MINIREVIEW

Slices and Sacs: Limitations on Metabolic and Functional Studies in Kidney Cortex and Intestine (43959A)

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Abstract. **While all experimental models, whether based on isolated preparations *in vitro* or on intact animals, possess characteristic limitations, reliance on simplified systems mandates special care. On the basis of both older and new evidence, the present review emphasizes problems associated especially with the use of renal cortical slices and everted sacs of the small intestine. Although these preparations have found renewed acceptance, the significance of results obtained is still at times interpreted without due regard for physiological reality. Sacs and slices will undoubtedly continue to prove useful in the study of biological processes, but their application must be predicated upon recognition of what information they can or cannot yield.**

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Analysis of metabolism and function outside the living animal has a long history. Early work on digestion by isolated gastric juice, for instance, shows that the power of what today are called *in vitro* techniques was recognized long ago. At a later stage, tissue homogenates as described by the German word *Brei* found extensive use in biochemical research. Next, more intact tissue preparations were introduced and became popular for the study of function in higher animals. For instance, Warburg and his collaborators in the 1920s established much of our present understanding of tissue metabolism with the aid of thin slices of liver, kidney, and other tissues. More recently, the efforts to minimize, where possible, use of living animals for research and testing led to renewed emphasis on *in vitro* preparations. They continue to serve as powerful experimental tools; their widespread application, however, requires at times a clearer understanding of their limitations.

As with any other simplified system, the need to extrapolate back to the intact animal may introduce considerable uncertainty. For instance, the demonstration that a nonspecific protein reagent like Hg^{++} can inhibit purified Na,K-ATPase (1) does not prove that this enzyme serves as prime target of the metal *in vivo*. The difficulties of extrapolation are exacerbated when the geometry of the isolated system or the properties of its constituents have been altered during isolation. This represents a second limitation on *in vitro* experiments; an example is the potential loss of functional polarity of epithelial cell preparations *in vitro*, as discussed below specifically in reference to renal cortical slices. A third problem may arise from differences between living animals and isolated systems in such characteristics as the composition of extracellular fluid, or the changes of substrate and inhibitor concentrations with time, or the chemical form in which toxicants like heavy metals are presented to the tissue.

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The result, as is well known, is that not all conclusions based on work with isolated systems can automatically be applied to the intact animal.

Some of these limitations were recognized early in the development of *in vitro* techniques; additional problems can be deduced from more recent findings. The aim of this review is to reanalyze the disadvantages specifically of renal cortical slices and of everted sacs of small intestine for the study of solute transport and metabolism.

Renal Cortical Slices

General. Reference has already been made to the early popularity of the tissue slice techniques for metabolic studies, especially in the hands of the Warburg school. Slices were originally cut freehand; this procedure was facilitated by the introduction of manual microtomes like the Stadie-Riggs microtome. In all cases, mean thickness of slices had to be maintained significantly below 0.5 mm in order to assure proper oxygenation and permit relatively unhindered solute exchange between the suspending medium and all cells in the tissue. The slices were usually cut into cold saline, and subsequently could maintain normal respiration at 37°C for an hour or longer.

In addition to their high rates of metabolism, such slices also retain the normal ability to accumulate high intracellular potassium in exchange for low sodium levels (2). Clearly, selected aspects of membrane function continue to be active in the slices. This is further illustrated in the work of Stern *et al.* (3) on accumulation of glutamic acid by brain slices, and by the accumulation of para-aminohippurate (PAH) against its concentration gradient in slices of the renal cortex (4). Quantitatively, of course, membrane function in slices is *a priori* likely to be affected by the membrane damage inescapably incurred by the cutting of cells; the thinner the slice the more significant such damage will be.

The reproducibility and viability of tissue slices were greatly improved by the more recent introduction of automated procedures by Smith *et al.* (5). These so-called precision-cut slices have been prepared from various tissues and remain viable for many hours. Their uniformity and stability will continue to make them a preferred system, especially for the analysis of tissue metabolism. The justified enthusiasm for this preparation must, however, not obscure the fact that, like renal cortical slices in general, the precision-cut cortical slices possess significant disadvantages for the study of renal metabolism and solute transport. Indeed, the intrinsic properties of renal slices, no matter how they are prepared, impose *a priori* limitations on their usefulness for studying renal function and metabolism *in vitro*. These limitations have been previously reviewed (6), and are restated here with additional emphasis on their implications for metabolic studies.

