expected to involve changes in the secretion of sodium-retaining hormones, the rate of production of aldosterone was estimated in rats with azotemia and in control rats. The rate of production of aldosterone, *in vitro*, was 0.85 ± 0.05 mg/100 g of adrenal/hour in rats with azotemia and 0.96 ± 0.08 mg/100 g of adrenal/hr in their paired controls. The similar rate of production in both groups indicates that changes in aldosterone secretion are not primarily responsible for the adjustments in sodium excretion in renal failure.

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Renal Tubular Secretion and Biosynthesis of Organic Acids*† (33686)

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The transtubular transport of organic acids in mammalian kidney involves at least a two-step process (1,2); the first step is from interstitial fluid to cell and the second step is from cell to lumen. Evidence of carrier-mediation in the first step of the process is readily obtained; however, evidence of method of transport in the second step is not readily available for study (2).

In the present investigation we took advantage of the well-established occurrence of a biosynthesis process of certain organic acids with the renal tubular cells (3, 4) to study the second step of transport process, as well as the interrelationship of these two processes, biosynthesis and tubular transport.

Experimental Methods. Mongrel dogs of both sexes were used. Using the *in vitro* kidney slice technique, the dog was ether anesthetized and one kidney was removed from the animal and packed in frozen saline. A circular core 15 mm in diameter was cut from the kidney in sagittal section and chilled for 5 min. It was then placed on a Riggs-Stadie slicer which was modified with a screw device for advancing the tissue and sections cut by hand from papilla to cortex. Each slice, averaging 0.3–0.6 mm in thickness, was alternately placed in Erlenmeyer flasks, each of which contained 5 ml of phosphate Ringer solution with either 0.075 m*M* *m*-aminohippuric acid (MAH) or 1.25 m*M* *m*-aminobenzoic acid (MAB) and 10 m*M* glycine. The flasks were gassed with O_2–CO_2 (95–5%) and incubated at 25°. One hr later the slices were removed, blotted, and homogenized with 5% trichloroacetic acid. The medium was centrifuged and diluted with trichloroacetic acid. Details of the procedure and preparation of Ringer

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solution were the same as described by Cross and Taggart (5) and us (3).

With the *in vivo* clearance technique, dogs under chloralose anesthesia were used. MAB or MAH was given to the animal intravenously in a prime dose and sustaining infusion. Creatinine was given for measurement of glomerular filtration rate. Urinary pH was titrated to 7.5–8.0 by intravenous administration of sodium bicarbonate. Usually 15–20 min were allowed for an equilibrium state to be reached. Three consecutive urine samples of 10-min periods each were collected. Blood samples were taken in the middle of each urine collection period. The protocol of the procedure was similar to that reported elsewhere. (6, 7).

The stop-flow technique as described by Malvin *et al.* (8) and Huang (6) on pentobarbitalized dogs was used to study the localization of biosynthesis and tubular transport of aminohippurate.

Analysis. Diazotization and coupling method (3, 5) was used in the determination of aminohippurate. Separation of aminobenzoate from its biosynthetic product, aminohippurate, was performed by ether–benzene extraction as described previously (3). The iodometric method was used to assay the concentration of Diodrast (7) and inulin concentration was determined by the method of Roe *et al.* (9). The radioactivity of PAH-^{14}C samles was determined by the method of Bray (10) with a Nuclear-Chicago liquid scintillation counter.

Results. 1. The site of biosynthesis and transport. Eight dogs were used for the *in vitro* studies of kidney slices from both the medulla and cortex. Figure 1 represents one of the experiments where the ordinate is the uptake of MAH by the slices expressed in terms of *S/M* ratio, or the rate of synthesis of MAH from MAB and the abscissa is the cumulative weight of the slices starting with the papilla toward the cortex. Results indicate that both the transport process of MAH (tissue uptake) and biosynthesis takes place mainly in the cortex.

Table I represents av. of nine *in vitro* experiments in which only cortical slices were

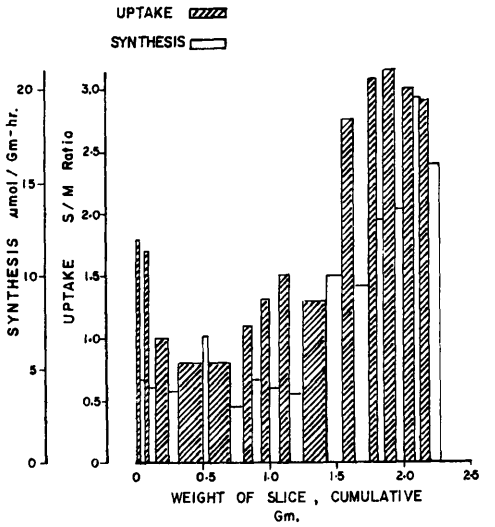

FIG. 1. Sites of transport and synthesis of MAH by kidney slices: the abscissa is cumulative weights of slices, beginning at the papilla; the ordinate is either the uptake of MAH, in term of *S/M* ratio, or the synthesis rate of MAH from MAB, μmoles/g-hr of wet tissue.

