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CHANGES OF SODIUM AND UREA CONCENTRATIONS IN THE RENAL PAPILLARY INTERSTITIAL FLUID ON DEHYDRATION OF RATS

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SUMMARY

1. The isolated renal papillae of a rat were centrifuged in a two tube assembly which allowed fluid from the tissue to separate into the lower tube.

2. The papillae were centrifuged for 15 min at 300 *g* and 1500 *g* consecutively.

3. After intraperitoneal injection of Na ^{131}I-diatrizoate, the activities of urine, and fluid samples obtained from the papilla, were compared. It was found at 1500 *g* that the median value for papillary fluid activity was 1·52 % of the activity of urine. This is evidence that the papillary fluid was virtually free from any contamination from the terminal collecting ducts.

4. It is considered that the fluid sample obtained from the papillae by centrifugation at 1500 *g* is a representative and reproducible sample of interstitial fluid.

5. The method was used to demonstrate changes in solute concentrations in the renal papillary fluid, following dehydration of rats.

INTRODUCTION

The technique of renal micropuncture has provided valuable information concerning the functions of the nephron in the cortex. This technique has also been applied to the study of the renal concentrating mechanism in the papilla (Gottschalk & Mylle, 1959; Jamison, Bennett & Berliner, 1967; Marsh, 1970). However, there are technical difficulties in micropuncture of the papillary loops of Henle. It appears that the surgical exposure of the whole papilla always causes the maximum concentrating ability of the kidney to fall, but nevertheless evidence was obtained of the existence of a sodium pump in the ascending limb of the loop of Henle by Jamison *et al.*

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(1967). However, other authors (Gottschalk, 1964; Morgan & Berliner, 1968; Marsh, 1970) were unable to demonstrate the presence of this pump.

Two other techniques have been used to investigate changes in solute concentration in the renal medulla. One, the melting point of frozen tissue slices (Wirz, Hargitay & Kuhn, 1951; Bray, 1960), is limited, since solute concentrations in different fluid compartments cannot be determined. The other, chemical analysis of whole tissue portions of the kidney (Levitin, Goodman, Pigeon & Epstein, 1962; Saikia, 1965; Valtin, 1966; Atherton, Green & Thomas, 1971), has been widely applied to the study of the corticomedullary solute concentration gradient with different states of hydration. However, this technique only measures solute concentrations indirectly by the comparison of total water to total solute, providing an average figure for a compendium of fluids from all compartments in the tissue. Dicker (1970) has recently considered the merits of the different techniques at some length.

A method recently described in brief (Lee, Lewis & Williams, 1971*a*), is designed to squeeze a fluid sample out of the isolated renal papilla. This paper describes the method of obtaining papillary fluid in more detail, together with experiments designed to show the proportion of collecting duct fluid present in the papillary fluids. Evidence is given supporting the view that this technique can provide a sample representative of papillary interstitial fluid, and the effects of dehydration on sodium and urea concentrations of this fluid are described.

METHODS

Method for obtaining papillary fluids

After stunning of a rat, the kidneys are exposed by free abdominal surgery. The renal vessels are clamped at the hilum with artery forceps and the kidneys are removed distal to the clamp. The renal papillae are then exposed by an eccentric sagittal section through each kidney and are cut free at the junction with the inner medulla. Both papillae are removed within 3 min of stunning the animal; the wet weight of each papilla from a 250 g rat is 2–3 mg. Rats are killed by rapid exsanguination following removal of the clamp on the renal vessels.

In these experiments, both isolated papillae from each rat were placed in the base of the upper of two autoanalyser pots (Technicon Ltd), one nestling in the other. The base of the upper pot had been perforated previously with about ten holes of approximately 0·5 mm in diameter, and the whole assembly was filled with light liquid paraffin (B.P.) cooled to 1° C. The mouth of the lower pot was reinforced with a soft wire band, to prevent splitting on centrifugation. Initial centrifugation at 300 *g* for 15 min (Gallenkamp Junior Bench Centrifuge) yielded 400–800 nl. fluid in the lower pot. The lower pot was replaced and a subsequent centrifugation of 1500 *g* for 15 min yielded a further 500–1000 nl. (Pl. 1). Fluids were removed from under the paraffin for analysis (Pl. 2) in micropipettes of about 300 nl. in size.

