Para-aminohippurate and Tetraethylammonium Transport in Fragments of Rat Renal Cortex[1] (37664)

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In 1950 Cross and Taggart (1) incubated rabbit kidney slices in a medium containing para-aminohippurate (PAH) and compared the concentration of PAH taken up by 1 g of slice (S) to the concentration of PAH left in 1 ml of medium (M). That active transport did take place was evidenced by an S/M ratio which greatly exceeded 1.0. Since these original studies, many have investigated transport of different types of compounds in kidney slices.

In the past, we have used this technique in rat experiments as one assessment of renal tissue viability (2–3). However, like others (4), we have noted large variations among the transport of individual slices, even those obtained from the same animal. The purpose of this report is to describe a simple *in vitro* procedure which, compared to slices, yields less variation in organic anion and cation transport within a given experiment.

Materials and Methods. Male Sprague-Dawley rats (150–250 g), allowed free access to rat chow and water, were sacrificed by a blow on the head; and the kidneys were removed rapidly and placed in cold saline. We cut cortical slices (0.4–0.5 mm) with a Stadie-Riggs microtome within 30 min after obtaining kidneys. Each slice was then bisected to give two halves which were paired for study as previously described (5). Tissue fragments were obtained by forcing cortex, diced into small pieces with scissors, through a nylon sieve (2 × 1.5 mm). This nylon sieve was obtained from the framework of a twin coil dialyzer (Travenol Lab Inc., Walton Grove, IL). The bottom of the sieve was scraped with a spatula to recover as much tissue as possible, and this was placed into cold oxygenated medium (1). The tissue was allowed to settle for 2 to 3 min and the supernatant was decanted. The fragments were washed twice by mixing with cold oxygenated medium.

The basic meedium used in the washings and incubation was that described by Cross and Taggart (1), a phosphate buffered sodium, potassium and chloride solution. To this we added both ^{14}C-tetraethyl ammonium bromide (TEA) (New England Nuclear) and ^{3}H-para-aminohippurate (PAH) (Amersham/Nuclear) to final concentrations approximating 10^{-5} *M*. For PAH and TEA studies, incubation was for 90 min at 24° on a Dubnoff metabolic shaker. After incubation, slices were removed, weighed and then placed in 5% trichloroacetic acid (TCA). The fragments were filtered with suction through circular filter paper (Whatman No. 40—2 cm radius). The filter paper and fragments were weighed together; and by means of a spatula, fragments were removed and placed in 5% TCA. The weight of the tissue placed in the TCA was determined by reweighing the filter paper and calculating the difference in weight. The slices and fragments were homogenized with a Teflon pestle, were centrifuged and 1 ml of the supernatant was used for counting. The incubation medium was added to 5% TCA and centrifuged prior to counting. The basic scintillation mixture was composed of Triton X-100 (Packard Nuclear) (1/3 vol), toluene (2/3 vol), 2-5-diphenyloxazole (5.5 g/liter) and 1,4-bis[2-(5-phenyloxazolyl)] benzene (0.125 g/liter). Double isotope β counting and

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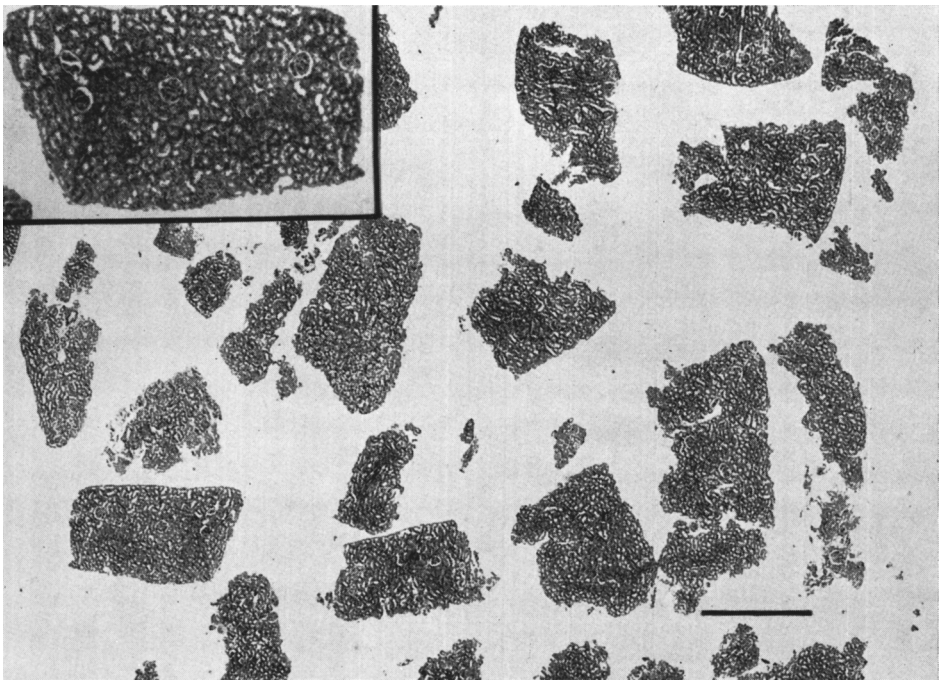

