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Aquatic Models for the Study of Renal Transport Function and Pollutant Toxicity

by David S. Miller*

Studies of renal cell transport mechanisms and their impairment by xenobiotics are often limited by technical difficulties related to renal tubule complexity. Problems include the juxtaposition of multiple tubule segments with different transport functions and severely limited access to the tubular lumen. Some limitations can be overcome by the careful selection of an appropriate aquatic experimental system. Two aquatic models for the vertebrate proximal segment are discussed here. The first is the kidney from certain marine flounder, which offers the following advantages: long-term viability, little tissue of nonproximal origin, and easy tubule isolation. Data are presented to demonstrate how studies with flounder kidney can be used to elucidate cellular mechanisms whereby different classes of toxic pollutants may interact. Results from these experiments indicate that the excretion of certain anionic xenobiotics can be delayed (1) by other anionic xenobiotics that compete for secretory transport sites and (2) by compounds that disrupt cellular ion gradients and energy metabolism needed to drive transport.

The second system is the crustacean urinary bladder, a simple, flatsheet epithelium. Bladder morphology and transport physiology closely resemble those of vertebrate proximal segment. Electron micrographs show a brush border membrane at the luminal surface, numerous mitochondria, and an infolded serosal membrane, while *in vivo* and *in vitro* transport studies show reabsorption of NaCl, nutrients and water and secretion of organic cations; organic anions are secreted in bladders from some species and reabsorbed in others. Moreover, since bladders can be mounted as flat sheets in flux chambers, studies with this tissue avoid the problems of complex renal tubule geometry and tissue heterogeneity that limit transport studies in proximal tubule.

The Comparative Approach

The kidney is a major site of environmental pollutant-mediated toxicity, with the consequences of impaired renal function being potentially life threatening. Thus, it is important to understand cellular mechanisms that underlie normal renal function and to determine how they are affected by nephrotoxic pollutants.

Important contributions to our knowledge of renal function and pollutant nephrotoxicity have been made by investigators using the comparative approach with aquatic animals. Two examples illustrate this point. In the 1920s, E. K. Marshall and co-workers (*1,2*) used the aglomerular kidney of the goosefish, *Lophius*, to demonstrate that tubular solute secretion is one of the fundamental processes utilized in urine formation. More recently, Trump and collaborators took advantage of the extended viability and structural simplicity of flounder renal tubular tissue to document the morphological changes that occur in proximal tubules exposed to transport inhibitors, metabolic inhibitors and heavy metals (*3*).

Investigators utilizing the comparative approach recognize that certain aquatic organisms provide preparations which, compared to those from warm-blooded animals, may exhibit extended viability, greatly simplified and occasionally exaggerated renal function, and a morphological simplicity which permits easier access to the processes under study. Clearly, the aquatic environment contains a wide range of organisms, all with specialized excretory organs or excretory cells performing many of the same functions as mammalian kidneys. This diversity provides ample opportunity for the comparative renal physiologist/toxicologist searching for simple model systems. For example, Forster (*4*) pointed out that even within vertebrates a wide spectrum of kidney types exist, some being aglomerular, some without loops of Henle, and some with no distal segment. Each of these deletions can be found among marine teleost fish, some of which lack all three specialized tissues.

Described here are two aquatic models for the renal proximal tubule: one, flounder renal tubule, is well established with a history of more than 30 years (*5–7*);

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the other, crustacean urinary bladder, has only recently attracted attention. Both have great potential to expand our understanding of basic renal transport mechanisms and how they interact with pollutants.

Renal Transport

Some general principles of renal function will be reviewed before these specific models are discussed. The methods used to study transport in renal epithelia will also be listed. Recent, comprehensive reviews of vertebrate (mostly mammalian) renal anatomy, biochemistry, physiology, and methodology are available (*8*).

Urine Formation

During the complex process of urine formation, animal renal systems perform two essential functions: (1) they remove potentially toxic waste products and xenobiotics from body fluids, and (2) they contribute to the maintenance of a constant and optimal internal environment, functioning as one component of an integrated system regulating body fluid composition and volume.

Figure 1 illustrates the relationships between small solute distribution in an animal's fluid compartments and mechanisms of urine formation. This scheme holds not only for endogenous solutes, but also for xenobiotics, some of which are nephrotoxins. Compartments may be separated by barriers that are semipermeable or selectively permeable to solute movement, or by no physical barrier other than diffusion distance. Solutes may exist in fluid compartment in both free and bound forms, the relative concentrations of each being determined by mass action relationships. Solutes move between compartments by a combination of bulk flow, simple diffusion, and carrier-mediated processes, the mechanism depending on the nature of the solute and the barrier. For the most part, microsolutes gain access to renal tissue in their free form through plasma and the extracellular fluid bathing the tissue. Important exceptions are those classes of microsolutes that are bound to proteins small enough to pass through the glomerular ultrafilter; heavy metal-metallothionein complexes are an example of these (*9*).