Tissue Morphology in Slices. Reliance on slice preparations presupposes that the tissue remains viable, as defined by continued normal metabolism, and by the ability to carry out basic cellular functions. Especially in the case of renal cortex, one of these functions is solute transport, in the directions either of secretion into the tubular lumen or of reabsorption out of the tubular fluid. This fact predictably leads to an important limitation of the usefulness of renal cortical slices in the study of membrane transport processes. Indeed, a viable slice, by definition, must be able to absorb salt and water from the tubular lumen as long as the lumen remains patent. In absence of glomerular filtration but continuing fluid absorption, there is no force to replace the fluid removed from the lumen, so that collapse of tubules in normal slices can be predicted.

There is considerable evidence to validate this prediction. Boyesen & Leyssak (7), for instance, could observe no patent lumina in normal cortex slices. Figure 1 contrasts the open tubules in fresh kidney with the occluded lumina in a slice after 15 min incubation. Precision-cut slices also, as could have been predicted from their active metabolism and consequently their presumably continued ability to absorb luminal fluid, were reported to contain few open tubules in the absence of metabolic inhibitors (8). However, when sufficient mercuric chloride (1 m*M*) was added to poison cells, thereby preventing normal salt and water uptake from the lumen, the tubules tended to remain open. The functional implications of such morphological findings point directly to a reduced role of apical cell membranes in cortical slices.

It is difficult to estimate how much of the fluid originally present in the lumen remains unabsorbed in a slice at steady state. This fact, in turn prevents the determination of intracellular volumes in slices. Indeed, addition of an extracellular volume marker like inulin to the suspending medium cannot assure its equilibration with luminal contents, so that the intracellular volume is likely to be less than the difference between total tissue water and inulin space. The implication of this fact, as further discussed below, lies in the difficulty of estimating transmembrane activity gradients.

Actually, even if the tubules remained open, the relatively long diffusion path from suspending medium through the cut ends of the tubules to the brush border, or possibly along intercellular channels, would significantly hinder access of solutes to the point of their absorption out of the lumen or secretion into the lumen. If cells in slices do take up a solute believed to react only with apical but not with basolateral cell

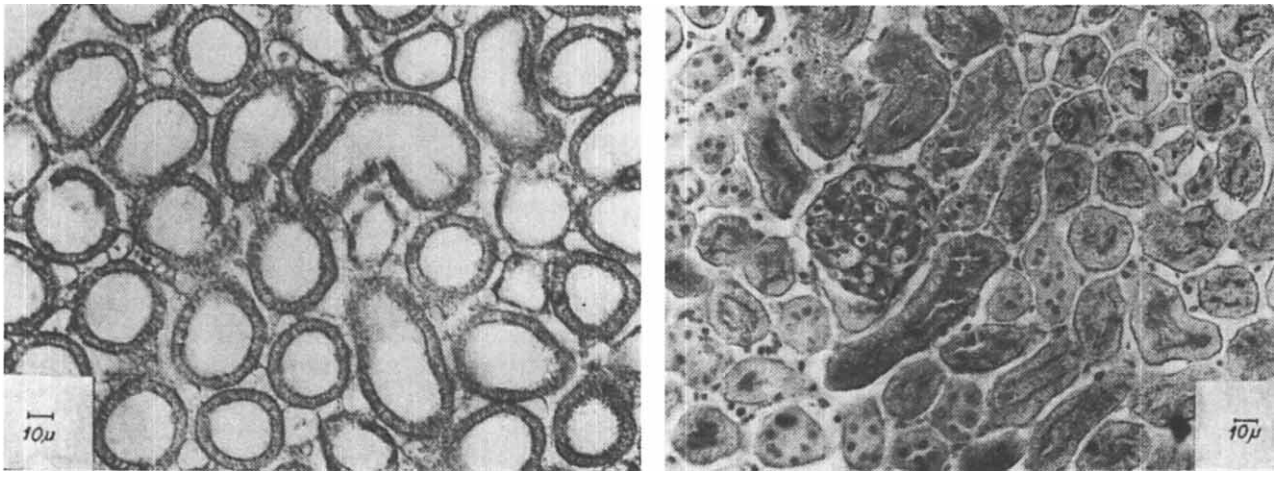

Figure 1. Collapse of renal tubules in slices (from Ref. 7, with permission). (left) Section of rat kidney frozen at instant of removal. (right) Section of thin slice incubated at 37°C for 15 min.

