules will undergo necrosis and the kidneys will express major features of Fanconi syndrome, which are proteinuria, aminoaciduria, glucosuria, phosphaturia and reduction in glomerular filtration rate. Repeated low exposures may also cause permanent kidney disease, leading to kidney stones and other health problems. Renal dysfunction will occur if the kidney Cd^{2+} concentration reaches about 200µg/g wet weight for long term exposure**.**

Another toxic effect of cadmium is renal cancer. In rodents, cadmium causes cancer in the kidney and other organs (Waalkes, Anver et al. 1999; Waalkes 2003). The cellular and molecular mechanisms implicated in cadmium carcinogenicity include activation of proto-oncogenes, inactivation of tumor suppressor genes, disruption of cell adhesion, and inhibition of DNA repair (Waalkes 2003; Waisberg, Joseph et al. 2003).

Cadmium adversely affects all four major pathways of DNA repair: (i) base-excision, (ii) nucleotide-excision, (iii) double strand break, and (iv) mismatch repair (Hartwig and Schwerdtle 2002; Waisberg, Joseph et al. 2003). Several investigators have demonstrated that cadmium induced expression of pro-oncogenes in renal cancer cell lines: c-*fos,* c-*Jun*, c-*myc*, and egr-1 in LLC-PK1 proximal tubular cells (Matsuoka and Call 1995), c-*fos,* c-*myc*, and c-*Jun* in rat kidney cell NRK 49F (Tang and Enger 1993), and c-*fos* in renal mesangial cells (Wang and Templeton 1998). In epithelial cell culture, cadmium disrupts cell junctions (Pearson and Prozialeck 2001) and may promote metastasis by disrupting cell-cell adhesion (Waisberg, Joseph et al. 2003).

Transport of cadmium across the apical membrane in the proximal tubule

In the kidneys, the proximal tubule is known as the major site of cadmium-induced toxicity. Using the isolated perfused tubule technique, Robinson, et al have indicated that all three segments (S_1, S_2 and S_3 segments) of the rabbit proximal tubule adsorb Cd^{2+} avidly when cadmium ions were perfused through the lumen in the form of $CdCl_2$. S_1 segments of the proximal tubule accumulated cadmium more rapidly and developed a more severe form of tubular injury (at concentrations greater than 500 µM) than the other two segments. Transport data could be collected in S_2 and S_3 segments, but not in S_1 segments due to the severe injury in S_1 segments. Their findings also showed that only about 10% of the cadmium taken up from the lumen was transported across the basolateral membrane into the bathing solution, indicating that 90% of the absorbed cadmium was retained in the proximal tubular epithelial cells. In transport experiments,

increasing the concentration of cadmium in the lumen caused an increase in the leak of the volume marker from the lumen into the bath. This is consistent with the finding that cadmium damaged tight junction by altering the localization of cadherin and catenin in the proximal tubule epithelium (Prozialeck, Lamar et al. 2003). Using free-flow micropuncture technique, Felley-Bosco E, et al studied the effect of Cd^{2+} on the superficial nephrons of Munich-Whistar type rats infused acutely with Cd acetate or Cd-DTPA. They found that most of Cd^{2+} ultrafiltered during Cd^{2+} administration is taken up by the convoluted part of the proximal tubule. Malgorzata M. et al also contribute to the finding that the proximal tubule is the major site for cadmium toxicity. They intoxicated the rats with cadmium administered in drinking water. The degree of kidney damage was evaluated biochemically and histopathologically. Their results turned out to further support the finding that the main absorptive parts are proximal convoluted tubules and straight tubules. These studies provide some potential insights into the proximal tubular transport and handling of cadmium ions.

Due to the deficiency in understanding of the transport of cadmium by target epithelial cells, several putative mechanisms for cadmium transport in the proximal tubule have been proposed. For the transport of Cd^{2+} at the luminal membrane of the epithelial cells of the renal proximal tubule, it is proposed that Cd^{2+} may interact with and compete for binding sites on membrane proteins involved in the transport of essential elements such as Ca^{2+}, Fe^{2+} and Zn^{2+}. For the transport of cadmium-sulfhydryl conjugates, cadmium-sulfhydryl conjugates may serve as molecular homologues of some amino acids, dipeptides, or other important homeostatic molecules, and compete for the transporters

that are involved in the uptake of these useful molecules. A third hypothesis was also suggested for the transport of cadmium. Endocytosis of proteins containing cadmium may be another mechanism by which cadmium gains entry into the epithelial cells. For example, cadmium ions may bind to MT, albumin or other proteins, and the conjugates are taken up by receptor-mediated endocytosis.

Since Cd^{2+} has a smaller ionic radius than Ca^{2+}, and Cd^{2+} has been shown to be an open channel blocker for L-type Ca^{2+} channels in excitable cells, Cd^{2+} can be thought of as a functional homologue of Ca^{2+} at certain types of Ca channels. Experimental findings indicated that Cd^{2+} may cross the hepatocyte membranes through L-type Ca^{2+} channels (Blazka and Shaikh 1991). In a pituitary cell line, one route of cadmium uptake in these cells is via voltage-gated dihydropyridine-sensitive calcium channels (Hinkle, Kinsella et al. 1987). Receptor-activated calcium channels may also allow Cd^{2+} to enter the cells, as suggested by the observation that Cd^{2+} inhibited Ca^{2+} inflow through the receptor-activated Ca^{2+} inflow system (Hughes and Barritt 1989).

However, many of these findings were obtained from culture cells bathed in protein-free physiological salt solutions, which does not represents the conditions in which cadmium is presented to the target epithelial cells *in vivo*. A significant contribution to the accumulation of Cd^{2+} in the renal proximal tubular epithelial cells by Ca^{2+} channels is also doubted because the voltage-gated Ca^{2+} channel blocker verapamil lacks any effect on accumulation of Cd^{2+} in cultured proximal tubular cells (Templeton 1990). But this finding still leaves the possibility that Cd^{2+} may be transported by non-voltage gated Ca^{2+} channel because the types of Ca^{2+} channels are variable in the kidneys.

The channels mediating Ca^{2+} entry across the plasma membrane of mesangial cells have been classified into three types: voltage-gated Ca^{2+} channels, receptor-mediated Ca^{2+} channels, and store-operated Ca^{2+} channels. In the distal tubules and collecting ducts of nephrons, the type of the channel mediating Ca^{2+} entry is a non-selective cation channel, TRPV5. In addition, the identity of calcium transport proteins in the renal proximal tubule remains unknown. Thus, the role of calcium channel in the uptake of Cd^{2+} at the apical and basolateral membranes of proximal tubule cells is a distinct possibility.

The divalent cation transporter (DCT1; also known as DMT1 or NRAMP2) has recently emerged as a potential candidate for Cd^{2+} transport in the renal tubules. DCT1 is a proton-coupled metal-ion transporter that is localized along several regions of the nephron, including the proximal tubule. This transporter is a 561-amino-acid protein comprising 12 putative transmembrane domains with intracellular NH_3 and COOH termini. DCT1 has a much higher expression level in proximal intestine than in the kidney, and more so in kidney than in the brain and other organs (Gunshin, Mackenzie et al. 1997). Its has an unusually broad substrate range which includes Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} as determined in *Xenopus laevis* oocytes designed to express this carrier protein (Gunshin, Mackenzie et al. 1997; Okubo, Yamada et al. 2003). Using specific affinity-purified anti-DCT1 polyclonal antibodies, Ferguson et al. discovered that DCT1 is located in the apical membrane of principle and intercalated cells of the collecting ducts, the thick ascending limbs of the loop of Henle, and the distal convoluted tubules (Ferguson, Wareing et al. 2001), suggesting that DCT1 mediates absorption of divalent metal ions in the distal nephron. The direct evidence for DCT1-

mediated Cd^{2+} uptake by Madin-Derby canine kidney (MDCK) cells, which are derived from the distal nephron, has been provided (Olivi, Sisk et al. 2001). However, the cellular localization of DCT1 in the proximal tubule is somewhat controversial. DCT1 has been reported to be localized in the cytosol of the proximal tubular cells (Ferguson, Wareing et al. 2001; Abouhamed, Gburek et al. 2006). By contrast, using the same specific affinity-purified anti-DCT1 polyclonal antibodies, Francois et al. found that DCT1 is expressed in the cortex and not in the medulla, and is present at the apical membrane of epithelial cells of the proximal tubule (Canonne-Hergaux and Gros 2002). In addition, most transport data for DCT1 have been obtained from *Xenopus* oocytes. Transport mediated by DCT1 is pH-dependent and optimal at pH 5.5 to 6.0 in *Xenopus* oocytes (Gunshin, Mackenzie et al. 1997), which is more acidic than is usually found in the luminal environment of the proximal tubule. Therefore, the disparity between the findings and the lack of *in vivo* transport data suggest the role of DCT1 in Cd^{2+} transport remains to be determined.

Cadmium and zinc are found together in natural deposits and both belong to the group IIB in the periodic chart, consequently they share some physico-chemical properties like charge and ionic radii. Therefore, they may behave antagonistically in biological system. Much of cadmium carcinogenicity may be due to its ability to substitute for zinc in biological reactions. Zinc is an essential metal required for the synthesis of DNA, RNA, and protein as well as for enzymatic activity of Zn-containing enzymes (Cousins, Blanchard et al. 2003). But cadmium is not required by any known biological reactions. A protective effect of Zn^{2+} on rat hepatocytes and porcine kidney LLC-PK(1) cells has been reported against the toxicity due to Cd^{2+} (Goering and

Klaassen 1984; Ishido, Suzuki et al. 1999; Jacquillet, Barbier et al. 2006). Using the microperfusion and cell culture technique, O. Barbier et al. found that injection of Zn^{2+} into the kidney increased Cd^{2+} recovery in urine (Barbier, Jacquillet et al. 2004), and Cd^{2+} uptake and Zn^{2+} uptake are competitively inhibited reciprocally (Barbier, Dauby et al. 2005). In animal models, zinc has been shown to reduce the carcinogenic effect of cadmium (Waalkes 2003). All of these findings support the antagonistic relationship between cadmium and zinc. Recently, a zinc-regulated human zinc transporter, hZTL1 (ZnT like transporter 1), has been identified in the kidney, brain, duodenum, jejunum, ileum, colon, mammary, spleen and liver of mice. The kidney has been shown to express hZTL1 mRNA at relatively high levels, and the liver expresses a relatively low level. hZTL1 is located in the apical membrane of enterocytes and the levels of hZTL1 expression were increased in response to increased zinc availability from a rich diet supply (Cragg, Christie et al. 2002). Although the direct evidence for the expression of ZTL1 in the luminal membrane of renal proximal tubular cells is lacking, it remains possible that ZTL1 may be involved in the transport of Zn^{2+} and Cd^{2+} in the proximal tubule. Another zinc transporter, rZip10, has been purified from rat renal brush border membrane (luminal membrane) of the proximal tubule (Kumar and Prasad 1999). Functional characterization of rZip10 was carried out by reconstituting it into proteoliposomes. The findings indicated that the purified 40-kDa rZip10 is involved in the uptake of Zn^{2+} into proteoliposomes, and this uptake was competitively inhibited by Cd^{2+} (Kumar and Prasad 2000), suggesting its role in Zn^{2+} and Cd^{2+} influx across the renal apical membrane of the proximal tubule.

Although the precise chemical forms of cadmium that are filtered at the glomerulus are not known currently, it is highly unlikely that cadmium is filtered and delivered to the luminal compartment of proximal tubules mainly in its free ionic form, Cd^{2+}. Due to the high affinity of Cd^{2+} for sulfhydryl groups, the major form of cadmium *in* vivo may be cadmium conjugates with sulfhydryl-containing amino acids, peptides or proteins such as L-cysteine, L-homocysteine, N-acetylcysteine, glutathione, albumin and metallothionein in plasma. These cadmium-sulfhydryl conjugates are hypothesized to serve as molecular homologues of some amino acids or peptides for amino acid or peptide transporters. The cadmium conjugates of L-cysteine (Cys-S-Cd-S-Cys) and L-homocysteine (Hcy-S-Cd-S-Hcy) have been hypothesized to serve as molecular homologues of L-cystine (Cys-S-S-Cys) and/or L-homocystine (Hcy-S-S-Hcy) and are transported by L-cystine and/or L-homocystine transport mechanisms (Zalups 2000). Some specific amino acid transporters have been reported to be the primary mechanisms for transporting mercuric conjugates of L-cysteine (i.e., Cys-S-Hg-S-Cys) across the luminal plasma membrane into proximal tubular epithelial cells (Cannon, Barfuss et al. 2000; Cannon, Zalups et al. 2001; Bridges, Bauch et al. 2004). Since cadmium and mercury are both group IIB metals, it is possible that sulfhydryl conjugates of Cd^{2+} may utilize one or more of the same mechanisms involved in the proximal tubular uptake for transport of mercury-conjugates. The cystine transporters are highly suspected in playing an important role in the luminal uptake of cysteinyl mercury or cadmium complex.

One heteromeric amino acid transporter, System b^{0+}, is newly discovered in the apical membrane of the proximal tubule and has a high affinity for L-cystine and L-

dibasic amino acids(Pfeiffer, Loffing et al. 1999; Fernandez, Carrascal et al. 2002). Thus, it is likely to be involved in the transport of cysteinyl mercury or cadmium complexes. System b^{0+} is composed of a heavy chain, rBAT, and a light chain, b^{0+}AT. The heavy chain is a type II membrane N-glycoprotein with a single transmembrane domain, and an intracellular NH_2 terminus, and an extracellular COOH terminus. The light chain is an unglycosylated and highly hydrophobic membrane protein bearing 12 putative transmembrane domains. Both the NH_2 and COOH terminals of the light chain are located inside the cell. The light chain and the heavy chain are linked by a disulfide bridge (Pfeiffer, Spindler et al. 1998). The light chain confers specific amino acid transport activity to the heteromeric complex (Reig, Chillaron et al. 2002). However, without association with the heavy chain, the light chain cannot reach the plasma membrane (Pfeiffer, Loffing et al. 1999). System b^{0+} is a tertiary active transport system that uses downhill efflux of the high intracellular concentration of neutral amino acids down their chemical gradient to counter of transport L-dibasic amino acids and L-cystine into the cell. To establish the high intracellular concentration of neutral amino acids, the function of the apical B^0AT1 and basolateral y^+LAT1-4F2hc and other undefined basolateral neutral amino acid transporters are required. L-cystine, once it enters the cell, is rapidly reduced to L-cysteine by cytosolic reducing systems. It has been reported that the absorption of L-cystine via system b^{0+} is facilitated by the intracellular reduction of cystine to cysteine (Busch, Herzer et al. 1994; Chillaron, Estevez et al. 1996; Pfeiffer, Loffing et al. 1999). Interestingly, the expression patterns of rBAT and b^{0+}AT along the proximal tubule are opposite. The rBAT expression pattern is $S_3 > S_2 > S_1$, while the

b^{0+}AT expression pattern is $S_1 > S_2 > S_3$ (Furriols, Chillaron et al. 1993; Chairoungdua, Segawa et al. 1999; Pfeiffer, Loffing et al. 1999; Mizoguchi, Cha et al. 2001). These findings suggested that there are two systems that mediate the apical transport of L-cystine: system b^{0+} is a high affinity, Na^+-independent L-cystine transporter. Another low affinity, Na^+-dependent L-cystine transporter is also suggested to function in the proximal tubule. But the identity of the low affinity L-cystine transporter is unknown. This low affinity cystine transporter may also be a potential candidate for the transport of cadmium-cysteine complex.