Three processes are involved in urine formation: ultrafiltration at the glomerulus, tubular reabsorption, and tubular secretion. Ultrafiltration is a sieving process in which plasma solutes pass through glomerular pores into the urinary space according to size. The passage of molecules 20–42 Å in radius is retarded; molecules with radii over 42 Å are excluded completely. Reabsorption is the removal of solutes and water from the ultrafiltrate, and secretion is the addition of solutes and water to the ultrafiltrate. These last two processes are facilitated by and regulated through specialized transport proteins located in the plasma membranes of the epithelium lining the renal tubule and urinary bladder. Xenobiotics interact with specific membrane-bound, transport proteins, and these interactions are

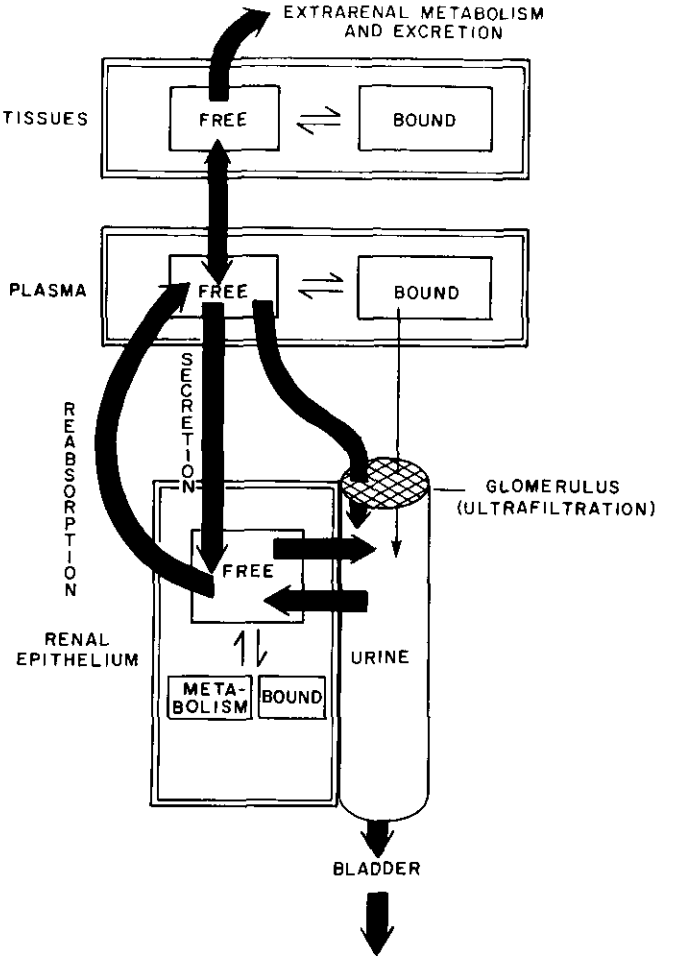

FIGURE 1. The relationships between microsolute distribution in body fluid compartments and mechanisms of urine formation. The nature of the barriers separating compartments determines both the transport mechanisms available and the molecular forms of the solute transported, i.e., free or free plus bound. Urine formation involves first ultrafiltration of plasma at the glomerulus, where plasma solute and water follow primarily an extracellular path. Through bulk flow and molecular sieving, water, microsolutes, and small macromolecules (with adsorbed microsolutes) pass into the urinary space, where the composition of the ultrafiltrate is modified further by reabsorption and secretion. Through reabsorption and secretion, the selective permeability characteristics of the epithelium lining the urinary space (i.e., the tubular and urinary bladder epithelium in vertebrates) play a major role in determining final urine composition. Transcellular and extracellular pathways are utilized. When cellular membrane barriers are crossed, both simple diffusion through the membrane and specific, carrier-protein-mediated routes may be involved. Extracellular (paracellular) pathways tend to be size-restrictive and cation-selective.

important determinants of toxicity—first, because transport proteins play a critical role in xenobiotic excretion, and second, because membrane proteins are sensitive targets of xenobiotic action.

Tubular Transport Mechanisms

The lipid cores of cellular membranes are hydrophobic; thus, small hydrophylic molecules, e.g., water, ions,

sugars, and amino acids, are poorly permeable. For rapid transport, polar solutes utilize protein-lined aqueous pores and specialized carrier proteins embedded in the plasma membranes. Local permeabilities along the renal epithelium are determined by many factors, including: (1) arrangements of specialized proteins, protein complexes, and lipids within cellular membranes, e.g., channels, carriers, and pumps; (2) the modification of their gating properties by hormones and secondary messengers; (3) available links to cellular energy supplies; and (4) extracellular (paracellular shunt) pathways, which are cation-selective. Examples of selected transcellular solute transport mechanisms are shown in Figure 2 and discussed further in the figure legend. Available evidence indicates that certain widespread environmental pollutants, e.g., many heavy metals, can selectively modify both passive and metabolically driven permeability characteristics of cell membranes (*10,11*). When this occurs in renal epithelia, the ability to remove toxic wastes and to regulate the composition of extracellular and intracellular fluid compartments may be impaired.