membranes, then the rate of uptake would be severely limited by the restricted diffusion just referred to. One such solute may be α-methylglucoside, which is accumulated by slices (9), but does not appear to react with basolateral membrane *in vivo* or *in vitro* (10). According to this argument, glucoside uptake by slices must reflect at least limited access of this solute to the brush border.

The inability of brush border membranes freely to contribute to solute turnover in slices was further suggested by the results of Murthy and Foulkes (11), who compared maximum steady-state levels of PAH achieved in regular slices and in short tubule fragments prepared by collagenase digestion. Normal PAH efflux in the direction of secretion was expected to be more pronounced in fragments (relatively free accessibility of brushborder) than in slices (luminal collapse, long path of diffusion, if any, from brushborder back into medium); as a consequence, higher PAH concentrations should be achievable in slices rather than in fragments. These predictions were fully confirmed by the results summarized in Table I (11).

When comparing slices with fragments, note that the fragments achieve lower steady-state slice/medium ratios for PAH, but more readily lose actively accumulated PAH or TEA (tetraethylammonium). Further support for the conclusion that brush border membranes do not significantly contribute to PAH turnover in slices is provided by the results of a more detailed kinetic analysis of PAH efflux (12). A maximum contribution of only 20% of total PAH efflux, and a probable contribution of much less, could be attributed to luminal cell membranes.

Table I. Accumulation and Efflux of Organic Solutes in Renal Slices and Tubular Fragments

	Accumulation (S/M)		Efflux (min^{-1})	
	Slices	Fragments	Slices	Fragments
PAH	12–24 (5)	4–9 (5)	0.002–0.020 (11)	0.74–1.30 (7)
TEA	—	—	0.003–0.27 (5)	0.25–0.71 (5)

Note. PAH, p-aminohippunate; TEA, tetraethyl ammonium. Results are shown as ranges, with the number of experiments given in parentheses. (Based on Ref. 11.)

In summary, considerations of tissue geometry in slices, and the pronounced polarity of tubular epithelial cells (see below), render renal cortical slices unsuitable for studying the process of solute transfer across the brush border, and in particular the process of solute reabsorption. As discussed below, slice accumulation of amino acids, for instance, is very likely to reflect primarily the activity of basolateral carriers, not the process of amino acid reabsorption at the brush border.

Polarity of Tubular Epithelial Cells. Given the geometrical restrictions on free membrane function in slices, the polarity of tubular epithelial cells may create additional problems in the interpretation of solute exchange between the suspending medium and cells in renal slices. Polarity of epithelial cells has long been recognized, especially in relation to electrolyte transport (13). Such polarity can readily be demonstrated also for organic solutes, as in the work of Silverman *et al.* (14) on monosaccharide transport in the dog kidney. This work showed pronounced differences between the ability of the two membranes to react with sugars. If, however, similar transport processes are active on both sides of cells, then even with free access of solutes to the brush border it would be difficult to separate the contributions of each membrane to total solute turnover in slices.

All this is well illustrated by the transport of amino

acids in slices. Rosenberg *et al.* in numerous publications (see e.g., Ref. 15) described the accumulation of natural amino acids in renal slices, and identified the process with that of proximal tubular absorption from the lumen. A difficulty with this identification is the fact that slices of renal medulla (16) and isolated glomeruli (17) (i.e. tissues little involved in amino acid reabsorption) also possess the ability to accumulate amino acids. It is of interest, also, that renal reabsorption of L-lysine could be separated from its cortical accumulation *in vivo* (18).

