Environmental Health Perspectives
Vol. 15, pp. 73-88, 1976

Use of the Tissue Slice Technique for Evaluation of Renal Transport Processes

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A detailed discussion of the tissue slice technique for evaluation of transport phenomena is presented. Information is given concerning the preparation of tissue slices and the advantages of this procedure over corresponding *in vivo* techniques. In addition, the relationship of the *in vitro* renal transport of organic substances to *in vivo* renal function is discussed in detail. Finally, certain pitfalls related to *in vitro* slice transport studies are presented.

The use of tissue slice preparations to evaluate physiological and biochemical phenomena is not new. For example, as early as the 1920's the tissue slice technique was used to examine tissue respiration. Warburg and colleagues (*1,2*) characterized this physiological function with both liver and tumor slices. These workers showed that the liver slice thickness optimal for respiratory activity was 0.5 mm or less, and that slice thickness was a major determinant for the efficiency of respiration. With tissue slices greater than 0.5 mm, oxygen penetration by diffusion (even in the presence of 100% oxygen) was found to be greatly retarded. In addition to respiration measurements far below the true values, the use of thick slices promotes enhanced deterioration of the tissue because many layers of cells never receive oxygen at all, or at such a low level that their viability is impaired.

The specific quantitative relationship is:

$$d = 8C_0D/A$$

where d is thickness, D is the diffusion constant for O_2, a is the rate of respiration (μl O_2 uptake/ml tissue-min), and C_0 is the O_2 concentration outside the slice (atmosphere). Let us assume an oxygen consumption of 5×10^{-2} μl/ml tissue-min and an oxygen diffusion coefficient of 1.4×10^{-5} sec/cm^2. If the gas phase is 100% oxygen, a calculated tissue thickness of 0.47 mm is obtained, indicating that tissues of this thickness or less can be oxygenated adequately. However, if air is used as the gas phase (i.e., 20% oxygen) reliable oxygenation will not take place with tissues thicker than 0.21 mm. Unfortunately, liver slices of about 0.2 mm in thickness are difficult to prepare and extremely friable. Slices slightly thicker (e.g., 0.3 mm) are much easier to prepare and less likely to be shaken apart during incubation.

Slice thickness can be measured rather simply, although in practice this is rarely done. Slices are floated over a ruled paper and trimmed so the areas can be measured readily. Slice volumes can be calculated from wet weights after assuming a specific gravity value. Then the thickness can be calculated from the volume and area. It was with analyses such as these performed on liver tissue that slice studies began. These data and concepts, however, are relevant to the renal slice studies to be discussed below. For example, the utilization of renal slices for transport studies necessitates the assumption that the slices are viable and capable of more or less normal respiratory activity, hence the use of renal slices of a proper thickness is critical.

One of the first attempts at studying renal transport processes with isolated tissues was made in 1930 by Chambers and Kempton (*3*).

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These workers grew chick mesonephros in tissue culture and observed dye accumulation in the culture. Phenol red added to the tissue culture vessels was found to be accumulated both in the tissues and in the tubular lumens, although most of the phenol red appeared to be in the lumens. No rigid quantification of the uptake was done, however, but on the basis of the crude observations it was estimated that the luminal concentrations exceeded that of the bathing solution by 20–30 times. Dye uptake was noted to be a proximal tubular function only. No dye was observed in either the distal tubular cells or the distal tubular lumens of isolated pieces of this nephron segment. Distal luminal coloration was noted only in those segments with intact connections to colored proximal tubules.

In 1948 Forster (4) first used renal slices for transport studies. Thin slices of frog kidney were prepared and bathed in a balanced salt solution to which was added a low concentration of phenol red. At various time intervals Forster removed the slices from the bathing solution and examined them with a microscope to determine the extent of luminal accumulation. For purposes of quantification, standard phenol red solutions were prepared and placed in capillary tubes with approximately the same diameters as frog nephrons. By this means direct color comparisons were possible between the standards and the tubular segments. This semiquantitative technique afforded a reliable estimate of a 1000-fold higher dye concentration in the tubular lumens than in the bathing solution. Further, Forster demonstrated that the accumulation was supported by metabolic energy ("active" transport), since inhibitors such as 2,4-dinitrophenol blocked or reduced the phenol red uptake.

With the availability of specific chemical and radiochemical analyses, uptake data may now be presented with considerable quantitative reliability. Usually data are expressed as the actual concentration of the material transported per gram of tissue, or as the so-called slice/medium or S/M ratio. This latter expression is probably more useful and involves computing the tissue concentration of the material under study m mole of substance/g of tissue) and dividing it by the bathing solution concentration (m mole/ml). If this ratio exceeds unity by an appreciable amount, it is thought to be indicative of an active transport process. A value of one or slightly less than one is usually interpreted to mean that the compound in the tissue got there by passive means.

Slice Preparation

For mammalian kidneys two methods have been used for the preparation of renal cortex slices. Free-hand slicing, as used by Forster (4), is used frequently. After removal of the capsule, the kidney is prepared for slicing as indicated in Figure 1. This diagram was prepared on the basis of the usual procedure employed for kidneys the size of those in the rabbit. Obviously, accommodation for organ size is needed when using dog or rat kidney instead of rabbit. A cube of tissue is placed on a moistened filter paper which is held onto a rubber stopper or similar support. A microscope slide is pressed firmly on the cortical surface of the cube and half of a double-edged razor blade (held in a hemostat) is drawn under the slide. With modest practice satisfactory tissue slices can be prepared quickly with this procedure.

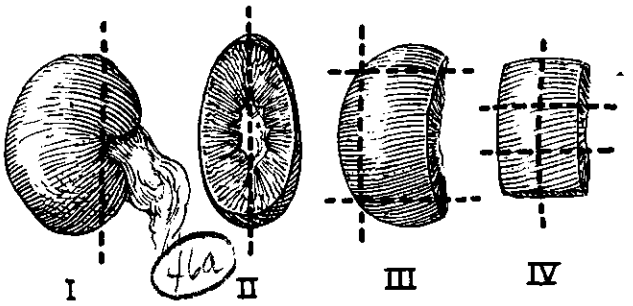


FIGURE 1. Preparation of kidney for making slices.

Because there is some difficulty in obtaining slices of uniform thickness, the Stadie-Riggs microtome was developed. This device allows the cutting of uniform slices by the application of the tissue cube of a constant pressure that is controlled mechanically. This is accomplished by allowing a Plexiglas block to rest on the tissue cube in a specially designed chamber. The surface of the tissue to be cut is housed in this chamber through which the cutting blade will be passed. The chamber is milled to permit slices of the desired thickness (either 0.25 mm or 0.50 mm) to be cut. A picture of a commercially available device is seen in Figure 2. In general, the preparation of slices with this procedure is slower than with the free-hand method, although probably the slices are of a more uniform thickness. Either of these procedures will serve for the preparation of slices of liver and kidney medulla as well as of kidney cortex.