used. Usually three or four cortical slices with a total weight of 150–200 mg were placed in a flask containing 5 ml of phosphate-Ringer solution with aminobenzoate and Diodrast or PAH and incubated for 1 hr at 25°. As shown, both Diodrast and PAH produce no significant change on renal biosynthesis of either MAB or PAB to the correspondent aminohippurate.

Four stop-flow experiments were done with MAB given to determine the site of biosynthesis and PAH-^{14}C given to determine the localization of tubular secretion. In all four cases no MAH was detected in the plasma samples; therefore, the MAH found in the urine was considered to have been synthesized in the kidney. The peak of U_{MAH} was identical with that of PAH-^{14}C. It can be concluded, therefore, that the processes of transport and biosynthesis take place in the same proximal tubular segment and probably in the same cells. Figure 2 presents one of the experiments.

2. The interaction of transport and conjugation. Three dogs were used in clearance determinations and the results are summarized in Table II. In the experiment with

TABLE I. Effect of Transported Substance on Biosynthesis by Renal Cortical Slices.

Organic acid in medium (mM)	Transported substance added			Renal synthesis of MAH or PAH from MAB or PAB (mean; μmoles/g-hr)	p
		(mM)	S/M ratio		
MAB, 1.25	None			12.7 ± 0.54 (16)[a]	
	Diodrast	0.30	11.3	13.3 ± 0.41 (8)	0.3
	Diodrast	0.60	6.3	13.4 ± 0.40 (8)	0.3
	Diodrast	0.90	2.1	12.3 ± 1.02 (8)	0.7
PAB, 1.25	None			4.31 ± 0.27 (6)	
	Diodrast	0.6	1.2	3.20 ± 0.6 (4)	0.1
	Diodrast	1.2	1.04	3.66 ± 0.65 (4)	0.3
	Diodrast	1.8	0.84	5.03 ± 1.19 (4)	0.5
	PAH	0.6	1.9	4.37 ± 0.58 (4)	0.9
	PAH	1.2	2.1	4.42 ± 0.61 (4)	0.8
	PAH	1.8	1.2	4.32 ± 0.23 (4)	0.9

[a] Standard error of the mean; the number of experiments is given in parentheses.

MAH it was shown that the Tm level had been reached. Administration of MAB showed either no effect or a slight depression of Tm_{MAH}.

Nine dogs were used in another series of experiments in which MAB or MAH was administered until a steady state was reached and then Diodrast was given to each animal in three separate dosages with sustaining infusion. As shown in Fig. 3, Diodrast caused a marked inhibition of tubular secretion of MAH and also a marked reduction in urinary excretion of synthesized MAH from the tubular cells. Similar results were obtained with the *para* isomers.

Discussion. The biosynthesis of aminohippurates from aminobenzoates occurring in the dog kidney has been shown *in situ* (3, 4, 11, 12), in perfused kidney (11, 12, 13), in slices (3, 14), and in brei having no discrete cells (13). Our results confirm these findings and indicate that this biosynthesis occurs mainly in the same region of the kidney, i.e., the cortex, in the same proximal part of the tubules and probably in the same cells as those which transport aminohippurates from interstitium to tubular lumen.

Our finding from the cortical slice studies showed that the loading of cells with substrates for both biosynthesis and transport does not appear to reduce the activity of each process. The resulting implication is that the