While amino acids are normally present in glomerular filtrate and are readily reabsorbed across the brush border, they also react extensively with basolateral membranes. Thus, working with isolated perfused renal tubules, Schafer and Barfuss (19) demonstrated amino acid uptake across basolateral membranes from the suspending medium, and concluded that this process represents active transport. Reaction of amino acids with basolateral membranes was demonstrated also *in vivo* (20), using the rapid renal transit technique of Silverman et al. (21). This technique compares artery-to-vein or artery-to-ureter transit times of test solutes with those of a glomerular marker following arterial bolus injections. Extensive evidence was obtained for basolateral amino acid carriers resembling in their specificity the apical carrier systems responsible for amino acid reabsorption. The physiological function of basolateral amino acid transport is presumably related to cell nutrition (6). Basolateral transport mechanisms also play a role in amino acid reabsorption (22). Because of the restricted accessibility of the brush border and the significant basolateral transport capacity, amino acid accumulation in renal slices is likely to result primarily from action at basolateral membranes, rather than from transport in the direction of reabsorption.

Use of renal slices for studying transport of other solutes also may raise problems of interpretation. For instance, heavy metals like cadmium and mercury are known to react with both apical and basolateral membranes *in vivo* (23); an estimate of the relative contributions of the two membranes suggested that two-thirds of Cd retained by the tissue *in vivo* originated from basolateral transport. In slices an even greater fraction of Cd uptake must reflect activity on the basolateral side. In other words, the process of luminal uptake (reabsorption) of such metals cannot be studied in slices.

The efficient accumulation of amino acids and heavy metals by slices is thus fully compatible with the conclusion that function at the basolateral membrane, as contrasted with apical membranes, proceeds freely in this system. Additional evidence for solute transport across basolateral membranes in slices is provided by the active accumulation of organic anions and cations. In particular, active uptake of paraaminohippurate (PAH) by slices was first studied by Cross and Taggart (4), and has since been further investigated in many laboratories. The active transport step in PAH secretion has been localized at the basolateral membrane (12). The important conclusion in the present context emphasizes the value of slices for studying basolateral transport phenomena. As a corollary, PAH efflux from slices to medium must result primarily from basolateral leakage; PAH secretion into the tubular lumen cannot be followed in slices (24). The same conclusion was drawn also from comparison of PAH efflux rates in slices and tubule fragments (Table I).

Estimation of Transport Gradients in Renal Slices. A common and unsolved problem met in the study of solute transport by renal slices is how to estimate cellular volumes of distribution in the tissue. Knowledge of intracellular, or preferably cytoplasmic, volume is required for the calculation of, for one, the concentration gradient against which PAH and other solutes can be accumulated. Total tissue water, comprising as it does interstitial, intracellular, and luminal fluids, does not provide a meaningful basis for assessing cellular accumulation in slices. Even if these volumes could be assessed *in vivo* (e.g., Ref. 25), there is, of course, no guarantee that the same values are applicable to slices.

An additional difficulty arises from the uncertainty of whether slices do maintain normal transmembrane electric potentials, especially following toxicant action. This becomes especially important when accumulation of an ionic substrate like PAH is used to assess the integrity of renal function. It is perfectly conceivable, for instance, that an agent might affect PAH transport as a result of its effect on membrane potentials rather than by direct interaction with the anion carrier system.

Active slices incubated under appropriate conditions may attain slice-to-medium (S/M) concentration ratios of PAH of 20 to 1 or even higher. Such ratios, of course, represent net PAH uptake, not the result of unidirectional influx; no direct conclusion on rates of active transport can be drawn from steady-state ratios. Thus, for instance, the observation of Suzuki *et al.* (26) that treatment of rats with Funomysin B1 reduces the S/M ratio of PAH in cortical slices should not automatically be attributed to a reduction in PAH influx. In addition, S/M ratios have no necessary bearing on actual transport gradients across the basolateral cell membranes. In the first place, the cell interior is normally approximately 60 mV negative to interstitial fluid, and almost 60 mV to intraluminal fluid. Assuming that slices maintain normal transmembrane potentials, the concentration gradient of PAH from medium to cells in the absence of active transport would equal

1:0.17 (25), not 1:1, as often implicitly assumed. Similarly, passive movement of PAH across the apical membrane would lead to a transmembrane gradient from cell to lumen of 0.17:1.