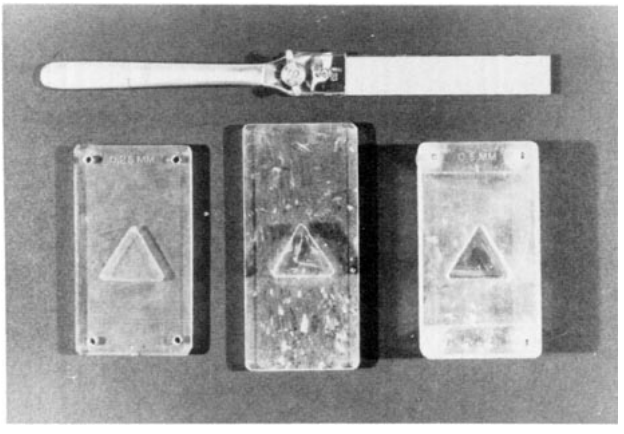


FIGURE 2. Stadie-Riggs tissue slicer.

Usefulness of the Slice Technique

Many of the reasons for the use of the slice technique in the study of renal transport processes have been detailed by Forster (*4*) and Cross and Taggart (*5*). This technique permits the study of specific transport functions without the unwanted influences of alterations in glomerular filtration rate or renal blood flow, either of which may affect the availability of metabolites, substrates, etc. Also the *in vitro* procedure permits the study of many noxious agents, e.g., potential nephrotoxins. In the intact animal, because such chemicals have generally deleterious, if not lethal effects, study of the specific actions is difficult if not impossible, e.g., the effects of sodium cyanide, sodium iodoacetamide, or 2,4-dinitrophenol. Precise control over various experimental parameters, e.g., the composition of the bathing solution, temperature, gas atmosphere, can be maintained with isolated tissue preparations. These factors cannot be controlled precisely but can be changed quickly permitting the rapid development of a new steady state.

Despite the relatively artificial conditions imposed by *in vitro* studies, one can still feel confident that the functions under study resemble those *in vivo*, and that the tissue has retained its integrity. In general, intact nephron segments do exist in the slice and present a more or less normal anatomical configuration in which the transport system can work. This is not so with the use of isolated renal cortical cells, or course, since the isolation procedure disrupts all anatomical relationships (*6*).

The renal slice system also is useful for studying effects of substances that act only in the intact animal. In pretreatment experiments the animals are sacrificed at various times after the pretreatment, the kidneys removed, and the uptake of the test substances evaluated (see below). This allows the determination of the time-course of effects on renal transport functions. Also this approach may yield information about mechanisms of action. For example, if a nephrotoxin disrupted renal transport only when administered to the intact animal, something has been learned about the offending chemical and possibly its mechanism of action.

Finally, there is at least one practical consideration worth mentioning. An enormous number of experimental variables can be examined in one experiment using tissue from one animal. For example, the renal cortical tissue from one rabbit is adequate for studying 10 to 15 experimental variables in duplicate. This latter factor is not an incidental one in these days of restricted research budgets.

Nature of the Transport Studied with Slices

Most of the renal slice transport studies have been done with renal cortex. These slices contain not only proximal tubular, but distal tubular tissue as well. Therefore if one is studying a function found in only one or the other of these nephron segments, the slice study works against the investigator. For example, if organic anion accumulation (an exclusively proximal tubular function) is investigated and the data are expressed as some function of tissue slice weight, then the apparent slice activity will be less than the actual activity.

Given the usefulness of the slice technique the next question concerns the nature of the transport processes studied with this procedure. Foulkes and Miller (*7*) have investigated this problem in considerable detail with respect to the cortical slice transport of organic acids. These workers attempted to investigate whether organic acid uptake represented cellular accumulation of the acid or accumulation in the tubular lumens. With isolated tubular preparations of fish (e.g., flounder, goldfish), accumulation in the lumens has been shown to be quantitatively important. Furthermore, cellular uptake of organic acids was separated from luminal accumulation by alterations in the inorganic electrolyte composition of the bathing solution. For example, the potassium ion was needed for cellular uptake of phenol red, but in the absence of calcium no dye was seen in the tubular lumens. Addition of calcium to the

bathing solution permitted visualization of the dye in lumens (*8*).

Mammalian preparations, however, apparently do not respond to alterations in electrolyte content of the bathing solution in this same way. There are effects of inorganic electrolytes on net uptake of organic acids by mammalian slice preparations (*7,9–11*), but none resemble the two-step effects noted with the flounder tubule. There is a potassium requirement for uptake, i.e., *p*-aminohippurate (PAH) uptake is lower in the absence of potassium than in its presence. Sodium is also needed for uptake of PAH and its dependence on the sodium concentration of the bathing solution is quantitatively like that for potassium. With phenosulfophthalein (PSP), however, a prominent sodium effect is noted, while the potassium requirement is relatively modest. Slice uptake of both of these acids is calcium-dependent, with PSP more sensitive to calcium concentration than PAH. The absence of the two-step transport noted in fish is further confirmed by the inability of investigators to visualize any dye in high concentration in the mammalian nephron.

Foulkes and Miller (*7*) attempted to evaluate the relative importance of luminal accumulation in the mammalian slice uptake process. These workers employed two basic experimental approaches. Slices of rabbit kidney cortex were preloaded with PAH and the runout was measured into a PAH-free balanced salt solution. Two runout phases were found, an initial rapid efflux and a subsequent, slower one. The initial rapid efflux did not constitute extracellular washout, since parallel measurements in which inulin space was measured indicated the initial PAH runout was from a compartment larger than the extracellular space. Furthermore, if inhibitors were employed during uptake, the initial efflux became equal to the inulin space. In the second type of experiment, PAH uptake rates were measured and also here two rates were noted. The first uptake phase was much more rapid than the second one.

Foulkes and Miller offered the model presented in Figure 3 to explain these data. In addition to an extracellular space this model presents two intracellular compartments. One compartment equilibrates rapidly and is unrelated to the inulin space. This component was demonstrated in both the uptake and efflux experiments by the presence of the rapid processes noted initially. The PAH accumulated into this compartment(s) was probably distributed throughout the intracellular

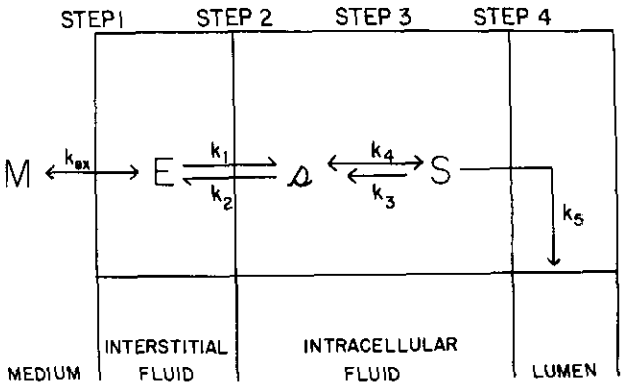


FIGURE 3. Foulkes and Miller model for *p*-aminohippurate transport in renal tubular cells. From Foulkes and Miller (*7*) with permission of the authors and the American Journal of Physiology.

water at a concentration equal to that in the bathing solution. The second intracellular fraction(s) is responsible for the high S/M ratios and was seen in the uptake and runout experiments by the secondary, relatively slow, kinetic components.

