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Effective use of renal cortical slices in transport and metabolic studies

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The uptake and metabolism of two water-soluble vitamins were measured in rat renal cortical slices, isolated tubules, and vesicles of the brush-border and basolateral cell membranes to determine (a) whether it is possible to produce slices that have open tubules and, (b) whether slices and tubules metabolize vitamins similarly Transport of ascorbic acid is sodium-dependent in slices and in brush-border vesicles but is sodium-independent in basolateral vesicles, suggesting that the brush-border membrane of slices is accessible to components of the bathing solution. Nicotinic acid was metabolized similarly (97-98%) in both slices and isolated tubules. Oxygen consumption by slices maintained in a closed chamber was constant as pO_2 decreased from 88% to 58%. Slices are concluded to be a suitable model for transport and metabolic studies providing that care is taken in their preparation and use.

Introduction

In spite of convincing claims by several investigators that they have been unable to make renal slices that are useful as a research tool [1–3], the apparent success of others in obtaining reliable information from slices [4] has prompted us to reevaluate the prospects of using renal slices in studies of vitamin transport and metabolism Because reports of slice studies are currently in the literature, a better understanding of the properties of the preparation will make it easier to draw conclusions about the transport process investigated For instance, if the sodium-dependent concentrative uptake of galactose in rat renal slices reported by Kleinzeller et al [4] is considered to be a property of the basolateral cell membrane, the observation would be difficult to reconcile with textbook description of tubular reabsorption of filtered sugars

The use of renal cortical slices for in vitro study of cellular transport and metabolism has been the subject of controversy for more than a decade At the present writing, certain peer-reviewed journals do not publish studies that used slices because it is believed that either (a) the tubular lumens in all slices are 'collapsed' and therefore the brush-border membrane is not accessible to components of the bathing medium or, (b) slices do not have adequate oxygen delivery to support normal metabolic processes Implicit in these objections is the assumption that all slices are created equal i e, renal slices from all animal species have identical properties, the numerous subtleties of performing studies with slices are inconsequential, and all investigators are equally adept at producing slices In that one or all of these assumptions might be in error, it is of interest that renal slices have several advantages over other preparations of renal tissues (a) they are quickly and conveniently obtained The equipment is inexpensive and can be used by the investigator after a short period of

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training, (b) they are obtained without lengthy periods of exposure to digestive enzymes which might alter or destroy the biologic property of interest, (c) they reflect a composite of transport and metabolism of substrates of interest and, (d) intact slices are more easily and rapidly manipulated between experimental conditions than dispersed preparations of tissue An additional reason for pursuing the use of tissue slices in general is that perfusion of intact organs with digestive enzymes is not always possible, as in the case of harvesting samples of human tissues at the time of surgery

Materials and Methods

Brush-border vesicles were prepared from rat kidney by a procedure slightly modified from Malathi et al [5] as described in more detail by Bianchi and Rose [6] The specific marker enzyme for the brush-border membrane was leucine aminopeptidase activity which increased to 14 7-fold the crude homogenate activity Basolateral vesicles were prepared by a procedure slightly modified from Sacktor et al [7] as described by Bianchi and Rose (unpublished data) The specific marker enzyme for the basolateral membrane was $(Na^+ + K^+)$-ATPase activity which increased 8 7-fold relative to the crude homogenate Rats of 200–250 g were anesthetized with Nembutal and the kidneys were perfused for 30 s with cooled Krebs-Henseleit - bicarbonate buffer prior to removal for preparation of isolated tubules and cortical slices The subsequent procedures in either preparation were performed with minimum delay at 0–4°C up to the preincubation step

The method of Guder [8] was used to isolate tubules Slices approximately 0 5 mm thick were cut from the whole kidney with the use of a Stadie-Riggs tissue slices Noncortical tissue was removed with a scalpel Care was taken to prevent tissue dehydration during all procedures

The possibility that cellular uptake of substrate by slices of rat renal superficial cortex represents transport properties of the basolateral membrane only was evaluated in conjunction with a detailed investigation of L-ascorbic acid/dehydro-L-ascorbic acid transport reported elsewhere [6,14] The rationale was based on our initial observation that both slices and intact tubules have Na^+-dependent uptake of L-ascorbic acid In that L-ascorbic acid is filtered in the mammalian glomerulus [9–11] but is not normally found in the urine in appreciable amounts, it can be concluded that L-ascorbic acid is reabsorbed in the kidney If the Na^+-dependent uptake could be attributed to the brush-border membrane only [12], a firm conclusion could be made about the accessibility of the tubular lumens in our preparations to components of the bathing media Slices, isolated tubules, and vesicles made from the brush-border and basolateral cell membranes are used for this evaluation