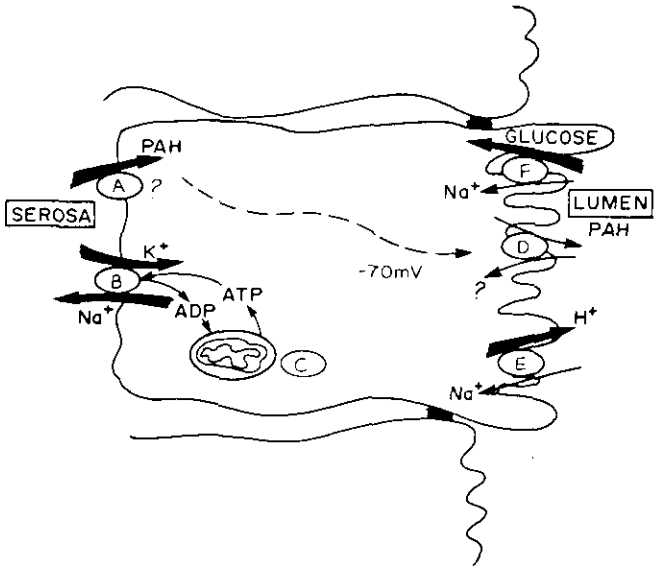

FIGURE 2. Some carrier-mediated, membrane transport mechanisms found in renal proximal tubular cells. Not all mechanisms known to exist in these cells are shown. Those depicted are related directly or indirectly to the overall process of organic anion (PAH) secretion. PAH is transported into the cell by a specific organic anion carrier (*A*) in the serosal membrane. This process is active, i.e., endothermic, in that cellular accumulation can occur in the face of steep chemical and electrical potential gradients. However, the mechanism coupling transport to cellular metabolism is poorly defined. It may involve coupling of PAH influx to the exothermic influx of Na^+ (symport). The extracellular-to-intracellular electrochemical potential gradient for Na^+ favors influx. Thus, it could provide the potential energy needed to move PAH into the cell. This gradient is maintained by the enzyme, Na,K-ATPase (*B*), which uses the energy liberated from the splitting of ATP to drive Na^+ out of the cell and K^+ into it. In turn, ATP is produced by *oxidative phosphorylation of ADP in the mitochondria* (*C*). PAH traverses the cell interior by simple diffusion and exits into the tubular lumen, utilizing a carrier protein (*D*) located in the luminal (brush border) membrane. This step is energetically downhill; however, studies in mammalian membrane vesicles show that PAH transport can be driven by an oppositely directed hydroxyl gradient (counter transport). Other carriers shown are Na^+-H^+ (*E*) and Na^+-glucose (*F*) systems. These draw on potential energy stored in the transmembrane Na^+ gradient to drive transport.

Transport Methodology

A wide variety of *in vivo* and *in vitro* techniques have been developed for studying renal transport mechanisms and the alterations caused by xenobiotics. These allow one to focus on many essential aspects of renal function at multiple organizational levels, from the whole animal down to the molecular; they are listed in Table 1. In general, moving the focus of study from a higher to a lower organizational level, e.g., from whole animal to isolated subcellular component, should provide more detailed mechanistic information. However, this may come at the expense of disrupted communication between the kidney and other organs or between organelles within the same cell. Thus, toxicological and basic findings in isolated systems should be related back to the intact tissue and the whole animal to assess their physiological significance.

Flounder Renal Tubule

Let us next consider some specifics of renal function in the first model system. The animals to be considered are marine flounder, specifically the southern flounder (*Paralichthys lethostigma*), a euryhaline teleost, and the winter flounder (*Pseudopleuronectes americanus*), a stenohaline marine teleost. Like all vertebrates, marine teleost kidneys are comprised of many functional units (nephrons) arranged in parallel. Compared to higher vertebrates, teleost nephrons are greatly simplified. In mammals, up to a dozen distinct tubular segments have been recognized. In the nephron of the marine southern flounder, Hickman and Trump (*12*) list, from the glomerulus down, a neck segment, two proximal segments, and a collecting duct. These are of unequal length, with almost 90% of the total tubule length

Table 1. Techniques used to study renal transport at different levels of tissue organization.

Level	Technique	References[a]
Whole animal	*Clearance*	(*19, 34, 36*)
Organ	Perfused organ (*in situ* or excised)	—
Tissue	Slices, isolated tubules, tissue sheets in flux chambers	(*5, 23, 28–30, 32, 37*)
Cellular	Cell culture	(*38*)
Organelle	Isolated membrane vesicles, isolated mitochondria	(*24, 26, 39, 40*)
Molecule	Purified carrier protein reconstitued in artificial membranes, purified enzymes	—

[a] When given, reference numbers refer to representative studies using aquatic species.

being proximal segment. Other marine flounder also show a preponderance of proximal tubular tissue, but some have a short distal segment as well, an example being the winter flounder.

Figure 3 shows the morphology of the isolated tubular mass preparation from winter flounder kidney. This simple but extremely useful preparation for studying transport was introduced by Forster (*5*) and has been used for more than 30 years. The tubule is lined by a continuous epithelium. When tubular masses are teased apart, the broken ends of the tubules close off, thus preserving the appropriate tissue polarity needed for the study of secretory transport, i.e., medium to tubular lumen. Beyenbach (*13*) has recently demonstrated the presence of a sheath of smooth muscle cells on the serosal surface of the tubules. This smooth muscle layer is most likely responsible for the constriction closing the cut ends. Figure 3 also shows the brush border membrane which lines the luminal surface of the epithelium. This extensive and regular amplification of the cell surface facing the urinary space is characteristic of the proximal segment in all vertebrates.

The preponderance of proximal tubular tissue and appropriate tissue polarity are only two reasons why flounder kidney is a good model for studying proximal tubular transport mechanisms. Others include: the ease by which tubules can be dissected free of the loose hematopoetic tissue in which they are embedded, extended tissue viability, and exaggerated secretory function *in vivo* and *in vitro*. Exaggerated secretory function *in vivo* results in part from the fact that these fish have an extensive renal portal circulation, and over a given time period, can clear solutes from a volume of blood nearly equal to the cardiac output (*14*). Exaggerated secretory function *in vitro* is due to functional retention of potent and specific transport systems expressed *in vivo*.

The present study only briefly considers overall renal transport function in marine teleosts; comprehensive reviews can be found in Hickman and Trump (*12*), Nishimura and Imai (*15*), and Pritchard and Renfro (*16*). Overall renal transport function in marine teleosts is summarized in Table 2. In general, NaCl and water are reabsorbed in a proportion that is nearly isoosmotic with plasma. Although there is recent evidence for NaCl and water secretion in winter flounder proximal segment *in vitro* (*13,17*), the physiological role of that process is unclear. The major osmoregulatory functions of the ne-

FIGURE 3. Micrograph of an isolated renal tubular mass from winter flounder kidney. The central tubular segment shows a morphology typical of vertebrate proximal segment (see text). The tubules were maintained in a physiological saline containing 10 μM CPR, a purple-red anionic dye. In the original color photograph, the CPR concentrations in the medium, cells and tubular lumen could be compared directly. They are only approximated in this black and white print. Data are from Kinter (unpublished).