On the basis of this model, these authors subjected their experimental data to a detailed kinetic analysis and determined the relative importance of step IV compared to the backflow component (k_2s) of step II in an attempt to solve the problem of the importance of luminal accumulation in tissue slices. These authors found that under all reasonable experimental conditions, the k_5S/k_2s ratio never exceeded 0.2. That is, no more than 20% of the PAH accumulated by the slice found its way across the luminal membrane. Therefore, net uptake studies with renal cortical slices, constitute an evaluation of transport across the peritubular membrane responsible for cellular accumulation, and do not represent luminal uptake as seen in isolated fish tubules.

Two additional comments are needed about this study. First, Foulkes and Miller were the first to develop the concept of two intracellular compartments for PAH. This concept was expanded by Farah, Frazer and Stoffel (*12*) and Welch and Bush (*13*) using somewhat different techniques. Secondly, it should be noted that the behavior of the isolated perfused tubule is different from that of the slice with respect to PAH transport. Tune, Burg, and Patlak (*14*) demonstrated significant, passive, luminal accumulation of PAH by the isolated, perfused, proximal tubule of the rabbit. No doubt luminal

accumulation occurs here because the lumen is open and fluid filled. In slices, lumens are frequently collapsed.

Uptake of Organic Acids

Having established that the renal slice technique is practical for the measurement of the uptake of certain substances, and that this process relates to cellular accumulation, can the procedure be used to evaluate parameters of renal physiology? The initial studies which attempted to establish the importance of this procedure for renal physiology were performed by Cross and Taggart (*5*) and Mudge and Taggart (*15,16*). Cross and Taggart established the basic criteria for organic acid accumulation by renal cortex slices. The companion study by Mudge and Taggart evaluated many of the same phenomena in the intact animal. Table 1 presents a qualitative comparison of data taken from these studies and others. These data assisted in the establishment

Table 1. PAH transport by dog kidney.

	In vitro slice uptake	*In vivo* tubular transport
Acetate	↑	↑
Lactate	↑	↑
Succinate	↓	↓
Fumarate	↓	↓
2,4-Dinitrophenol	↓	↓
2,4Dinitro-6-phenylphenol	↓	↓
Probenecid	↓	↓
Carinamide	↓	↓
Iodopyracet	↓	↓
Penicillin	↓	↓

of the predictive value of the slice technique for identification of the existence of renal anion secretion in the intact animal. For example, acetate enhanced slice uptake of PAH and increased the maximal transport rate T_m for PAH in the intact animal. Lactate did likewise. Both succinate and fumarate blocked slice uptake and decreased T_m. A whole host of "competitors", e.g., probenecid, carinamide, iodopyracet, penicillin G, reduce both *in vivo* secretion and slice accumulation. Hence, the *in vitro* transport system had an exact parallel *in vivo*. In other words, when measuring the uptake of PAH or a variety of other organic anions, by renal cortex slices, *the study serves as an in vitro counterpart for* the *in vivo* tubular secretory process.

The relationship between *in vivo* tubular secretory activity and *in vitro* renal transport can be amplified if certain species differences are examined. The urographic agent, diatrizoate, is an iodinated organic acid and as such might be expected to undergo active tubular secretion by the kidney (*17*). In Table 2 are presented the diatrizoate S/M ratios as measured in two mammalian species, rabbit and dog. The rabbit renal

Table 2. ^{125}I-diatrizoate uptake by renal cortex slices of dog and rabbit.[a]

		S/M ratio	
		Dog	Rabbit[b]
–	–	0.6	3.0
Succinate	–	0.5	0.9
Lactate	–	0.6	3.6
Acetate	–	0.7	4.0
Acetate	DNP, 10^{-4}M	0.5	0.7
Acetate	Probenecid, 10^{-4}M	0.5	0.7
Acetate	Ipodate, 10^{-4}M	0.5	0.7
Acetate	Tyropanoate, $10^{-4}M$	0.6	0.7

[a] Measurements were made after 3 hr incubation at 25° C in an atmosphere of 100% oxygen.

[b] All values are significantly different from control (no additions), $p<0.05$.

cortex slices appeared to accumulate diatrizoate by a process not unlike that for PAH, i.e., the classical organic anion system. This was substantiated by the effects of the various substrates and inhibitors, e.g., succinate depressed while acetate and lactate enhanced diatrizoate accumulation; probenecid, ipodate and tyropanoate blocked diatrizoate uptake as did the metabolic inhibitor 2,4-dinitrophenol. Contrast these results with those obtained with dog slices. With renal cortical slices from this animal, no net accumulation was noted under any experimental condition and, of course, no effects of substrates on inhibitors were noted. From these slice data the prediction is that the rabbit actively secretes diatrizoate while the dog does not.

This prediction was examined with the stop-flow technique as well as standard clearance procedures, but only the stop-flow data are presented here. In Figure 4 are the mean stop-flow patterns before and after probenecid obtained from five dog experiments. Net secretion did not occur in any segment of the nephron, and the anion competitor, probenecid, failed to alter *these already negative results.* In Figure 5 is presented a typical stop-flow pattern for the rab-

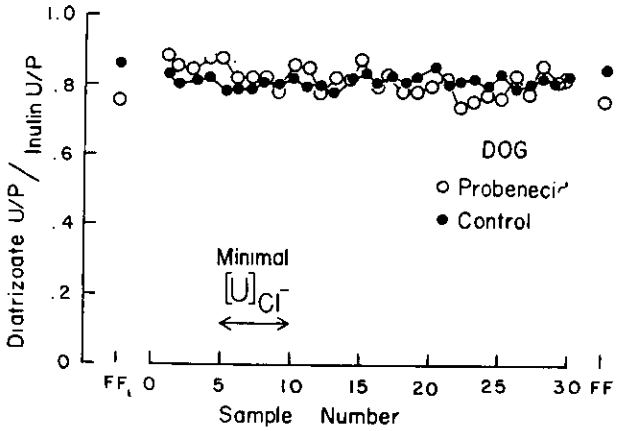


FIGURE 4. Dog stop-flow pattern for diatrizoate before and after probenecid. These stop-flow patterns are the means of five experiments. From Mudge et al. (*17*) with permission of Nephron.

