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Effects of Buffer and Temperature on pH Dependence of *p*-Aminohippurate Transport in Rabbit Kidney Slices[1] (40235)

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The transport of organic ions in mammalian kidney cortical slices is influenced by the pH of the incubation medium, often displaying a pH optimum at which either the rate of uptake or the degree of steady-state accumulation is maximal. However, a literature survey indicates that the values of pH optimum for the transport of *p*-aminohippurate (PAH), an organic anion that is used most widely to study the transport mechanism, are quite variable ranging from approximately 7.0 (1–3) to 8.0 or above (4, 5). The reasons for this wide variation are not known, although Bowman *et al.* (5) suspected that the species difference may be partly responsible. A careful analysis of the literature revealed that the low pH optimum may be associated with the use of phosphate or bicarbonate buffer and the high pH optimum with propanediol, an amine buffer. Recently, temperature has also been shown to alter the pH dependence of organic anion transport in rat kidney slices (6), and of certain enzyme systems such as the toad skin Na-K-activated adenosine triphosphatase (7) and the goldfish M_4 isozyme of lactate dehydrogenase (8). Hence, the present investigation was undertaken to systematically evaluate the effects of buffer and temperature on the pH dependence of the PAH transport system in the rabbit kidney.

Materials and methods. Adult New Zealand rabbits weighing from 2 to 4 kg were used. After the rabbit was sacrificed, the kidneys were immediately removed and placed in an ice-cold 0.12 *M* NaCl-0.02 *M* KCl solution (preincubation medium). The kidney was decapsulated and then perfused with 30–40 ml of the above solution to remove the blood. Renal cortical slices (0.3–0.5 mm thick and 70–100 mg) were prepared using a Stadie-Riggs tissue slicer, and were stored in the ice-cold preincubation medium for 20 min. Approximately 150 mg of the slices were then transferred to a flask containing 10 ml of a modified Cross-Taggart incubation medium (see below) and 70 μM PAH. The temperature of incubation medium was set at 17, 25, or 37°, with corresponding incubation periods of 60, 40, or 20 min. All preincubations and incubations were performed under 100% O_2, and the incubations were carried out in a Dubnoff metabolic shaker with the temperature regulated to within ±0.1°.

Three different buffers were used: 5 mM phosphate, 10 m*M* Tris(hydroxymethyl)-aminomethane (generally referred to as "Tris"), or 10 m*M* *N*-Tris(hydroxymethyl)-methyl-2-aminoethanesulfonic acid (generally referred to as "TES"). The last two buffers are amine buffers that are widely used in biological research. These buffers have not only different p*K*′ values, but also different temperature coefficients of p*K*′ ($\Delta pK'/\Delta T$), as shown in Table I. The basic incubation medium contained 125 m*M* NaCl, 5 m*M* KCl, 1.5 m*M* $CaCl_2$, and 10 m*M* Na acetate. The initial pH was adjusted to 6.6, 7.0, 7.4, 7.8 or 8.2 for the phosphate series, and to 7.0, 7.4, 7.8, 8.2, 8.6 or 9.0 for the Tris and TES series. The pH of the medium was adjusted by the ratio of basic to acidic forms of each buffer. Minor deviations from the desired pH values were corrected by adding either 0.12 *N* HCl or 0.15 *N* NaOH. The pH was always adjusted at the experimental temperature.

At the end of incubation, the slices were immediately removed from the medium, blotted and weighed. A portion of the slice was

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TABLE I. pK' AND ITS TEMPERATURE COEFFICIENTS ($\Delta pK'/\Delta T$) FOR WATER AND THREE BUFFER SUBSTANCES.

	pK' (Temp.)	$\Delta pK'/\Delta T$ (°C)
Water (Ref. 15)	14.0 (25°)[a]	−0.017
Phosphate (Ref. 16)	6.9 (25°)[b]	+0.0056
TES (Ref. 17)	7.5 (20°)	−0.020
Tris (Ref. 17)	8.3 (20°)	−0.031

[a] pKw.
[b] pK_2'.

TABLE II. RATE CONSTANTS FOR PAH EFFLUX AS FUNCTIONS OF MEDIUM pH AT 25°.