FIG. 1. Low magnification view of the renal cortical fragments showing average size and glomerular contents. Scale marker is 1 mm (14.5×). Insert: Tubular lumina are open and the epithelium is well preserved. 7 glomeruli are shown (25.0×).

quench correction were performed on a Packard counter, Model 2420.

To express the *in vitro* accumulation of para-aminohippurate into kidney slices and fragments, an S/M or T/M ratio was used. This is defined as the ratio of the amount of material estimated by the counts per minute in 1 g of tissue slice (S) or fragmented tissue (T) to the amount of material estimated by the counts per minute in 1 ml of media (M) present after incubation. To minimize variations among individual slices and to correct for daily variations in the procedure, the S/M quotient, which is defined as the relative value of the S/M ratio based on a control determined at the same time, was used. In this study, a single slice approximately 50 mg in weight was halved. One half was arbitrarily placed in the "experimental flask" while the other half was incubated in the "control flask." The S/M quotient is the ratio of the S/M of the experimental flask compared to the S/M of the control flask, and in our case since we are studying duplicates, should approximate 0.

Oxygen consumption was measured on kidney tissue, preweighed in the manner described above, on a Gilson differential respirometer (submarine) at 37°. Readings were obtained at 10 min intervals for 90 min. Results are expressed as microliters per milligram of wet weight per hour. Samples of the fragments were fixed in 4% buffered formalin and processed routinely for light microscopy. For electron microscopy the fragments were fixed in 2.5% buffered glutaraldehyde, postfixed with 1% osmic acid, embedded in Epon 812, sectioned with Porter & Blum ultramicrotome MT-2, stained with uranyl acetate and lead citrate, and examined with Siemens Elmiskop 101.

Results. Morphology. Light microscopy. The renal parenchyma shows excellent preservation (Fig. 1). Mild compression artifacts are noted at the edges of some fragments. The tubular epithelium are intact in most areas with the tubular lumina open. Each fragment in the 6-μm sections has an average of 5–6 glomeruli by light microscopy and so would contain approximately 11–15