Micropipettes were prepared by cutting portions from standard 1 μl. micropipettes (Drummond Scientific Instrument Co. Ltd.). The cut ends were squared using a

grinding stone and the micropipettes were sealed with wax or epoxy resin into glass bulbs inside which pressure adjustments could be made for drawing up papillary fluids (Lee *et al.* 1971*a*). Micropipettes were previously calibrated for size, by measurement of the dilution of standard solutions of NaCl (0–500 m-equiv/l.) drawn up by the pipette and then washed into 2 ml. of double deionized water. The resulting concentration was measured by flame photometry. Calibration by this method did not differ (< 3%, 95% confidence limits) from calibration by comparison of the weight of the pipette to the weight of the standard 1 μl. pipette from which it was sectioned.

The papillary fluid was drawn up into a micropipette and the column of fluid was examined for continuity using an eyepiece of 10 × magnification. If the fluid column was continuous it was blown into an appropriate eluent for analysis and the pipette lumen was rinsed with this eluent. Any sample in which liquid paraffin had entered the pipette was discarded, and the pipette was washed sequentially with petroleum spirit (b.p. 100–120° C) and acetone; for routine cleaning the micropipette was washed with deionized water.

Proportion of collecting duct fluid in papillary sample

The substance Na ^{131}I-diatrizoate (Radio Chemical Centre, Amersham) was chosen to label the collecting duct fluid, since diatrizoate, like inulin, may be used to measure glomerular filtration rate (Chinard, Enns, Goresky & Nolan, 1965) and in normally hydrated animals it achieves a high concentration in the collecting duct compared to the remainder of the papilla. Each rat was given 250 μc of this chemical by I.P. injection, in order to give a high plasma level, and because of the ease of administration to conscious animals. However, the rise of activity in the blood following I.P. injection was first determined in unconscious animals.

Three male Wistar rats were anaesthetized by I.P. injection of sodium pentobarbitone (36 mg/kg body wt.); anaesthesia was maintained by further injection of pentobarbitone as necessary. The carotid artery on the left side of each rat was cannulated with polyethylene tubing (PP 50, Portex Ltd.) filled with heparinized saline. At intervals (5, 10, 15, 30, 45 min; 1, 2, 3, 4, 5 hr) following I.P. injection of Na ^{131}I-diatrizoate, small aliquots of about 0·2 ml. blood were taken from the carotid cannula of each rat into heparinized syringes. After the removal of each sample the cannulae were washed with heparinized saline, to prevent clotting. Aliquots of whole blood (0·1 ml.) were measured into 2 ml. Perspex counting vials, and radioactivity measured by well-type iodide crystal scintillation counter. Every sample was counted to at least 100 K disintegrations.

The activity of plasma was always high 30 min after I.P. injection of the tracer (Text-fig. 1, results) and therefore the eight conscious Wistar rats in which the papillary activity was obtained were stunned 30 min after the injection. From each rat, both papillae were removed as described, a blood sample was taken from the exposed inferior vena cava and lengths of ureter near to the renal pelvis were ligated about 1 cm apart and removed. Papillae were dried on the external surface with blotting paper, and papillary fluids obtained by centrifugation. Ureters were washed on the external surface to remove any activity, and blotted; each was then placed under paraffin in a top autoanalyser pot, cut several times with a pair of ophthalmic scissors, and centrifuged only at the lower speed to yield urine in the lower pot. Blood was centrifuged in heparinized tubes immediately after removal from the animals, and 5 μl. aliquots of plasma washed into 2 ml. water in Perspex counting vials. Small volumes of papillary fluids and ureter urine were measured using the calibrated micropipettes, and also washed into 2 ml. water in counting vials. Activities

were again assessed by crystal scintillation counter to at least 10 K disintegration. Any sample with activity of less than twice background was rejected.

Dehydration of rats

In these experiments papillae were obtained from Wistar rats weighing 225–250 g. Before killing, six rats were allowed free access to food and water, and six rats were dehydrated for 24 hr, but had free access to food. All rats were killed between 10.00 and 11.00 B.S.T., avoiding possible diurnal rhythm in the countercurrent system, and the papillae were centrifuged. Fluid from the slower centrifugation was discarded, and that from the faster centrifugation was analysed for sodium and urea by the methods previously described (Lee *et al.* 1971*a*). Samples of urine were not collected from the animals.