Calculation of transport or activity gradients further presuppose definition of intracellular volumes of solute distribution. Reference may be made in this connection to the attempt (25) to estimate *in vivo* the steady-state activity gradient for PAH from cytoplasm to interstitial fluid. Two assumptions needed to be made: (i) the transmembrane potential across both basolateral and apical membranes approximates 60 mV, cytoplasm negative, and (ii) the cytoplasmic volume, excluding the volume of intracellular organelles, equals 0.13 ml/g, as determined from the volume of distribution of 3-O-methylglucose. On this basis, the steady-state activity gradient from cytoplasm to interstitial fluid in the absence of secretion (stop-flow kidney) was calculated at 6:1. The corresponding value in slices at present cannot be accurately evaluated because of the uncertainty about the residual volume in tubular lumina. While this should be small in normal slices, it might be significant in presence of inhibitors of active transport or in anoxia. Without information on luminal volumes, cytoplasmic volumes in slices cannot readily be estimated.

In summary, the significance of alterations in PAH accumulation in slices in presence of noncompetitive inhibitors is unclear if, as is likely, such agents affect transmembrane electric potentials and volumes of tissue fluid compartments. In other words, slice experiments may not be able to pinpoint the site of a functional lesion, and are therefore limited in their ability to serve as an experimental model for sensitive and quantitative evaluation of renal functional integrity.

Metabolic Implications of Cell Polarity. The conclusion that metabolic substrates or end products cannot readily cross the brush border into and out of cells in slices implies limitations also for the study of renal metabolism. This fact may be illustrated with the renal handling of Cd-metallothionein. Tubular epithelial cells react with this metalloprotein only at the brush border, where a carrier system specific for anionic proteins mediates their uptake from the lumen into cells (27); no evidence was found for reactions of metallothionein with basolateral membranes (28). It follows that slices are not good models for the study of the renal handling of metallothionein or of its nephrotoxicity. Metabolic studies with renal slices are useful only to the extent that substrates, inhibitors, and metabolites can freely cross basolateral membranes.

Summary. Slices of renal cortex cannot readily yield quantitative information on many aspects of renal function. These aspects include not only membrane solute transport, as has long been realized, but also frequently renal metabolism. Study of events at the brush border is made difficult because of the limited access from the suspending medium. It follows that solute uptake from the medium on the whole does not reflect normal reabsorption, and that efflux of a solute from slices into the suspending medium should not be equated to its transfer across the brush border. Basolateral transport processes, in contrast, are more amenable to study in slices. However, steady-state accumulation of, for instance, PAH provides only limited information about the functional integrity of the intact kidney. One of the reasons for this limitation lies in the difficulty of estimating the transport gradient from medium into slice. Even for metabolic studies, reliance on slices requires assurance that restricted access to the brush border does not prevent normal uptake of metabolic substrates into cells, and possibly also extrusion of metabolic products from cells. Like all experimental models and techniques, the renal cortical slice preparation possesses advantages and limitations. As a result, and in spite of their great potential for testing many questions, slices cannot serve to provide meaningful answers to a variety of other questions.

Everted Sacs of Intestine

General. Intestinal solute transport has been extensively studied, usually in the direction of absorption. Early experiments were carried out with segments of intestine isolated *in vivo* with intact blood supply. Realization of the critical importance of oxygenation for the maintenance of a viable mucosa led Fisher and Parson (29) to develop an *in vitro* preparation of the small intestine exposed to oxygenated solutions both through the lumen and on the serosal side; at no time was the intestine subjected to anoxia. In the search for a simpler isolated preparation, Wilson and Wiseman (30) devised the popular everted sac technique. Here only the mucosa is directly in contact with oxygenated incubation medium, while the serosal fluid is isolated inside the closed sac, where it can be sampled independently from the mucosal medium. Absorption in this system is equated to the transmural movement of solutes from the medium into the serosal fluid trapped in the sacs. Significant amounts of solute may be retained and/or metabolized in the tissue, so that disappearance of a solute from the mucosal fluid does not necessarily constitute absorption.