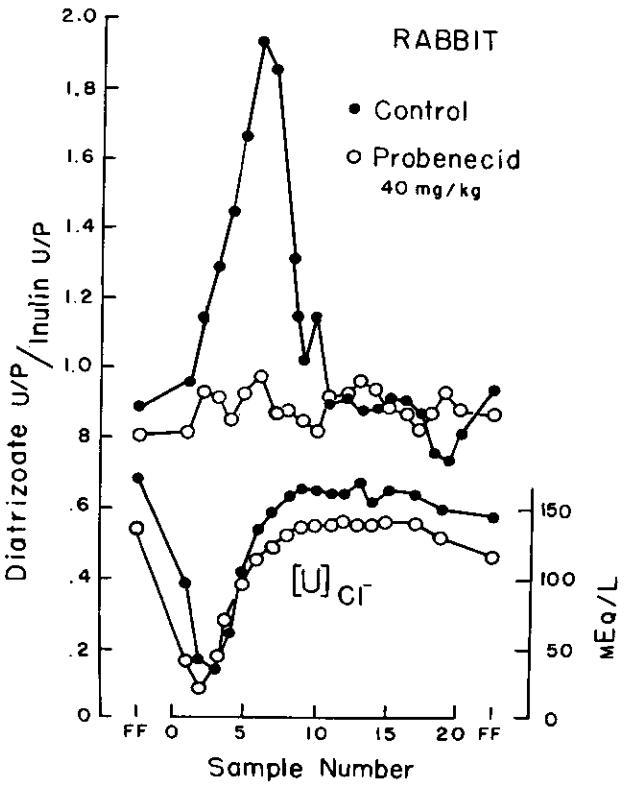


FIGURE 5. Rabbit stop-flow pattern for diatrizoate before and after probenecid. Distal tubular region marked by chloride minimum. From Mudge et al. (*17*) with permission.

bit. The distal region is marked by the chloride concentration minimum at samples 2-3. In the control part of the experiment diatrizoate secretion was noted in a region proximal to the distal dip, presumably the proximal tubule. The secretory peak, as urine: plasma ratio for diatrizoate divided by the urine: plasma ratio for inulin, was reduced from U/P:U/P of about 2.0 to about 0.9 by probenecid, which further confirms the participation of diatrizoate in the renal anion transport process. These *in vivo* experiments, therefore, support completely the predictions made on the basis of the *in vitro* slice experiments, i.e., diatrizoate undergoes active tubular secretion in the rabbit, but not in the dog. Results similar to these have been reported for the cholangiographic agent, iodipamide, by Berndt and Mudge (*18*).

There are some difficulties, however, with the correlation of *in vivo* secretion with *in vitro* slice accumulation of a given organic acid. In a series of papers dealing with two cholecystographic agents, iopanoic acid and iophenoxic acid, Mudge, Berndt and their colleagues (*19-22*) demonstrated the transport of both compounds by dog and rabbit renal cortical slices. Iophenoxic acid was accumulated to high S/M ratios, showed a potassium dependence, and stimulation by acetate, as well as depression by probenecid and other competitors. These data were taken to mean that iophenoxic acid was transported by the PAH mechanism. In the intact dog, however, minimal secretory activity was noted. In fact, tubular reabsorption predominated. Whether the lack of agreement between the *in vivo* and *in vitro* data is a reflection of complications associated with metabolism, biliary excretion, and/or protein binding of iophenoxic acid or due to a failure in the basic concept is not entirely clear. It is likely, however, that complications related to protein binding are the influencing factors, since the albumin binding of iophenoxic acid is extraordinary.

Foulkes and Miller (*7*) attempted to determine the effects of several experimental variables on PAH uptake in terms of their kinetic model. For example, a characteristic of the organic anion uptake process is a requirement for potassium. Foulkes and Miller found that potassium stimulated PAH uptake at step II, i.e., the step responsible for rapid equilibration with the extracellular compartment. Also it appeared that in the absence of potassium step III was affected, i.e., a rapid efflux was noted. Acetate was found to stimulate both step II and step III. Probenecid was interpreted to block step II, not step III,

because these authors found no enhanced PAH runout when this agent was used. Of course, although this analysis helped localize the cellular sites of action of a number of experimental variables, no information was obtained concerning the specific mechanisms of these actions.

Another aspect of this problem further confirms the association between the *in vitro* technique and *in vivo* renal activity. The absence of tubular transport processes in the newborn is well documented. *In vitro* slice studies by several workers (*23-30*) demonstrate the development of organic anion transport in renal tissue of the newborn of several species.

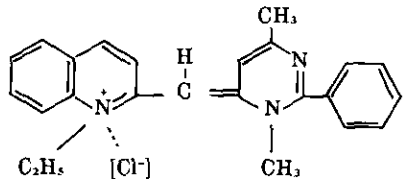
Work in Hook's laboratory showed that the anion transport system was inducible, i.e., pretreatment of the pregnant animal with organic acids such as penicillin, caused a rapid development of anion transport in the newborn or fetal kidneys. This was a specific process in that pretreatment with organic acids did not induce the development of organic cation transport. In recent studies Ecker and Hook (*31*) demonstrated with isolated tubules that the development of anion transport follows a somewhat different temporal pattern than that seen in slices, but the immaturity of the newborn kidney was substantiated. It appears, therefore, that it is possible to isolate intact nephron segments from neonatal renal tissue that does not transport organic anions, but with proper pretreatment, can be induced to do so.

Uptake of Organic Bases

The work of Farah and Rennick (*32*) and Peters (*33*) building on the original observations of Sperber (*34*) has led to an understanding of the renal organic cation transport system. Initially these studies dealt with *N*-methyl nicotinamide and tetraethylammonium, but have subsequently been expanded to include several important pharmacological agents such as hexamethonium (*35-37*) and mepiperphenidol (*38*).

Many of the slice studies were similar in design to those for the original PAH transport studies. That is, net accumulation was examined, and the effects of various physiological factors as well as competitors were tested. The uptake process was shown to be an energy requiring one and although substrate studies have been performed, the details are not clearly established except that acetate probably stimulates uptake. The slice experiments also demonstrated that the uptake process was confined to cortical tissue. Subsequent stop-flow studies confirmed those observations and showed the secretory activity was confined to the proximal tubule. In any event, there is little doubt in the minds of most nephrologists that *in vitro* accumulation of substances such as tetraethylammonium (TEA), or *N*-methylnicotinamide (NMN) are also a demonstration of an *in vivo* secretory process.

At least one specific inhibitor of this transport process is known. This compound is a basic cyanine dye, No. 863.



1′-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2′cyanine chloride

This compound produces a reversible inhibition of organic base secretion *in vivo* and blocks accumulation of these compounds by the renal cortical slice.

Detailed structure activity relationships concerning this inhibitor have not been pursued, but an attempt at examination of the nature of the base carrier has been undertaken using certain alkylating agents, e.g., ethylenimines such as phenoxybenzamine. Farah, Ross, and others (*39-42*) found that phenoxybenzamine and related compounds blocked base transport irreversibly, presumably by binding to the cation carrier. The base receptor or carrier could be protected by first reacting it with a transportable cation, and then treating the tissue with phenoxybenzamine in the presence of the transportable base. This permitted tying up nonspecific binding sites, after which the transportable cation was removed from the tissue by repeated washings. Specific binding sites (i.e., carrier sites) could then be reacted with labeled phenoxybenzamine. This binding was irreversible and allowed the use of the label to identify the binder material during attempts at isolation. The carrier appears to be a protein although detailed identification of its composition, the nature of the binding process, identification of specific molecular binding sites, etc. has not been forthcoming. Of course, the possible effects of phenoxybenzamine binding on these characteristics is a further complication

that will have to be addressed. Incidentally, similar techniques and procedures have been modified to study the organic anion binder in renal tissue (*43-45*). Many of these procedures, however, did not involve the use of irreversible binders.

