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INTRA- AND EXTRACELLULAR SOLUTE CONCENTRATIONS IN THE INNER ZONE OF A MAMMALIAN KIDNEY

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In the renal papilla of antidiuretic desert rodents it was found that the osmolality of the papillary tissue was lower than that of the urine by 250 to 1000 mOsm. The osmolality of tubular fluid pressed from the cut end of the excised papillae exceeded the osmolality of the papilliary tissue from which it had been pressed (Zell and Schmidt-Nielsen, submitted to the Amer. J. Physiology).

This investigation was undertaken to determine which compartment within the renal papilla had the lower osmolality, the interstitial or the intracellular, and to measure the distribution of electrolytes and urea between these compartments. The cat was chosen as an experimental animal

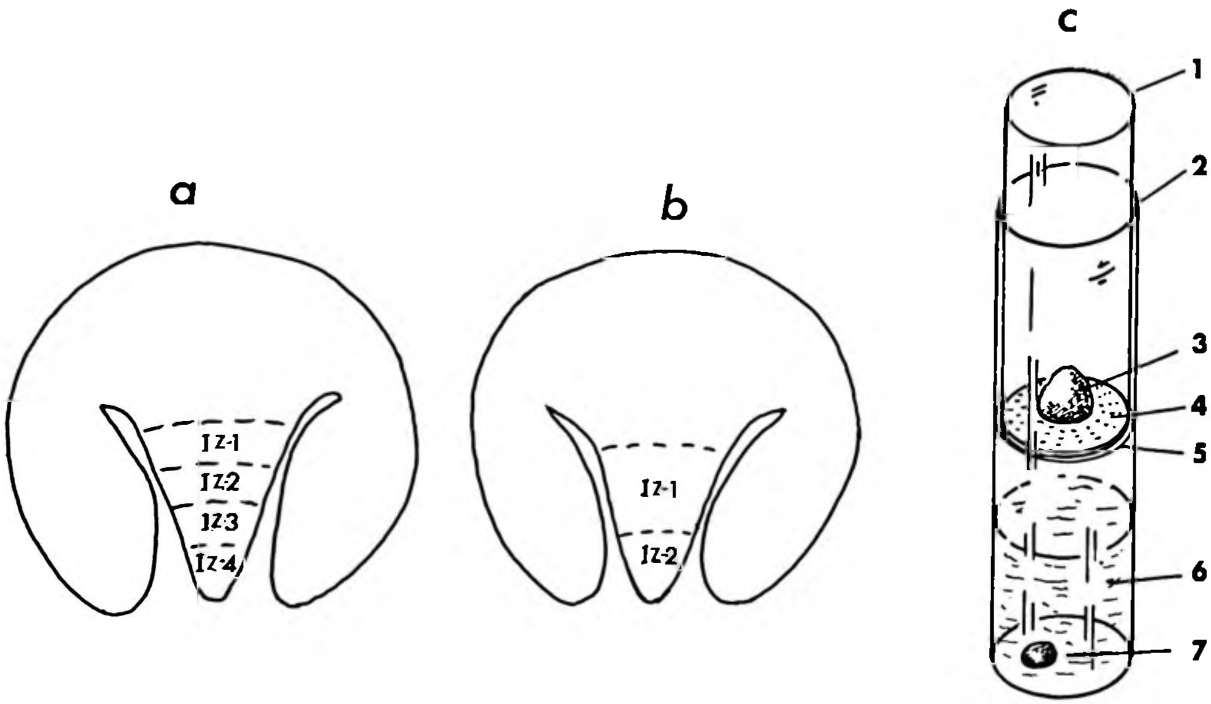

Figure 1

a. The way the inner zone (IZ) was cut into four pieces. These results are not presented separately in this report.
b. The way the inner zone was cut in the experiments presented here.
c. The polyethylene tubes used for centrifugation of tissue. The tissue (3), covered with a film of oil, is placed on the perforated bottom (4) of the inner tube (1), which rests on a support (5). The outer tube (2) contains oil (6). The fluid spun out of the tissue (7) collects on the bottom of the outer tube.

because of its large size and because of the relatively good concentrating ability of its kidneys (maximum urine concentration about 3000 mOsms). It was first necessary to determine a) if extracellular fluid could be removed by centrifugation without breaking the cells, and b) if a fluid could be obtained which represented the average interstitial fluid with respect to the extracellular marker (PEG) and with respect to the ionic and osmotic composition.

