fect was obtained either during the first or second hour. Polythiazide produced an almost equal increase in excretion of sodium and chloride. The increase in excretion of potassium was approximately 1/10 of that of sodium. In acute experiments on rats polythiazide was found to be 10 times more potent than trichlormethiazide in its natriuretic effect but only 0.4 times as potent in its kaliuretic effect. Polythiazide at dose levels as high as 10 mg/kg i.v. did not depress glomerular filtration rate. Polythiazide is a carbonic anhydrase inhibitor *in vitro*. *In vivo*, polythiazide produced no or only minimal increases in excretion of bicarbonate in animals with normal acid-base balance. The drug was effective as saluretic agent in dogs with experimental metabolic acidosis or alkalosis. Antihypertensive activity of polythiazide was clearly demonstrated in rats and dogs with experimental hypertension. The antihypertensive effect had a slow onset and became evident on the second day of treatment.

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Demonstration of Countercurrent Diffusion Exchange in the Vasa Recta of the Renal Medulla.* (26781)

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Man and certain animals are capable of producing urine hypertonic to plasma as a means of conserving body water. It is currently held that, under the influence of antidiuretic hormone, the collecting ducts become more permeable to water and probably to urea. Urine passing through the collecting duct equilibrates with the hypertonic interstitium of the renal medulla and papilla, becoming concentrated with respect to plasma (1). Not only is the medulla hypertonic, but a gradient of osmolality(2) and solute concentration (sodium, urea, chloride, creatinine) has been demonstrated, increasing from the base of the medulla to the papillary tip(1,3,4). Micropuncture studies have

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shown that fluid in the tip of the Henle loop, plasma in the vasa recta, and urine in the collecting ducts are all hypertonic to plasma and are of approximately the same osmolality at any one level in the concentrating kidney(5,6). This gradient is presently considered maintained by an active sodium pump, primarily in the loop of Henle, with countercurrent diffusion exchange of sodium, urea, water and other diffusible solutes in the vasa recta(1,6). Some sodium also probably enters the medulla from the collecting ducts (7).

The close approximation of the descending and ascending limbs of the vasa recta in the outer medulla, with blood flowing in opposite directions, could allow countercurrent exchange of diffusible substances to which the vessels are permeable(1,3). This exchange would serve to decrease the effective flow of interstitial diffusible solute out of the papilla, by shunting it from the ascending to descending limbs of the vasa recta. At the same time water would be partially excluded from the papilla by exchange from the descending to ascending limbs. This would serve to maintain the high solute concentrations in the papilla. Since the process is passive, diffusible solute present in the renal arterial blood should be short circuited from descending to ascending limbs of the vascular bundles. This would effectively decrease their incorporation rate into the deeper papillary areas.

The following experiment was devised to determine whether a rapidly diffusible ion to which capillaries are permeable *viz.* Rb^{86+}, is in fact incorporated more slowly into the renal papilla than a relatively nondiffusible substance, I^{131} human serum albumin.