The L-cystine transport system may also be responsible for the uptake of cadmium conjugates with glutathione (GSH). Glutathione is a tripeptide consisting of glutamate, cysteine and glycine; therefore, it may form conjugate with cadmium (G-S-Cd-S-G). But it is not likely that G-S-Cd-S-G is taken up as an intact complex due to the existence of brush-border enzymes, glutamyltransferase and cysteinylglycinase in the luminal membrane of proximal tubular cells. The function of glutamyltransferase is to cleave the glutamylcysteine bond in molecules of glutathione, while the function of cysteinylglycinase is to cleave cysteinylglycine bond in molecules of glutathione. When G-S-Cd-S-G is filtered into the proximal tubular lumen, it is most likely degraded rapidly in the tubular lumen to cysteinylglycine *S*-conjugate of cadmium (Gly-Cys-Cd-Cys-Gly) by ?-glutamyltransferase, and then to the cadmium-cysteine conjugate (Cys-S-Cd-S-Cys) by cysteinylglycinase. The resulting cadmium-cysteine conjugates are transported by the cystine transport system into the epithelial cells.

The structure of the cadmium-cysteine conjugate also resembles that of a dipeptide. In the proximal tubule, PepT2 mediates the high-affinity low capacity transport of small peptides (i.e., sequence-independent di- and tripeptides) as well as the various peptide-like drugs at the apical membrane. PepT2 is an electrogenic, sodium independent symporter that cotransports di- and tripeptides with protons. An inwardly directed electrochemical gradient of protons and the intracellular hydrolysis of di- and tripeptides to single amino acids drive more di- and tripeptides to be absorbed via PepT2 into the epithelial cells. Due to the structural similarity between cadmium-cysteine conjugate and dipeptide, cadmium-cysteine conjugates may compete for the transport via PepT2 in the proximal tubule.

Endocytosis has been postulated as an important mechanism for the uptake of Cd^{2+}-protein complexes such as Cd-MT and Cd-albumin. Metallothionein and albumin are low molecular weight proteins that contain multiple cysteine residues. However, only a small percentage of the cadmium in plasma that is bound to albumin is likely filtered into lumen of the proximal tubule, mainly because the glomerular-sieving coefficient for albumin is very low (Zalups and Ahmad 2003). Therefore, cadmium conjugate with albumin may not be presented to the apical membrane of the proximal tubule. Cd-MT conjugates are formed in hepatocytes in response to exposure to cadmium. Complexes of Cd-MT are released from necrotic hepatocytes and are delivered via systemic circulation to the kidney. Cd-MT can be filtered freely at the glomerulus due to its small size (a molecular mass of approximately 6-7 kDa). Generally, the glomerular filtration barrier allows the molecules having a molecular weight of less than 66kDa to pass through.

Absorbed Cd^{2+} is initially taken up into the liver, where Cd^{2+} can bind to metallothionein and be stored. However, some of CD-MT complexes are released from necrotic/apoptotic hepatocytes into the plasma, from where Cd-MTs are easily filtered through the glomerulus into the lumen and subject to absorption by renal proximal tubular cells. Recent studies assume that Cd-MT is absorbed at the apical membrane of proximal tubular cells by receptor-mediated endocytosis and sorted to the lysosomal compartment. In the lysosomes, Cd-MT may be degraded by acidic proteases and the free Cd^{2+} is transported into the cytoplasmic compartment from the lysosomes via some kind of transporters which may be DCT1 transporters. This model is supported by data demonstrating that acute uptake of Cd-MT by the kidney in rat is blocked by low molecular weight proteins, including ß2-microglobin, a ligand that binds the receptor protein, megalin (Bernard, Ouled Amor et al. 1987). However, the efficiency of Cd-MT absorption is controversial. Using the *in vivo* microperfusion technique, Felley-Bosco and Diezi reported that Cd-MT is not absorbed very efficiently along the proximal tubule *in vivo* (Felley-Bosco and Diezi 1987), but Dorian *et al* demonstrated that Cd-MT is rapidly and efficiently taken up by the S_1 and S_2 cells of the proximal tubule (Dorian, Gattone et al. 1992). While Barfuss and Zalups showed that Hg-MT was not absorbed at all in S_1, S_2, and S_3 segments isolated and perfused *in vitro* (Zalups, Cherian et al. 1995).

Transport of cadmium across the basolateral membrane in the proximal tubule

Unfortunately, by far, very little attention has been given to the possibility of cadmium uptake across the basolateral membrane of the proximal tubule. Therefore, the

mechanisms for basolateral cadmium transport are poorly understood. Some findings demonstrated that accumulation of cadmium in the LLC-PK1 cells following basolateral exposure to cadmium occurred (Prozialeck and Lamar 1993; Liu, Liu et al. 1994; Kimura, Endo et al. 1996). However, although their findings tend to indicate that cadmium can interact with the basolateral membrane and be transported into the cultured cells, the findings could not reflect the manner by which cadmium is taken up at the basolateral membrane *in vivo*. Recently, using the stop-flow technique, Zalups has provided strong *in vivo* evidence for basolateral uptake in the kidneys, and this uptake is stimulated by co-administration of cadmium with cysteine or glutathione. But they are not certain that what specific segments of nephron are involved in the process (Zalups 2000).

Zalups' findings suggested that Cd^{2+} present in the blood may be transported across the basolateral membrane as conjugates of cysteine or other non-protein sulfhydryl-containing molecules. However, the specific transport mechanisms have not been identified. One potential candidate for this transport is OAT1. OAT1 has been reported to function in the transport of Hg^{2+} in the form of Cys-S-Hg-S-Cys, Hcy-S-Hg-S-Hcy, and NAC-S-Hg-S-NAC across the basolateral membrane into the proximal tubular cells via a mechanism of molecular mimicry (Aslamkhan, Han et al. 2003; Zalups, Aslamkhan et al. 2004; Zalups and Ahmad 2005). Since cadmium-sulfhydryl conjugates are similar to those of Hg^{2+} in structure, it is hypothesized that OAT1 may be involved in the basolateral transport of cadmium conjugates into the epithelial cells.

Kaur *et al* found that zinc transport across the renal basolateral membrane vesicles was time and concentration-dependent, and competitively inhibited by cadmium (Kaur,

Sharma et al. 2006). In addition, overexpression of a zinc transporter ZnT1 has been found in cultured baby hamster kidney cells (Palmiter and Findley 1995). These findings suggested that Cd^{2+} in the blood may gain entry into the renal cells via a Zn^{2+} transporter in the basolateral membrane.

Adaptive and protective responses of proximal tubular cells to Cd^{2+} toxicity

Robinson *et al.* has demonstrated that less than 10% of the Cd^{2+} absorbed at the luminal membrane of the proximal tubule is subsequently transported across the basolateral membrane, indicating that more than 90% of the transported Cd^{2+} is sequestered within the epithelial cells (Robinson, Barfuss et al. 1993). In the cytosol of the epithelial cells, Cd^{2+} binds to sulfhydryl-containing amino acids such as L-cysteine, peptides such as GSH, or to proteins such as metallothionein, which protects the intracellular environment from oxidative damage by Cd^{2+}. Therefore, if a very low concentration (micromolar) of Cd^{2+} is applied to the cells, not all the cells are proned to damage by Cd^{2+} intoxication. In addition, the low concentration of Cd^{2+} will initiate repair processes to counterbalance the toxicity caused by cadmium. In other words, the cells will make more intracellular scavengers for Cd^{2+}. When the amount of cadmium exceeds the ability of the kidney cells to produce binding proteins that keep the cadmium biologically inactive, serious kidney damage may occur.

Recent studies illustrate that an important adaptive and protective response to toxic cadmium exposure is induction of metallothionein synthesis. Under normal conditions, metallothioneins occur at a very low level in the circulation. When exposed to

chronic Cd^{2+} intoxication, production of metallothioneins is increased greatly. In the human body, large quantities of metallothioneins are synthesized primarily in the liver and kidneys. Metallothioneins are a group of low molecular weight (mammalian forms, 6-7kD), intracellular metal-binding proteins that serve as a storage depot for Fenton metals, such as copper, zinc and iron. Metallothioneins contain numerous sulfhydryl groups due to their high L-cysteine content (20 cysteine residues), and have a high affinity for sulfhydryl-reactive metals such as mercury and cadmium. Metallothioneins even have higher affinities for mercury and cadmium than for Fenton metals such as zinc (Hamer 1986). Therefore, as mercury or cadmium binds to metallothionein, and is restricted from entering the mitochondria, zinc is released from metallothionein. The free, ionized zinc is toxic if permitted to accumulate and produce ROS. However, the released Zn^{2+} can bind to a metal regulatory element on the promoter region of the metallothionein gene and turn on the synthesis of metallothionein (Hamer 1986). The increased level of metallothionein provides increased binding capacity for both zinc and toxic cadmium, thereby protecting from cadmium toxicity and maintaining normal zinc function. The important role of metallothionein in cadmium cellular disposition and detoxification has been reported. Mammalian cell lines transfected with the greatest number of copies of the metallothionein gene expressed the highest levels of metallothionein. In the mean time, a relatively large number of those mammalian cells survived exposure to cadmium in culture media (Burnam, Palmiter et al. 1987). Metallothionein-null mice, genetically engineered to have inactivated metallothionein genes, died within three days of exposure to cadmium in drinking water, while control (normal) mice did not exhibit any symptoms

of cadmium toxicity (Masters, Kelly et al. 1994). Metallothionein also decreased cadmium elimination through the bile (Klaassen 1978), and increased cadmium sequestration in the liver (Liu, Liu et al. 1996), thus, reducing the amount of cadmium available to injure other critical organs.

A second cadmium-induced protective response is induction of glutathione synthesis. Glutathione is the primary intracellular antioxidant and conjugating agent. It has been reported that short or long-term exposure to cadmium resulted in a significant increase in the activity of glutathione S-transferase and glutathione level (Vogiatzis and Loumbourdis 1998; Casalino, Sblano et al. 2004; Casalino, Sblano et al. 2006; Yannarelli, Fernandez-Alvarez et al. 2007).

The other adaptive and protective responses induced by low concentration of Cd^{2+} include up-regulation of detoxifying proteins such as P-glycoprotein (Thevenod, Friedmann et al. 2000), multidrug resistance protein 2 (MRP2) (Terlouw, Graeff et al. 2002), or stimulation of the expression of the tumor suppressor gene for p53 (Achanzar, Achanzar et al. 2000). The detoxifying multidrug resistance P-glycoprotein pump and MRP2 are members of the superfamily of ATP-binding cassette (ABC) transporters. They are expressed in the luminal membrane of proximal tubule cells, and export cytotoxic substances and a variety of drugs. The increase in expression of detoxifying proteins in the luminal membrane in response to long term exposure to Cd^{2+} are expected to transport Cd^{2+} out of cells into urine. However, Frank Thevenod *et al* reported that although upregulation of P-glycoprotein is associated with anti-apoptotic protection for proximal tubular cells against Cd^{2+}-mediated stress (Thevenod, Friedmann et al. 2000),

their results strongly argue against the hypothesis that P-glycoprotein is involved in Cd^{2+} transport out of the proximal tubular cells. Therefore, the function of detoxifying proteins in cadmium toxicity remains to be determined. p53 is also known as tumor protein 53. p53 is a transcription factor that regulates and guarantee the correct cell cycle, thus suppressing cancer. Therefore, upregulation of p53 by low levels of cadmium can help reduce cadmium toxicity.

Possible measures that protect against cadmium toxicity

As discussed above, after being absorbed from the intestine and the pulmonary system, most cadmium accumulates and is retained in the body for a long time and very little is excreted via the urine or feces. Therefore, if we find ways to impair its absorption pathway, thereby favoring its renal clearance, its accumulation and toxicity may be reduced. Currently, some methods have been suggested or applied for the treatment of cadmium toxicity.

Heavy metal chelators. Heavy metal chelators used to chelate Cd^{2+}, Hg^{2+}, and Pb^{2+} include ethylenediaminetetraacetic acid (EDTA), triethylenetetramine (TETA), deferoxamine, deferiprone, meso-2,3-dimercaptosuccinic acid, 2, 3-dimercaptopropane-1-Sulfonate (DMPS) and diethyldithiocarbamate. A chelator is a molecule with two or more electronegative groups that can form stable co-ordinate covalent bonds with metal cations. The complexes are relatively large and difficult to absorb, and consequently are excreted by the body. The chelator's electronegative groups include -OH, -SH, or -NH. The efficacy of a chelator depends on the number of electronegative groups available for

metal binding. In general, the greater number of the electronegative groups, the more stable and huge the chelator-metal complex, and the more heavy metals are excreted. The efficacy of heavy metal chelator is enhanced by some amino acids such as methionine, but the mechanism is not known. In Europe, heavy metal chelators have been used as medical therapy for heavy metal toxicity. For example, DMPS ($C_3H_7O_3S_3Na$) has been used to cure acute and chronic toxicity caused by mercury, lead, arsenic, copper, antimony, chromium, and cobalt. However, since most chelators also complex essential divalent cations such as Ca^{2+}, Mg^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+} and increase their excretion, the use of heavy metal chelators is still considered experimental in U.S.A.

D-Cysteine. Since the heavy metal chelators have side effects, it is better to find natural compounds to chelate Cd^{2+}. It has been assumed that all D-amino acids except D-aspartic acid cannot be transported by amino acid transport systems as their L-counterparts. Therefore, the sulfhydryl-containing D-amino acids may be good natural chelators for Cd^{2+}. Using *in vivo* [109]Cd microinjection of the proximal tubule in the rat, Olivier Barbier *et al* found that D-cysteine was possible to increase the urinary excretion of [109]Cd (unpublished). But the role of D-cysteine still needs to be further confirmed because recently, it is reported that some amino acid transporters can accept small neutral D-amino acids as substrates to a certain degree (Fukasawa, Segawa et al. 2000).

Inhibitors and blockers of renal luminal transport of cadmium. As discussed previously, Cd^{2+} may compete with essential metal (Fe^{2+}, Zn^{2+} and Ca^{2+}) transport, and cadmium-sulfhydryl conjugates may compete with amino acid (L-cystine) transport mutually. Therefore, if these hypotheses can be verified *in vivo*, cadmium toxicity could

be countered by administering and these metals or amino acids and increase the renal clearance of cadmium. However, the efficiency of these treatments needs to be determined and the side effects induced by the acute administration of high concentration of essential elements or amino acids need to be carefully analyzed.

CHAPTER III: MATERIALS AND METHODS

Animals

Female New Zealand rabbits (1-2kg) were used in the present study. All animals were allowed at least two days of acclimation prior to any experimentation. Water and a commercial laboratory diet for rabbits were provided *ad libitum* during all phases of the study.

Composition of Bathing and Perfusing Solutions

In all experiments, the solution bathing the perfused tubular segments consisted of simple electrolyte solutions. This bathing solution contained the following: 140 mM Na^+, 140 mM Cl^-, 5 mM K^+, 1.3 mM Ca^+, 0.6 mM Mg^{2+}, 0.6 mM SO_4^{2-}, 2mM NaH_2PO_4, 1 mM D-glucose, and 0.5 mM L-glutamine. The pH was adjusted to 7.4 by the addition of 1N NaOH solution. Final osmolality was adjusted to 290 mOsmol /kg of H_2O by the addition of either doubly distilled and deionized water or NaCl. To evaluate the net absorption of cadmium or sulfhydryl conjugates of cadmium, $^{109}Cd^{2+}$ (0.588Ci/mg, Amersham) was added to the perfusate. The vital dye FD&C Green 3 (809Da) (NEELICERT) was placed in the perfusate at a concentration of 250nM to visually determine toxic effects of the Cd^{2+} or Cd-conjugates. $[^3H]$-L-glucose (14.6 Ci/mmol; American Radiolabeled) was added to the perfusing solution as a volume marker and a leak indicator. $[^3H]$-L-glucose is used as a leak indicator because it does not adhere to cell membrane, is not transported, does not penetrate tight junctions or cell membranes and is water soluble. The perfusing solution is identical to the bathing solution except the 2mM NaH_2PO_4 was replaced by 2mM HEPES because it was found that HPO_4^- or HPO_4^{2-} precipitated $^{109}Cd^{2+}$ in solution. For

experiments designed to determine if Fe^{2+} can compete with Cd^{2+} at the site of certain transporters, 10 µM $FeCl_2$ along with 100 µM ascorbic acid (to prevent oxidation) were added to the perfusate. In addition, this latter perfusate was adjusted to a pH of 6.8 to assure maximum DCT1 activity.