After the second centrifugation, papillae (which still contained about 60 % of their total water) were washed in petroleum spirit and acetone to remove paraffin. They were desiccated to dry weight under reduced pressure and then weighed by tare on an electronic balance, accurate to $\pm 0{\cdot}01$ mg. This procedure ensured that comparable portions of papilla had been obtained in the two groups.

Statistics

Since the distribution of most of the results appeared to be skewed, they have been analysed using rank-order statistics. However, the papillary dry weights were symmetrically distributed and have been analysed parametrically.

RESULTS

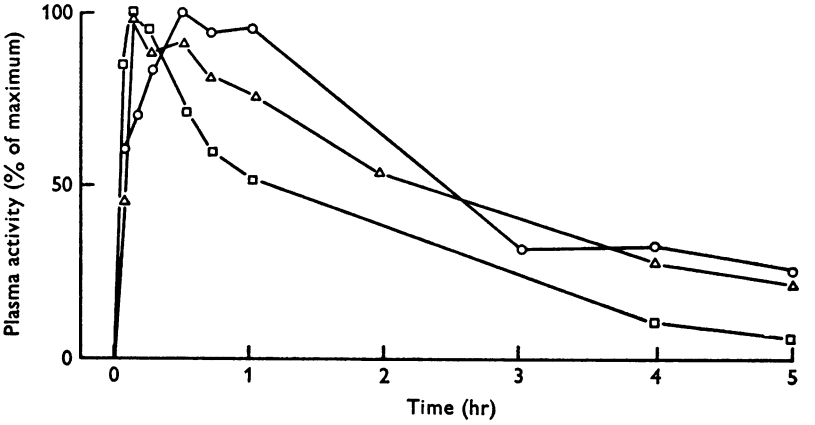
Activity of Na ^{131}I-diatrizoate in blood following intraperitonal injection

The graphs of activity of diatrizoate in blood, expressed as a percentage of the maximum achieved following intraperitonal injection, is shown in Text-fig. 1, for each of the three rats. The initial rise in activity was rapid and the maximum was reached within 15–30 min. Activity remained about 70 % of maximum for 30–100 min. After 5 hr the activity was always below 30 % of maximum, and the fall of activity with time between the fourth and fifth hour was relatively constant in the three rats. The decline in activity at this stage was probably due to the renal clearance of the diatrizoate.

Activity of papillary fluids

The activities of papillary fluids and ureter urine expressed as a ratio of the plasma activity of each rat are expressed in Table 1. This method of representation is chosen because of fluctuations in the absolute value of plasma activity between rats. The median activity of urine was 185 times higher than that of plasma (range 72–450). This wide variation is probably due to differences of renal function in conscious, unrestrained rats (see heading to Table 1). In the fluid from the first centrifugation median activity was 10·15 times higher than that of plasma (range 2·92–50·6).

However, in the second centrifugation the range was only 1·58–5·15, with a median activity of 2·73 times higher than that of plasma. Table 2 shows the data from Table 1 recalculated to show the activity of papillary fluid expressed as a percentage of the activity of ureter urine. This value represents the maximum percentage of collecting duct fluid in each sample.



Text-fig. 1. Graph to demonstrate the change of Na ^{131}I-diatrizoate activity in the plasma of three anaesthetized rats (○, □, △), for 5 hr following I.P. injection of the tracer at time 0.

TABLE 1. Ratio of activity of Na ^{131}I-diatrizoate in papillary fluids and in urine, compared to plasma. In these laboratories the urine osmolality of conscious, normally hydrated rats varies between 700 and 2200 m-mole/l. Furthermore the daily solute excretion varies between 5 and 10 m-mole/100 g body wt. This variation would account for the wide fluctuation in the urine/plasma ratio of the diatrizoate shown in this Table

Centrifugation at...	300 *g*	1500 *g*	300 *g*
Rat no.	Papillary fluid/plasma	Papillary fluid/plasma	Urine/ plasma
H 1	5·78	2·95	150
H 2	4·26	1·87	365
H 3	50·60	1·97	185
H 4	15·43	1·58	450
H 5	10·60	2·50	304
H 6	20·20	3·38	124
H 7	2·92	3·74	185
H 8	9·70	5·15	72
Median values	10·15	2·73	185

Median contamination by collecting duct fluid to fluid from the first centrifugation was 3·67 % (range 1·17–27·35 %) whereas the median percentage in the second fraction was only 1·52 % (range 0·35–7·15 %). From Table 2 the skew nature of the distribution can be seen.

