Influence of Extracellular and Intracellular Factors on Hippurate Uptake by Rat Kidney Cortex: Acid–Base Effects[1] (34980)

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Organic anion transport is an important part of normal homeostasis. This transport system has been studied in many ways (1–3). During the course of several studies using the rat kidney slice technique to evaluate organic anion transport, it was noted that acidosis changed the ability of kidney slices to accumulate organic anions. The present set of experiments were conducted to amplify the various factors affecting renal organic anion transport during acidosis and alkalosis.

Methods. Male Sprague-Dawley strain rats, 100–150 g were used in all experiments. Control nontreated and acidotic rats were allowed free access to water and rat chow. Acute acidosis was produced in rats by the gastric instillation of 4 mmoles of NH_4Cl in 2.0 cc of H_2O. These rats were used 4 hr later. Chronic acidosis was produced by the gastric instillation of 2.0 mmoles NH_4Cl twice a day for at least 4 days. Acute alkalosis was produced by the gastric instillation of 5.0 mmoles sodium bicarbonate. Chronic alkalotic rats had gastric instillation of 5.0 mmoles sodium bicarbonate twice a day for 4 days. The alkalotic rats also had access to 1.5% sodium bicarbonate drinking water. Controls for each group were intubated in the same manner as the treated rats with the instillation of an equivalent volume of saline.

Blood was collected from all animals by cardiac puncture under light ether anesthesia. Animals were sacrificed by a blow to the head, and the kidneys were placed in iced saline. Cortical tissue was cut on a Stadie Riggs microtome within 10 min of death.

The basic incubation medium was that described by Cross and Taggart (1). It consisted of a phosphate-buffered sodium, potassium, and calcium chloride solution. ^{131}I hippurate was added to a concentration of approximately 2×10^{-5} *M*. Unless otherwise stated, the pH of this medium was 7.3–7.4. When an acid medium was needed, 0.1 *N* HCl was added; and when an alkaline medium was desired, 0.1 *N* NaOH was added to the medium. Gamma counting was performed in a Packard well-type gamma counter.

Results are expressed as S/M ratios; that is the ratio of the counts per minute per gram of wet weight of tissue to the counts per minute per milliliter of incubation medium. Statistics were calculated by either the Student *t* test or paired analysis as indicated in the results.

Results and Discussion. The studies were divided into three parts. First, the effect of media pH on ^{131}I hippurate accumulation in nontreated rat kidney cortical slices was investigated. Second, the effect of sera from alkalotic or acidotic rats on nontreated rat kidney slice hippurate accumulation was evaluated; and finally, the ability of kidney slices from acidotic and alkalotic rats to accumulate hippurate was studied.

Figure 1 is a histographic presentation of the effects of media pH on ^{131}I hippurate accumulation. It is seen that at the pH values investigated, the maximum accumulation of hippurate occurred around a pH of 6.5. At pH 5.5, 7.5, and 8.5, hippurate accumulation was similar with no statistical difference noted between these groups.

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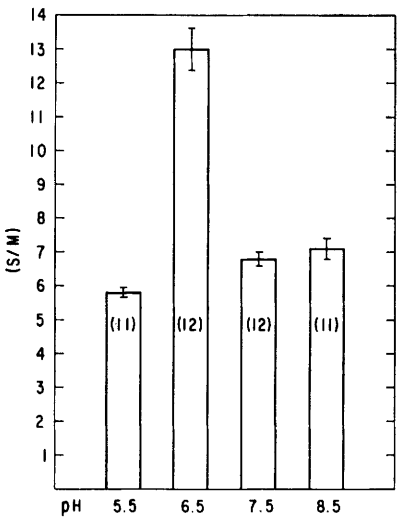

FIG. 1. Rat kidney cortical slices incubated in ^{131}I-hippurate at various media pH. Results are expressed as S/M ratios as defined in methods. Numbers in parentheses equal the number of experiments run at each pH. I = SEM.

Changes take place in many constituents in the serum in response to acute and chronic acid–base changes (2, 4, 5). To determine how such changes in sera might effect hippurate accumulation, the next set of experiments was performed. Slices from nontreated rats were halved. Each half was incubated in a 10% (v/v) concentration of sera from either alkalotic or acidotic rats. The final pH of the media was within 7.3–7.4. As can be seen in Fig. 2, in every case but one, hippurate accumulation was greater in the slice pair incubated with acid sera ($.01 < p < .001$).

Finally, slices from acidotic and alkalotic rats were incubated at pH 7.3–7.4. In this series of experiments a slice from a nontreated rat, run on the same day with the same media, was used as control. The results are presented in Table I. It is noted that slices from acute and chronic acidotic rats accumulate significantly less hippurate when compared to control. Slices from chronically alkalotic rats have a significantly increased ability to accumulate ^{131}I hippurate when compared to control. No difference is noted between control rat kidney slice hippurate accumulation and acutely alkalotic rat kidney slice hippurate accumulation. Acute alkalosis is difficult to produce; and so, this preparation may be no different from the control rat—a fact reflected in similar hippurate accumulation.

In this study we have shown that (1) a relatively acidic pH optimum exists in the rat kidney slice around which hippurate accumulation is maximal, and (2) that acidosis and alkalosis alter factors in both serum and tissue that affect hippurate accumulation, and affect it in an opposite manner.

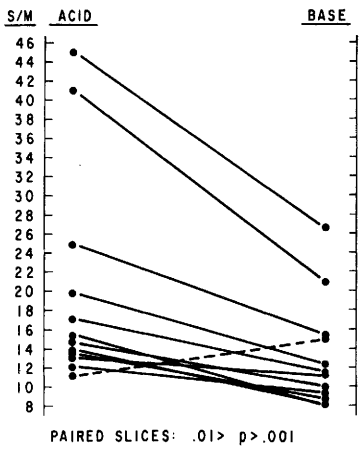

FIG. 2. ^{131}I-hippurate accumulation of nontreated rat kidney cortex slice pairs incubated in 10% acid (v/v) or 10% base (v/v) sera. Results are expressed as S/M ratios as defined in text.

TABLE I. ^{131}I Hippurate Accumulation in Kidney Slices from Alkalotic or Acidotic Rats.

Diagnosis	S/M ± SEM	Control S/M ± SEM	p
Acute acidosis	8.4 ± 0.52 (9)[a]	16.5 ± 1.72 (9)	<.001
Chronic acidosis	7.9 ± 0.42 (9)	14.5 ± 1.04 (11)	<.001
Acute alkalosis	11.8 ± 1.10 (12)	12.3 ± 1.59 (12)	NS
Chronic alkalosis	25.9 ± 1.73 (10)	16.8 ± 1.23 (10)	<.001

[a] Numbers in parentheses represent number of rats in each group.

Summary. The studies presented show that the accumulation of hippurate and presumably other organic anions is modified by a complex of several intra- and extracellular events during changing acid–base states. These extra- and intracellular events may act in seemingly opposite ways to determine the final sum total of hippurate accumulation by the slice.

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