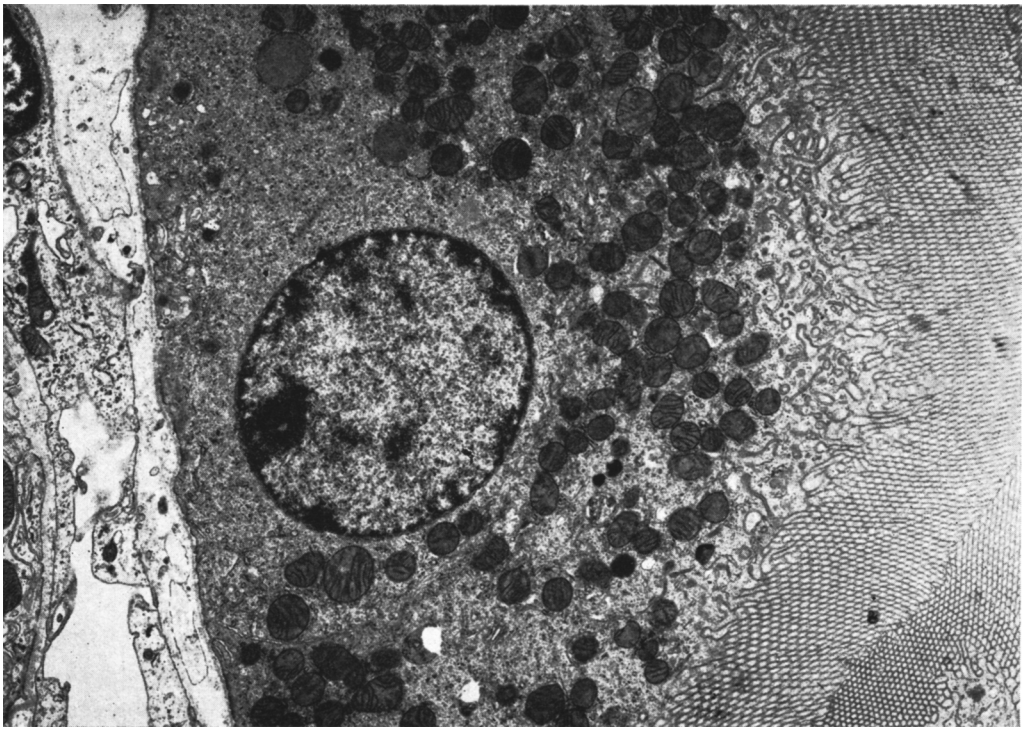

FIG. 2. Ultrastructure of a proximal convoluted tubular epithelial cell after 90 min incubation showing normal brush border, well preserved mitochondria and normal nucleus (10,200×).

glomeruli in this cube of tissue. Electron microscopy: the ultrastructure of the tubular epithelium after 90 min of incubation also show remarkable preservation (Fig. 2). The brush borders are intact and normal appearing in most areas. There is reduction in the numbers of the mitochondrial matrix granules, but no swelling of the matrix or the crystae is noted. The perinuclear cysterna is not dilated and the chromatin materials are normally distributed.

PAH and TEA transport. We studied 28 slice pairs to determine the range of S/M quotients (Fig. 3) for PAH and TEA transport. For PAH, the quotient was +2.8 ± 3.2% (SEM) a figure not significantly different from 0. However, the differences in quotients ranged between +29 and −29%. For TEA transport, the quotient was −3.2 ± 4.1% (SEM) with even a wider range of +30 to −50%. Since these are slice pairs, comparisons are made on slices incubated and analyzed simultaneously. When one compares the variation among fragments studied on the same day by comparing the difference in each individual flask with the overall average for the day, the greatest range in any of 6 experiments depicted in Fig. 3 for PAH transport was from +11 to −8% and for TEA transport +18 to −21%, a much narrower range than that seen for slice pairs.

The above studies were performed using a 90 min incubation time. To determine when transport in fragments reached a steady state condition, *i.e.,* when influx of PAH and TEA equaled efflux, the studies depicted in Fig. 4 were performed. A plateau for PAH T/M was seen by 30 min. TEA transport did not reach a steady state until after 1 hr.

The overall uptake of PAH in slices was 8.8 ± 2.1 (SEM) and for fragments was 5.9 ± 0.1 (SEM) and the uptake of TEA in slices was 12.8 ± 4.2 (SEM) and for fragments was 14.8 ± 0.2 (SEM). In the presence of acetate and serum (5% v/v), the uptakes of both PAH and TEA increased (Table I).

Oxygen consumption. In 9 flasks containing slices, the oxygen consumption averaged 3.48 ± 0.06 μl/mg/hr (SEM) while in 9

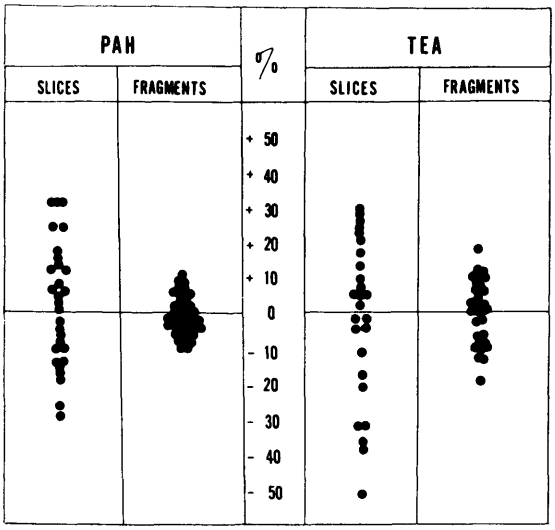

FIG. 3. Comparison of PAH and TEA transport in paired slices and in tissue fragments. Results for slice pairs are expressed by the S/M quotient and in each flask of tissue fragments as the percentage of the overall average on a given day (6 experiments). For slices $n = 28$ pairs and for fragments $n = 30$ flasks.

flasks with fragments the oxygen consumption averaged 3.45 ± 0.11 μl/mg/hr (SEM).