The organic cation transport process is as specific as the organic anion process. This specificity is emphasized by the data presented in Figures 6 and 7. The tissue uptakes (plotted as percentage of control) in Figure 6 demonstrate that PAH accumulation was blocked by various organic acids, e.g., probenecid and penicillin, while tetraethylammonium uptake was unaffected. Conversely, the effects of various organic bases (Fig. 7) show a specific depression of tetraethylammonium uptake with no effects on PAH accumulation.

As with the organic anions, the slice uptake process for organic cations can serve a useful predictive function, although the situation is not as clear cut. There appears to be little doubt that this transport function is located in the proximal tubule, and is of a highly specific nature for which there are reversible and irreversible inhibitors. These assertions are supported by abundant *in vivo* and *in vitro* data.

Some difficulties arise, however, when transport of organic cations by different species is examined (*35,37,47*). For example, although hexamethonium (C-6) is accumulated to significant levels by cat renal cortex slices *in vitro*, the C-6/inulin clearance ratio has been reported to be 1.0 or less. The specific situation in the cat is complicated further by the fact that *in vivo* cat kidneys attain tissue:plasma C-6 concentration ratios of 40-70. There are two species, however, in which slice uptake does serve as a useful predictor of *in vivo* renal activity. Rabbit slices fail to accumulate C-6 or decamethonium (C-10). *In vivo* Young et al. (*47*) found the C-6 clearance equal to that of inulin and Christensen (*48*) reported the C-10 clearance to be 85% of that for inulin. At the other extreme the chicken actively secretes C-6 (*49*) and chicken slices show high S/M ratios (*37*).

Species specificity is also emphasized by purely *in vitro* studies. For example, the rat kidney cortex slices transport both TEA and NMN well, but hexamethonium not at all. The rabbit kidney cortex transports TEA, but not NMN or hexamethonium. The cat kidney cortex slices transport all three about equally effectively (*37*).

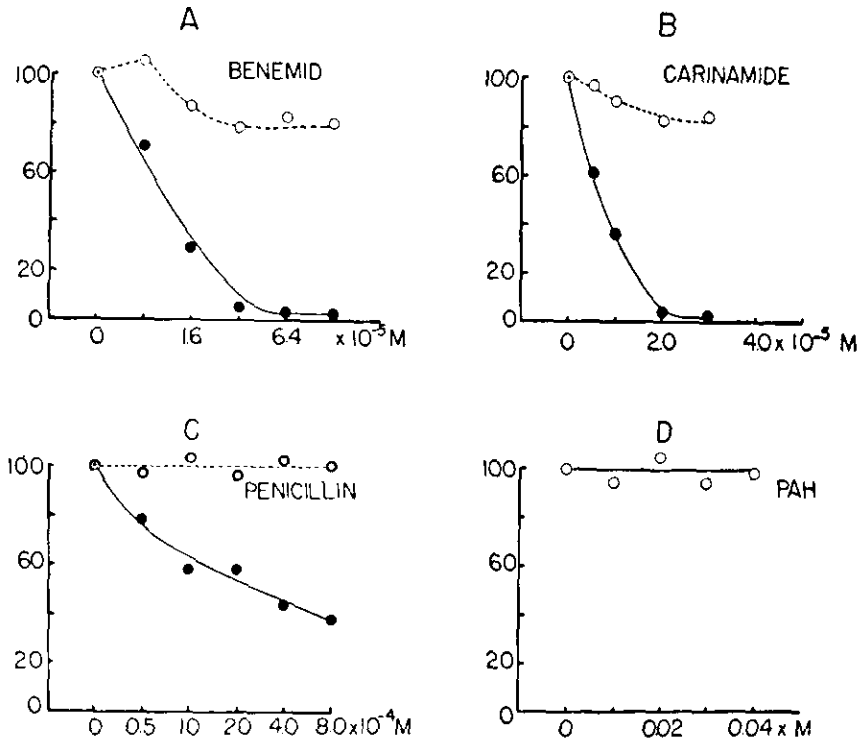


FIGURE 6. Effects of various acids on uptake of (•) PAH and (○) TEA by dog renal slices. Data are given as percent of control. From Shideman et al. (*46*) with permission of the authors and University of Wisconsin Press.

Uptake of Sugars

Although some evidence has accumulated to indicate that glucose may undergo bidirectional transport (*50-54*), most of the transport is in the reabsorptive direction in the intact animal. Glucose is completely filtered at the glomerulus and is virtually totally removed from the tubular fluid in its passage through the nephron. Most of this activity occurs in the proximal tubule, although some is found in other nephron segments (*55*). Hence whatever slice uptake is noted with glucose or other sugars, it is likely to represent an *in vitro* model for *in vivo* reabsorption, rather than secretion as noted with other organic substances.

Krane and Crane (*56*) attempted to demonstrate D-glucose uptake by rabbit renal cortex slices. The failure to do so was attributed to the rapid metabolism of glucose in the slice system. However, these workers did show S/M ratios greater than one for D-galactose. This uptake was reduced by anaerobiosis, uncouplers of oxidative phosphorylation, phlorizin and glucose.

More recent studies from Kleinzeller's laboratory confirmed these experiments (*57,58*). In addition the dependence of galactose uptake on sodium ion was demonstrated (*59,60*). Galactose efflux from renal cortex slices has also been interpreted to be carrier mediated since it is blocked by phlorizin. Also the efflux phenomenon is more sensitive to temperature than is uptake (*61*), at least with α-methylglucoside.

Kleinzeller's laboratory has devised a method whereby glucose transport can be measured directly. In experiments where substrates that promoted gluconeogenesis were present, exogenous glucose uptake could be measured. S/M ratios of 3-4 were reported. The glucose uptake was saturable, energy-dependent and sensitive to ouabain and phlorizin.

