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In vitro Distribution of Inulin and PAH in Dog and Sheep Kidney Cortex and Medullary Slices.* (31209)

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Measurements of the renal blood flow using PAH have generally ignored the fate of PAH in the medullary blood flow. It has been suggested that the medullary segments of the renal tubules do not extract PAH, and therefore the measurements of the renal blood flow as determined by the clearance of PAH reflect only the renal cortical flow(1,2). The experiments reported here are in accord with this concept, for they have demonstrated that the medullary cells *in vitro* did not extract and concentrate PAH in a system in which cortical cells effectively concentrated the hippurate. Concurrent estimates of the inulin space were performed in order to determine the extent of intracellular concentration of PAH by kidney cortex and medullary slices *in vitro*.

Methods. All experiments were performed on hydropenic dogs or sheep. Each animal was anesthetized with pentobarbital sodium and exsanguination was accomplished by cutting through the jugular veins and carotid arteries. The kidneys were quickly removed, cut in half along the longitudinal axis and placed into iced beakers. Slices of cortex and medulla were prepared with the Stadie-Riggs tissue slicer. Approximately 250 mg of cortex or medullary slices (239-271 mg) were incubated in 6 ml of Krebs-Henseleit solution for 2 hours following equilibration with 95% O_2-5% CO_2. The medium contained sodium acetate 10 mM, PAH 48 μg/ml and inulin-carboxyl-C^{14}, 0.5 microcurie per 6 ml of medium. At the conclusion of the incubation period the slices were quickly removed from the incubation medium, dipped twice into 30 ml of distilled water, blotted lightly on Whatman #2 filter paper, reweighed and transferred into centrifuge tubes containing 2 ml of water. The incubation medium also was transferred to centrifuge tubes and the centrifuge tubes were immediately placed into a boiling water bath for 3 to 4 minutes.

PAH in the tissue extract and in the medium was determined according to the method described by Smith and colleagues(3). PAH was determined with and without hydrolysis with 2 N HCl in tissue extracts and medium from 3 dog experiments; in the remaining 3 experiments no hydrolysis was performed. PAH was determined on both hydrolyzed and non-hydrolyzed tissue extracts and medium from all samples in the sheep experiments.

The inulin space (ECF) was determined according to methods outlined by Rosenberg, Downing and Segal(4). For determination of total tissue water, approximately 250 mg cortex and medullary slices were incubated in Krebs-Henseleit solution containing sodium acetate 10 mM and PAH 48 μg/ml. These slices were washed in distilled water, at the conclusion of the 2-hour incubation period, blotted on Whatman #2 filter paper, reweighed and placed in weighed beakers. The beakers were placed in an oven at 100°C for 15 to 18 hours and then reweighed.

The intracellular fluid of cortex and medullary slices and the concentration of PAH in the intracellular fluid were determined according to the following formulae:

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Total tissue water = final wet weight—dry weight

ICF = Total tissue water—ECF

$$[PAH]_{ICF} = \frac{\mu g \text{ PAH in total tissue water—ECF} \times \mu g \text{ PAH/ml medium}}{\text{ml ICF}}$$

$$PAH \frac{ICF}{M} = \frac{\mu g \text{ PAH/ml ICF}}{\mu g \text{ PAH/ml medium}}$$

Results. The inulin space (ECF) of dog and sheep kidney cortex and medullary slices is shown in Table I. These results are comparable to the determinations of the inulin space in rat kidney cortex slices reported by Rosenberg, Downing and Segal(4) in dog kidney cortex and medullary slices by Ullrich and colleagues(5).

TABLE I. Inulin Space (ECF) of Dog and Sheep Kidney Cortex and Medullary Slices.

Species	Tissue	Inulin space (ECF) ml	% Final wet wt
Dog	Cortex (N = 8)	.062 ±.005	27.6 ± 2.14
	Medulla (N = 8)	.138 ±.009	46.5 ± 3.47
Sheep	Cortex (N = 7)	.059 ±.0023	27.3 ± 1.10
	Medulla (N = 7)	.115 ±.0069	44.9 ± 3.12

The data in this and subsequent Tables are given as the average ± 1 S.E.M. N refers to number of animals from which kidney tissue was taken.

PAH distribution in dog and sheep kidney cortex and medullary slices is summarized in Tables II and III. Recoveries of PAH from dog kidney tissues were the same whether the determinations were performed prior to or following hydrolysis with 2 N HCl. Thus dog kidney tissues did not form a PAH conjugate *in vitro*. Sheep kidney cortex slices, however, conjugated 13% of the total PAH (Table IV); medullary slices from sheep kidneys did not conjugate PAH. The nature of the conjugate was not identified, but is presumed to have been para-acetyl amino hippurate (PAAH). The distribution of total, free and conjugated PAH in sheep kidney cortex slices is shown in Table IV. Since medullary slices did not conjugate PAH, the data are tabulated under the column "free." The time course of PAH conjugation and uptake by sheep kidney cortex slices has been illustrated in Fig. 1.