Table 2. Overall renal solute handling in marine teleosts.

Reabsorbed	Secreted
Amino acids, sugars	Calcium, magnesium
Proteins	Sulfate, phosphate
NaCl and water	Organic anions
	Organic cations

phron involve secretion of the multivalent inorganic ions, Ca^{2+}, Mg^{2+}, SO_4^{2-} and PO_4^{3-}. Other important functions are related to organic solute transport. As a rule, carbohydrates, amino acids, and proteins are reabsorbed whereas charged (anionic and cationic) metabolic wastes and xenobiotics are secreted. However, there are some apparent exceptions: winter flounder renal tubules secrete both the sugar, 2-deoxygalactose (*18*) and the amino acid, phenylalanine (*19*).

It is clear that teleost kidney, like kidneys from other vertebrates, is a major site of heavy metal, phenoxyacetic acid herbicide, and organochlorine accumulation, and that accumulation can lead to morphological damage, enzyme inhibition and impaired transport (*3,7,16*). With *in vivo* dosing, the mechanistic bases for the observed effects remain largely unknown or uninvestigated. However, *in vitro* dosing studies have focused on mechanisms of pollutant toxicity; some of these are discussed further here.

Organic Anion Secretion

The present discussion focuses on the secretory transport system for organic anions. In all vertebrates studied, this system is localized to the proximal segment. Available data on the substrate specificities, energetics, and molecular mechanisms of organic anion transport in vertebrate kidney have been reviewed recently (*20–22*). Reviews of organic anion transport in flounder renal tissue have also been published (*6,7,16*). Substrates for the transport system include a wide variety of aromatic and aliphatic carboxylic and sulfonic acids. Among these are neurotransmitter metabolites, phenols, DDA [bis(*p*-chlorophenyl) acetic acid], the polar metabolite of DDT and its major excreted form, phenoxyacetic acid herbicides, some metabolites of polycyclic aromatic hydrocarbons, and the glycine, sulfate, and taurine conjugates of many xenobiotics. One model substrate used to study renal transport mechanisms is *p*-aminohippuric acid (PAH), the glycine conjugate of *p*-aminobenzoic acid; another is the anionic dye, chlorophenol red (CPR).

Figure 3 shows the steady-state accumulation of anionic dye in flounder renal tubules incubated in a medium with 10 μM CPR. Although the features are more evident in the original color photograph, Figure 3 does show the relative CPR concentrations in incubation medium, epithelium, and tubular lumen as a step gradient of gray tones. This is a striking visual demonstration of the active excretory transport of an organic anion by winter flounder renal tubules. The dye concentration appears to be somewhat higher in the cells than in the medium [this point was demonstrated unequivocally in Kinter's (*23*) microspectrophotometric studies of living tubular tissue], but it is clearly orders of magnitude higher in the luminal fluid. Indeed, MacKenzie et al. (*14*) have shown that *in vivo* urine-to-plasma concentration ratios for CPR can exceed 1000, and that *in vitro* tubular tissue-to-medium ratios can be as high as several hundred.

How are these xenobiotics excreted so efficiently? Figure 2 shows a plausible molecular model for *p*-aminohippuric acid (PAH) transport, constructed from available flounder and mammalian data (*6,7,16,20–22*). A portion of the renal epithelium is shown, with the brush border, serosal membrane, and cell interior drawn as a coordinated, transepithelial transport machine. Two membrane-bound, organic anion carrier proteins are depicted, one on each side of the epithelial cell. The serosal carrier can drive PAH into the cell against chemical and electrical potential differences (the cell interior, being ~ 70 mV more negative than the extracellular fluid, repels anions). It is probably powered directly or indirectly by the energy stored in the cellular Na^+ gradient (extracellular Na^+ concentrations exceed intracellular by nearly an order of magnitude), but the actual coupling mechanism has not been resolved in fish and has been only partially resolved in mammals (*20,21*). The Na gradient is maintained by the enzyme Na,K-ATPase, which splits ATP and uses the potential energy liberated to drive Na out of the cell and K into the cell. ATP is provided to the enzyme primarily through oxidative phosphorylation in the mitochondria.

The serosal organic anion "pump" causes PAH to accumulate in the cytoplasm (Fig. 2). Thus, exit from the cell into the tubular lumen can be driven by both chemical and electrical potential gradients. The second carrier protein, localized in the brush border (luminal) membrane, facilitates exit into the tubular lumen (*24*). There is evidence from studies with mammals that luminal efflux can be energetically linked to hydroxyl uptake (*21*); this aspect of PAH transport has not yet been examined in fish kidney.

This rather complicated cellular mechanism for excreting organic anions provides several possibilities for interaction with pollutants. Two examples follow.

Excretion of Benzo(a)pyrene (BP) Metabolites

Pritchard and Bend (*25*) injected radiolabeled BP and two of its oxidative metabolites into southern flounder and studied *in vivo* renal clearance of label. Measuring renal clearance allows one to determine if a compound undergoes net tubular reabsorption or net secretion. The clearance is the calculated volume of plasma that would have to be completely cleared of solute per unit time to account for the total amount excreted; this is a virtual volume, calculated from urine and plasma concentrations and urinary rate. Two caveats must be considered before the data can be interpreted. First, clearance values were not corrected for binding of solutes to plasma proteins. If extensive binding occurred, the true

clearance values would have been substantially higher than those reported. Thus, the values reported by Pritchard and Bend are conservative estimates of renal secretion. Second, since total excreted label was measured, the chemical forms appearing in urine may not be known for every experiment. For labeled benzo(a)pyrene-7,8-dihydrodiol (^{3}H-BP-7,8-dihydrodiol), most of the label was shown to be excreted as anionic conjugates, primarily as sulfates and glucuronides.