In addition to glucose and galactose many other sugars have been studied *in vitro* (*58,60*). D-Fructose and α-methyl-D-glucoside are accumulated by renal cortex slices to the same extent as galactose, and by a sodium-dependent system. D-Xylose, 6-deoxy-D-glucose, and 6-deoxy-D-galactose are also taken up, presumably by the

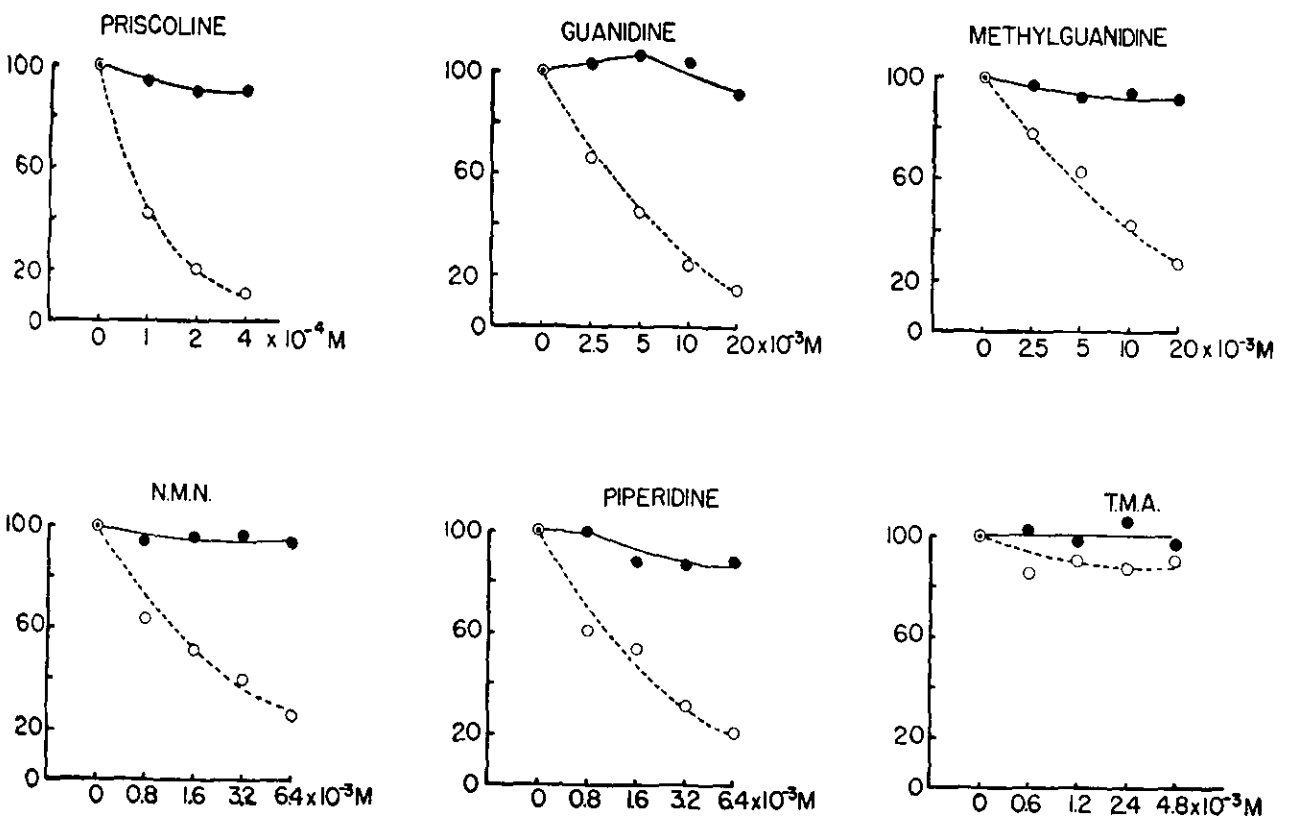


FIGURE 7. Effects of various bases on uptake of (•) PAH and (○) TEA uptake by dog renal slices. Data are given as percent of control. From Shideman et al. (*46*) with permission.

same system, but to a lesser degree. 3-O-methyl-D-glucose, D-arabinose and L-arabinose are not accumulated by renal slices. Two other sugars, 2-deoxy-D-glucose and 2-deoxy-D-galactose are taken up by an active transport system, but not the same one that handles the other sugars. This latter system is both sodium-independent and phlorizin-insensitive.

Most of the sugars mentioned above have also been examined under *in vivo* conditions. This gives us the opportunity to determine whether or not a correlation exists between *in vitro* slice uptake and *in vivo* reabsorption by the nephron. The relationship is presented in Table 3. Most of the *in vitro* data are those of Kleinzeller and his colleagues (*57,58,60*), but the *in vivo* data come from many other sources (*53,54,65-67*). In general, those sugars that are actively reabsorbed *in vivo* distribute in the renal cortex slice with high slice:medium ratios. There are exceptions, e.g., 6-deoxy-D-galactose is accumulated *in vitro* but not reabsorbed *in vivo*, D-xylose is not accumulated *in vitro*, while the *in vivo* data are inconsistent, i.e., Shannon (*65*) demonstrated reabsorption, while Silverman et al. (*66,67*) failed to do so.

With the organic acids, such as PAH, the *in vitro* accumulation is correlated with *in vivo* renal tubular secretion. In the case of sugars, it appears *in vitro* uptake is correlated with *in vivo* reabsorption. At least most workers agree that reabsorption is the predominant tubular event, hence it seems unlikely that slice uptake represents secretion.

Uptake of Amino Acids

Extensive studies utilizing renal cortical slices have been reviewed by Segal and Thier (*68*), and will not be discussed here. Most of the *in vitro* studies have been with cystine and the basic amino acids, although other transport processes do exist. For example, the nonmetabolizable, neutral amino acid, α-aminoisobutyric acid (AIB), has been found to be accumulated well by rat renal cortex slices. The transport of AIB, however, is species specific, and for example, the use of the rabbit is virtually precluded because of the poor transport.

Table 3. *In vivo* **reabsorption compared to renal cortical slice uptake of sugars.**[a]

Sugar	*In vitro*[b]	*In vivo*[c]
D-Glucose	+ +	+
L-Glucose	–	–
D-Galactose	+ +	+
D-Fructose	+ +	+
D-Mannose	+	+
3-*O*-Methyl-D-glucose	–	–
6-Deoxy-D-galactose	+	–
2-Deoxy-D-glucose	+ +	+
D-Xylose	+	+

[a] Modified from Mudge et al. (*62*).

[b] Tissue to medium distributions > 2.0 are indicated by double plus (++); values between 1.0 and 2.0 by plus (+); values < 1.0 by minus (–).

[c] Reabsorption is indicated by plus (+); no reabsorption by minus (–). No distinction is made between species.

Uptake of Inorganic Electrolytes

A variety of studies have been undertaken to evaluate the tissue accumulation of both the normally occurring electrolytes, e.g., sodium, potassium, as well as exogenous substances, e.g., thiosulfate. These studies have not been confined to just renal tissue, of course. Many studies on the steady-state electrolyte distribution have been performed with brain slices, for example, along with evaluations of substrate requirements for the maintenance of normal electrolyte content. In addition, the effects of potassium on brain slice respiration has been studied. The subsequent comments here, however, will deal only with kidney.

Most of the renal studies have been done with cortical tissue and two general types of experimental protocols have been used. The first of these involved allowing fresh slices to attain a steady state electrolyte distribution in the presence of a balanced salt solution, after which a tracer amount of labeled potassium, for example, was added to the incubation medium. Then the accumulation of the labeled ion was followed with time or in the presence of an inhibitor, etc. (*69*).