The new technique yielded considerable information on transmural movement of such solutes as sugars and amino acids. However, from the beginning of the widespread use of everted sacs concern was expressed about the influence of submucosal tissue on the apparent absorption process. Indeed, both the length of the diffusion path through this tissue and possible interactions between absorbed solutes and constituents of submucosal tissues may significantly delay their

movement into the serosal fluid. Other, perhaps more subtle, problems also arise, restricting the usefulness of the everted sac technique for studying intestinal absorption; they are reviewed in this section.

Diffusion Pathways through (Transcellular) or between (Intercellular) Cells. For a solute that crosses the mucosa along transcellular pathways, apical uptake may be defined as step I of overall absorption (31), step II then describes the basolateral extrusion of this solute. This two-step series model is, of course, not applicable to the extent that a solute follows intercellular diffusion pathways, paralleling the cellular uptake.

In studying intestinal handling of cadmium, for example, cellular uptake as mandatory step 1 in absorption can be inferred from observations such as saturability, specificity, sensitivity to inhibitors, and trapping by the intracellular protein metallothionein (32). According to these criteria, Cd absorption *in vivo* follows a transcellular route. It does not necessarily follow that other solutes utilize the same pathway, or that Cd moves across the intestinal wall in the same manner in the intact animal and in everted sacs. This possibility is further discussed below in Artefacts of Preparation. In any case, the good fit of the series model to absorption in the intact animal can be no guarantee of an equally good fit to everted sacs, as assumed, for instance, in the work of Ohta *et al.* (33).

An instance where this assumption is clearly incorrect is seen in the effect of the chelator EDTA on Cd transport in everted sacs (34). Presence of EDTA on the mucosal side stimulated appearance of the metal in serosal fluid, while decreasing its retention in the tissue. The suggested explanation, that EDTA mobilizes intracellular Cd, conflicts with the finding that in this tissue the chelator reacts primarily with extracellular metal (35). In fact, 1 m*M* EDTA strongly inhibits the binding of Cd to the brush border membrane (36), the required first step in Cd absorption. Uptake of Cd by everted sacs is 80% inhibited in presence of 5 m*M* EDTA (35). If step I of absorption is greatly depressed, no stimulation of step II in series can be expected. In this instance, therefore, Cd movement in sacs cannot be reconciled with the series model. Sahagian *et al.* (37) had earlier implicitly used a parallel model in their study of transmural movement of heavy metals in the rat intestine.

A subtle effect of EDTA on intestinal solute uptake is seen also in its stimulation of phenol red accumulation from the intestinal lumen (38). Apparently, EDTA opens Ca-gated aqueous channels in the mucosa, permitting increased solute penetration. The additional finding reviewed below that everted sacs are more permeable to urea than is the tissue *in vivo* reflects a higher density of aqueous channels and points to preparation artefacts independent of EDTA. The likelihood must be considered, therefore, that in sacs, even in absence of EDTA or similar compounds, a fraction of Cd removed from the lumen may utilize intercellular pathways, parallel to rather than in series with the normal cellular uptake step. This would differentiate the mechanism of Cd absorption in sacs from that in the living animal where cellular uptake normally constitutes the mandatory first step in absorption (39).

Study of Step I in Sacs. In spite of the possibility of increased leakage of solutes between mucosal cells in everted sacs, this preparation has proven very useful for the study of step I of absorption (i.e., the reaction of absorbed solutes with, and their transfer across, the apical membrane of mucosal epithelial cells). It will suffice here to refer, for instance, to the knowledge gained on the mechanism of step I of Cd absorption in the rat intestine (39). This appears to consist of electrostatic binding of the metal to the membrane, followed by an internalization step that is temperature dependent but insensitive to metabolic inhibitors; membrane fluidity may here play an important role. Because of the required time resolution (seconds), everted sacs of jejunum proved to be the model of choice in these experiments.

Step II and the Role of Submucosal Tissue in Sacs. In sacs, passage of a solute through the mucosal epithelium must be followed by diffusion through the submucosal layers into the serosal fluid. The length of this diffusion path obviously greatly exceeds that from mucosa into lymph or blood in the normally perfused organ. Reaction of solutes with submucosal tissue will, of course, further slow the rate of their movement across the intestinal barrier.