Tubular Dissection Solution

The tubular dissection solution was a sucrose/phosphate buffer containing 125mM sucrose, 13.3mM anhydrous monosodium dihydrogen phosphate (NaH_2PO_4), and 56 mM anhydrous disodium monohydrogen phosphate (Na_2HPO_4). The pH was adjusted to 7.4 by the addition of either 1N NaOH or HCl. The osmolality was adjusted to 290 mOsm/Kg of water by the addition of either water or NaCl.

Procedure for Obtaining Segments of Proximal Tubules

On each day of experimentation, a rabbit was anesthetized with a combination of 33mg/kg ketamine (FORT DODGE) and 33mg/kg xylazine (LLOYD). When the rabbit reached deep anesthesia (as determined by the corneal reflex) the abdominal wall was cut and the kidneys were rapidly removed and placed in the cold (4^oC) aqueous sucrose-phosphate buffer solution (dissection solution). The kidneys were then quickly sliced into 1-2mm thick coronal sections, using a single-edge razor blade. The sections were stored in the same sucrose-phosphate buffer solution on ice for up to next 8 hours. The S_1, S_2, and S_3 segments of the proximal tubule were dissected from the coronal sections. The S_1 segments were identified as convoluted tubules dissected from the outer cortical regions of the kidney slice. The S_2 segments were identified as

straight portions of the proximal tubule spanning the entire thickness of the cortex, while the S_3 segments were identified as the last 1 mm of the proximal straight tubule which was attached to the easily identifiable thin descending limb of Henle's loop and located in the outer stripe of the outer medulla.

Method for Perfusing Segments of Proximal Tubules

Each dissected tubule was transferred to a Lucite perfusion chamber and was suspended between two sets of pipettes. One set of pipettes including the holding pipette and the perfusion pipette was used to perfuse the suspended tubule, whereas the other set including the collection pipette and the constant volume pipette was used to collect the perfused fluid (Figure 4). Each tubular segment was warmed from room temperature to $37^{o}C$ over 15 min prior to the beginning of an experiment. The perfusion rate was maintained, on average, at 7-10 nl/min, with constant hydrostatic pressure. Because of differences in tip diameters of the perfusion pipettes used in the present study, the hydrostatic pressure needed to perfuse the segments of proximal tubules at 7-10 nl/min varied between 15 and 50mmHg. Each perfused tubule was monitored for any changes in tubular diameter resulting from abnormally high intraluminal pressure. The perfused fluid (perfusate) was collected from the lumen into a constant volume pipette (designed to accurately collect 30-50nl). The bathing fluid surrounding the outside basolateral surface of the perfused tubule was pumped into one end of the bathing chamber at a rate of approximately 0.3ml/min and was aspirated continually out of the other end, and collected into scintillation vials at 5-min intervals. The volume fluid held in the perfusion chamber was approximately 0.3ml, thus the bathing solution was exchanged about once per minute.

Collection of Samples

The fluid exiting from each perfused tubular segment and corresponding bathing solution sample were collected for each perfused tubule to measure the rates of lumen-to-bath flux (fmol min^{-1} (mm tubule length)$^{-1}$) of ^{109}Cd and the volume marker, ^{3}H-L-glucose. The constant-volume pipette was used to collect collectate samples, which were immediately added to 4 ml of scintillation fluid. The bathing solution was routinely collected and analyzed for the appearance of ^{109}Cd and the volume marker (fmol min^{-1} (mm tubule length)$^{-1}$). The aspirated bathing solution was collected in 8-ml scintillation vials that were configured with a vacuum trap. These scintillation vials were changed every 5 min during experiments. To each vial, 4 ml of scintillation fluid (Opti-Fluor; Packard Instrument Company, Downers Grove, IL) was added. The collectate and bathing fluid samples were then counted in a Beckman 5800 scintillation counter (Beckman Instruments, Fullerton, CA) to quantify of the amount ^{109}Cd and ^{3}H present in each sample using standard isotopic separation methods.

Harvesting of Perfused Tubular Segments

To calculate the cellular content of cadmium in the isolated perfused segments of proximal tubules, it is necessary to harvest the perfused tubule at the end of each experiment. The tubular segment was quickly harvested (about one second) with the aid of a pair of fine forceps by perfusing it at the perfusion end and pulling it free while it was being perfused. The tubule then was removed from the bathing solution and placed in 10 μl of 3% (W/V) TCA (trichloroacetic acid). The TCA precipitated the larger proteins leaving the tubule opaque-white

and rigid while releasing the cytosolic contents into the TCA solution. After a few minutes, the tubular segment (TCA-precipitable fraction) was removed from the TCA solution, placed into a vial with 4 ml of scintillation fluid and later analyzed by scintillation counting with standard isotopic methods for the contents of ^{109}Cd and the volume marker, [^{3}H]-L-glucose. Like wise the TCA solution (TCA-soluble fraction, presumably cytosolic content) data was obtained. The TCA-soluble fraction permitted the approximate calculation of the cellular content of ^{109}Cd (μM).

Calculations

Lumen-to-cell disappearance flux. Transport of cadmium in lumen-to-cell transport experiments was determined by measuring the rate at which ^{109}Cd disappeared from the luminal fluid. This disappearance flux (J_D) (fmol min^{-1} (mm tubule length)$^{-1}$) measurement was calculated by equation #1:

$$1) \qquad J_D = (V_P[Cd^{2+}]_P - V_C[Cd^{2+}]_C)/L$$

Where $[Cd^{2+}]_P$ and $[Cd^{2+}]_C$ are the concentrations (fmol nl^{-1}) of ^{109}Cd in the perfusate and collectate, respectively. $[Cd^{2+}]_P$ and $[Cd^{2+}]_C$ were determined from the specific activity of ^{109}Cd. L is the length (mm) of the perfused tubular segment. V_C is the collectate collection rate (nl min^{-1}), which was calculated from the time required to fill the constant volume pipette. V_P is the perfusion rate (nl min^{-1}) and was calculated by equation #2:

$$2: \quad V_P = V_C([VM]_C/[VM]_P)$$

Where $[VM]_C$ and $[VM]_P$ are the concentrations (cpm nl^{-1}) of the Volume Marker (^{3}H-L-glucose) in the collectate and perfusate respectively. The final $[VM]_C$ was determined by adding the amount of [^{3}H]-L-glucose (cpm min^{-1} x collectate collection time) that leaked into the bathing solution during the collection period to the collectate [^{3}H]-L-glucose, and for intact tubular segments, this is very little. [^{3}H]-L-glucose is not transported by the glucose transporters in the renal proximal tubules, therefore, the little leak is caused by the intercellular passage of [^{3}H]-L-glucose through the various junctional complexes. The generally accepted normal lumen-to-bath leak of the volume marker, [^{3}H]-L-glucose, is about 1% of the total volume marker being perfused into the lumen. If the leak is greater than the normal rate, it can be assumed that the perfused tubular segment has been structurally compromised, and it becomes justifiable to discard the data from that experiment.

Cell-to-Lumen ratio of ^{109}Cd. The cell/lumen concentration ratio of ^{109}Cd was calculated using the following equations, 3, 4 and 5:

$$3: \quad [Cd]_{Tubule} = cpm_{Cd} \div V_{Cell}$$

$$4: \quad V_{Cell} = 0.7p\,(r_0^2 - r_i^2)\text{ x } L$$

$$5: \quad Cell/Lumen = [Cd]_{Tubule}/[^{109}Cd]_{Lumen}$$

Where Cd_{Tubule} (cpm/nl) is the intracellular content of ^{109}Cd in perfused tubular segment; cpm_{Cd} is the amount (cpm) of ^{109}Cd in the perfused tubular segment (only TCA-soluble fractions) while V_{Cell} (nl) is the volume of the perfuse segment and L is the length (μm) of the tubular segment; 0.7 is the fraction of cellular volume that is water (Tune and Burg 1971); **r** is inside or outside

radii of the perfused segment; $[^{109}Cd]_{Lumen}$ is the mean concentration of ^{109}Cd in the luminal perfusing solution.

Statistical Analysis

A minimum of five tubules were perfused under each experimental condition. Moreover, data for each parameter assessed was obtained from tubular segments isolated from at least two animals. For each perfused tubule, the three J_D measurements of ^{109}Cd were averaged. These averaged values (5-10) for J_D were used to compute the overall mean and standard error of the mean for each experimental condition. The same analysis sequence was use for the Cell/Lumen concentration ratios. After an analysis of variance (ANOVA), the mean values of the various groups were tested for differences using the conservative Bonferroni test. Values were assumed to be significantly different when $P<0.05$.

CHAPTER IV: RESULTS

Effect of Fe^{2+} on the lumen-to-cell transport of Cd^{2+}

To determine if Fe^{2+} has an effect on the luminal transport of Cd^{2+}, 10μM FeCl$_2$ and 100μM ascorbic acid were added to the pH 6.8 perfusate with 0.73μM ^{109}CdCl$_2$, Fig. 5. The disappearance flux (JD) and cell-to-lumen concentration ratio of ^{109}Cd are 0.60 ± 0.04 fmol min^{-1} (mm tubular length)$^{-1}$ and 20 ± 2.15, respectively Comparative (control) studies were performed with 100μM ascorbic acid, pH 6.8 and no Fe^{2+}. The disappearance flux (J_D) and cell-to-lumen concentration ratio of ^{109}Cd are 1.04 ± 0.06 fmol min^{-1} (mm tubular length)$^{-1}$ and 37.86 ± 1.47, respectively. Compared to the control group, the presence of Fe^{2+} significantly reduced the J_D of Cd^{2+} by 42%, and cell-to-lumen concentration ratio of Cd^{2+} by 49%.

Effect of Zn^{2+} on the lumen-to-cell transport of Cd^{2+}

To investigate whether Zn^{2+} can affect luminal absorption of Cd^{2+}, 20μM ZnCl$_2$ was added to the perfusate with 0.73μM ^{109}CdCl$_2$. As shown in Fig 6, the J_D of Cd^{2+} in the presence of Zn^{2+} is 1.62 ± 0.24 fmol min^{-1} (mm tubular length)$^{-1}$, and the cell-to-lumen concentration ratio is 154.36 ± 23.26. The J_D of Cd^{2+} in the control group is 3.13 ± 0.14 fmol min^{-1} (mm tubular length)$^{-1}$, and the cell-to-lumen concentration ratio is 223.8 ± 22.7. Compared to the control group, Zn^{2+} significantly decreased the J_D of Cd^{2+} by 48% and the cell-to-lumen concentration ratio by 31%.
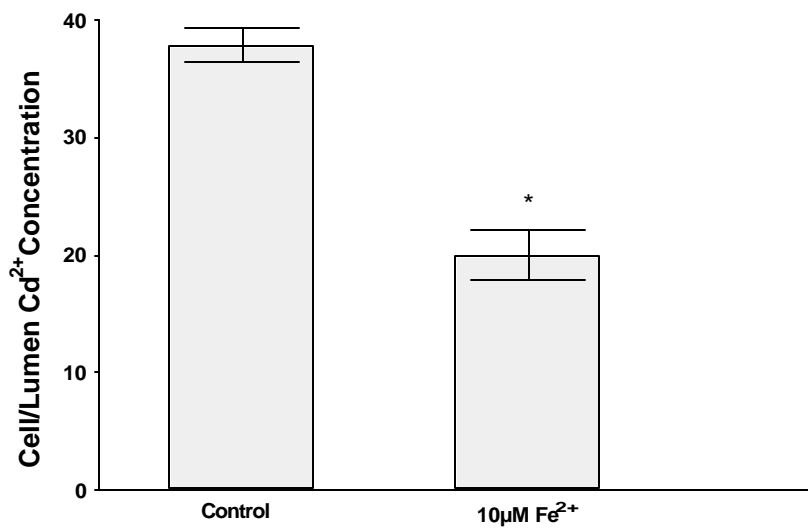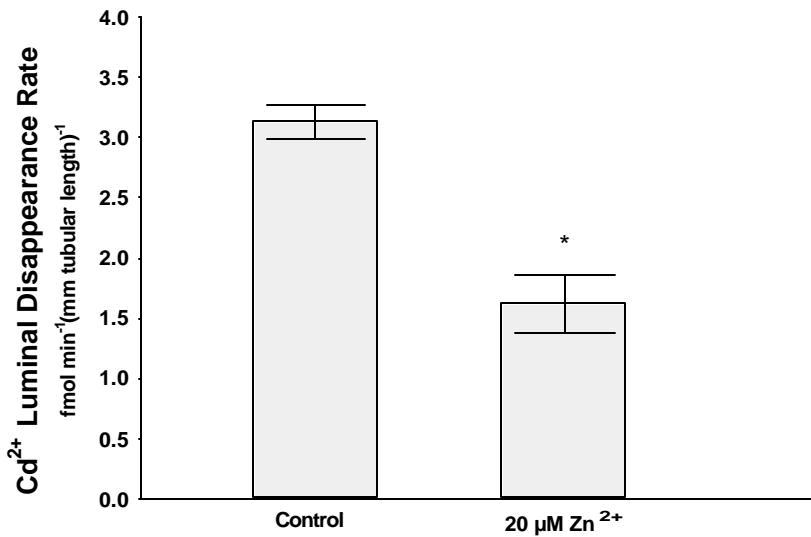
A
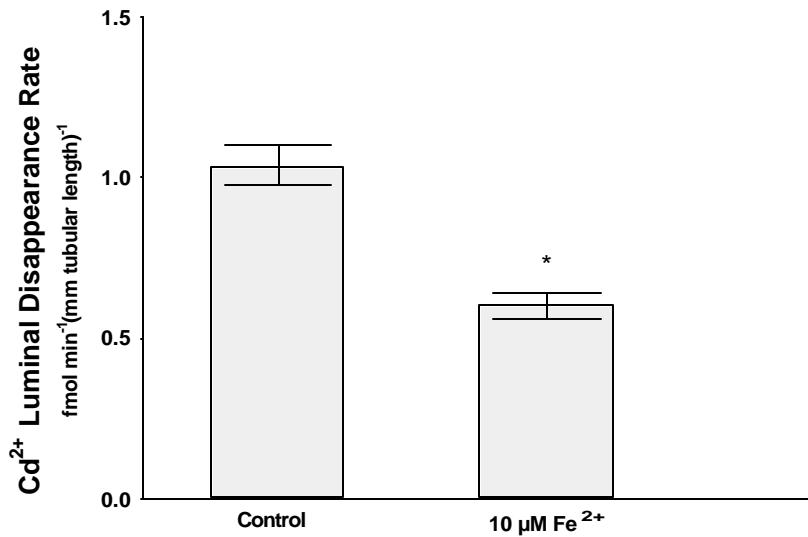
B