Table 3 shows that renal benzo(a)pyrene (BP) clearance was lower than 7-hydroxybenzo(a)pyrene (7-OH-BP) clearance, which in turn was much lower than BP-7,8-dihydrodiol (7,8-diol) clearance. The BP clearance was lower than the measured glomerular filtration rate, since the clearance ratio is less than unity. The ratio for 7-OH-BP was somewhat higher than unity, indicating perhaps weak secretion, and the ratio for the 7,8-diol was substantially greater than unity, indicating strong net secretion. Subsequently, it was shown that renal clearances for the two BP metabolites were reduced substantially by dosing fish with probenecid (*25*), the classical competitive inhibitor of the organic anion transport system. Taken together, the data demonstrate that the two metabolites are secreted (mostly as conjugates) by the organic anion system, but that BP is probably not.

The results of additional experiments (*25*) in which the clearance of radiolabeled 7,8-diol was measured in controls and fish pretreated for 60 min with the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) are shown in Figure 4. Data are given as 7,8-diol clearance over the glomerular filtration rate. Controls show the high renal clearance ratios expected of this BP metabolite. In contrast, fish pretreated with 2,4-D show dramatically reduced clearance. Although the mechanism of inhibition was not characterized further, one plausible explanation is that the herbicide, a known substrate for the organic anion transport system in vertebrates, competitively inhibits excretion of the BP metabolite. Thus, competition for a renal transport protein delayed the excretion of the toxic metabolite.

In vivo individual renal transport systems can handle many substrates simultaneously. Thus, there is potential for competitive interactions between compounds that share the same transport carriers or that utilize the same (possibly limited) energy sources to power active, excretory transport.

Table 3. Renal clearance of benzo(a)pyrene (BP) and two of its metabolites in southern flounder.[a]

Compound	Clearance, mL/hr	Clearance/GFR[b]	
BP	0.2	0.3	Reabsorption
7-OH-BP	0.6	1.3	Secretion (weak)
7,8-Diol	9.0	14.2	Secretion

[a] Data from Pritchard and Bend (*25*).
[b] GFR, glomerular filtration rate.

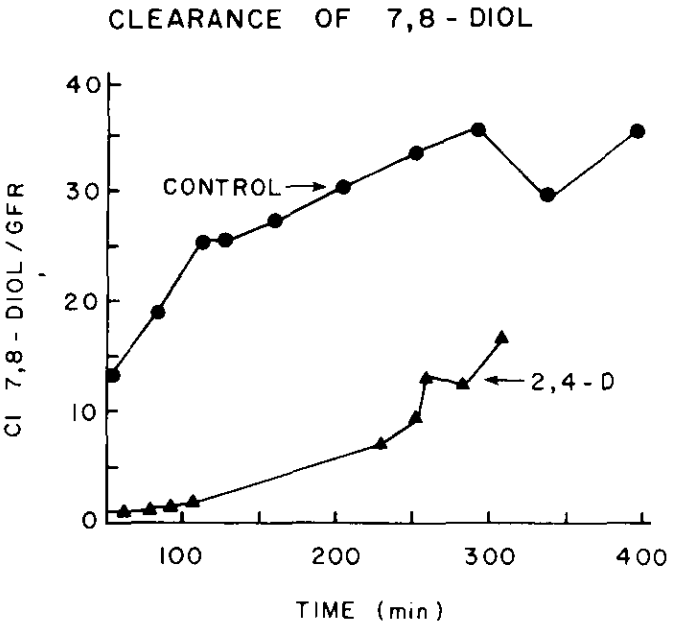

FIGURE 4. Renal clearance of BP-7,8-diol equivalents in control and 2,4-D pretreated southern flounder. Fish were given 2.5 μmole/kg ^{3}H-BP-7,8-diol at time zero. Pretreated fish were given 25 μmole/kg 2,4-D 1 hr before 7,8-diol injection. Taken from Pritchard and Bend (*25*).

Sites of $HgCl_2$ Action

The second example demonstrates how the flounder tubule can be used to identify cellular sites of heavy metal nephrotoxicity; the data cited are from the author's laboratory from unpublished and previously reported studies (*26,27*). The focus is on the organic anion transport system in winter flounder and its use to identify the site(s) of mercuric chloride action. It should be remembered that any reduction in transport through this pathway can affect the animal's ability to excrete the wide range of pollutants and metabolites that are substrates for the organic anion system.

Table 4 contains the results of *in vitro* dose-response studies in which the concentrations of several metal salts that would cause 50% inhibition of organic anion (PAH) transport in isolated winter flounder tubules and Na,K-ATPase activity in tubule homogenates were determined. All metal salts tested reduced both PAH uptake and enzyme activity, and, for a given salt, the order of effectiveness was about the same for both processes. This is not surprising, since inhibitors of the ATPase, e.g., ouabain, are also known to inhibit organic anion transport in flounder tubules (*26,28*). For the selected group of metal salts shown in Table 4, I_{50} values range from submicromolar to millimolar, with Hg and Pt appearing to be the most potent inhibitors.