Method. Six hydropenic mongrel dogs each weighing approximately 10 kg were anesthetized with about 25 mg/kg of thiopental sodium or Na-ethyl-(1-methyl-propyl)-malonyl-thio-urea (Inactin).‡ The right carotid artery was exposed, a polyethylene catheter inserted into the vessel, and the tip advanced to the root of the aorta. One femoral artery was catheterized for collection of blood samples. Through an abdominal incision both renal pedicles were isolated and a loose ligature placed about each. Each ureter was isolated and a small polyethylene catheter threaded to the renal pelvis, from approximately 6 cm below the pedicle. After allowing time for stabilization of the animal, a mixture of I^{131} albumin and $Rb^{86}Cl$ in normal saline, containing a small amount of nonradioactive rubidium, was infused into the aortic root. A constant infusion pump delivered approximately 15 μc/sec of $Rb^{86}Cl$ and 10 μc/sec of I^{131} albumin. Twenty seconds after beginning the infusion, the renal pedicles were simultaneously ligated. The kidneys were then removed, and immediately placed in a dry ice-acetone mixture. Continuous femoral arterial blood samples were collected each second for 25 seconds during infusion. Duplicate pieces of inner medulla (papilla), outer medulla (carefully excluding the cortico-medullary border zone) and cortex, were cut from the frozen kidneys and weighed. Rb^{86+} and I^{131} activity in these pieces, as well as that in aliquots of arterial plasma was determined, employing a well-type scintillation counter and gamma ray spectrometer. Tissue radioactivities for Rb^{86+} and I^{131} were expressed as counts per gram of tissue (wet weight), and plasma radioactivity as counts per milliliter of plasma. After correcting for collecting catheter time delay, mean arterial blood plasma Rb^{86+} and I^{131} radioactivity concentrations were determined by summing the plasma concentrations from zero time until time of ligation and dividing by this time interval. The ratio of tissue radioactivity to average arterial plasma radioactivity for Rb^{86+} and I^{131} was individually calculated, multiplied by 100 and designated "V", "apparent volume of distribution," in ml/100 g of tissue. Ratios of the apparent volume of distribution of Rb^{86+} (V_R) to that of I^{131} (V_I) were determined for each piece of tissue (V_R/V_I). Timed urine specimens were collected from each ureter for osmolality and volume flow determinations. Urine to plasma osmolal ratios varied from 2.0 to 3.6. Arterial pressure was monitored by means of a strain gauge transducer and direct writing recorder throughout the procedure. Mean pres-

‡ Promonta GmbH, Hamburg, Germany.

TABLE I. Ratio of "Apparent Volume of Distribution" of $Rb^{86}Cl$ and I^{131} Albumin (V_R/V_I) in the Kidney in 20 Seconds.

Kidney No.	Inner medulla (papilla)	Outer medulla	Cortex
1 R	.197	.700	3.19
L	.216	1.03	3.59
2 R	.789	2.40	7.33
L	.566	1.55	5.64
3 R	.484	3.03	6.68
L	.441	2.46	7.18
4 R	.725	1.05	3.29
L	.641	.737	3.16
5 R	.322	1.93	6.49
L	.239	1.5	2.9
6 R	.64	2.6	7.2
L		1.8	4.9
Mean ± S.D.	.48 ±.20	1.73 ±.55	5.13 ±1.74

sure remained above 105 mm Hg in all animals.

Three additional hydropenic dogs were similarly prepared but $Na^{22}Cl$ was used in place of $Rb^{86}Cl$ and the kidneys were ligated in 15 seconds. Only cortex and papilla were analyzed in these animals.

Results. The ratios of Rb^{86+} to albumin volumes of distribution in 20 seconds (V_R/V_I) averaged 0.48 ± 0.20 for the papilla, 1.73 ± 0.55 for the outer medulla and 5.13 ± 1.74 for the cortex (Table I). It is apparent that less than one half as much rubidium as albumin was incorporated into the papilla in 20 seconds, while approximately twice as much rubidium appeared in the outer medulla, and 5 times as much in the cortex. Similarly, the Na^{22+} to albumin volumes of distribution in 15 seconds (V_{Na}/V_I) averaged 0.64 ± 0.16 for the papilla and 2.05 ± 0.45 for the cortex.