Seventeen mature, young cats were used. Two were studied during diuresis, the rest during anti-diuresis. ^{14}C-labeled PEG was used as an extracellular marker. One or two hours following the injection the cat was anesthetized by placing it in a container filled with pure CO_2 gas. Thirty seconds later it was decapitated to stop renal circulation and glomerular filtration abruptly. Both kidneys were removed within one to two minutes after death. The inner zone of the renal medulla was exposed. In some cases it was cut into two pieces and in other cases into four pieces, as shown in Figures 1a and b. Then either of two procedures was followed. In procedure 1 the pieces were placed in a depression slide under oil and tubular and vascular fluid was gently pressed out until tiny separate drops of fluid appeared in the oil. The tissue was then quickly placed in a polyethylene tube as shown in Figure 1c and centrifuged in a Sorvall refrigerated centrifuge, first at 12,000 rpm for five minutes and then either 12,000 rpm for 35 minutes or at 18,500 rpm for 35 minutes. In procedure 2 the tissue was not placed under oil but was centrifuged first at 4,000 rpm for five minutes, then at 12,000 rpm for five minutes and then again at either 12,000 or 18,500 for 35 minutes. Between each centrifugation the bottom part of the tube containing the fluid centrifuged out of the tissue was replaced so that the fluid collected at the different centrifugations was kept separate. Following the centrifugation the tissue was quickly frozen in dry ice and acetone. The fluid collected at the bottom of the centrifuge tubes under oil was removed quantitatively and weighed in test tubes containing 50 µl water. The tissue was removed from the dry ice and acetone and blotted. A piece from each tissue section was weighed and dried to constant weight for determination of water content. The rest of the tissue was weighed in test tubes containing 0.5 ml of water. These tubes were then reweighed and immersed in a boiling water bath for two minutes. The test tubes with tissue were placed in a refrigerator for 18 to 24 hours prior to the analysis. All solutions were analyzed for sodium, potassium, chloride, and urea. Osmolality was determined by the Ramsay method on undiluted samples of blood and extracellular fluid. In the tissue samples this was determined on the boiled samples.

The volumes of fluid removed by centrifugation are presented in percent of total tissue fluid in Table 1. The amount of fluid that could be pressed out or that could be removed at 4,000 rpm was four to five percent of the total amount of tissue fluid. The total amount of fluid that could be removed by centrifugation was found to be 29 percent of the tissue fluid in IZ-1 and 35 percent in IZ-2.

The potassium concentration of the fluid was used as a criterion to determine if cell breakage had taken place. Consecutive centrifugations and increasing gravitational force (Table 2) did not cause any significant difference in the potassium concentration of the fluid that could be spun out of the tissue. The potassium concentration from IZ-2 was slightly but significantly higher than potassium concentration in IZ-1.

The PEG concentration in fluid obtained at 17,000 g was slightly but not significantly higher than the fluid obtained at 41,000 g from the same tissue (Table 2). The fluid removed at 1,900 g or the fluid pressed out under oil was not significantly different from the fluid obtained after centrifu-

TABLE 1

FLUID REMOVED BY CENTRIFUGATION IN PERCENT OF TOTAL TISSUE FLUID

	Pressed out	17,000g 5 min	41,000g 35 min	Total
IZ 1	4.2 ± 0.9 (9)	6.9 ± 0.8 (9)	13.2 ± 1.3 (7)	29.1 ± 2.5 (7)
IZ 2	4.9 ± 0.6 (10)	14.7 ± 1.8 (9)	15.2 ± 1.3 (7)	35.0 ± 2.5 (6)
P	n.s.	<0.001	n.s.	n.s.