The effects of mercuric chloride exposure *in vitro* on PAH accumulation by winter flounder tubules and on tubular Na and K concentrations are shown in Figure 5. Data are given as the percent of the paired control

Table 4. Heavy metal inhibition of winter flounder renal Na,K-ATPase and organic anion transport.[a,b]

Metal salt	Concentration for 50% inhibition, mM	
	ATPase	Transport
Mercuric chloride	0.00075	< 0.001
Cadmium chloride	0.4	~ 0.5
Potassium chromate	7	1
Potassium dichromate	2.5	1
Uranyl nitrate	0.3	~ 0.7
Lead (II) chloride	> 10	—
Chloroplatinic (IV) acid	0.001	0.01

[a] I_{50} values determined from dose-response curves for ouabain-sensitive, Na,K-ATPase activity in tubular homogenates and for 1–2 hr 10 μM PAH uptake by renal tubular masses. Control Na,K-ATPase activity averaged 5 μmole Pi/mg protein/hr; control PAH uptake averaged about 100 pmole/mg tissue, which is equivalent to an uncorrected tissue-to-medium concentration ratio of 10.

[b] Data from Miller (*26*), Guarino et al. (*27*), and Miller (unpublished data).

value at the exposure time indicated. Inhibitory effects were both time and dose dependent. Mercury (1 mM) caused inhibition of PAH transport, a decline in tissue K, and an elevation of tissue Na. At this high concentration, significant effects could be seen within about 1 min. With exposure times of 1 to 2 hr (not shown), significant effects were apparent with Hg concentrations of 1 μM and lower.

Based on these data and others in which $HgCl_2$ effects were measured on transport in isolated membrane vesicles, transport in metabolically poisoned tubules, tubule Na,K-ATPase activity (as ouabain-dependent respiration), and total tubule respiration rates, cellular sites responsible for Hg inhibition of organic anion transport could be determined. These are shown in order of apparent decreasing sensitivity to $HgCl_2$ in Table 5. It is not surprising that the most sensitive sites, the ATPase and ion channels regulating Na and K passive permeabilities, are on the serosal membrane, since this is the first cellular structure encountered by Hg. Hg also probably enters cells to gain access to mitochondria and inhibit respiration. However, the serosal organic anion carrier protein itself is relatively insensitive to Hg in spite of its exposed location; preliminary experiments with isolated brush border membrane vesicles suggest that the luminal carrier protein is also relatively insensitive.

These findings agree well with those of Trump and collaborators (*3*), who showed that following $HgCl_2$ exposure *in vivo* or *in vitro* flounder renal tubule cell ultrastructure was altered in a manner consistent with disrupted ion and volume regulation. It is not clear from the data presented here or from the data of Trump and coworkers whether impaired cellular ion regulation also contributes indirectly to the inhibition of oxidative phosphorylation or whether inhibition of respiration is caused solely by direct interactions between Hg and mitrochondria.

The flounder tubule-Hg data show a second manner by which two classes of pollutant may interact within

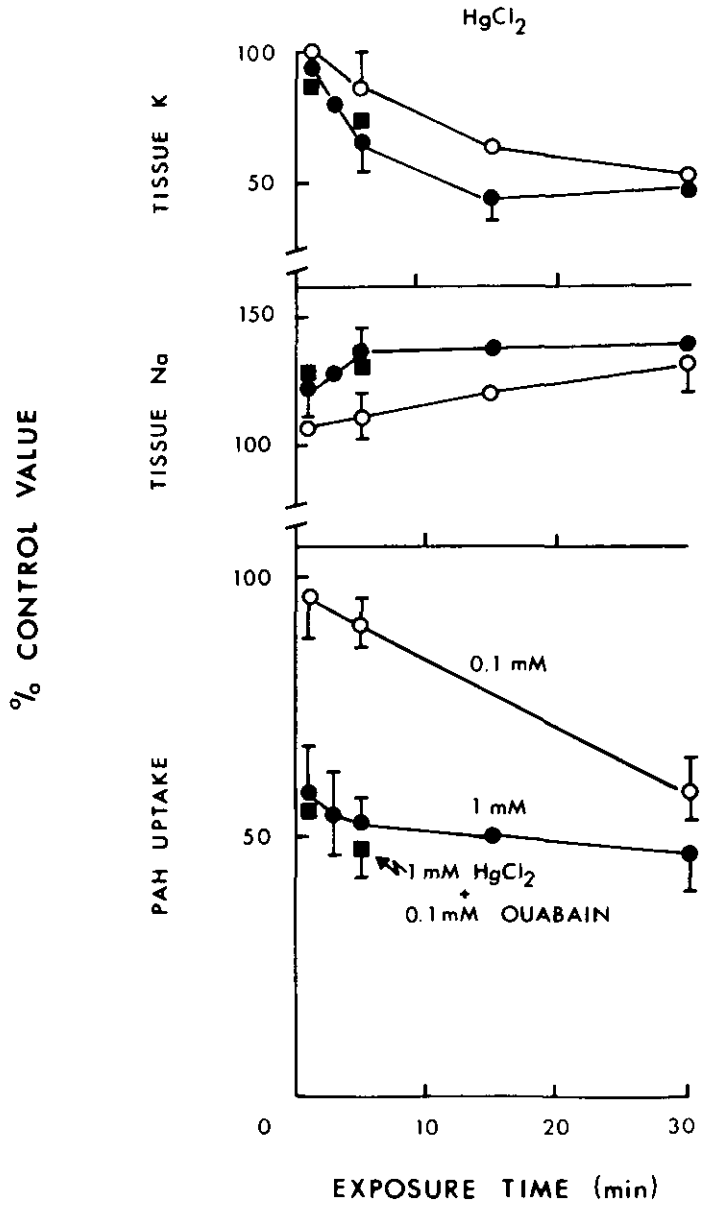

FIGURE 5. Effects of exposing winter flounder renal tubular masses to $HgCl_2$ *in vitro*. Tissue was incubated in physiological saline containing radiolabeled PAH, without (control) or with 0.1 or 1.0 mM $HgCl_2$. At the times indicated, tubular masses were removed for ion analyses and measurement (liquid scintillation counting) of PAH accumulation. Data are given as percent of control values. Control tissue (cellular plus luminal fluid compartments) averaged: for Na, 82 mEq/L tissue water; for K, 61 mEq/L of tissue water. Control PAH uptake ranged from 7 pmole/mg tissue at 1 min to 70 pmole/mg tissue at 30 min. Taken from Miller (*26*).