Figure 5: The effect of Fe^{2+} on the lumen-to-cell transport (A) and cell-to-lumen concentration ratio (B) of Cd^{2+} in isolated S_2 segments of the proximal tubule of the rabbit perfused with 0.73 µM Cd^{2+} (at 37ºC). Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference, P< 0.05.
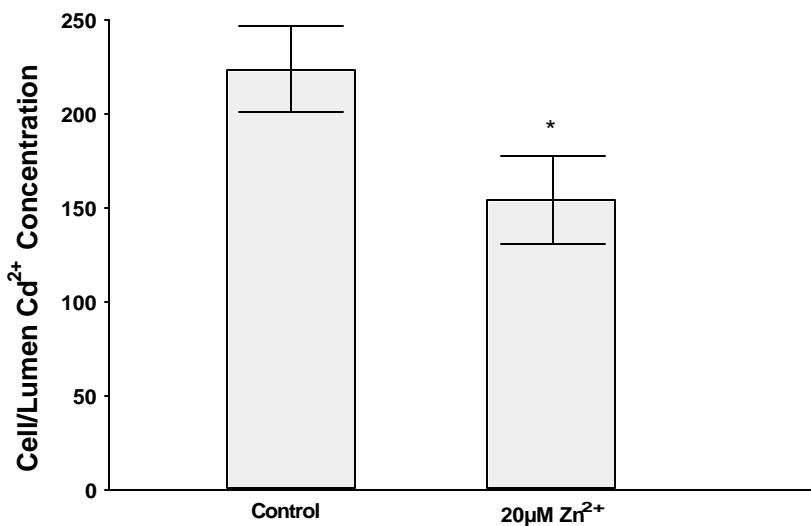
A

B

Figure 6: The effect of Zn^{2+} on the lumen-to-cell transport (A) and cell-to-lumen concentration ratio (B) of Cd^{2+} in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 µM Cd^{2+} (at 37ºC). Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference, P< 0.05.
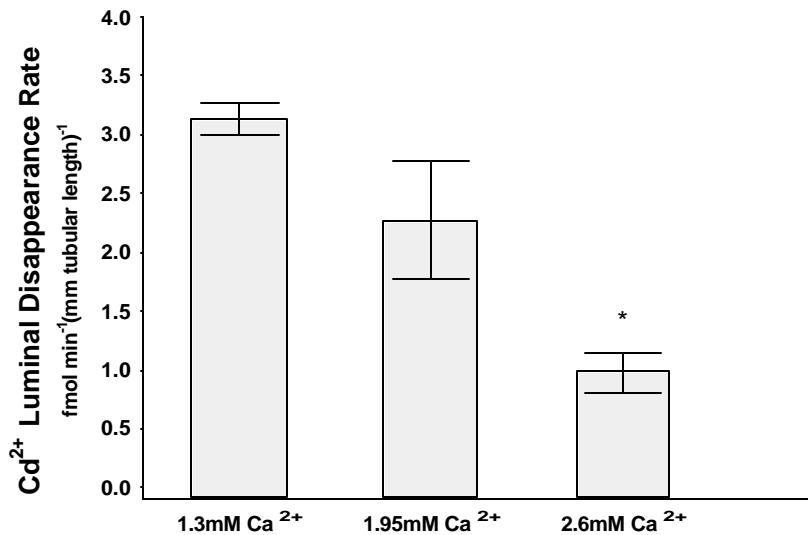
Effect of Ca^{2+} on the lumen-to-cell transport of Cd^{2+}

To determine whether a calcium channel is a mechanism for Cd^{2+} entry into the renal epithelial cells, the concentration of Ca^{2+} in perfusate was adjusted from 1.3mM (control perfusate) to 1.95mM or 2.6mM. The J_{DS} at these concentrations were 3.13 ± 0.14, 2.28 ± 0.50 and 0.98 ± 0.17 fmol min^{-1} (mm tubular length)$^{-1}$, respectively, Fig.7. The presence of high concentrations of Ca^{2+} in the perfusate decreased the J_D of Cd^{2+} (27% reduction at 1.95mM and 69% at 2.6mM). The cell-to-lumen concentration ratios at these three concentrations were 223.8 ± 22.7, 179.8 ± 30.7 and 124.2 ± 25.9. High concentrations of Ca^{2+} in the perfusate decreased the cell-to-lumen concentration ratio of Cd^{2+} (19.7% reduction at 1.95mM and 45% at 2.6mM). When the concentration of Ca^{2+} in perfusate was adjusted to be less than that in control perfusate (1.3mM), the tubules expressed an acute visual toxicity, such as blebbing of the luminal membrane and cellular swelling (data not shown), consequently, transport data could not be obtained.

Effect of calcium channel blockers on the lumen-to-cell transport of Cd^{2+}

L-type calcium channel blockers, 100μM verapamil or 100μM diltiazem, were used to test for any effect they might have on the absorption of Cd^{2+}, Fig.8. The data showed that 100μM verapamil did not have any significant effect on Cd^{2+} transport, while diltiazem slightly reduced the J_D of Cd^{2+} by 26.7%, from 1.05 ± 0.05 to 0.77 ± 0.02 fmol min^{-1} (mm tubular length)$^{-1}$, and the cell-to-lumen concentration ratio by 19%, from 45.1 ± 5.29 to 36.5 ± 2.57.
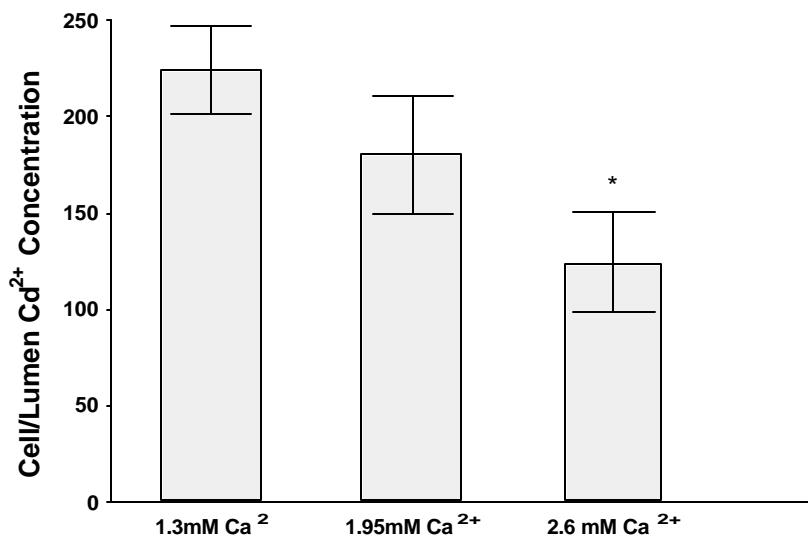
A.

B

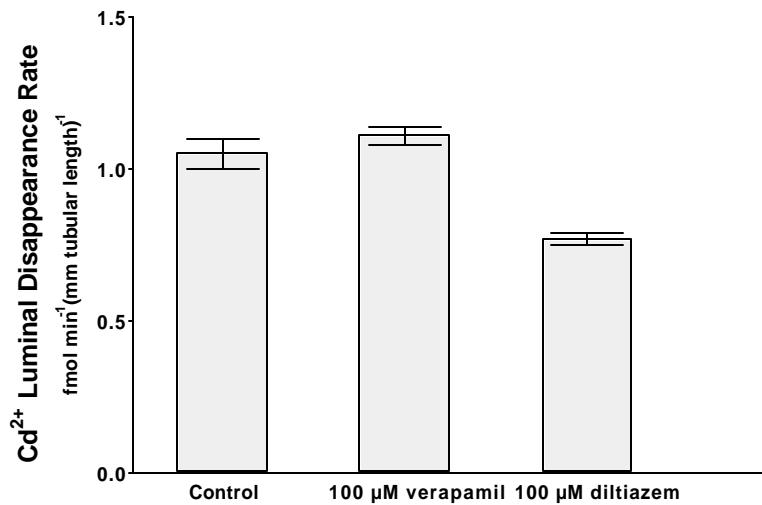**Figure 7:** The effect of Ca^{2+} on the lumen-to-cell transport (A) and cell-to-lumen concentration ratio (B) of Cd^{2+} in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 μM Cd^{2+} (at 37ºC). Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference, P< 0.05 compared to control, 1.3 mM Ca^{2+}.

A

B

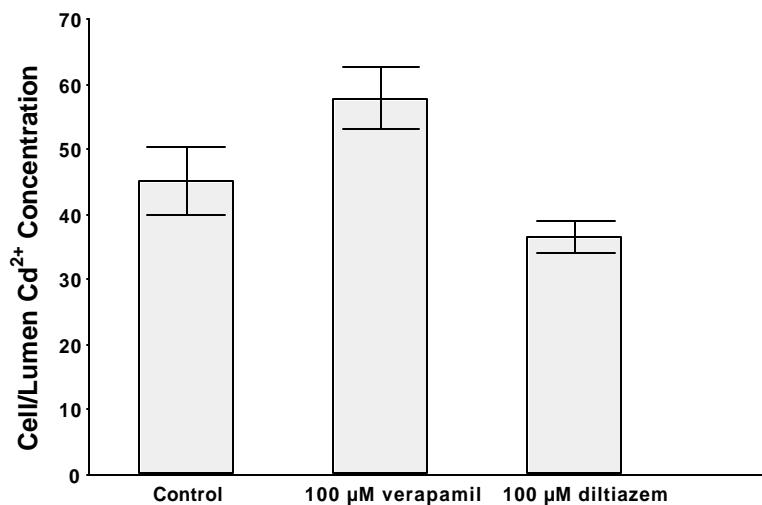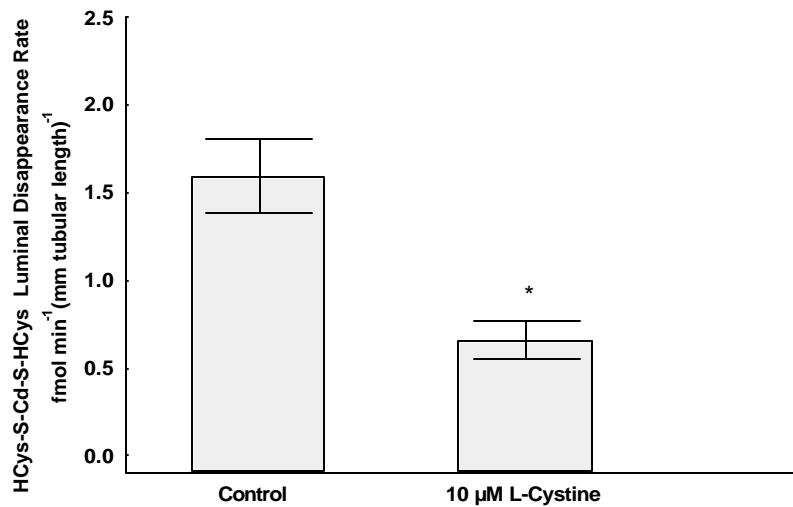**Figure 8:** The effects of verapamil and diltiazem on the lumen-to-cell transport (A) and cell-to-lumen concentration ratio (B) of Cd^{2+} in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 μM Cd^{2+} (at 37°C).   Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference from control, P< 0.05.

Effect of L-cystine on the lumen-to-cell transport of cadmium conjugates of sulfhydryl-containing molecules

When 10μM L-cystine was added to the perfusate, it reduced the J_D of cadmium-homocysteine conjugate (HCys-S-Cd-S-HCys) by about 58%, from 1.59 ± 0.21 to 0.66 ± 0.11 fmol min^{-1} (mm tubular length)$^{-1}$, and the cell-to-lumen concentration ratio by 41%, from 157.3 ± 13.5 to 92.7 ± 15.8, Fig.9.

When co-perfused with cadmium-N-acetylcysteine conjugate (NAC-S-Cd-S-NAC), L-cystine reduced the J_D of NAC-S-Cd-S-NAC by about 38%, from 0.96 ± 0.06 to 0.60 ± 0.02 fmol min^{-1} (mm tubular length)$^{-1}$, and the cell-to-lumen concentration ratio by 10%, from 36.5 ± 1.65 to 33.05 ± 1.38, Fig. 10.

A.

B.

Figure 9: The effect of L-cystine on the lumen-to-cell transport (A) and cell-to-lumen concentration ratio (B) of HCys-S-Cd-S-HCys in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 μM HCys-S-Cd-S-HCys (at 37ºC). Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference, P< 0.05.

A.

B.

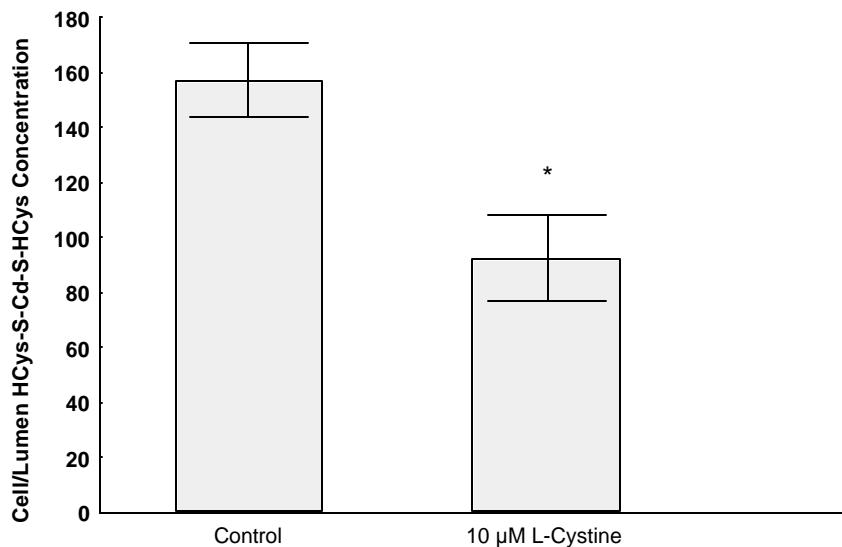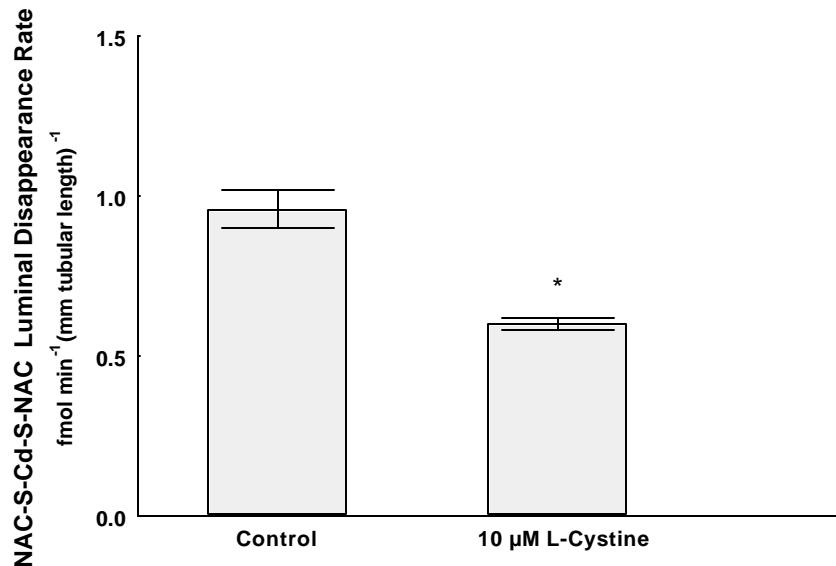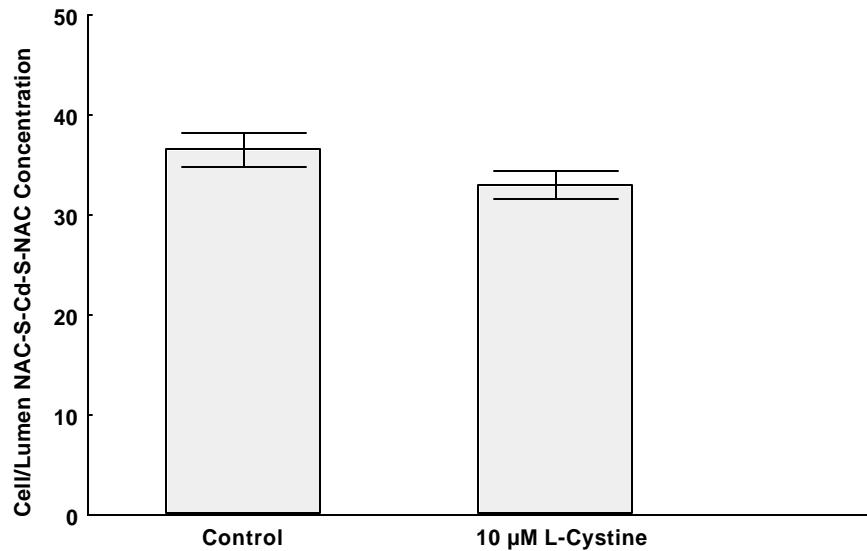**Figure 10:** The effect of L-cystine on the lumen-to-cell transport (A) and cell-to-lumen concentration ratio (B) of NAC-S-Cd-S-NAC in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 µM NAC-S-Cd-S-NAC (at 37ºC). Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference, P< 0.05.