Discussion. The ascending and descending limbs of the vasa recta, comprising the vascular bundles of the outer medulla, could act either as conduits carrying blood to and from the papilla, or as permeable vessels acting as countercurrent diffusion exchangers. If they were merely conduits, then no Rb^{86+} could escape into the interstitium of the outer medulla and the ratio V_R/V_I in the outer medullary tissue could only be 1.0. The papillary tissue in this instance would also have a V_R/V_I ratio of 1.0, for no Rb^{86+} could escape prior to reaching this area. Furthermore, none could accumulate in excess of albumin, since the 20 second perfusion time is substantially less than the papillary circulation time (8). However, as the results indicate, the observed ratio of V_R/V_I in the papilla was always less than 1.0. This circumstance can only mean that some Rb^{86+} has escaped from the descending vessels, prior to entering the papilla. That the descending and ascending limbs of the vasa recta are permeable to Rb^{86+} is further substantiated by the average V_R/V_I ratio of 1.75 found in the outer medulla. Such a situation suggests diffusion of Rb^{86+} into the interstitium, while the albumin remains intravascular. In 2 kidneys, 1R and 4R (Table I) the observed outer medullary V_R/V_I ratios were 0.70 and 0.74, *i.e.*, there was less Rb^{86+} than albumin in these pieces. The segment removed from the medulla in these cases was probably far enough distal to the origin of the vascular bundles at the base, to have allowed some diffusion loss of Rb^{86+} from the blood prior to its reaching the tissue counted.

The accumulations of Rb^{86+} and I^{131} in the cortex cannot be interpreted in the same manner as those in the papilla because of the very rapid blood flow in this area(8). The large V_R/V_I ratios do, however, indicate permeability of the cortical capillaries to the Rb^{86} ion.

In the 3 dogs in which Na^{22+} was used, the findings are similar to that found with Rb^{86+} and indicate that the Na^{22} ion was also effectively excluded from the papilla.

These results, plus the anatomic arrangement of the medullary vessels, demonstrate that countercurrent exchange can occur in

TABLE II. Ratio of "Apparent Volume of Distribution of $Na^{22}Cl$ and I^{131} Albumin (V_{Na}/V_I) in the Kidney in 15 Seconds.

Kidney No.	Inner medulla (papilla)	Cortex
7 R	.82	1.80
L	.77	2.14
8 R	.43	1.64
L	.67	2.80
9 R	.54	1.9
Mean ± S.D.	.64 ± .16	2.05 ± .45

the vascular bundles. There is a marked similarity, as also demonstrated by the electron microscope, between vascular structure and arrangement in the medulla to that in the swim bladder of certain deep sea fish(9, 10). In the latter, countercurrent diffusion exchange of gases, primarily oxygen does take place(11).

The findings of solute and osmolal gradients in the papilla, and especially the high concentrations in the vasa recta, as shown by the micropuncture studies of Wirz(5) and confirmed by Gottschalk(6) have been explained by countercurrent diffusion exchange in these vessels. Recent micropuncture studies by Thurau, Sugiura, and Lilienfield(12) have demonstrated albumin concentrations in the vasa recta at the tip of papillary loop nearly 3 times greater than those in the straight vessels at the base of the papilla. These results are interpreted to indicate a proximal water shunt by countercurrent diffusion. White, Tosteson and Rolf(13) showed that THO was incorporated more slowly into the inner medulla than Na^{22+}, again indicating a proximal water shunt in the vascular bundle. Lassen and Longley working with rats have shown that Kr^{85}, Na^{24+} and I^{131} iodoantipyrine were effectively excluded from the papilla as compared to the cortex(14). Furthermore, the efficiency of exclusion seemed to be proportional to the expected diffusibilities of these substances, *i.e.*, the more rapidly diffusible were more effectively excluded from the papilla.

Summary. The accumulations of Rb^{86+} and I^{131} albumin were compared in the cortex, outer medulla and papilla of 6 hydropenic anesthetized mongrel dogs. Only one-half as much rubidium as albumin appeared in the papilla in 20 seconds of perfusion, while almost twice as much appeared in the outer medulla and 5 times as much in the cortex. In 3 hydropenic dogs perfused with $Na^{22}Cl$ and I^{131} albumin the Na^{22+} was similarly excluded from the papilla. These data suggest that the vasa recta do indeed function as countercurrent exchangers to maintain the high renal medullary solute concentrations and gradient, necessary to conserve body water.

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