Buffer	Medium pH	K (min^{-1})
Phosphate (4)[a]	6.6	0.043 ± 0.002[c]
	7.4[b]	0.049 ± 0.002
	8.2	0.055 ± 0.003[d]
TES (3)	7.0	0.046 ± 0.002
	8.0[b]	0.064 ± 0.004[d]
	9.0	0.054 ± 0.005
Tris (3)	7.0	0.039 ± 0.002
	8.0[b]	0.045 ± 0.004
	9.0	0.053 ± 0.006

[a] Number of experiments.
[b] Mean optimal pH for uptake.
[c] Mean ± SE.
[d] Significantly different ($P < 0.05$) from the corresponding value at medium pH 6.6.

then dried for 24 hr at 90° in an oven for the gravimetric determination of the tissue water content. The remaining slices were homogenized in distilled water for the determination of PAH. The pH of the medium was again measured at the incubation temperature immediately after removal of the slices, and this pH will be referred to as the final pH. The medium was then analyzed for PAH. The transport function was evaluated by the rate of PAH uptake per unit tissue weight as determined at the end of incubation.

The effect of medium pH on the efflux of preaccumulated PAH was studied only at 25°. Renal slices were first incubated for 1 hr in a medium containing a trace amount of [^{14}C]PAH and 70 μM PAH, after which slices were rinsed for 20 sec in PAH-free medium in order to remove PAH adhering to the tissue surface. The slices were then transferred at 1-min intervals through a series of 15 chambers containing PAH-free incubation medium and bubbled with oxygen. The rate of efflux was then determined over a 15-min period using a method described by Ross *et al.* (9). Three levels of pH were selected for efflux studies on the basis of the pH dependence of PAH uptake; below optimal, near optimal, and above optimal (Table II). In a given experiment, the same pH was used for both preaccumulation and efflux of PAH. The composition of basic (PAH-free) incubation medium was the same as described earlier.

The pH of the medium was determined with a Radiometer pH meter (Model 27) using a combined pH electrode (Radiometer Model GK 2321 C). The system was calibrated at the incubation temperature. The concentration of PAH was determined by the method of Smith *et al.* (10). The [^{14}C]PAH activity in the slice and medium was determined with a Beckman LS 350 liquid scintillation spectrometer as described elsewhere (11). Unless stated otherwise, the statistical significance was evaluated by unpaired Student *t* tests.

Results. Although the volume of incubation medium in the present work was 10 ml as compared to usual 2 to 3 ml in conventional slice experiments, there were small changes in the medium pH during incubation, as noted by previous investigators (4, 5). In general, there was a tendency for the medium pH to shift toward neutrality. For instance, when the initial pH was 6.6 in phosphate buffer, the medium pH increased by approximately 0.1 pH unit during incubation. On the other hand, when the initial pH was 9.0 in TES or Tris buffer, an average reduction of medium pH by 0.4 was noted. These changes in medium pH during incubation tended to be greater as the temperature increased. Moreover, the changes in medium pH during incubation were less marked in Tris buffer as compared to the two other buffers.

Because of these shifts in medium pH during incubation, the pH dependence of PAH uptake was evaluated as a function of the final pH. In all experiments the slice to medium (S/M) PAH concentration ratio was above 1.0. Since preliminary experiments showed that the slice uptake of PAH had not reached a steady state during the incubation period at each temperature, the rate of PAH uptake ($\mu g \cdot min^{-1}/g$ wet tissue) was calculated as a measure of the rate of transport. As shown in Fig. 1, the rate of PAH uptake was clearly dependent on both pH and temperature. In general, the rate of PAH uptake increased with temperature and clear pH optima (where the rate of uptake reached a peak) were present at higher temperatures (25 and 37°). At 17°, the pH optimum was clearly demonstrable with Tris buffer but not with other buffers. Overall, the pH optimum at a given temperature was lowest with phosphate buffer (approximately 7.4 for phosphate vs 8.1 for both TES and Tris buffers at 25° ($P < 0.001$). The pattern of the temperature dependence of pH optimum was similar in all three buffer systems. The pH optimum decreased significantly ($P < 0.005$) in all three buffers as the temperature increased from 25 to 37°, with the average $\Delta pH/\Delta T$ of −0.049 $\mu M/°C$ (range: −0.043 to −0.062). However, the possible shift in pH optimum as temperature decreased from 25 to 17° was difficult to assess because of unclear pH optima with phosphate and TES buffers at 17°.

The total tissue water content determined at the end of incubation varied randomly between 74 and 78%, independent of the medium pH, temperature, and buffer species.

The average values of the rate constant for the efflux of preaccumulated PAH at three different pH's are shown in Table II. In general, the efflux rate tended to increase as the medium pH increased without displaying any pH optimum.