Effect of amino acids on the lumen-to-cell transport of cadmium-cysteine conjugate

Figure 11 shows that 10μM L-cystine and 10μM L-arginine reduced the J_D of cadmium-cysteine conjugate (Cys-S-Cd-S-Cys) by about 55% and 50%, and the cell-to-lumen concentration ratio by about 64% and 20%, while 10μM L-aspartic acid or 10μM L-glutamate had no effect on the transport of cadmium-cysteine conjugate. When 0.73 μM cadmium-cysteine conjugate was co-perfused with 10μM L-methionine, the J_D of cadmium-cysteine conjugate was increased by about 56% and the cell-to-lumen concentration by about 129%.

Effect of Gly-Sar on the lumen-to-cell transport of cadmium-cysteine conjugate

To test the effect of dipeptide on lumen-to-cell transport of cadmium-cysteine conjugate, 10μM Gly-Sar (glycylsarcosine) was co-perfused with 0.73μM cadmium-cysteine conjugate. Both the J_D of the cadmium-cysteine conjugate and the cell-to-lumen concentration ratio were not significantly changed, Fig.12.

Effect of Zn^{2+} on the lumen-to-cell transport of cadmium-cysteine conjugate

To determine the stability of the cadmium-cysteine bond, 20μM Zn^{2+} was co-perfused with 0.73μM cadmium-cysteine conjugate. There was no decrease in both the J_D of the cadmium-cysteine conjugate and the cell-to-lumen concentration ratio, Fig. 13.

A.
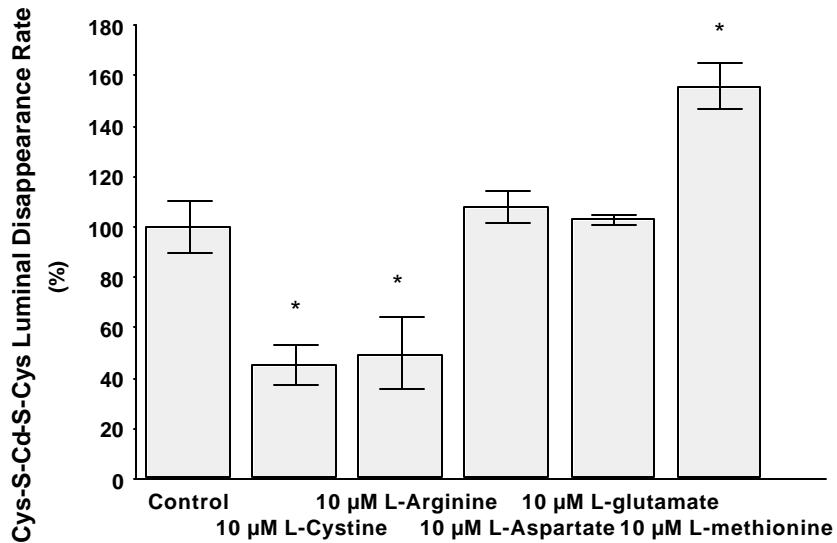
B.

Figure 11: The effects of relevant amino acids on the lumen-to-cell transport (A) and cell-to-lumen concentration ratio (B) of Cys-S-Cd-S-Cys in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 µM Cys-S-Cd-S-Cys (at 37ºC). Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference from control, P< 0.05.

A.

B.

Figure 12: The effect of the dipeptide, gly-sar, on the lumen-to-cell transport (A) and cell-to-lumen concentration ratio (B) of Cys-S-Cd-S-Cys in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 µM Cys-S-Cd-S-Cys (at 37ºC). Each value represents the mean ± SE for a sample size of five or six.

A.

B.

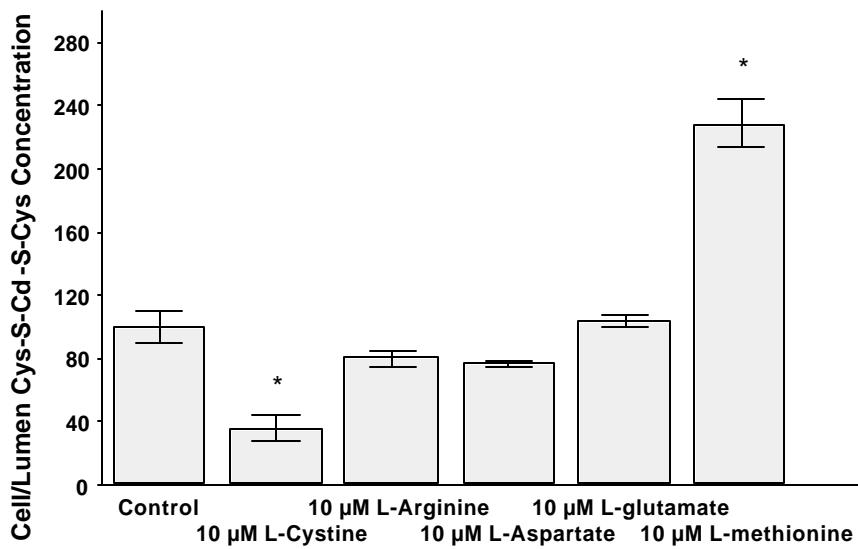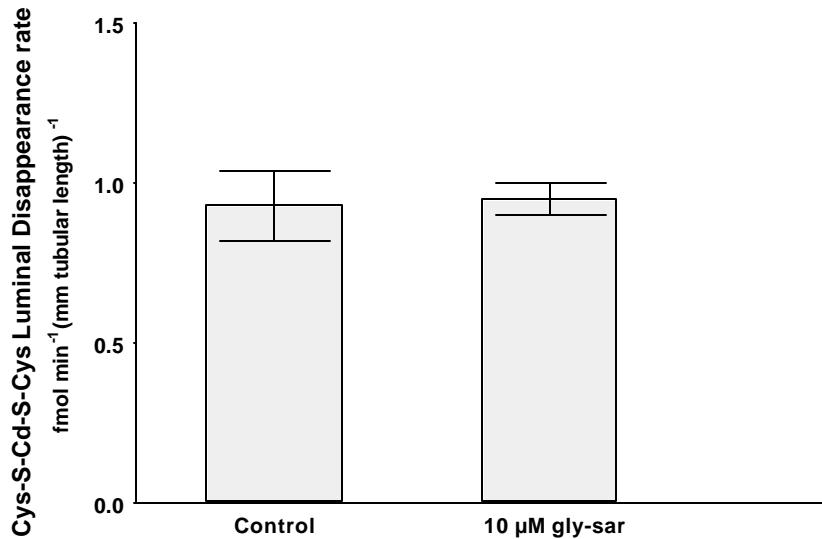**Figure 13:** The effect of zinc on the lumen-to-cell transport (A) and cell-to-lumen concentration ratio (B) of Cys-S-Cd-S-Cys in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 μM Cys-S-Cd-S-Cys (at 37ºC). Each value represents the mean ± SE for a sample size of five or six.

Effect of temperature on the lumen-to-cell transport of Cd^{2+}

To assess whether the transport of Cd^{2+} is modulated by temperature, the S_2 segments of the proximal tubules were perfused with Cd^{2+} at 37 $^\circ$C, 22 $^\circ$C and 11 $^\circ$C (Fig. 14). The J_Ds of Cd^{2+} at these temperatures were 3.13 ± 0.14, 0.41 ± 0.11 and 0, fmol min^{-1} (mm tubular length)$^{-1}$. The corresponding cell-to-lumen concentration ratios of Cd^{2+} were 223.8 ± 22.7, 79.5 ± 5.46 and 12.27 ± 0.92. Significant decreases in the J_D (87% reduction at 22 $^\circ$C and 100% at 11 $^\circ$C) and cell-lumen concentration ratio (64.5% reduction at 22 $^\circ$C and 94.5% at 11 $^\circ$C) of Cd^{2+} were observed in response to these reduction in temperature.

Effect of temperature on the lumen-to-cell transport of cadmium-cysteine conjugate

To assess whether the transport of cadmium-cysteine conjugate is modulated by temperature, the S_2 segments of the proximal tubules were perfused with 0.73μM Cys-S-Cd-S-Cys at 37 $^\circ$C, 22 $^\circ$C and 11 $^\circ$C. The J_Ds of cadmium-cysteine conjugate at these temperatures were 2.92 ± 0.31, 0.70 ± 0.16 and 0, fmol min^{-1} (mm tubular length)$^{-1}$ while the corresponding cell-to-lumen concentration ratios of cadmium-cysteine conjugate were 282.3 ± 55, 52.5 ± 9.3 and 11.55 ± 2.46. Significant decreases in the J_D (76% reduction at 22 $^\circ$C and 100% at 11 $^\circ$C) and cell-lumen concentration ratio (81.4% reduction at 22 $^\circ$C and 95.9% at 11 $^\circ$C) of cadmium-cysteine conjugate were observed to the reduction in temperature, Fig. 15.

A

B

Figure 14: The effect of temperature on the lumen-to-cell transport (A) and cell-to-lumen concentration ratio (B) of Cd^{2+} in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 μM Cd^{2+}. Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference from all other groups, P< 0.05.
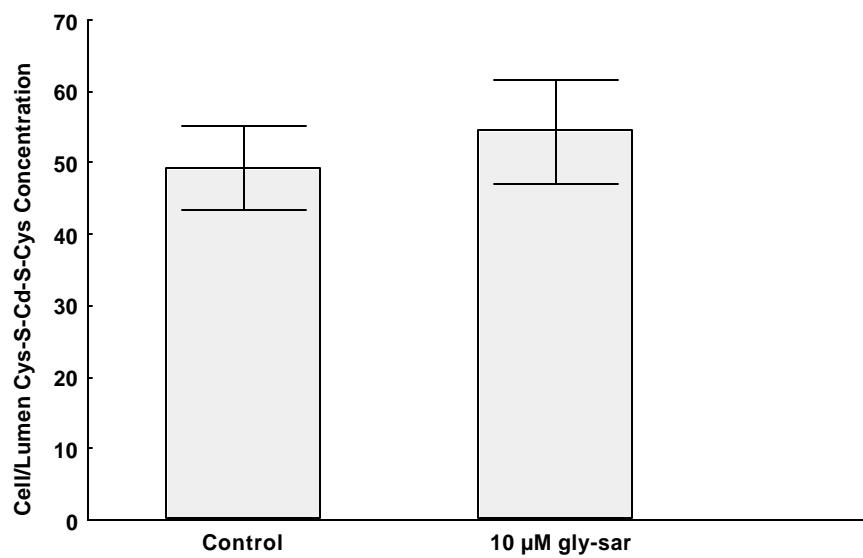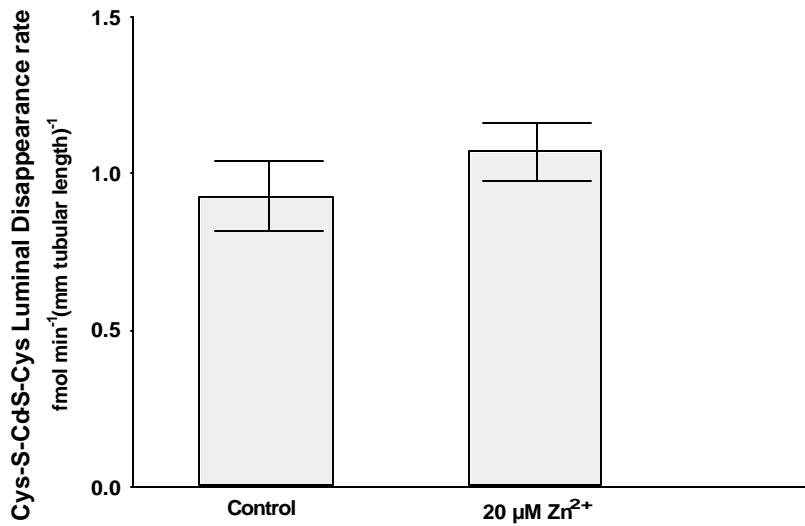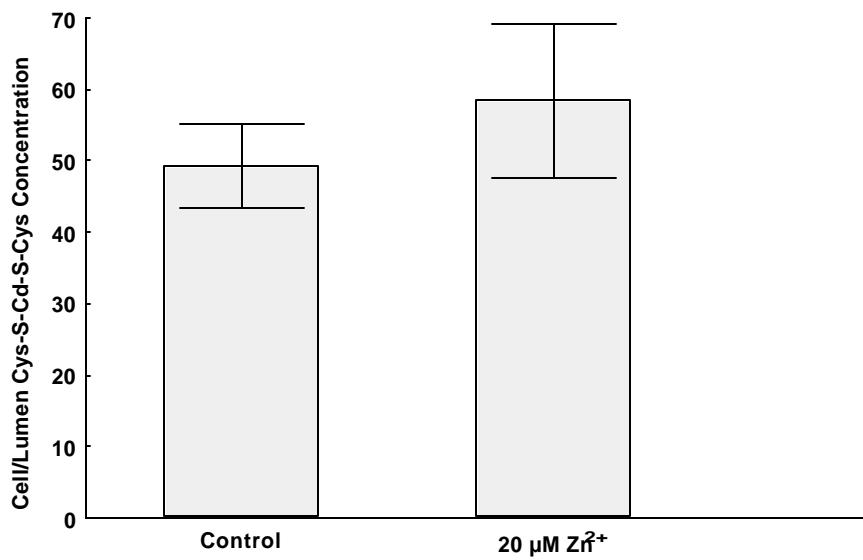
A

B

Figure 15: The effect of temperature on the lumen‑to‑cell transport (A) and cell‑to‑lumen ratio (B) of Cys-S-Cd-S-Cys in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 µM Cys-S-Cd-S-Cys (at 37ºC).   Each value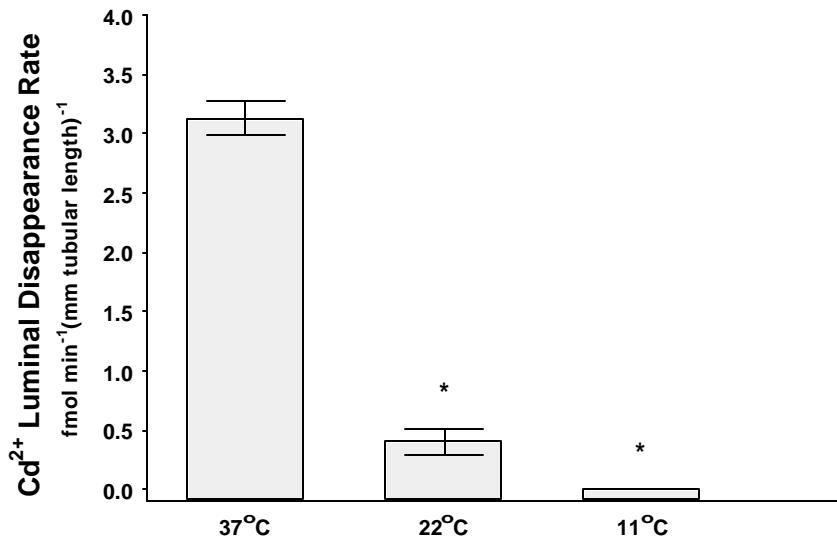 represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference from all other groups, P< 0.05.