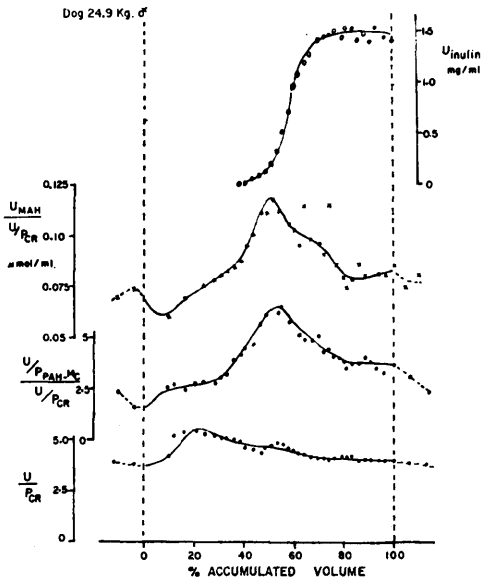

FIG. 2. Localization of transport and synthesis of MAH by stop-flow technique: 35 sequent urine samples were collected at 4 sec each after the release of clamp; the abscissa is the percentage of accumulated urine volume; U_{inulin}= the concentration of inulin in the urine sample; $U_{MAH}/(U/P_{CR})$ = concentration of synthesized MAH appearing in the urine sample and corrected from the water reabsorption, the peak of the concentration is corresponding to the proximal section: $(U/P_{PAH})/U/P_{CR})$ = ratio of the urine to plasma ratio of PAH and creatinine, the peak is also corresponding to the proximal section. U/P_{CR} = urine to plasma ratio of creatinine. All curves were drawn by inspection.

TABLE II. T Value Determination in Dogs.[a]

Dog no.	Wt. (kg) and sex	Compound given: Prime (mmoles/kg)	Compound given: Infusion (μmoles/min)	Urine flow (ml/min)	C_{CR} (ml/min)	MAH: P_{MAH} (μmoles/ml)	MAH (μmoles/min): UV	MAH (μmoles/min): T
1	14.5 ♀	MAB, 0.1	14.6	1.6	35	0	3.4	
				2.0	36	0	5.13	
				2.6	37	0	5.33	
		MAB, 0.1	29.5	2.7	37	0	5.29	
				3.2	38	0	5.36	
				3.0	38	0	5.23	
		MAB, 0.2	58.4	2.9	38	0	5.26	
				2.7	35	0	5.69	
				2.6	25	0	5.26	
2	15.8 ♂	MAH, 0.25	80	3.2	24	0.53	29.4	+17.0
				2.9	23	0.47	28.3	+18
				2.7	24	0.45	30.8	+21.0
		MAH, 0.1	110	2.7	23	0.6	28.3	+15
				2.6	23	0.73	36.4	+20
				2.5	22	0.80	34.4	+18
		MAH	110					
		+MAB, 0.1	16	2.5	22	0.8	35.8	+20
				2.3	23	0.83	36.9	+19
				2.0	25	0.88	38.9	+17
		MAH	110					
		+MAB, 0.1	32	2.0	23	0.9	35.5	+16
				2.1	25	0.9	38.8	+18
				2.3	27	0.89	42.2	+20
		MAH	110					
		+MAB, 0.2	64	2.2	26	0.93	42.6	+20
				2.2	26	0.98	47.1	+23
				2.2	26	1.04	47.0	+23
3	15.0 ♀	MAH, 0.35	80	3.0	28	0.58	43.8	+28
				3.2	24	0.58	42.0	+29
				2.8	28	0.58	53.3	+28
		MAH, 0.1	102	2.6	28	0.74	46.7	+26
				2.4	28	0.79	48.3	+28
				2.2	28	0.74	46.7	+27
		MAH	102					
		+MAB, 0.1	15	2.0	28	0.75	38.8	+19
				1.8	26	0.76	41.3	+23
				1.8	29	0.73	43.3	+24
		MAH	102					
		+MAB, 0.1	30	1.8	29	0.74	39.6	+20
				1.8	29	0.76	40.3	+20
				1.9	30	0.74	43.3	+23
		MAH	102					
		+MAB, 0.2	60	2.0	29	0.74	41.3	+21
				2.0	30	0.76	42.1	+20
				2.0	31	0.83	44.2	+20

[a] C_{CR} = creatinine clearance; UV = excretory rate or (urine concentration) × (urine flow); P = plasma concentration; $T = UV - 0.94 \times P \cdot C_{CR}$ = transport rate.