EXTRACELLULAR FLUID VOLUME IN PERCENT OF TOTAL TISSUE WATER

		All
IZ 1	53.6 ± 3.5 (9)	54.0 ± 2.3 (18)
IZ 2	56.7 ± 2.8 (9)	

TABLE 2

K CONCENTRATION (mM/L) IN FLUID OBTAINED BY CENTRIFUGATION OF INNER ZONE OF THE MEDULLA

	1900 g (5 min)	17000 g (5 min)	41000 g (35 min)	P
IZ 1	20.6 ± 2.0 (9) 5.9	26.4 ± 2.4 (9) 7.1	22.4 ± 1.8 (7) 4.7	n.s.
IZ 2	28.0 ± 3.0 (10) 9.4	30.0 ± 2.0 (9) 6.1	31.1 ± 3.3 (7) 8.7	n.s.
P	<0.05	n.s.	<0.05	

RATIO OF PEG CONCENTRATION IN FLUID OBTAINED AT DIFFERENT g

1900/41,000	17,000/41,000
1.04 ± 0.18 (7)	1.14 ± 0.05 (12)
0.48	0.17

TABLE 3

ANTIDIURETIC

Concentrations in mM

	IZ-1		IZ-2		Urine
	Intracell.	Extracell.	Intracell.	Extracell.	
Meas. Osm	1155 ± 108 (13)	892 ± 47 (13)	2230 ± 106 (15)	1605 ± 70 (15)	2348 ± 1156 (9)
Calc. Osm	797 ± 52 (12)	801 ± 29 (12)	1621 ± 76 (15)	1430 ± 66 (15)	2197 ± 122 (9)
Urea	348 ± 27 (12)	307 ± 17 (17)	1031 ± 63 (15)	774 ± 50 (17)	1471 ± 117 (9)
Na	181 ± 15 (13)	254 ± 7 (17)	230 ± 18 (14)	333 ± 15 (17)	183 ± 36 (9)
Cl	169 ± 10 (13)	231 ± 8 (16)	234 ± 11 (16)	323 ± 14 (16)	135 ± 29 (9)
K	76.4 ± 2.6 (14)	25.4 ± 1.6 (17)	86.6 ± 3 (16)	25.1 ± 1.5 (17)	208 ± 17 (9)

DIURETIC

Concentrations in mM

	IZ-1		IZ-2		Urine
	Intracell.	Extracell.	Intracell.	Extracell.	
Meas. Osm	837.7 ± 31 (4)	588 ± 25 (4)	915 ± 85 (4)	621 ± 60 (4)	413 ± 101 (2)
Calc. Osm	392 ± 10 (4)	464 ± 16 (4)	402 ± 11 (4)	483 ± 14 (4)	267 ± 124 (2)
Urea	36.1 ± 2.1 (4)	47.5 ± 13.9 (4)	69.9 ± 13.2 (4)	69.3 ± 22 (4)	70.4 ± 21.7 (2)
Na	30 ± 5 (4)	200 ± 12 (4)	107 ± 7 (4)	200 ± 5 (4)	9.8 ± 3.4 (2)
Cl	121 ± 3 (4)	169 ± 9 (4)	94 ± 7 (4)	171 ± 6 (4)	40.6 ± 36.8 (2)
K	62.5 ± 8.1 (4)	25.4 ± 2.5 (4)	72.3 ± 8 (4)	23.1 ± 2.7 (4)	40.2 ± 18.7 (2)

TABLE 4

EXTRACELLULAR/INTRACELLULAR
Concentrations

	IZ 1	IZ 2
Urea	0.818 ± 0.034 (12)	0.723 ± 0.030 (15)
Measured osm	0.855 ± 0.090 (13)	0.724 ± 0.046 (14)
Calculated osm	1.004 ± 0.068 (13)	0.860 ± 0.032 (14)
URINE/INTRACELLULAR OF IZ 2		
Measured osm	1.06 ± 0.06 (10)	

gation at 41,000 g. This ratio showed more variability (higher S.D.) as expected since this first fluid was a mixture of the fluid from collecting ducts and intratubular fluid. Fluid obtained at 17,000 g was taken to represent the average interstitial fluid with respect to the extracellular marker and with respect to the ionic and osmotic concentrations. Using these values and the concentrations measured in the tissue we can now calculate the extracellular volume of the tissue (Table 2), as well as the intracellular concentrations. Intracellular concentrations were calculated from the equation

$$IC_x = \frac{T_x - (It.\,Fl._{PEG}/T_{PEG}) \times It.Fl._x}{1 - (It.Fl._{PEG}/T_{PEG})}$$

where IC_x = concentration of x in intracellular water
T_x = concentration of x in tissue water
It.Fl. = interstitial fluid