the kidney. By disrupting cellular ion regulation and energy metabolism, this heavy metal salt could delay the energy-dependent excretion of anionic toxicants.

Crab Urinary Bladder

The last topic to be discussed is the development of a new aquatic model for the renal proximal tubule, the

Table 5. Cellular processes affected by $HgCl_2$ in winter flounder renal tubules.[a]

Sensitivity	Process
Most sensitive	Serosal plasma membrane Na,K-ATPase Plasma membrane cation permeability Mitochondrial respiration
Least sensitive	Serosal membrane organic anion carrier

[a] Conclusions drawn from data presented in Miller (*26*) and from unpublished experiments.

crustacean urinary bladder. Renal tubule geometry makes difficult the study of transepithelial transport, since access to the urinary space is severely limited. Ideally, one could avoid this problem by slitting the tubule down its length to produce a flat sheet that could be studied in a flux chamber. This is not practical because of the small size of the tubule. Thus, one searches for model epithelia that are geometrically simpler, but functionally analogous.

The model tissue should possess five important properties. First, it should be a flat sheet epithelium so that it can be mounted and studied in flux chambers under closely controlled conditions and with detailed knowledge of the composition of the solutions bathing both surfaces. Second, it should be a single cell thick so that transport mechanisms can be localized unambiguously. Third, it should contain a single cell type to facilitate preparation of isolated cellular components for biochemical studies. Fourth, it should "look" like a transporting epithelium, with amplified surface area, structural polarity and abundant mitochondria. Finally, it should exhibit transport characteristics similar to one of the renal tubular segments.

Urinary bladders from decapod crustaceans seem to qualify by these criteria as models for renal proximal tubule. They are simple epithelia, one cell thick, with a single cell type, a columnar cell. An electron micrograph of the bladder from *Cancer magister* is shown in Figure 6. It is oriented so that the urinary space is at the top right and the serosa at the bottom. The following ultrastructural features, which are also characteristic of vertebrate renal proximal tubule cells, should be noted: a well-developed brush border at the luminal surface, numerous mitochondria, and a greatly infolded serosal membrane. To be sure, there are other morphological features not found in proximal tubule, for example, numerous vacuoles whose function is currently unknown; nevertheless, at first glance, this tissue looks like a transporting epithelium.

In collaboration with Dr. Charles Holliday, I have been characterizing the transepithelial transport properties of bladders from several species of crabs and one lobster. Our findings, along with some from other laboratories, are summarized in Table 6, which lists the many functional analogies between crustacean urinary bladder and vertebrate proximal tubule. Among these are the ability of the bladder to reabsorb nutrients and to transport organic anions and cations (*29–33*), processes localized solely to the proximal segment in ver-

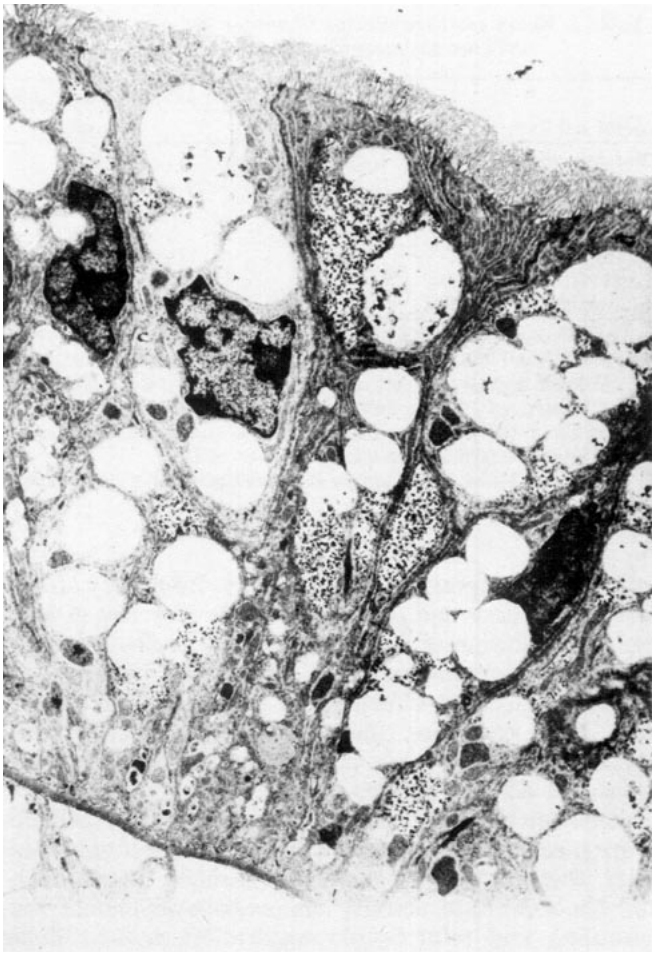

FIGURE 6. Electron micrograph of urinary bladder tissue from *Cancer magister*. The tissue is oriented so that the urinary space is at the top right and the serosal membrane is at the bottom. From Holliday (unpublished).