Slices and isolated tubules were incubated 20 min at 37°C in the presence of 1 4 μM [^{14}C]ascorbic acid in either Ringer or Tris-substituted buffer In both preparations uptake was reduced approx 80% in the absence of Na^+

The Na^+-dependence of L-ascorbic acid uptake was evaluated in brush-border and basolateral vesicles prepared as described above Vesicles of either source were preequilibrated in 300 mM mannitol, 20 mM Hepes/Tris (pH 7 0) Incubation in the presence of 65 μM [^{14}C]ascorbic acid was performed at 20°C in 100 mM mannitol, 20 mM Tris-Hepes, and either 100 mM NaCl or 100 mM KCl Na^+-dependent uptake of L-ascorbic acid was clearly a property of only the brush-border membrane (Fig 1) The resulting conclusion that brush-border membrane vesicles and slices share a common transport property allows the deduction that slices can be made that have open tubules This deduction is easily reconciled with the earlier finding of concentrative, Na^+-dependent galactose uptake in slices, which appeared to conflict with textbook descriptions of renal function under the assumption that slices had collapsed tubules and therefore transport only at the basolateral cell membrane

The utility of slices in the study of vitamin metabolism was evaluated in conjunction with a more extensive investigation of renal handling of nicotinic acid (Schuette, S A and Rose, R C, unpublished data) Slices and isolated tubules were incubated 20 min at 37°C in Ringer that contained 2 2 μM [^{14}C]nicotinic acid The tissue samples were extracted and analyzed for nicotinic acid and its anticipated products of metabolism as de-

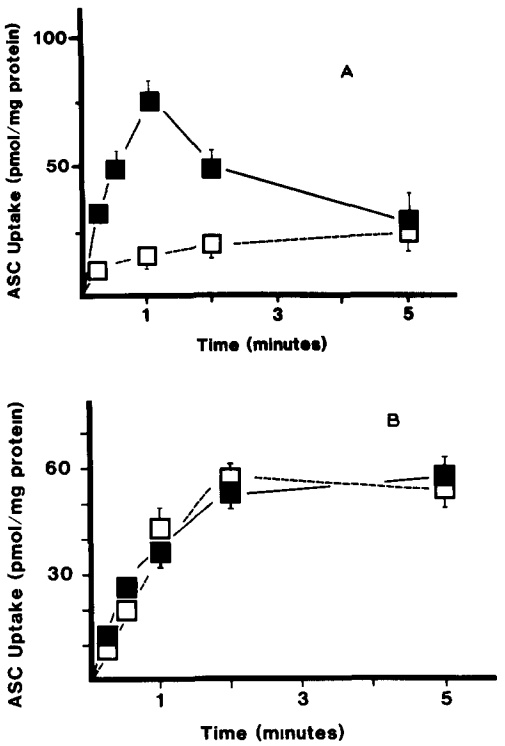

fig 1 Uptake of L-ascorbic acid (ASC) in brush border (A) and basolateral (B) membrane vesicles of rat renal cortex The vesicles were preincubated 60 min in 300 mM mannitol and 20 mM Tris-Hepes (pH 7 0) Incubation was performed at 20°C with 65 μM [^{14}C]ascorbic acid in the following medium 100 mM mannitol, 20 mM Tris-Hepes (pH 7 0) and either 100 mM NaCl (■) or 100 mM KCl (□) Bars represent S E of four determinations in (A) and three determinations in (B)

scribed above The results from studies on slices and isolated tubules are presented in Fig 2 Both tissue preparations metabolized at least 97% of recently accumulated substrate

According to the rationale of Balaban et al [3], the adequacy of oxygenation in a tissue preparation in vitro can be evaluated by measuring the O_2 consumption over a range of oxygen tensions If O_2 consumption diminishes at reduced O_2 tensions, O_2 delivery might be a rate-limiting step in metabolism and it can be assumed that the tissue is anoxic to some degree Freshly obtained slices of rat renal cortex were incubated at 37°C in Ringer that was equilibrated with air Oxygen consumption was determined with a standard Clark oxygen electrode The results (Fig 3) show that O_2 consumption was linear with time from

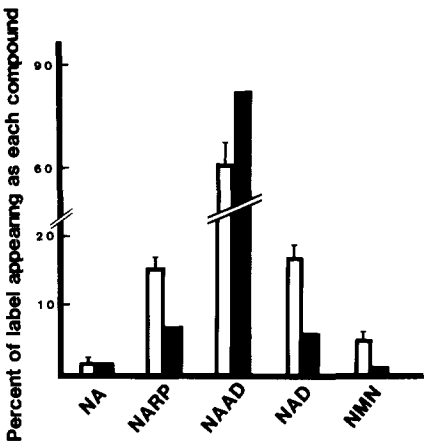

Fig 2 Identification of nicotinic acid and its metabolites extracted from tissue slices (shaded) or isolated tubules (open) exposed to [^{14}C]nicotinic acid Incubations were 20 min in 37°C Ringer with [^{14}C]nicotinic acid present at 2 2 μM In either preparation 97–98% of the radioactivity eluted with metabolites of nicotinic acid Values represent the amount of ^{14}C-label found in fractions collected from the column effluent of HPLC analysis NA nicotinic acid, NARP, nicotinic acid ribonucleotide, NAAD, nicotinic acid adenine dinucleotide and NMN, nicotinamide mononucleotide Values represent means ± S E of five determinations, or representative observations