Discussion. Since the original work by Cross and Taggart (1), kidney slices have been used frequently to obtain *in vitro* data on organic anion and cation transport. While much has been learned through this technique, large numbers of slices must be studied in any given experiment because of difficulty in reproducing results among individual slices.

Why do slices vary so much in their uptakes of PAH? There are probably many reasons, *e.g.*, (a) transport differences between animals (b) transport differences in tissue from different areas and depths of the cortex in the same animal, and (c) the varying thickness of the slices themselves. In regard to the latter, Wedeen and Weiner (6, 7) has shown through autoradiography that PAH transport occurs to only a certain slice depth. Therefore, an S/M which is based on tissue weight would be theoretically higher in thinner slices. To minimize many of these variations, we have bisected slices, using one half under control conditions, the other half under test conditions and have expressed our results as an S/M quotient, a concept originally suggested by Koishi (4). He defines the S/M quotient as the relative value of the S/M ratio of the test half slice compared to the control half slice S/M. Even by this procedure where tissue from approximately the same area of the same kidney is handled at the same time, we have had wide variation among our slice pairs in PAH and TEA transport (see Fig. 3).

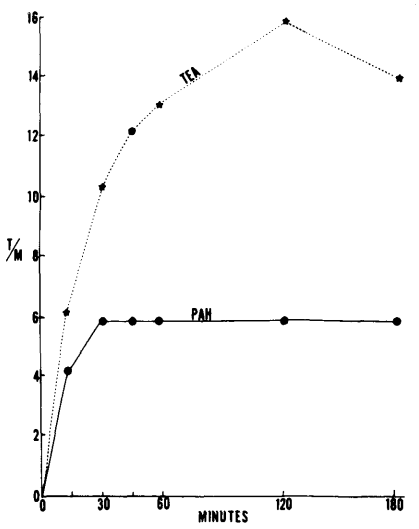

FIG. 4. Plot of average T/M ratio for PAH and TEA over 3 hr in rat kidney fragments. A minimum of 5 observations was made for each point.

TABLE I. Effects of Acetate and Calf Serum on PAH and TEA in Renal Fragments.

Additions	No.[c]	PAH T/M	TEA T/M
None	6	5.6 ± 0.2	12.2 ± 0.3
Acetate (10 m*M*)	6	7.1 ± 0.1[a]	14.3 ± 0.4[b]
Calf serum (10%, v/v)	6	9.3 ± 0.2[b]	16.9 ± 0.5[b]

[a] $p < 0.02$.
[b] $p < 0.01$.
[c] Number of flasks.

One alternative has been to isolate tubules and glomeruli from rabbit kidneys with the enzyme collagenase as described by Burg and Orloff (8). While this *in vitro* method requires extra time in preparation compared to slices, it does allow for more reproducible results in transport studies (9). However, the majority of our studies are on rats rather than on rabbits, and tubular isolation becomes somewhat more involved in these smaller animals. Rather than a direct injection of collagenase into the renal artery, collagenase must be given into the aorta after various peripheral vessels have been tied. Further, the yield of tubules per rat kidney by this digestion procedure is small.

To obviate these problems, we utilize renal fragments obtained by forcing diced cortex of rat kidneys through a nylon mesh. This requires no appreciable increase in preparatory time compared to the slice method. On a weight basis, these fragments (containing an average of 11 to 15 glomeruli) appear to be in good condition after incubation by morphologic criteria, consume oxygen at a similar rate as slices, reach steady state T/M for PAH within 20 min and steady state for TEA after 2 hr. In addition, fragments, like slices, are stimulated by the addition of 5% v/v serum (5, 8) and acetate (1). Their major advantage, however, is that within a given experiment, the T/M ratios for PAH and TEA have less variation among replicates, certainly less than that seen in paired studies (see Fig. 3). Obviously, this allows us to obtain more meaningful data in comparing *in vitro* transport of PAH and TEA under various conditions.

Summary. To develop a simple *in vitro* procedure which, compared to slices, yields less variation in organic anion and cation transport, we used fragments of renal cortex. Each fragment contained 11–15 glomeruli and, after 90 min of incubation, showed normal brush border and well-preserved epithelium. With this technique, there was less variation in transport studies within a given experiment, and fragments responded in the same manner as slices to addition of acetate and serum to the medium.

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