Effect of DMPS on the lumen-to-cell transport of Cd^{2+}

Addition of 200μM 2, 3-Dimercaptopropane-1-Sulfonate (DMPS) to the bathing solution caused no significant difference in the J_D of Cd^{2+}, but decreased the cell-to-lumen concentration ratio by 80%, from 37.52 ± 5.48 to 7.47 ± 1.31, Fig.16. Addition of 30μM DMPS to the perfusate decreased the J_D of Cd^{2+} by 39%, from 25.77 ± 1.80 to 15.67 ± 0.77 fmol min^{-1} (mm tubular length)$^{-1}$, and the cell-to-lumen concentration ratio by 61%, from 37.52 ± 5.48 to 14.7 ± 1.21. When the concentration of DMPS was increased to 200μM in the perfusate, the J_D of Cd^{2+} was decreased by 94.6%, from 25.77±1.80 to 1.4±0.76 fmol min^{-1} (mm tubular length)$^{-1}$, and the cell-to-lumen concentration ratio by 95.2%, from 37.52 ± 5.48 to 1.81 ± 0.68. Either addition of DMPS to the bathing solution or to the perfusate abolished the slight toxicity resulting from the 20μM luminal Cd^{2+}.

Effect of DMPS on the lumen-to-cell transport of cadmium-cysteine conjugates

To examine whether DMPS can affect the transport of cadmium-cysteine conjugates, 30μM DMPS was added to the perfusate with 20μM Cys-S-Cd-S-Cys. The J_D for Cys-S-Cd-S-Cys was reduced by 62%, from 29.84 ±1.78 to 11.37 ± 1.24 fmol min^{-1} (mm tubular length)$^{-1}$ and the cell-to-lumen concentration ratio by 68%, from 40.7 ± 5.36 to 13 ± 1.76, Fig. 17. When 20μM L-cystine was co-perfused with 30μM DMPS there was no reduction in the transport of the L-cystine.
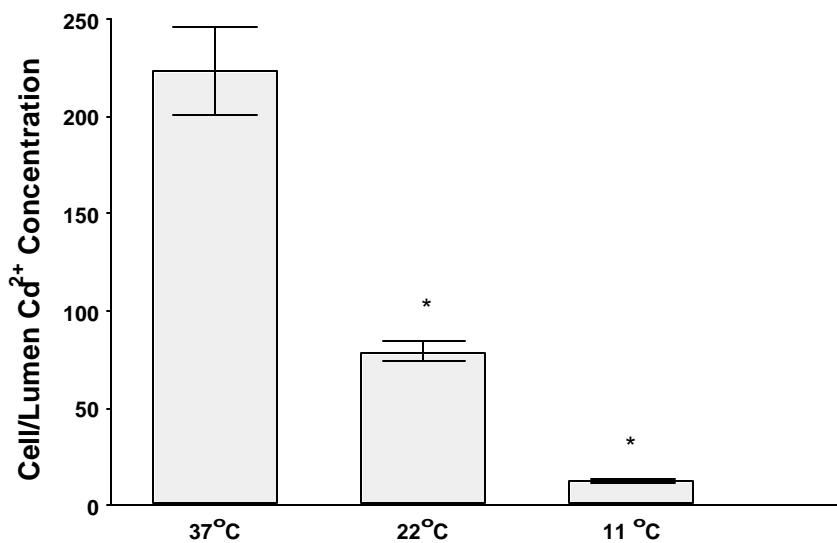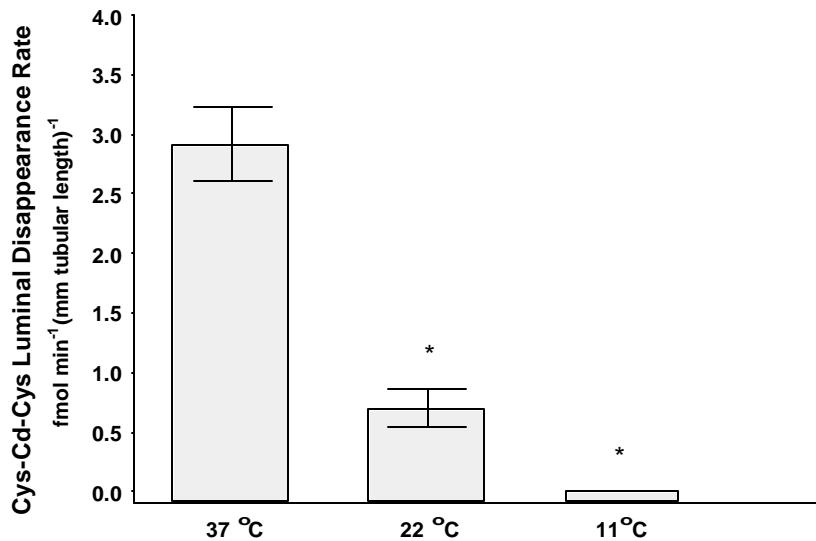
A.

B.

Figure 16: The effect of DMPS on the lumen-to-cell transport (A) and cell-to-lumen ratio (B) of Cd^{2+} in isolated S_2 segment of the proximal tubule of the rabbit perfused with 20 µM Cd^{2+} (at 37ºC). Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference from all other groups, P< 0.05.
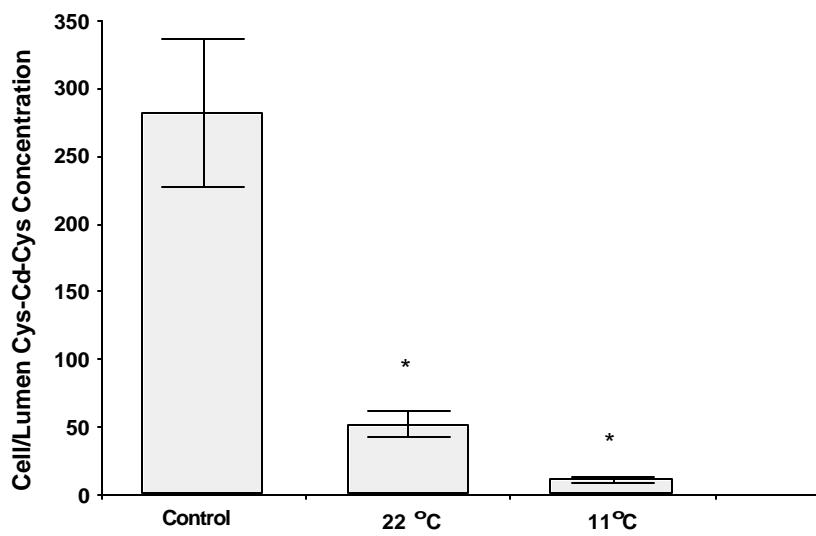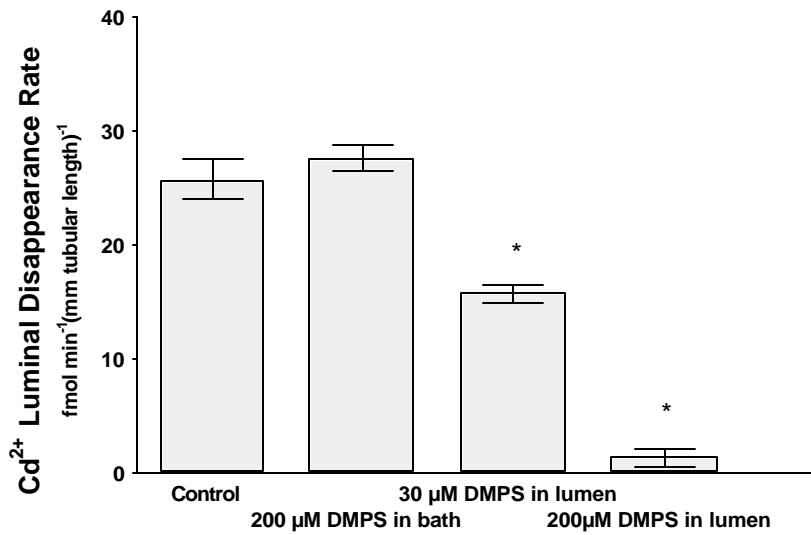
A.

B.
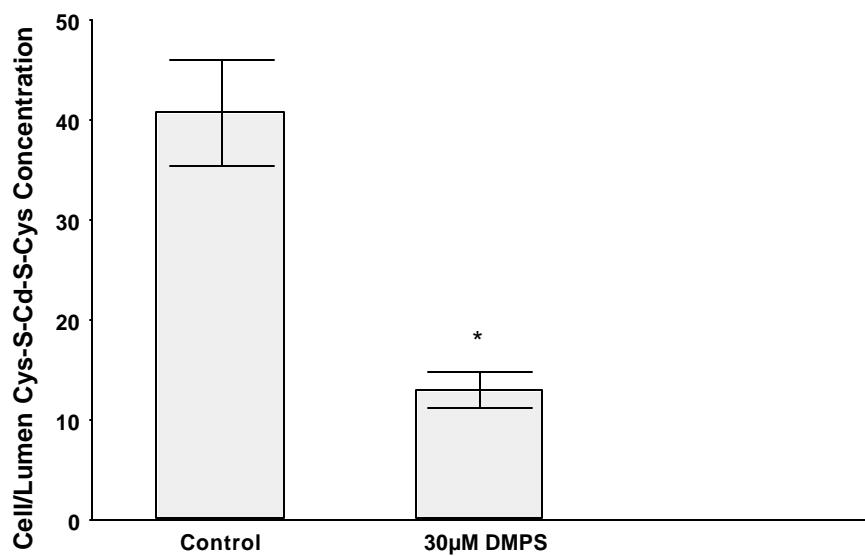
Figure 17: The effect of DMPS on the lumen-to-cell transport (A) and cell-to-lumen ratio (B) of Cys-S-Cd-S-Cys in isolated S_2 segment of the proximal tubule of the rabbit perfused with 20 µM Cys-S-Cd-S-Cys (at 37ºC). Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference, P< 0.05.

Effect of *D*-cysteine on the lumen-to-cell transport of Cd^{2+}

To determine whether *D*-cysteine can change lumen-to-cell transport of ^{109}Cd, 0.73µM Cd^{2+} was co-perfused with 2µM *D*-cysteine. As shown in Figure 18, The J_D for *D*-Cys-S-Cd-*D*-S-Cys was 1.86 ± 0.07 fmol min^{-1}(mm tubular length)$^{-1}$ and the cell-to-lumen concentration ratio was 125.6 ± 12.63, while the J_D for Cd^{2+} was 1.05 ± 0.05 and the cell-to-lumen concentration ratio was 45.1 ± 5.29. Compared to the control group (Cd^{2+}), D-cysteine significantly increased the disappearance flux of ^{109}Cd by 77% and cell-to-lumen concentration ratio by 178%.

Acute cellular toxicity of cadmium

Cd^{2+} and cadmium-sulfhydryl conjugate were transported into the epithelial cells, which resulted in substantial cadmium accumulation in the cytosol of the epithelial cells as shown by the cell-to-lumen concentration ratios in all experiments when perfusate Cd^{2+} concentration was 0.73 µM. No visual evidence of acute cellular toxicity such as cellular swelling, blebbing of the luminal membrane, and cellular vital dye uptake, was noted in S_2 segments. However, when it was necessary to perfuse with 20µM Cd^{2+} there was slight cellular swelling at the perfusion end of the tubule for approximately 40µm along the tubule but there was no vital dye uptake and only an occasional bleb from the luminal membrane of this affected region.
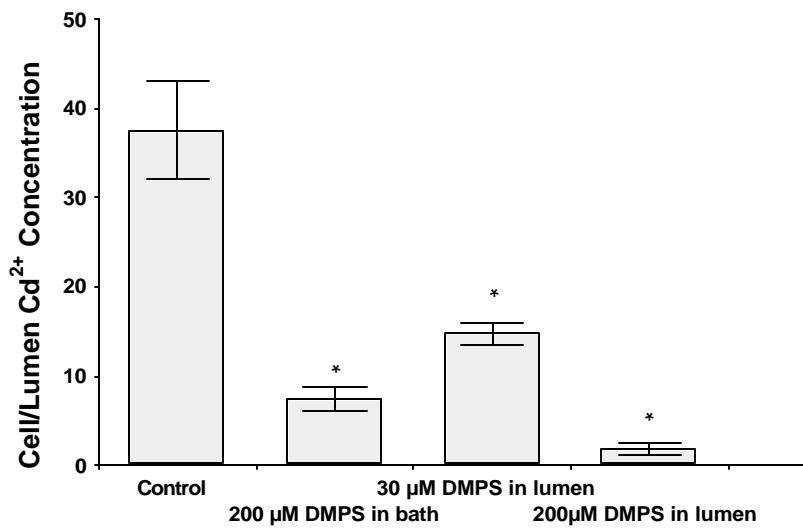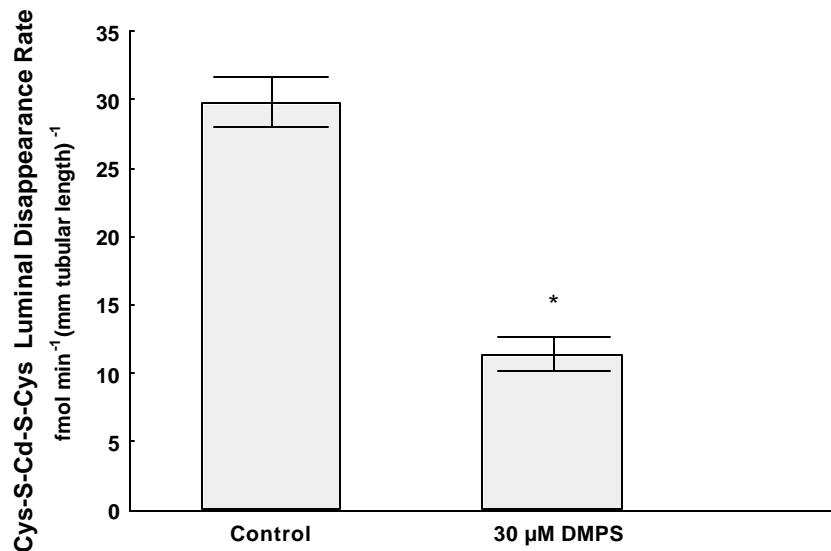
A.