The more common technique is depicted in Figure 8 taken from the paper of Mudge (*70*). In this procedure the renal cortex slices are bathed in a large volume of isotonic sodium chloride solution either at room temperature or in the cold. This promotes the loss of potassium (and other substances) from the tissue with an approximate equal accumulation of sodium. As can be seen from Figure 8, most of the tissue electrolyte imbalance occurred during the preparation of the slices and early in the leaching process. Even leaching for several hours did not deplete the tissue potassium stores entirely. Once this partially potassium-depleted tissue was added to a balanced salt solution and the temperature elevated, the tissue electrolyte content was promptly restored to near the fresh tissue values.

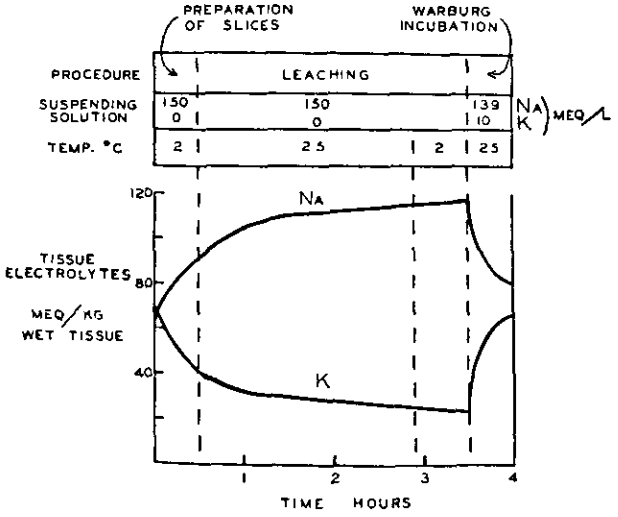


FIGURE 8. Renal cortex levels of sodium and potassium during slice preparation and incubation. From Mudge (*70*) with permission of the author and the American Journal of Physiology.

Mudge (*70,71*) utilized this procedure to evaluate the characteristics of potassium accumulation by renal cortex slices. In addition he examined the effects of a large number of inhibitors, e.g., organic mercury compounds. Other workers (*72,73*) studied the effects of temperature on potassium uptake with the details of substrate requirements. Also the effects of potassium loading and hormone pretreatment in rabbits were examined. Cohen and his colleagues (*74-76*) have evaluated the details of many of these problems in an attempt to relate the electrolyte transport to substrate accumulation under *in vivo* as well as *in vitro* conditions. The significance of these relationships is still unclear, however, although the substrate-electrolyte interactions may represent an example of coupled transport.

Furthermore, the overall meaning of the *in vitro* electrolyte transport studies is difficult to evaluate if an attempt is made to relate them to normal renal physiology. In the case of organic anions, and probably organic cations, it is possible to equate renal slice uptake to active tubular secretion of these substances by the intact animal (see above). With the sulfate ion, for which an attempt has been made to correlate the *in vitro* and *in vivo* studies, the situation appears more complicated. Deyrup and Ussing (*77*) first reported that the sulfate ion was accumulated by rat renal cortex slices. This uptake process appeared to be an active one, with specific temperature and substrate requirements, etc. (*78-81*). No evidence exists, however, for *in vivo* secretion of this ion. In fact only tubular reabsorption has been found with stop-flow studies (*82*). Deyrup has proposed, therefore, that slice uptake of sulfate is related to tubular reabsorption of the ion. A comparison of the characteristics of *in vivo* reabsorption with *in vitro* slice uptake is presented in Table 4. Glucose depressed sulfate T_m in the dog (*83*) and sulfate accumulation by rat slices, an effect that can be reversed by phlorizin. Also several amino acids depressed both functions (*84*).

Thiosulfate, a close chemical relative of sulfate, is also accumulated by rat renal cortex slices. It is not entirely clear whether or not the uptake process for this ion is the same as for sulfate although detailed comparisons have been attempted (*80,85,86*). It is interesting to note, however, that there is unequivocal evidence for thiosulfate secretion. For example, the male dog and the female after cortisol pretreatment can be shown, by stop-flow analysis, to secrete thiosulfate in the proximal region of the nephron (*87*). Experiments on *in vivo* renal handling of thiosulfate in the rat are not available.

Probably, however, there is no relationship of *in vivo* transport of the inorganic electrolytes, sodium, potassium, or chloride by renal tissue to that noted *in vitro*. All tissues strive to maintain normal electrolyte and fluid balance, and *in vitro* studies which relate to sodium, potassium, and water movement probably are demonstrations of these functions. This issue is not settled, however, and data may be accumulating to change this point of view (*88*). However, except for a few workers, most would agree that the *in vitro* slice measurement of electrolyte transport does not have an *in vivo* counterpart in terms of overall renal function. Nevertheless, a variety of chemicals, e.g., nephrotoxins, are capable of disrupting normal electrolyte and water distribution within the renal tissue, and this can be evaluated using the renal slice technique under *in vitro* conditions. To determine actual intracellular concentrations of electrolytes it is

Table 4. Comparison of renal sulfate handling.

	Uptake by slices	Tubular reabsorption, T_m
Glucose	↓	↓
Glucose and phlorizin	—	—
Arginine	↓	↓
Alanine	↓	↓
Taurine	↓	↓

necessary to measure the inulin space as well as concentrations of the ions. Usually the latter is done with flame emission photometry, while conventional chemical or radiochemical procedures assist in the determination of tissue inulin values. Inulin space (extracellular space) and intracellular electrolyte concentrations are calculated by standard procedures (*89*). From an experimental point of view these slice studies have proven quite useful for the evaluation of the effects of various nephrotoxins on renal electrolytes, whether added *in vitro* to fresh slices, or administered to intact animals.

Efflux Experiments

All of the above experiments were concerned with the net accumulation of the substances. These studies do not allow the evaluation of unidirectional movement of the compounds under study. Farah's group (*12*) devised a technique for study of the one-way washout of PAH and other compounds from renal cortex slices. The slices were preloaded under the usual experimental conditions, after which they were rinsed free of extracellular contamination and then exposed for short periods of time to a series of beakers containing PAH-free media. From the amount of PAH in the runout samples and in the tissue at the end of the experiment efflux curves such as those in Figure 9 can be constructed. This figure from the work of Farah et al. (*12*) shows that the slow phase of the efflux process is first order and that the rate of runout increases as the time interval of exposure to the PAH-free medium is reduced. That is, at short time intervals, e.g. 30 sec and 1 min, the efflux is essentially constant, which indicates that reaccumulation of PAH from the solution in a given beaker is minimal over that time period. Farah et al. further evaluated PAH reaccumulation during the runout experiment by adding ^{14}C-labeled PAH to a runout beaker and then analyzed the tissue for radioactivity after it had been exposed to the solution for the appropriate runout time period. With a 30-sec exposure it was calculated that less than 5% of material lost from the slices was reaccumulated. This further confirms that this procedure measures essentially unidirectional movement of PAH.