88% down to 58% of the initial O_2 partial pressure Afer the bathing solution was again equilibrated with atmospheric O_2, the original rate of O_2 con-

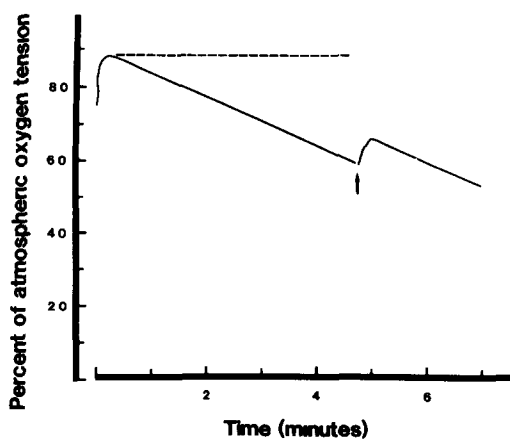

Fig 3 Oxygen consumption of a rat renal cortical slice The tissue was incubated at 37°C in physiological buffer equilibrated with air The chamber was open to atmosphere briefly at the time shown by the arrow Redrawn from original tracing Dashed line is signal drift in the absence of tissue

sumption resumed, suggesting that the tissue was maintaining constant viability over the duration of the study Application of CN resulted in a prompt cessation in O_2 consumption These results suggest that the renal slices used in the present study have adequate oxygenation

The present study presents data that can be used to evaluate whether slices of cortex might be useful in determining the properties of renal vitamin transport and metabolism The physiochemical properties of water-soluble vitamins suggest that they would be filtered in the renal glomerulus, the low urinary losses suggest that extensive reabsorption occurs It is clear that a reabsorptive process can be investigated in intact tissue in vitro only if tracer vitamins added to the bathing medium have access to the tubular lumen and the brush-border membrane In that slices, tubules, and brush-border vesicles, but not basolateral vesicles, demonstrate Na^+-dependent ascorbic acid uptake, it is concluded that the Na^+-dependent uptake of ascorbic acid in slices represents a brush-border process The similar (97–98%) metabolism of nicotinic acid in slices and isolated tubules suggests that the two preparations have similar metabolic capacities Because conversion of nicotinic acid to nicotonic acid adenine dinucleotide and the other products indicated in Fig 2 are ATP-consuming processes, it is concluded that both tissue preparations have sufficient ATP available that energy is not a major limiting factor in the handling of nicotinic acid The oxygen consumption data support this conclusion

There are clearly contrasting conclusions regarding the suitability of renal slices for transport and metabolic studies between the affirmative findings reported here and by Kleinzeller et al [4] versus the negative reports of Wedeen and Weiner [1], Balaban et al [3], Arthus et al [13], and Cole et al [2] It would be of interest to examine differences in experimental design that might account for these differences

Wedeen and Weiner incubated slices of rat kidney in the presence of ^{14}C or ^{3}H-labeled inulin or sucrose The authors found that both of these substances, which are considered to diffuse into freely accessible extracellular spaces, were excluded from the proximal tubules lumen It should be pointed out that most of this study was performed in a medium that contained 36 mM K^+ The cells of various epithelia exposed to such elevated K^+ concentrations are electrically depolarized, swollen, and have other physiologic alterations Also, bicarbonate was not included in the bathing medium Bicarbonate is reabsorbed by the tubule and plays an important role there in fluid reabsorption and cellular metabolism Thus, it must be anticipated that these tissue slices did not have the same physiological properties as those used by most other investigators in kidney slice studies When Wedeen and Weiner changed from K^+-buffer (no bicarbonate) to Krebs-Ringer-bicarbonate buffer containing K^+ at the physiologic concentration of 5 7 mM, the tissue inulin space changed from 22% to 26% This mean increase of 18% in inulin space might represent the difference between open tubules and closed tubules Unfortunately, no histology was reported using slices incubated in Krebs-Ringer It must be concluded that the data of Wedeen and Weiner are of little help in estimating whether slices incubated under more physiologic conditions have open or closed tubules