The role of submucosal tissue is particularly relevant to the transmural movement of heavy metals in everted sacs. Like other protein reagents, these metals may be expected to react with constituents of the submucosa. This expectation is fully confirmed by the work Endo *et al.* (40) on Hg transport in everted sacs of rat intestine. Nayak and Benet (41) had succeeded in removing the epithelium from the small intestine by treatment with a high concentration of EDTA (0.1 *M*) for 45 min. Under those conditions, EDTA removes the mucosal epithelium but leaves behind morphologically intact muscle and serosal layers. Endo *et al.* (40) reported that after removal of the mucosa in this manner, the amount of Hg accumulated in the tissue as much as doubled during 30 min of exposure to 10 μ*M* Hg in the mucosal medium. We must conclude, therefore, that submucosal tissues can trap large amounts of metals. As a result, even if these metals followed primarily a transcellular pathway of absorption in sacs, this preparation can provide little information on step II of metal absorption (i.e., their basolateral extrusion).

Artefacts of Preparation. In the present context, where questions are raised about the adequacy of everted sacs for the study of intestinal absorption, a particularly relevant finding is that preparation of the sacs may itself increase the density of aqueous channels in the membranes (42); this is illustrated in Table II. The rationale for this conclusion rests on the plausible assumption that urea permeates only through aqueous channels.

Note that urea uptake in Table II is normalized by that of ethanol. We have previously demonstrated how the passive fractional absorption of a lipid- and water-soluble compound like ethanol can serve as measure of the size of the absorbing area (43). In other words, dividing urea flux by that of ethanol expresses in arbitrary units the urea flux per unit area, or the intrinsic urea permeability of the tissue. According to this criterion, everted sacs are clearly more urea permeable than is the parent tissue. The increased density of aqueous pores, in turn, raises the possibility that transmural movement of solutes in sacs may not follow the same pathways as in the intact animal. These results thus raise additional questions about the applicability of everted sacs for studying intestinal absorption of selected solutes.

Summary. Everted sacs provide a convenient technique for the study of a viable mucosa *in vitro*. Reliance on this preparation, however, must take into account both the occurrence of functional artefacts, as well as the anatomical limitations of the preparation. An important artefact is the loss of some intrinsic permeability properties of the tissue. Nevertheless, while everted sacs cannot usefully serve as model for transmural absorption of solutes such as heavy metals which are trapped in submucosal tissue, they do offer great advantages for studying events at the apical membrane of mucosal epithelial cells.

Conclusions

Renal cortical slices and everted sacs of the small intestine offer great convenience for the study of solute transport in these organs, but at times at the cost of uncertainty about the physiological significance of the results. Because of the limited accessibility of the brush border in slices, they cannot serve as appropriate models for the quantitative analysis of solute transport at luminal cell membranes. This specifically includes processes such as reabsorption from, and secretion into, the tubular lumen. In addition, the significance of metabolic studies and inhibitor action in slices may be limited for compounds normally taken up only at the apical side of tubule cells. For instance, Cd-metallothionein, unlike Cd itself, does not react with basolateral cell membranes. The toxicity of these two forms of Cd can therefore not be compared in renal slices. In contrast, many aspects of basolateral transport processes such as that of PAH have been successfully elucidated in slices.

Limitations on the use of everted sacs of the small intestine for the study of the overall process of intestinal absorption in part also arise from morphological factors. In particular, the need for absorbed solutes to traverse a relatively large submucosal tissue is likely significantly to affect transmural movement of certain solutes like heavy metals, characterized by their high and relatively nonspecific protein affinity. Indeed, trapping of metals in muscle and serosal layers of everted sacs has been well documented. In addition, there is evidence that artefacts may arise during tissue preparation and alter its permeability; similar changes have been observed in presence of reagents like Ca chelators.

Table II. Intrinsic Urea Permeability of Rat Jejunum

Mature rats	Everted sacs
0.18 ± 0.06 (n = 6)	0.75 ± 0.07 (n = 5)

Note. Values (X ± SD) express in arbitrary units the exponential urea efflux from jejunal perfusate *in vivo* or mucosal fluid *in vitro*, as normalized by absorbing area (see text). (Based on Ref. 41.)

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