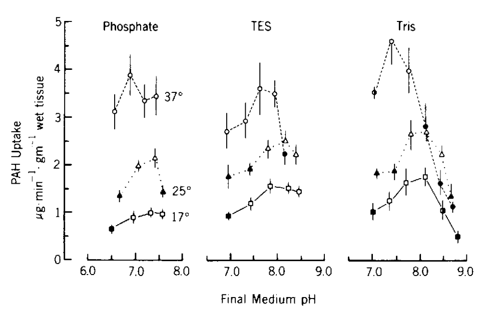

FIG. 1. The slice uptake of PAH as a function of the final medium pH at 17°, 25°, and 37°. Each point represents the mean (±1 SE) of five experiments. Open symbols indicate values not significantly different, solid symbols significantly different ($P < 0.05$), and semi-solid symbols marginally different ($0.05 < P < 0.10$), from the corresponding maximal uptake value observed at the pH optimum.

In two experiments carried out at 25° with TES buffer, the PAH uptake was followed while the slices were first incubated at either high pH (9.0) or low pH (6.6) for 90 min and then transferred to a medium with optimal pH (8.1) for an additional 90-min incubation. In both experiments, the S/M ratio of PAH at the end of 90-min incubation at high or low pH was approximately 10 as compared to the corresponding control (optimal pH) value of 17. Upon transfer of the slices from low to optimal pH, S/M increased rapidly and reached the control level in 30 min. However, it took 60 min for the similar recovery when the slices were transferred from high to optimal pH. These findings indicate that an incubation of slices at high or low pH for 90 min at 25° does not seem to induce any irreversible damages.

Discussion. Three buffer species with different pK′ and $\Delta pK'/\Delta T$ were used in the present work (Table I) to study if the pH optimum is related to the buffer used. In general, the pH optimum was consistently higher in experiments with amine buffers (TES and Tris) than with phosphate buffer at all temperatures tested (Fig. 1). For instance, at 25° which is most widely used for studies with the kidney slice, the pH optimum was approximately 8.1 with Tris and TES buffers, and 7.4 with phosphate. These results are consistent with those reported in the literature in that the pH optimum for PAH transport appears to be within the physiological pH range with phosphate or bicarbonate buffer (1–3) while it is as high as 8.0 or above with propanediol, an amine buffer (4, 5).

The reasons for such a dependence of pH optimum on buffer species are not clear. However, the differences in pK' are not responsible for this phenomenon, since the pH optimum values are substantially different from the corresponding pK' values except in the case of Tris. Tris is known to readily penetrate the cell membrane through a nonionic diffusion mechanism and participate in the intracellular buffering (12). As a result, the intracellular pH tends to increase when Tris is present in the medium, and this may be related to the higher pH optimum. Evidently, more studies are needed to monitor the change in the intracellular pH and correlate it with the rate of transport of PAH.

In the phosphate buffer, a decrease in Ca^{2+}

activity at the higher pH's could cause a reduction in PAH uptake and an artificially low pH optimum. To test this possibility the pH optimum was determined in the absence of calcium in the medium. Under these conditions uptake was not significantly reduced and the pH optimum was essentially unchanged.

In any event, it is very important to point out again that maintenance of the medium pH at around 7.4 with amine buffers does not provide a condition for the maximal transport of PAH.

In contrast to the marked dependence of PAH uptake on the medium pH, the efflux rate of preaccumulated PAH was relatively independent of the pH (Table II). The present work also indicated that the inhibitory effect of low and high pH on PAH uptake is reversible. No significant changes in the efflux rate and the oxygen consumption of the slice over a pH range of 5–8.5 were observed by previous workers (4, 13).

Interestingly, the magnitude of change in the pH optimum with temperature between 25 and 37° ($-0.049\ \mu/°C$) was not correlated with $\Delta pK'/\Delta T$ of three buffers, indicating that the changes in pK′ of the buffer with temperature are not responsible for this shift in pH optimum with temperature. Recently, Park and Solomon (6) reported a similar shift in pH optimum with temperature for PAH and phenol red uptake in the rat kidney slice. However, when the uptake was evaluated as a function of the $(OH^-)/(H^+)$ ratio of the medium, a maximal uptake was observed at a constant $(OH^-)/(H^+)$. The toad skin Na-K-activated ATPase also displays a similar pH-temperature interaction (7).

The reasons for this temperature dependence of the pH optimum are not known at present. It is probably related to the temperature-dependent changes in the degree of ionization of proteins (14) involved in the transport of PAH, but it is virtually impossible even to speculate the mechanism because of the complex nature of the transport system. Nevertheless, it is important to note the pattern of interaction between the pH and the temperature in studying the effect of temperature on the transport system.

Summary. The present investigation on the pH dependence of PAH uptake in the rabbit kidney cortical slice has shown that (i) there exists a pH optimum at which the rate of uptake is maximal, (ii) the pH optimum varies with the buffer used, and (iii) the pH optimum varies with the temperature.

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