From several experimental studies, the following model for the renal cortical cell handling of PAH was developed. The efflux process was contributed to by: (a) the activity of an intracellular PAH concentrating mechanism, (b) a transport

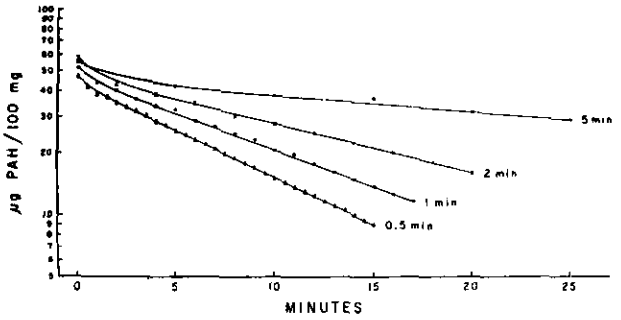


FIGURE 9. *Efflux of PAH from preloaded slices of dog* kidney cortex. From Farah et al. (*12*) with permission of the authors and the Journal of Pharmacology and Experimental Therapeutics.

process involved in the removal of PAH from the tissue and (c) a passive efflux process.

Probably the availability of PAH for diffusion out of the tissue is inversely related to the activity of the intracellular PAH concentrating mechanism. Acetate and lactate were shown to slow the efflux, and it was suggested they act by enhancing the activity of this step. Metabolic inhibitors, which decreased the concentrating activity, increased the efflux rate. Also competitors (depending on the concentration used) might increase the runout rate by promoting the release of PAH from the site of accumulation. Whether or not this intracellular site of accumulation is equivalent to PAH binding is not clear, although no evidence of quantitatively important tissue binding of PAH is available.

The transport process (b) must also be involved in explaining the effects of several substances on PAH efflux. For example, several competitors (probenecid, iodopyracet) can increase runout when they are present in low concentration and decrease runout in high concentration. This dual effect is thought to reflect an inhibition of the "carrier-mediated" efflux process by high concentrations of inhibitors rather than an effect on the concentrating mechanism. This is especially convincing when it is noted that probenecid blocks the runout from a metabolically inhibited tissue since the effect here cannot be related to the accumulation process.

Certain difficulties are encountered with this explanation, however. For example, a decrease in temperature produces a decrease in efflux. If the temperature effect is mostly mediated through an alteration in accumulation process, which is presumed to be the metabolically dependent one, it might be expected that the decreased temperature would increase efflux. No doubt this means

that the temperature effect is a complicated one, perhaps, with a relatively greater effect on the efflux mechanism than on the accumulation process. The mechanism for such an effect is unknown, although similar observations have been made with respect to sugar transport (*61*).

It is worth noting that this model proposed by Farah is similar to that suggested by Foulkes and Miller (*7*). That is, an accumulation process exists which is responsible for the high S/M ratios and is distinct from an active flux mechanism responsible for promoting intracellular and extracellular equilibration.

Another approach utilizing a similar technique was undertaken by Welch and Bush (*12*). By their kinetic analysis these workers demonstrated the presence of two intracellular compartments for PAH, only one of which was freely diffusable. This pool accounted for about 20% of the total tissue PAH. The addition of large concentrations of PAH to tissues preloaded with PAH-^{14}C, caused an increase in the freely diffusable pool to about 50% of total tissue PAH. Although there are problems with relating these studies to those of Farah (e.g., differences in the magnitude of the efflux rate constants), it is likely that Welch and Bush have offered a kinetic demonstration of the model suggested by Farah. The freely diffusable pool appears to be the equivalent of Farah's outward transport process and the nondiffusable pool the kinetic equivalent of Farah's intracellularly bound PAH. Interestingly, however, to date no direct demonstration of quantitatively significant PAH binding by renal cortical tissue has been forthcoming.

Runout studies also have been undertaken with the organic bases (*90*), but the data are not as clear cut nor the explanations of the data as convincing as with the organic acids. For example, no effect of metabolic inhibitors was noted on the *N*-methylnicotinamide (NMN) efflux process although these same inhibitors block net uptake of NMN. This is interpreted to mean that the runout process is independent of metabolism, i.e., passive, so that even though a metabolic inhibitor interferes with the concentration process there is no appreciable alteration of the efflux. These data, of course, stand in marked contrast to those for PAH. It was possible for NMN to resemble PAH efflux if the latter experiments were performed at 3°C, i.e., when no active accumulation of PAH occurred.

It may prove that the efflux technique is a more powerful one than the measurement of net uptake for sorting out subtle differences in the behavior of certain compounds. The characteristics of the uptake processes for PAH and uric acid (*10,91*) in the rabbit renal cortex slice system appear identical. Both acids are affected qualitatively in the same way by various factors under these *in vitro* conditions, despite differences in their renal handling *in vivo* (*92*). Examination of the efflux data, however, reveal differences in the handling of these substances. Although certain factors affect the runout of both compounds in an identical manner, there are striking differences. For example, acetate and probenecid do not affect urate efflux while both alter PAH runout. The K^+ ion has no effect on PAH efflux but is responsible, at least in part, for slowing urate efflux. Perhaps these differences are the *in vitro* counterparts of the discrepancies noted with the *in vivo* studies.

Concluding Remarks

In general the preceding comments summarize the state of the art and give an introductory look at the more useful aspects of the slice technique. Some minor variations in the use of slices have been offered, but in general have not proven too useful. For example, Burg and Orloff (*93*) devised a chamber in which they mounted a single, large, tissue slice. Each side of the slice could be superfused and the entire chamber fitted into a well counter. This procedure allowed continuous monitoring of, for example, ^{42}K uptake by the tissue. This procedure, however, did not offer any new information, relating to tissue electrolyte uptake, than had been learned with more conventional and technically simpler slice procedures.

Net accumulation experiments like those reported herein also have been done with liver slices and kidney medulla. Despite the *in vivo* evidence for an organic acid secretory system in the liver, the *in vitro* data are not as convincing. With organic bases (e.g., procaineamide ethobromide), Solomon and Schanker (*94*) demonstrated active uptake by liver slices. Attempts at demonstrating specific organic acid uptake have not been successful, however. For the most part the uptake of acids, such as bromsulfophthalein appears to be the result of nonspecific binding rather than a specific transport process (*95*). Such binding has been noted with some compounds in renal tissue slices, but the incidence of such occurrences is low. Nonetheless this is an aspect of renal slice uptake which cannot be ignored and should be entertained as a

possible uptake mechanism unless proven otherwise. For example, part of the accumulation of 2,4-dinitrophenol is by such a mechanism, although specific accumulation is also noted (*96,97*).

Finally, the slice technique when used for transport studies is most powerful if the movement of foreign organic compounds is studied. The investigations involving naturally occurring substances almost always become complicated by metabolic considerations. This is not an insurmountable problem, but it does tend to complicate a straight-forward transport study. In fact even with certain drugs, one has to be aware of this possible complication and consider metabolite formation as a possible uptake mechanism.

Much of the author's experimental work included herein was done collaboratively with Dr. G. H. Mudge while on the faculty of Dartmouth Medical School. Some was done during the tenure of an Established Investigatorship of the American Heart Association, 1964-1969. The work was supported by various grants from the National Institutes of Health, American Heart Association, New Hampshire Heart Association, and the Life Insurance Medical Research Fund.

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