B.
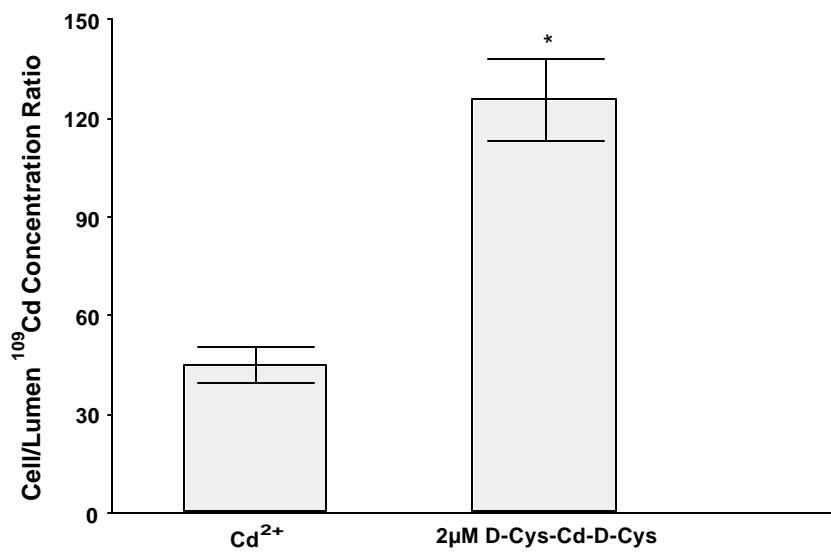
Figure 18: The effect of D-Cysteine on the lumen‑to‑cell transport (A) and cell‑to‑lumen ratio (B) of Cd^{2+} in isolated S_2 segment of the proximal tubule of the rabbit perfused with 0.73 μM Cd^{2+} (at 37°C). Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a significant statistical difference, P< 0.05.

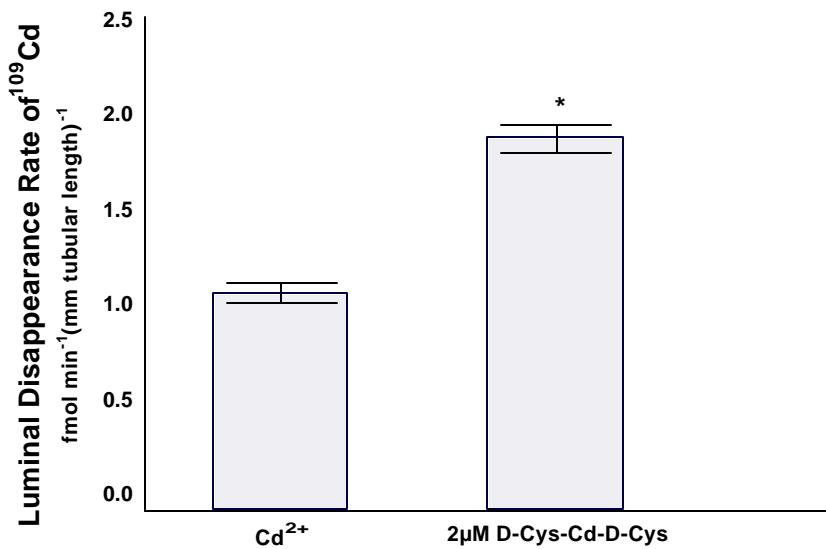**Comparison of transport of cadmium-cysteine conjugates in S_1, S_2 and S_3 segments**

Perfusate containing 0.73μM Cys-S-Cd-S-Cys was perfused through the lumen of S_1, S_2, and S_3 proximal tubular segments. In S_1 segment, the J_D of Cys-S-Cd-S-Cys was 1.23 ± 0.08 fmol min^{-1} (mm tubular length)$^{-1}$ and the cell-to-lumen concentration ratio was 76.83 ± 14.09. In S_2 segment, the J_D of Cys-S-Cd-S-Cys is 2.92 ± 0.31 fmol min^{-1} (mm tubular length)$^{-1}$ while the cell-to-lumen concentration ratio was 282.3 ± 55.0. In S_3 segment, the J_D of Cys-S-Cd-S-Cys was 3.09 ± 0.25 fmol min^{-1} (mm tubular length)$^{-1}$ while the cell-to-lumen concentration ratio was 250.3 ± 25.3. The J_D of Cys-S-Cd-S-Cys in S_2 segment was comparable to that in S_3 segment, but significantly more than that in S_1 segment, Fig.19.

Comparison of transport of L-cystine in S_1, S_2 and S_3 segments

Perfusate containing 20μM ^{14}C-L-cystine was perfused through the lumen of S_1, S_2, and S_3 proximal tubular segments. In S_1 segment, the J_D of was 112 ± 3.8 fmol min^{-1} (mm tubular length)$^{-1}$ while the cell-to-lumen concentration ratio was 53.2 ± 7.8. In S_2 segments, the J_D of was 138 ± 4.0 fmol min^{-1} (mm tubular length)$^{-1}$ while the cell-to-lumen concentration ratio was 232 ± 24. In S_3 segment, the J_D of -cystine was 248 ± 14.7 fmol min^{-1} (mm tubular length)$^{-1}$ while the cell-to-lumen concentration ratio was 176 ± 27.6. The J_D of L-cystine progressively increased from the S_1 to S_3 segment, Fig.20.

A
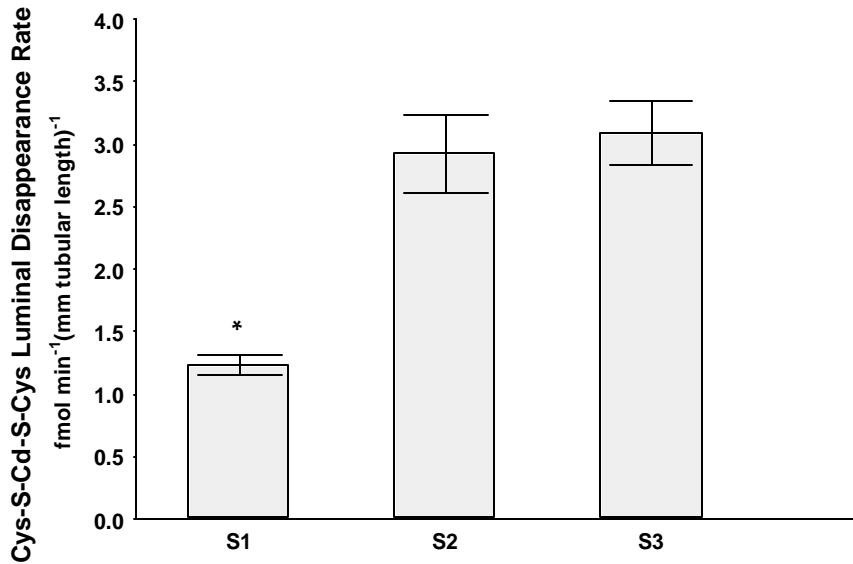
B.
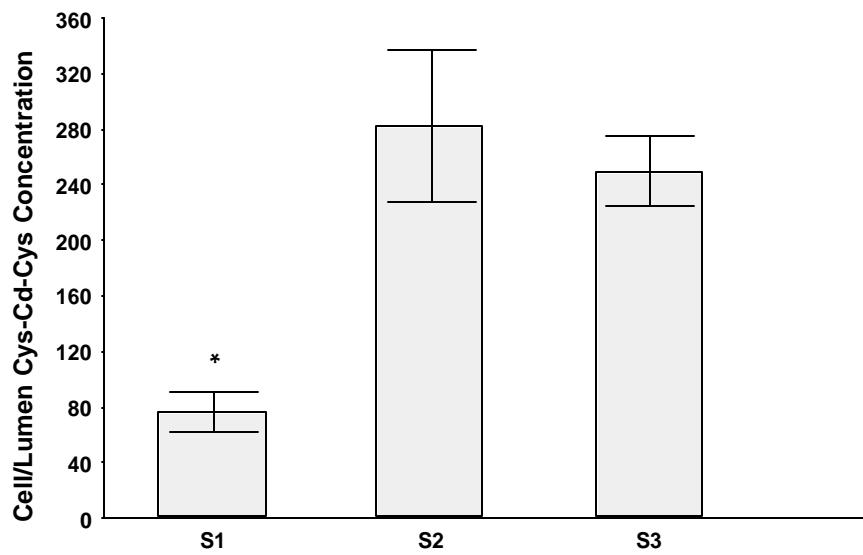
Figure 19: The differences in the transport rate (A) and cell-to-lumen ratio (B) of Cys-S-Cd-S-Cys in S_1, S_2 and S_3 proximal tubular segments (at 37ºC). Each value represents the mean ± SE for a sample size of five or six. . The "*" indicates a significant statistical difference, $P< 0.05$.

A.
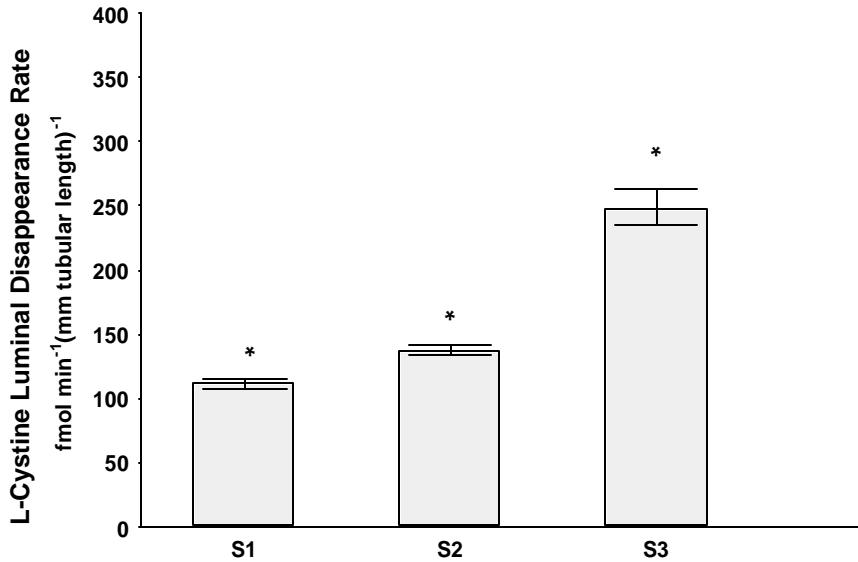
B.
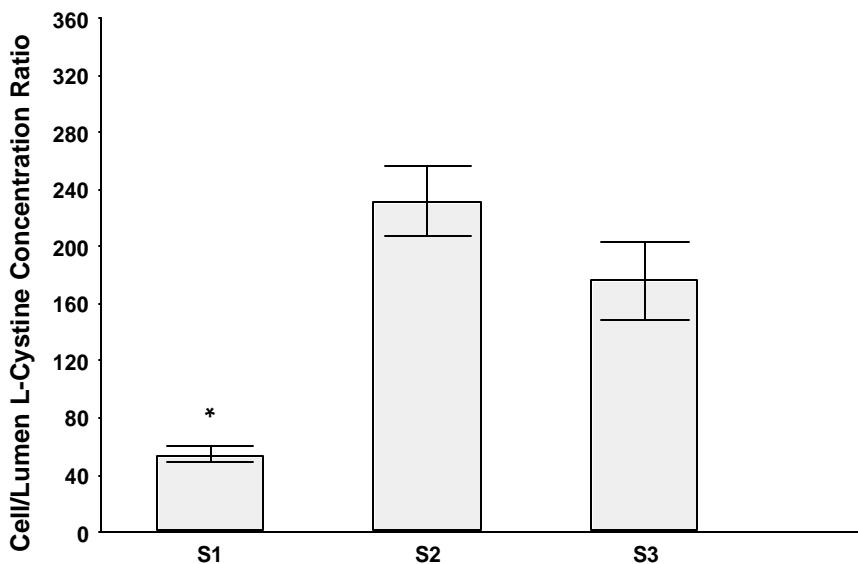
Figure 20: The differences in the transport rate (A) and cell-to-lumen ratio (B) of L-Cystine in S_1, S_2 and S_3 proximal tubular segments (at 37ºC). Each value represents the mean ± SE for a sample size of five or six. The "*" indicates a statistical different compared to the other two groups, $P < 0.05$

CHAPTER V: DISCUSSION

The purpose of the present study is to investigate the transport systems that are involved in the uptake of ionic cadmium and cadmium-sulfhydryl conjugate at the apical membrane of the rabbit renal proximal tubule.

Ionic cadmium may share the membrane proteins involved in the transport of essential elements, such as iron, zinc and calcium, into target epithelial cells. DCT1, a newly discovered metal ion transporter, was recently described as a major mechanism for cellular uptake of Fe^{2+} and other divalent metals (Gunshin, Mackenzie et al. 1997). DCT1 accepts a broad range of metal ions, favoring the divalent cations Fe^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+}, Ni^{2+}, and Pb^{2+}, but notably Ca^{2+} is not transported by this DCT1. However, the reports are not consistent concerning the cellular location of DCT1 in the proximal tubules. It has been reported that DCT1 is either located in the apical membrane or cytosol of the proximal tubule. In the present study, the role of DCT1 in luminal uptake of ionic cadmium is examined in the S_2 segments of the proximal tubules. The data indicated that Fe^{2+} inhibited the uptake of cadmium across the apical membrane significantly [Fig.5]. This result agreed with the finding of Francois et al. that DCT1 may be involved in the translocation of divalent cations at the brush border of kidney proximal tubules (Canonne-Hergaux and Gros 2002). However, this did not agree the conclusion made by Ferguson et al that DCT1 is mainly expressed in the cytosol rather than at the apical membrane of the proximal tubule (Ferguson, Wareing et al. 2001).

As for the role of zinc transporters in Cd^{2+} transport, the data from the current study showed that Zn^{2+} caused an inhibition in cadmium transport [Fig.6]. In light of recent findings, it is likely that the ZTL1 transporter is involved in the luminal transport of cadmium. ZTL1 is a recently discovered Zn^{2+} transporter in the luminal membrane of epithelia (Cragg, Christie et al. 2002). Using RT-PCR, the highest expression level of ZTL1 was detected in kidney compared to all mouse tissue analyzed (Cragg, Christie et al. 2002).

In order to test the possibility of Ca^{2+} transport systems being involved in Cd^{2+} transport, the S_2 segments of the proximal tubules were co-perfused with Cd^{2+} and with different concentrations of Ca^{2+}. When the concentration of Ca^{2+} was increased from 1.3mM (a physiological concentration of unbound ionic calcium) to 1.95mM and 2.6mM, respectively, 27% and 69% reduction in luminal disappearance transport rate of Cd^{2+} was observed [Fig.7]. These data showed that the transport of Cd^{2+} was inhibited by increased Ca^{2+} concentrations, which implied that the Ca^{2+} transport mechanism may also be involved in Cd^{2+} uptake in the proximal tubule. However, the molecular nature of the apical entry mechanism for Ca^{2+} in the proximal tubule has remained obscure to date. Some studies tend to support the hypothesis that Ca^{2+} diffuses from lumen to blood via paracellular pathway in the proximal tubule (Fromter and Gessner 1974). But the temperature-dependent experiment in the present study indicated that the paracellular pathway did not play a significant role in Cd^{2+} uptake because practically no Cd^{2+} was absorbed at low temperatures [Fig.14]. The temperature-dependent experiments also suggests that some calcium channel may be involved in the transport of Cd^{2+} if one takes

in to account that it is now known that the TRP channel family is temperature-dependent and more specifically the Ca^{2+} transporting channels TRPV5 and TRPV6 (Hoenderop, Nilius et al. 2005) Some studies reported DHP-sensitive calcium channels present in renal proximal tubule cells, and these channels express some characteristics of L-type calcium channels (Zhang and O'Neil 1996; Zhang and O'Neil 1996). To further determine whether voltage-activated, L-type calcium channels could account for the reduction in Cd^{2+} uptake in response to the high concentration of Ca^{2+}, 100μM verapamil or diltiazem, voltage-gated calcium channel blockers, was co-perfused with Cd^{2+} through the lumen of the tubule. The data showed that verapamil had no effect on cadmium lumen-to-cell transport, which is consistent with the data obtained by Templeton (Templeton 1990). Though diltiazem caused a 26.7% reduction on cadmium lumen-to-cell transport [Fig. 8], the inhibition is not statistically significant. These data demonstrated that the Ca^{2+} transport proteins involved in Cd^{2+} lumen-to-cell transport in these experiments are not voltage-gated calcium channels. These might be some TRP channels or calcium-selective channels that resemble the classified voltage-dependent calcium channels in part. In addition, the tubules were also perfused under the low calcium concentrations of less than 1.3mM, however, the tubules couldn't survive under the lower level of Ca^{2+}, and the reasons are not certain. Possibly, the low levels of calcium compromised the tight junctions of epithelial cells.