Arthus et al [13] found that brush-border enzyme markers are somewhat more accessible to substrates of the bathing media in isolated tubules than in slice preparations It must be concluded that the tubular lumens in slices prepared by this research group are somewhat restricted, but not prevented, from having contact with components of the bathing solution Although this reference is sometimes cited as evidence that most transport in slice preparations occurs at the basolateral membrane, the underlying reasoning is clearly fallacious If nature has placed the transport apparatus of a particular substrate in the brush border membrane only, and if the substrate has access to the tubular lumen, then the slice will represent brush-border transport Thus, Arthus et al [13] is most appropriately cited in favor of the possibility that renal slices can be used to study brush-border transport

Cole et al [2] attempted to evaluate whether mouse renal cortical slices take up sulfate by a brush-border membrane mechanism Their rationale was to compare the Na^+-dependence of sulfate uptake in slices and in brush-border vesicles In

the latter preparation, sulfate accumulated by a saturable mechanism that was dependent on a Na^+ gradient (outside greater than inside), although it was not possible to determine from the manuscript what concentration gradient of Na^+ was initially imposed The Na^+-dependence of sulfate uptake in slices was determined in a sucrose medium Slices were incubated in sucrose/Tris-Hepes (pH 6 5) for 60 min at 37°C Cations were added to the sucrose medium as chloride salts K^+ was present at concentrations of 0–80 mM Extracellular K^+ concentrations of 0 and 20–80 mM are quite nonphysiologic and not frequently used in biological studies when the investigator seeks to maximize tissue viability and normal transport/metabolic properties The most physiologic K^+ concentration used was 5 mM Na^+ was either absent or was present at 20 mM in media that contained K^+ at 0 or 20 mM In that Na^+ was added only to a final concentration approx 15% of physiologic buffers, and only in media that contained sucrose at 280 mM and K^+ at nonphysiologic levels, the finding that Na^+ did not stimulate uptake of sulfate appears to apply only to tissue incubated under the unique circumstances of the study Because the slices in Ref 2 underwent a 60 min incubation at a relatively acidic pH (6 5), it is quite possible that they had become metabolically inert No control studies were performed to assess tissue viability It is apparent that these lengthy studies on mouse tissue are of limited value in predicting the success of short-term incubations of renal cortical slices from other animal species under more physiologic conditions

Balaban et al [3] have attempted to evaluate the usefulness of tubules and slices of rabbit kidney in transport and metabolic studies They found that isolated tubules in a closed chamber maintained constant O_2 consumption over a wide range of O_2 tensions whereas the O_2 consumption of slices decreased as p_{O_2} declined They reasoned that the declining O_2 consumption by slices indicated that insufficient O_2 reached inner parts of the tissue as the study progressed However, no control experimental procedure was employed, as in the present study where it was shown that reequilibration at the original O_2 tension yielded the original rate of O_2 consumption Thus, it is possible that the tissue slices of Balaban et al [3] which had been preincubated 60 min at 37°C, were losing viability and would have shown decreasing O_2 consumption even at a constant O_2 tension It is inevitable that tissue viability decreases with time under the conditions in vitro The most that can be concluded from this observation is that rabbit renal slices incubated in excess of 60 min have suboptimal O_2 consumption This observation has unknown relevance to the use of renal slices incubated for shorter periods of time

Balaban et al [3] also advised against the use of renal slices on the basis of their lower ATP content and cytochrome redox levels when prepared as described above Again, however, it appears that either the 45 min preincubation procedure, the initial 10–15 min perfusion of kidneys with Ringer, or the delay and possible trauma encountered due to measuring the thickness and uniformity of each slice might have led to the diminished tissue viability noted by the investigators of Ref 3 In any event, it is not clear whether the slices of Ref 3 had normal vitamin transport function, and how those findings apply to the use of renal slices by other investigators

Although we consider invalid the claim that all renal slices are metabolically deficient regardless of incubation conditions, it is of interest to consider the consequence of using for transport studies slices that have some inert inner areas In an evaluation of cumulative uptake of a substrate (e g , Fig 1 in Ref 4) the inert cells can be assumed to have no greater substrate content than expected to occur at equilibrium conditions, i e , the medium concentration Thus, the total amount of substrate found in the tissue would be distributed in a smaller amount of cellular water than one calculates on the basis of extracellular space markers and tissue water content To the extent that some of the tissue in Fig 1 in Ref 4 might have been inert, the reported tissue/medium ratio of sugar would be an underestimate of the actual value in transporting cells Thus, major conclusions of the study would not be affected

A direct assessment of how slices might be used in a transport/metabolic investigation has been made in the present study in which concentrative transport of ascorbic acid and energy-dependent metabolism of nicotinic acid is demonstrated The positive findings do not allow one to suggest, of

course, that renal slices should be used to the exclusion of isolated tubules and membrane vesicles, but rather that slices may be used advantageously in conjunction with other approaches As with any scientific tool, care must be taken to optimize the experimental conditions

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