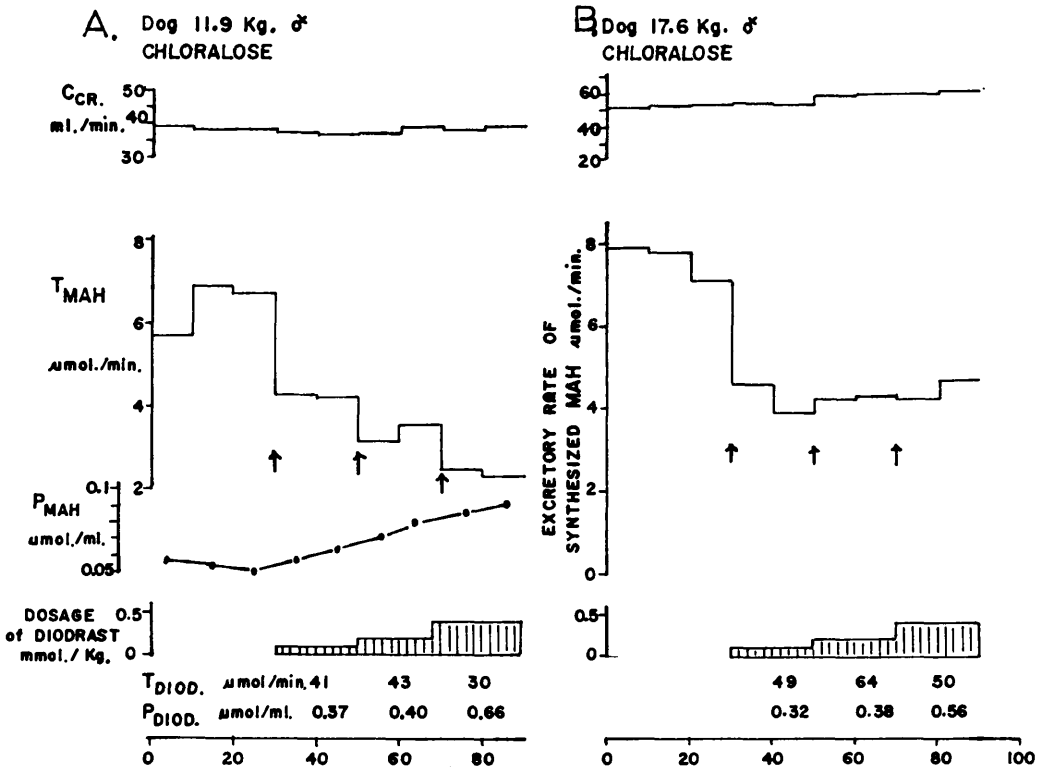

FIG. 3. Competition at the two cellular borders: (A) experiment with administered MAH where the tubular secretory rate, T_{MAH} is inhibited by Diodrast which competes with MAH at the first transport step; (B) experiment with administered MAH, where the urinary excretory rate of synthesized MAH is decreased by Diodrast which competes at the luminal border. Diodrast was administered at the arrows by a prime and sustaining infusion. Its dosage, secretory rate ($T_{Diodrast}$) and plasma concentration are presented at the bottom part of the figure.

process for intracellular accumulation of transported substance does not interfere with the process of biosynthesis. Also implied is that the entrance of aminobenzoate from the peritubular fluid into the tubular cells is not by a sharing of the same carrier transport system as that for the entrance of Diodrast or PAH into the tubular cells. However, our results from the cortical slice technique does not reveal whether there is an interaction between the transported substrate and biosynthesized aminohippurate at the luminal border site. From his studies on rabbit cortical slices, Foulkes (2) also left open the question of whether the transport step from cell to lumen is by an active process or carrier-mediated. Hong and Forster studied the transport of chlorphenol red by renal tubules of flounder fish and found that the cell-to-lumen transport process is energy demanding and can be competitively inhibited by other organic acids and certain metabolic inhibitors (15, 16).

Our data as shown in Fig. 3 indicate that with the administration of aminohippurate, a progressive decrease in transtubular movement occurs as the increasing levels of Diodrast in interstitial fluid compete with aminohippurate for the carrier at the first step transport process. Since Diodrast does not compete with the synthesis process as evidenced by the cortical slice technique, the indications are that the first step process is not a limiting factor for the entrance of aminobenzoic acid into the cells and for the synthesis of the aminohippurate but that the reduction of urinary excretion of synthesized aminohippurate by the administration of Di-

odrast must be due to competition between the transported Diodrast and the synthesized aminohippurate in the second step transport process from cell to lumen.

We therefore conclude that the second step transport process from cell to lumen in mammalian kidney is similar to that in flounder kidney, namely, the process is carrier-mediated; and also that the carrier is saturated at a *T*m level but does not distinguish between movement with or against a gradient.

Summary. With the *in vitro* kidney slice technique, the *in vivo* steady state and stop-flow technique, we have studied the sites and the interrelationship of tubular secretion and biosynthesis of organic acids and found that both occur in the cortex, in the same proximal segment and probably in the same cells. Addition of transported substance, such as PAH or Diodrast, in the medium does not alter the rate of biosynthesis. Simultaneous administration of aminobenzoic acid does not augment, but exhibits either no effect or a slight decrease of tubular transport maximum of the aminohippurate. However, administration of Diodrast would markedly depress the urinary excretion of aminohippurate either by administration or by biosynthesis. Our results indicate that the second step of the transport process, from cell to lumen, is carrier-mediated, and that this carrier can be saturated at a *T*m level.

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