rather than the thymus, may play a more important role in the pathogenesis of the murine autoimmune disease.

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Action of Alkali Metals on Papillary-Cortical Sodium Gradient Of Dog Kidney.* (32308)

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It is generally accepted that a countercurrent transfer mechanism is responsible for the concentrating process in mammalian kidney. Evidence in favor of this view has been reviewed(1). As a further generality, it seems that sodium transfer out of the ascending limb of the loop of Henle is the "single effect" which underlies the countercurrent system. The following observations support this hypothesis: 1) A gradient in sodium concentration is found between papilla and cortex and the gradient is related to the final osmolality of the urine(2,3,4). 2) In micropuncture studies, it has been found that fluid collected from an ascending limb is usually hypo-osmotic to fluid collected from an adjacent descending limb(5). 3) Under free flow conditions, the ascending limb is electronegative by 8-10 mv to the interstitium(6,7).

Several observations, however, are difficult to explain if sodium movement out of the ascending limb is the only mechanism responsible for urinary concentration. 1) Using the Gertz technique, Gottschalk found that a fluid droplet was reabsorbed from the descending limb but not from the ascending limb(8). This result would indicate solution transfer in the opposite direction to that predicted if no *ad hoc* assumptions are invoked. 2) Although Marsh and Solomon did not find collapse of the descending limb droplet under all stop-flow conditions, they were unable to detect the establishment of any significant electrochemical gradients in either ascending or descending limbs under stop-flow conditions utilizing the Gertz technique(7). 3) Thurau and Henne have found volume flow in the ascending limb to be greater than that in the descending limb(9).

If, in fact, sodium transport is the only factor responsible for establishing the corticopapillary concentration gradient, further insight into this problem might be gained from a study of the effects of alkali metals on the concentrating process. In many transport systems alkali metals interact, and more specifically, lithium competes with sodium

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(10). If such interaction did occur, one expects that administration of alkali metal salts would result in two consequences: 1) Papillary-cortical gradients for the ion administered should be established; 2) A reduction of the sodium gradient should accompany the establishment of the gradient for the alkali metal ion. The experiments to be described were designed to test whether intravenous infusion of lithium, ribidium and caesium ions would lead to these predicted results.

Materials and methods. Dogs of either sex, weighing over 12 kg, were anesthetized with nembutal. Through a midline approach, both kidneys were exposed and cleared of perirenal fat in preparation for removing them for slice studies. The ureters were cannulated with plastic tubing. The abdominal incision was clamped and body temperature maintained during the remaining period of an experiment with an electric heating pad. An isotonic solution was infused *via* a femoral vein. Solutions were one of the following: a) saline; b) 10% mannitol in saline; c) 150 mM lithium chloride; d) 150 mM ribidium chloride, or e) 150 mM caesium chloride. Infusion rates were varied and ranged from 2 cc/min to 5 cc/min. After a 60- to 90-minute equilibration period, 2 consecutive 10-minute urine samples and 3 blood samples were obtained so that a blood sample was drawn at the beginning and end of each period. The midline incision was then opened and a hemostat used to clamp the renal artery and vein and the ureter. The kidney was removed and quickly sliced. After another equilibration period, urine and blood were again collected and the second kidney removed and sliced.

After each kidney was removed, a wedge was cut by extending the cleft along the inner medulla through the outer medulla and cortex; the capsule remaining on the wedge was removed. The wedge was then cut into the following sections: 1) approximately 2 mm of the outermost part of the medulla to represent papilla; 2) the remainder of the outer medulla; 3) inner medulla, and 4) cortex. Each section was then divided into 4 parts. Each part was blotted on filter paper and 2 were placed on preweighed aluminum foil to be used for determination of water contents; the other 2 were placed in preweighed vials for electrolyte analysis. The vials were sealed with parafilm and the foils and tissues were held in a covered petri dish in order to provide a closed environment and to reduce evaporation.

Water contents were determined by loss of weight found after a drying period of 24 hours at 105-110°C. For electrolyte analysis, 5 ml of water was added to the vials; these were then weighed. The tissue was subjected to ultrasound (Bronwill Biosonik) for 20 minutes with the vials held in a beaker containing ice. Despite cooling, there was some evaporative loss. For this reason, the vials were reweighed after washing the ultrasound probe. Dilutions were calculated on the basis of the final volume. Tissues were extracted in the cold overnight. Protein was precipitated from aliquots of the resulting suspension using 5% trichloracetic acid. After centrifugation and filtration, alkali metals were estimated by direct reading flame photometry using a Beckman flame attachment. Chloride was estimated using an Aminco titrator. Recovery studies for all ions showed that recovery ranged from 75-110% with average recoveries for each ion being 102 or 103%.

All data are expressed either as tissue content in mEq/kg wet weight, or as "apparent" concentrations in mEq/kg water. There is really no reason to prefor one expression over the other since one does not know how the ions are distributed through the various fluid compartments of the tissue slices. These variables were chosen, however, since they are the ones most frequently used in slice analysis studies.

Results and discussion. Table I summarizes the tissue analysis data for lithium, rubidium and caesium. Both tissue content (mEq/kg wet weight) and apparent concentration (mEq/kg tissue H_2O) are shown. Data on sodium, potassium and chloride of control experiments are not shown since in 12 kidneys cortico-papillary gradients for these ions are essentially the same as found

TABLE I. Papillary-Cortical Gradients for Alkali Metal Ions.