TABLE II. Distribution of PAH in Dog Kidney Cortex and Medullary Slices.

Tissue	$PAH \frac{ICF}{M}$
Cortex (N = 6)	23.4 ± 1.44
Medulla (N = 6)	.4 ± .03

TABLE III. Distribution of PAH in Sheep Kidney Cortex and Medullary Slices.

Tissue	$PAH \frac{ICF}{M}$ Total	Free	Conjugated
Cortex (N = 7)	9.0 ± .99	6.9 ± 1.40	25.4 ± 5.20
Medulla (N = 7)	————	.91 ± .073	————

TABLE IV. Concentration of Medium PAH Prior to and Following 120 Minute Incubation with Slices of Sheep Kidney Cortex.

Initial PAH conc (μg/ml) (N = 7)	PAH concentration after 120 min incubation (N = 7) Total (μg/ml)	Free (μg/ml)	Conjugated (μg/ml)
42.6 ± .87	37.2 ± .76	31.7 ± 1.47	5.5 ± 1.04

Discussion. These *in vitro* experiments demonstrated that medullary cells were incapable of concentrating PAH in a system in which cortex cells achieved considerable intracellular concentration of the hippurate. PAH clearances, therefore, would represent the effective renal cortical blood flow if these *in vitro* results do indeed mirror events *in vivo*. Kidney cortex and medullary slices were taken from 2 species of domestic animals, the dog and the sheep, in order to demonstrate that this was not a species specific phenomenon.

The patterns of PAH distribution into the kidney tissues of these 2 species were qualitatively similar, but dog kidney cortex achieved concentrations 2.5 times greater than sheep kidney cortex. Na acetate, 10 mM, was added to the incubation since this concentration of acetate has been shown to promote maximum PAH and PAAH uptake by kidney cortex slices *in vitro*(6). Thus, the quantitative differences between dog and sheep

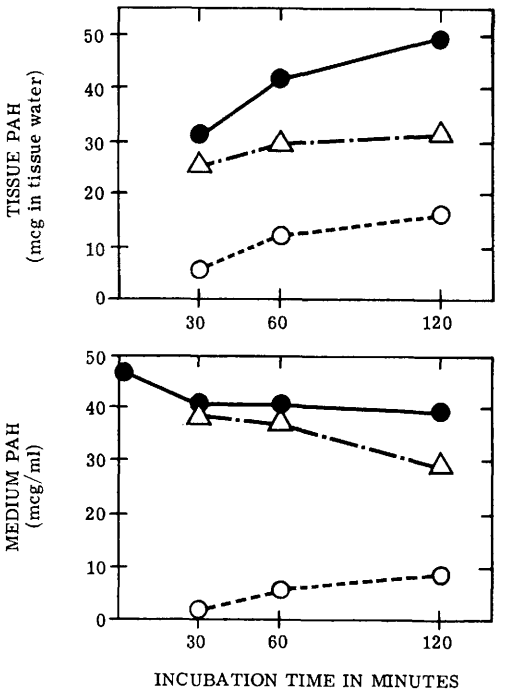

FIG. 1. Exp. 424. Uptake of PAH by sheep kidney cortex slices *in vitro*, and medium concentration of free and conjugated PAH. ——●—— total PAH; —·—△—·— free PAH; — — — ○ — — — conjugated PAH.

kidney cortex slices were not the result of inadequate substrate.

The concentration of the PAH conjugate by sheep kidney cortex was comparable to the concentration of PAH observed in dog kidney cortex slices. It would appear, therefore, that the PAH conjugate was preferentially retained within the intracellular fluid of the sheep kidney cortex cells. The presence of the PAH conjugate in the medium indicated that the conjugate passed from the cells into the ECF, and possibly was subject to bidirectional transport. The PAH conjugate may have competitively inhibited either the uptake and/or concentration of the free PAH.

Summary. PAH uptake by dog and sheep kidney cortex and medulla was studied *in vitro* with simultaneous determinations of the inulin space. Dog and sheep kidney cortex slices concentrated PAH within a calculated intracellular fluid space. Under similar conditions medullary slices from both the species were incapable of concentrating PAH within the intracellular fluid. Sheep but not dog kidney cortex slices formed a conjugate (presumably PAAH) which was preferentially concentrated and may have competitively inhibited the uptake and/or concentration of free PAH.

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