Table 6. Membrane transport characteristics common to crustacean urinary bladder and vertebrate renal proximal tubule.

Reabsorption of sugars and amino acids
Reabsorption of NaCl and water
Secretion of organic cations
Secretion of organic anions (some crustacean species, others reabsorb; see text)
Classified as electrically "leaky" epithelia

[a] Data supporting these conclusions may be found in the following reviews: for proximal tubule (*8*); for crustacean bladder (*33, 41*).

tebrate kidney. Except for two interesting differences, the ion dependencies, substrate specificites, and kinetics of organic anion and cation transport in crustacean bladder and vertebrate proximal tubule are very similar. One difference is that the transport of organic anions and cations is powered by glycolytic metabolism in crustacean bladder (*29,31,32*), but by aerobic metabolism in vertebrate kidney (*22*). The other difference is reflected in the data presented in Table 7. With few

exceptions, vertebrates secrete both organic anions and cations in the proximal segment (*20*). This generalization does not appear to hold for crustacean bladder. Table 7 gives secretory-to-reabsorptive flux ratios measured *in vitro* for the model organic anion, PAH, and the model organic cation, tetraethylammonium (TEA). As shown by flux ratios greater than unity, bladders from all four species secrete the organic cation. In contrast, the direction of net organic anion transport appears to be species dependent. Half of the species surveyed secrete PAH and half reabsorb.

pq: Tables 6 and 7 here?

The differences in the direction of net bladder organic anion transport between species are also expressed *in vivo*. In decapod crustacea, the bladder is the terminal element of the antennal gland, which functions as a kidney analog. The gland contains an ultrafiltration site, the coelomosac; an extensive labyrinth, capable of solute transport; in some species, a true tubular segment; and the extensive urinary bladder (*33*). These are arranged in series. Thus, for a given solute, renal function *in vivo* is expressed as the sum of the contribution of all antennal gland components.

Clearance experiments in two crabs, *C. borealis* (bladder secretes PAH *in vitro*) and *C. irroratus* (bladder reabsorbs *in vitro*), show that both species exhibit net secretion of the model substrate, PAH (*34*), and the herbicide 2,4-D (Guarino, Holliday, and Miller, unpublished data). Renal clearances for *C. irroratus* are lower than for *C. borealis*. This is consistent with bladder reabsorption in the former and secretion in the latter. Moreover, transport experiments in which the bladder was functionally isolated *in vivo* demonstrate strong, carrier-mediated PAH reabsorption in *C. irroratus*, but not in *C. borealis* (*31,32,34*). Species with reabsorbing bladders also utilize a nonrenal route of excretion, through the hepatopancreas. The 2,4-D data show that only unmetabolized herbicide is found in the urine of both species. In *C. irroratus* only, an unidentified metabolite was excreted through the nonrenal pathway. The metabolite is most likely an anionic conjugate of 2,4-D (*35*).

In addition, our data suggest that bladder transport may be a physiological variable, changing with an animal's reproductive and molt state. Some evidence for this is shown in Table 7. Most of the year, we were only able to obtain male, intermolt animals for studies of bladder function. However, in some instances we could collect enough data from females to draw limited conclusions about male-female differences. In Homarus, there were no differences in rates of organic anion transport (*33*), but bladders from females exhibited 50% greater flux ratios for organic cations than males (Table 7). In Menippe, organic anion flux ratios in males were twice those in females. Of potentially greater importance, limited data on premolt and postmolt crabs suggest that rates of organic anion transport may be correlated with molt state (*29*; and unpublished data). This finding implies that bladder organic anion transport function plays some role in the molt cycle and that transport is under hormonal control, potentially providing a simple model for the study of the mechanisms by which hormones modulate epithelial transport activity.

The crustacean urinary bladder—a simple, model epithelium—has provided for the first time ready access to both the serosal and luminal membrane transport mechanisms for organic anions and organic cations in an intact renal tissue. Using a variety of *in vivo* and *in vitro* techniques and working at multiple levels of tissue organization, we are beginning to characterize carrier specificities and energetics and to determine how different model and xenobiotic substrates for transport might interact at each site and how such interactions could relate to overall effects and physiological regulation in the integrated cellular system.

Table 7. Organic ion transport in crustacean urinary bladder.[a]

	Flux ratio (secretory/reabsorptive)	
	Organic anion	Organic cation
Cancer irroratus	0.04	186
Cancer borealis	4.0	65
Menippe mercenaria	7–14 (F–M)	—
Homarus americanus	0.3	10–15 (M–F)

[a] Flux ratios calculated from unidirectional flux data for the model organic anion, PAH, and the model organic cation, TEA. To obtain fluxes, sheets of bladder tissue were mounted in paired Lucite chambers and radiolabeled substrate was added to the Ringer's solution bathing one surface of the tissue. The appearance of label in the solution bathing the other surface was monitored using liquid scintillation counting. Original data can be found in the literature (*29, 31–33*). Flux ratios greater than unity indicate net solute secretion; those less than unity indicate net reabsorption. For both solutes, in all species tested, flux ratios were significantly different from unity ($p < 0.01$). Data from intermolt males in both *C. borealis* and *C. irroratus*, but from intermolt males (M) and females (F) in the two other species.

Conclusion

Presented here are data showing how aquatic organisms can contribute to our understanding of both normal renal function and function impaired by environmental pollutants. These are further examples of how a comparative approach to biological problems often can lead to advances that may not have been possible using more conventional renal preparations.

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