Ion species	N	Cortex		Outer medulla		Inner medulla		Papilla	
		Content	Conc.	Content	Conc.	Content	Conc.	Content	Conc.
Lithium	12	10.6 ± 1.3	12.9 ± 1.7	20.5 ± 2.5	24.8 ± 3.0	23.5 ± 2.6	27.4 ± 3.1	*27.3 ± 4.9	*32.6 ± 5.8
Rubidium	8	17.4 ± 2.9	*23.9 ± 3.9	19.3 ± 3.4	*22.4 ± 5.0	14.4 ± 2.1	17.0 ± 2.5	14.3 ± 1.6	17.4 ± 2.0
Caesium	9	18.6 ± 1.4	23.6 ± 2.7	14.6 ± .7	18.1 ± 1.1	7.1 ± .8	8.3 ± .9	6.0 ± .6	7.2 ± .7

N = No. of kidneys analyzed.
* Where data are marked with an asterisk, the value is the mean of N-1 samples because of a loss of the samples of the tissue designated.
All values are given as the mean ± standard error.

by other workers studying gradients in dogs(4,11,12).

The data demonstrate that lithium behaves like sodium and is concentrated by the medulla into a cortico-papillary gradient. Since plasma lithium concentrations in these studies varied between 3 and 15 mEq/l, it would seem that the counter-current system is more effective in concentrating lithium than it is for sodium. In contrast to lithium, the two alkali metal ions, Rb^+ and Cs^+, which are accumulated by cells(10), show a reverse gradient to Li^+ and Na^+. Tissue slice studies are not adequate, however, to establish that there is no concentration of Rb^+ and Cs^+ in vasa recta plasma or thin loop fluid. Although K^+ also shows the reverse gradient(11,12), micropuncture studies have established that this ion is concentrated in these two fluids(7). It is of interest that the steepness of the reverse gradient is increased as ionic size increases.

The process of establishing suitable control conditions for determining if alkali metals interfere with cortico-papillary sodium gradients poses some difficulty. Infusion of lithium, rubidium or caesium leads to a moderate diuresis. Diuresis *per se* results in "washout" of the counter-current gradient (13). It, therefore, becomes important to compare ion concentrations at the same level of diuresis. The control data were obtained by determining the papillary sodium concentration as a function of urine osmolality under conditions of varying degrees of osmotic diuresis induced by mannitol. Such data are shown as the open circles in Fig. 1. Data obtained when lithium was the diuresing substance are shown as the crosses in Fig. 1. No change in the relationship between papillary sodium content and urine osmolality was evident.

One may question whether the tissue slice method is precise enough to detect such changes. In the control data, the greatest spread of tissue sodium at a small increment of osmotic pressure (the 5 points at U_{osms} between 540 and 640) is less than 60 mEq. At this relatively low urine osmolality, tissue lithium tends to be high, 35-60 mEq/kg H_2O. One would expect then that if lithium

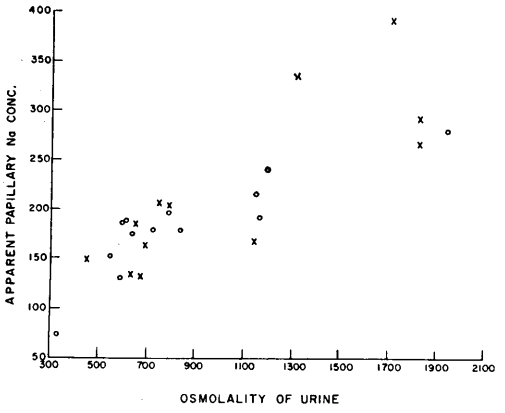

FIG. 1. Relationship between urine osmolality and apparent papillary sodium concentration. Open circles show data obtained in control experiments. Crosses show data obtained under conditions of lithium infusion.

replaced sodium in the concentrating process that a statistically significant difference in sodium concentration would be detectable by slice studies.

These studies are further complicated by the fact that plasma sodium concentrations were not the same in all dogs and ranged from 121-165 mEq/l. To account for this variable, the data were reanalyzed on the basis of whether lithium had any effect on the ratio of papillary to plasma sodium determined as a function of urine osmolality. This analysis, as well as analysis of data from dogs infused with Rb or Cs, also showed no apparent effect of alkali metals.

As a result, it is concluded that lithium does not alter medullary sodium concentration despite the fact that lithium is concentrated by the counter-current system. These observations leave open several possibilities of which the most likely seem to be: 1) Sodium transport by the ascending limb is the primary mechanism involved in concentrating the urine, and the transport system does not show competitive behavior among alkali metal ions. If such, in fact, is the case, it would require that establishment of the lithium cortico-papillary gradient requires a secondary process. 2) Cortico-papillary gradients for both sodium and lithium are secondary to some other process, perhaps the movement of water as would be suggested by Thurau's results(9).

Summary. Anesthetized dogs were infused with isotonic solutions of LiCl, RbCl or CsCl and the cortico-papillary ion gradients of the kidneys were studied by tissue analysis. Lithium content increases from cortex to papilla. Tissue content for rubidium and caesium decreases from cortex to papilla. None of the alkali metals changed the relationship between papillary urinary sodium content and urine osmolality.

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