Since cadmium has a high propensity to bind to the sulfhydryl groups, the cadmium conjugates with sulfhydryl-containing amino acid molecules are considered the main form of cadmium *in vivo* in contrast to free Cd^{2+}. In the present study, the lumen-to-

cell transport of cadmium-cysteine conjugate (Cys-S-Cd-S-Cys), cadmium-homocysteine conjugate (Hcys-S-Cd-S-Hcys) and cadmium-N-acetylcysteine conjugate (NAC-S-Cd-S-NAC) was investigated. The molecular structure of these conjugates is very similar to that of the amino acid L-cystine (Cys-S-S-Cys). Therefore, due to the structural similarity, these cadmium-sulfhydryl conjugates may mimic L-cystine and compete for the transport via L-cystine transporters. The data from the current study confirmed this hypothesis. Addition of L-cystine to the perfusate with Cys-S-Cd-S-Cys, Hcys-S-Cd-S-Hcys or NAC-S-Cd-S-NAC reduced the transport of these conjugates significantly [Fig.9,10,11]. The transporter involved in the transport of these conjugates might be the amino acid transporter, system b^{0+}. System b^{0+} is a heterodimeric amino acid transporter that is composed of a light chain, $b^{0+}AT$, and a heavy chain, rBAT (Palacin, Estevez et al. 1998; Palacin, Fernandez et al. 2001). It is expressed in the luminal membrane of the epithelial cells in the proximal tubule (Furriols, Chillaron et al. 1993; Pfeiffer, Loffing et al. 1999). System b^{0+} has a high affinity for L-cystine and is responsible for the uptake of L-cystine across the luminal membrane (Palacin, Estevez et al. 1998; Palacin, Fernandez et al. 2001). The active absorption of L-cystine through System b^{0+} is a counter-transport that is driven by high intracellular concentration of neutral amino acids together with the membrane potential and intracellular reduction of L-cystine to L-cysteine. It has been reported that system b^{0+} is involved in the uptake of the mercury conjugates, Cys-S-Hg-S-Cys (Bridges, Bauch et al. 2004). To examine whether system b^{0+} is involved in the transport of cadmium-sulfhydryl conjugates, L-arginine, L-aspartic acid, L-glutamate or L-methionine was co-perfused with Cys-S-Cd-S-Cys. L-arginine, a substrate for system

b^{0+}, inhibited the transport of cadmium-sulfhydryl conjugates, whereas L-aspartic acid and L-glutamate which are not the substrates for system b^{0+} did not affect the uptake of this conjugate. The neutral amino acid, L-methionine, increased the transport of Cys-S-Cd-S-Cys presumably because accumulation of L-methionine in the cell trans-stimulated the uptake of Cys-S-Cd-S-Cys into the cell through system b^{0+} [Fig.11]. These results further support the hypothesis that cadmium-sulfhydryl conjugates behave as a functional molecular homolog of L-cystine for system b^{0+}.

In addition to L-cystine, the molecular structure of Cys-S-Cd-S-Cys also resembles that of dipeptides. Peptide transporter 2 (PepT2) is localized in the apical membrane (Palacin, Nunes et al. 2005), and it has been reported to transport the conjugate of histidine-Zn-histidine (Piersol *et al.*, 2006). In the present study, in order to investigate the role of PepT2 in cadmium transport, Gly-Sar, a non-metabolizable dipeptide substrate for PepT2, was added to the perfusate with Cys-S-Cd-S-Cys. The data demonstrated that Gly-Sar did not affect the disappearance flux of cadmium from the lumen [Fig.12], which suggested that PepT2 is not involved in the transport of Cys-S-Cd-S-Cys.

Although most agreed that cadmium has a high affinity for sulfhydryl groups, the stability of the bond between cadmium and sulfhydryl groups is still in doubt. In the present study, it was hypothesized that if the bond is weak, ionic cadmium will be released from the conjugates easily, and the transport of the released cadmium will be inhibited by Zn^{2+}, as evidenced by the inhibition of Cd^{2+} transport by Zn^{2+} reported above in this study. However, the data from the current experiment indicated that Zn^{2+} did not

significantly change the transport of Cys-S-Cd-S-Cys [Fig.13], which suggests that cadmium-sulfhydryl conjugates are formed with tight bonds to Cd^{2+}.

Additional evidence for transport mechanisms being involved in the uptake of ionic cadmium and conjugates was revealed by the effects of temperature on the transport of ionic cadmium and Cys-S-Cd-S-Cys. The transport of Cd^{2+} and Cys-S-Cd-S-Cys appears to be temperature-dependent processes [Fig.14, 15]. Reduction in temperature caused a significant decrease in the transport of Cd^{2+} and Cys-S-Cd-S-Cys, which excluded the possibility that the loss of cadmium from the lumen of the tubule was due to nonspecific binding. Thus temperature experiments further supported the conclusion that disappearance of either Cd^{2+} or cadmium-sulfhydryl conjugates from the luminal fluid was due to transport processes not to nonspecific binding to the luminal membrane.

The toxicity caused by heavy metals can be reversed by heavy metal chelators, such as 2,3-Dimercaptopropane-1-sulfonate (DMPS). A DMPS molecule has two active sulfhydryl sites that can form complexes with cadmium. The cadmium-DMPS complex is large and difficult to be absorbed by the renal proximal tubules, thus DMPS may reduce the toxicity caused by cadmium. It has been reported that when DMPS is added to the bathing solution, it prevented the toxicity caused by mercury (Hg^{2+}) in the lumen of the proximal tubules (Zalups, Parks et al. 1998). In the present study, the tubules were perfused with a high concentration (20μM) of Cd^{2+} in order to cause toxicity. Slight cell swelling at the perfusion end of the tubule was observed. When 200μM DMPS was added in the bathing solution, the toxicity was abolished, and the intracellular accumulation of Cd^{2+} was reduced, but the lumen-to-cell transport rate was not changed

significantly. The possible explanation for these results may be due to the secretion of Cd^{2+}-DMPS conjugate from the cytoplasm across the luminal membrane into the lumen. While Cd^{2+} was being transported into the cell across the luminal membrane DMPS was simultaneously being transported into the cell across the basolateral membrane. Inside the cell, DMPS chelated Cd^{2+} and formed a conjugate. This conjugate might be exported into the lumen across the luminal membrane, thereby decreasing the intracellular accumulation and not changing luminal Cd^{2+} concentration. This evidence suggests the possibility of a secretary mechanism for the Cd^{2+}-DMPS located at the luminal membrane.

When 30µM DMPS was added in the perfusate with 20 µM Cd^{2+}, the luminal transport of cadmium was reduced, but not eliminated. We had hypothesized that 30µM DMPS would have eliminated the transport of all Cd^{2+} because one DMPS molecule has two sulfhydryl groups, and could have bound all Cd^{2+}. When the concentration of DMPS added in the perfusate was increased to 200µM, the luminal transport of cadmium was reduced to almost zero [Fig.16]. This data seemed to indicate that although DMPS appears to be an efficient chelator for Cd^{2+}, its affinity for Cd^{2+} is not as high as that for Hg^{2+} (Zalups, Parks et al. 1998).

Since most Cd^{2+} probably binds to sulfhydryl-containing proteins *in vivo,* the effect of DMPS on the transport of cadmium-sulfhydryl conjugate was investigated in the present study. 30µM DMPS significantly reduced the transport of Cys-Cd-Cys by 62% [Fig.17]. This inhibition was more pronounced than that of 30µM DMPS on the transport of Cd^{2+}. The possible explanation lies in the size of the putative complex. Due to the two

active sulfhydryl sites, DMPS may have an attraction to, and can bind, heavy metals stronger than proteins that can bind heavy metals. It is likely that DMPS removed Cd^{2+} from L-cysteine and formed the conjugates with Cd^{2+} (DMPS-Cd^{2+}) or with L-cysteine and Cd^{2+} (Cys-Cd-DMPS-Cys). Due to the addition of DMPS, the size and morphology of the conjugate was much bigger than the conjugate formed only by DMPS and cadmium (DMPS-Cd). The bigger size and shape made it more difficult for the tubule to absorb the conjugate. This result provided the evidence that DMPS can efficiently aid the body in getting rid of cadmium via urinary excretion even if Cd^{2+} is conjugated to sulfhydryl groups of various compounds.

Theoretically, D-cysteine can act as a biological chelator for Cd^{2+}. Like its counterpart, L-cysteine, D-cysteine has a sulfhydryl group available to bind Cd^{2+}, and it has long been believed that D-amino acids can not be transported through the amino acid transporters. Therefore, it was expected that the cadmium complexed with D-cysteine probably would not be absorbed by the renal epithelial cells. However, our data indicated that D-cysteine increased the absorption of Cd^{2+} significantly [Fig.18]. Although the reason for the increase is not certain, our data has ruled out the role of D-cysteine in being an effective chelator. Apparently, some amino acid transport systems can transport D-amino acids.

Robinson et al. have demonstrated that Cd^{2+} is avidly taken up in the proximal tubule, especially in S_1 segment. However, it was shown that S_1 segments are very vulnerable to the toxic effects of Cd^{2+}; consequently no transport data was obtained (Robinson, Barfuss et al. 1993). In addition, other reasons for using the S_2 segments in

this study is the fact that the S_2 segments are straight, easier to dissect and perfuse than S_3 segments, therefore, S_2 segments were chosen for the current study of Cd^{2+} transport. But it was unknown which segment is most suitable for transport studies of cadmium-sulfhydryl conjugates. In the current study, the transport rates of Cys-S-Cd-S-Cys in S_1, S_2, and S_3 segment were compared at the same perfusate concentration, 0.73 μM. The transport rate of Cys-S-Cd-S-Cys in S_2 segment was comparable to that in S_3 segment, while the transport rate of Cys-S-Cd-S-Cys in S_1 segment was about 58% less than that in S_2 and S_3 segments [Fig.19]. This pattern of transport axial heterogeneity of Cys-S-Cd-S-Cys follows the distribution pattern of system $b^{0, +}$-rBAT complex, it primarily being located in S_2 and S_3 segments rather than S_1 segment. In addition, the present study indicates the pattern of transport of L-cystine follows the same axial pattern as Cys-S-Cd-S-Cys transport, greater transport in the latter S_2 and S_3 segments [Fig.20], adding credence to the hypothesis that cadmium conjugates are transported by the same transport mechanism as L-cystine.

The current study clearly demonstrated that cadmium could be transported at the luminal membrane of the S_2 segment of the proximal tubule by multiple mechanisms, depending on the form in which it is presented to membrane. Ionic cadmium, Cd^{2+}, seems to utilize the zinc transporter, ZLT1, the iron transporter, DCT1 (?), and some type of calcium-selective channel to be transported into renal proximal tubular epithelial cells. Alternatively, cadmium conjugates of L-cysteine appear to be transported by L-cystine transporters (system b^{0+}). Both the absorption of ionic cadmium and cadmium conjugates of L-cysteine can be reduced by the heavy metal chelator, DMPS [Fig.21]. This

knowledge permits the development of strategies for treatment against cadmium gaining access to the cytoplasm of the proximal tubular cells in order to prevent or minimize its nephrotoxic effects during conditions of cadmium exposure.
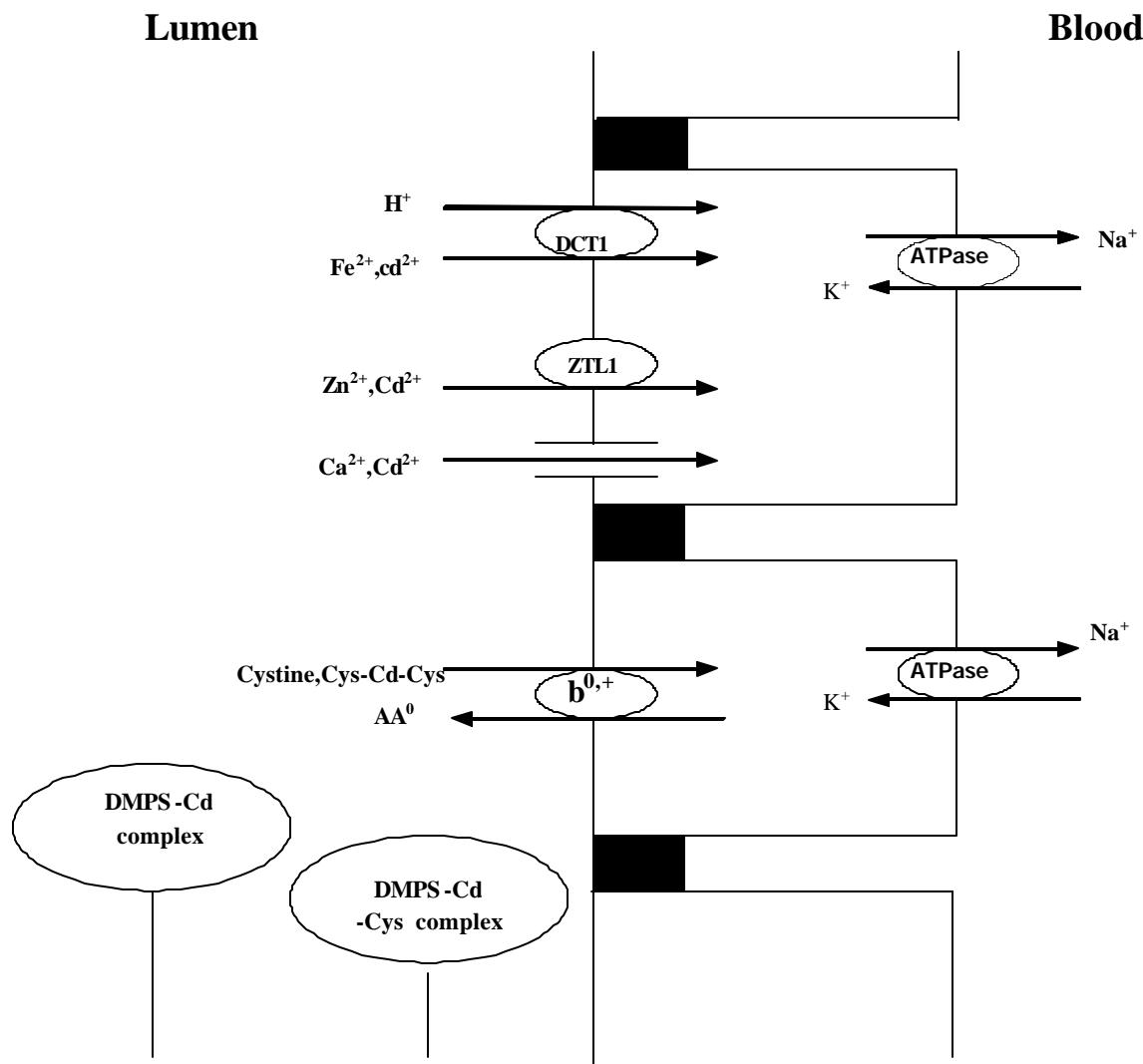

Figure 21: Mechanisms proposed to be involved in the luminal uptake of cadmium by the proximal tubular cells.

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