Intracellular potassium concentration (Table 3) was quite low compared to other tissues -- 75 to 87 mM per liter tissue water in the inner zone of the kidney of antidiuretic as well as diuretic cats. Extracellular potassium concentration was low in both antidiuretic and diuretic cats, and independent of the urine potassium concentration. Intracellular sodium concentration was considerably lower than the extracellular sodium concentration in antidiuretic cats. Intra- and extracellular sodium concentrations were independent of urine sodium concentrations. The chloride concentrations followed the same pattern as the sodium concentrations. Intracellular urea concentration and osmolality were consistently higher than in extracellular fluid in antidiuretic cats but not in diuretic cats. The ratio between extra- and intracellular urea concentrations in the antidiuretic cats was 0.72 in IZ-2 and was identical to the ratio between measured osmolality in extra- and intracellular fluid (Table 4). The measured osmolality of the urine was not significantly different from the measured intracellular osmolality (Table 4) whereas the interstitial fluid had a lower osmolality than that of the urine and of the cells. In diuretic cats on the other hand the intra- and extracellular urea concentrations were not significantly different.

These findings show that in antidiuretic cats the papillary tissue as a whole has a lower osmolality than that of the urine and they show further that it is the extracellular compartment in the papilla which has the lower osmolality. Functionally this finding is difficult to explain since water presumably leaves the collecting duct by moving from a region with higher water activity to a region with lower water activity. Our current working hypothesis is that a hydrostatic pressure difference exists between collecting duct fluid and interstitial fluid causing the activity of the water in the compartments to be such that water can move down its gradient from collecting duct lumen to the interstitium. Experiments of the type reported in another paper (Schmidt-Nielsen, Patel and Patel, Bulletin, MDIBL, this issue) have shown that the papillary tissue as well as the salt gland tissue can withstand a hydrostatic pressure of 21 Atm without cell breakage.

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FUNCTIONAL CORRELATES OF THE DOGFISH RECTAL GLAND DURING *In vitro* PERFUSION

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In a previous study (Bull. MDIBL 12: 45 and 50, 1972) it was shown that during *in vitro* perfusion of the dogfish rectal gland with shark Ringer's solution the secretion of sodium remained relatively constant for at least two hours and was proportional to the perfusion flow rate and/or perfusion pressure. Sodium was secreted against an electrochemical gradient of 5.7 ± 1.4 mV (mean ± SE) and chloride against a gradient of 13.6 ± 1.2 mV. Using this model further studies were performed to analyze the composition of glandular fluid and to determine functional correlates of electrolyte secretion.

In these experiments rectal glands were perfused at a rate of approximately 4.0 ml per minute using gravity flow at a hydrostatic pressure of 30 mmHg. The perfusion solution was gently bubbled with 99 percent O_2 and one percent CO_2 in most experiments. In studies involving the influence of pH on sodium excretion either 100 percent O_2 or 95 percent O_2 and five percent CO_2 was used.

The composition of the perfusion solution (P) and glandular fluid (GF), under control conditions, is shown in Table 1. Sodium composition in GF was 475 ± 7 mEq/L and the concentration of chloride was 487 ± 9 mEq/L, indicating gradients (GF/P) across the glandular epithelium of 1.57 ± 0.05 and 1.53 ± 0.05, respectively. Associated with a high concentration gradient for sodium the level of urea in glandular fluid was approximately 14 percent of the perfusate concentration. There was no difference in total CO_2 content of glandular fluid compared to perfusate, and glandular fluid maintained a slightly but significantly lower pH.

It was of interest to compare the rate of secretion in these *in vitro* experiments with levels observed in collections from free swimming fish (Science 131: 670, 1960). In perfusion studies the flow rate of glandular fluid was 3.6 ± 0.5 μl/min/g of wet gland weight and sodium secretion averaged 1.73 ± 0.27 μEq/min/g. These levels compare reasonably well with rates observed *in vivo* as recalculated from Burger's data, 11 μl/min/g (glandular flow rate) and 6 μEq/min/g (sodium secretion rate.)