TABLE 2. Activity of papillary fluids expressed as a percentage of the activity of urine (calculated from Table 1). These results are a more detailed account of those published in abstract form by Lee *et al.* (1971*a*)

Centrifugation at...	300 *g*	1500 *g*
Rat no.	Papillary fluid/urine	Papillary fluid/urine
H 1	3·85	1·97
H 2	1·17	0·51
H 3	27·35	1·06
H 4	3·43	0·35
H 5	3·49	0·82
H 6	16·29	2·73
H 7	1·58	2·02
H 8	13·47	7·15
Median values	3·67	1·52

Effect of dehydration on papillary solute concentrations

Table 3 shows the changes in the concentration of the main papillary solutes, induced by dehydration. The median sodium concentration in the papillary fluid of normally hydrated rats were 272·5 m-mole/l. (range 250–290); the urea concentration was 289·5 m-mole/l. (range 210–447). After 24 hr dehydration the median sodium concentration rose significantly to 372·5 m-mole/l. (range 280–420; $P < 0{\cdot}002$ Mann–Whitney U test) and the urea concentration rose to 598·5 m-mole/l. (range 503–757; $P < 0{\cdot}001$).

TABLE 3. Sodium and urea concentrations (mM) in second papillary fluid fraction. The sodium and urea concentrations in the papillae of normally hydrated rats, and dehydrated rats, are given in this Table. The rats were resting quietly in their cages before being stunned

Group 1*			Group 2†		
Rat no.	[Na^+]	[Urea]	Rat no.	[Na^+]	[Urea]
N 1	290	425	D 1	420	576
N 2	250	320	D 2	400	503
N 3	255	233	D 3	280	597
N 4	270	447	D 4	393	600
N 5	275	210	D 5	309	757
N 6	280	259	D 6	352	725
Median values	272·5	289·5		372·5	598·5
Papillary dry weights in mg (mean ± S.D.)		0·75 ± 0·18			0·80 ± 0·12
		$n = 12$			$n = 12$

* Group 1, normally hydrated rats.
† Group 2, dehydrated for 24 hr.

It can be seen from Table 3 that there was no significant difference between the average papillary dry weights ($P > 0{\cdot}2$, t test) of the two groups.

DISCUSSION

In the experiments in the conscious rat there is evidence that diatrizoate does not leave the lumen of the nephron, since the median urine/plasma ratio of 185 is of the expected magnitude. The diatrizoate activity in papillary fluids relative to urine (Table 2) indicates the maximum possible contamination of the papillary fluids by the collecting ducts, assuming that activity in the papilla higher than that of plasma is due to the collecting ducts, and that the activity of the terminal collecting duct fluid is equal to that of ureter urine. The fluid obtained from the ureter by centrifugation has been shown to be indistinguishable from urine on the basis of the concentration of diatrizoate, and also by analysis of the solute concentrations (J. Lee and P. Williams, unpublished). Although this method of obtaining urine is both cumbersome and difficult, it provides a good approximation to the terminal collecting duct fluid of the conscious rat.

The amount of activity present in the papillary fluid from the first centrifugation (Table 2) is either high or low, a result that would be obtained if the terminal collecting ducts were either empty or full at death, without an intermediate phase. The low activity of the papillary fluid fraction obtained at the faster centrifugation is taken to indicate little contamination from the collecting ducts (median 1·52 %); this source of contamination is removed, when present, by the first centrifugation. Having eliminated the possibility that collecting duct fluid contributes significantly to fluid from the faster centrifugation, it is necessary to consider the alternative sources, intracellular fluid and extracellular fluid (including the loops of Henle, interstitium and vasa recta plasma).

Intracellular fluid probably does not contribute greatly to the sample obtained as at least 55–60 % of the total tissue water still remains in the tissue after centrifugation, which would be approximately equal to the intracellular space. The small force of 1500 *g* may be insufficient to force out the intracellular fluids, and also diffusion of solutes between cells and interstitium is minimized by cooling the paraffin.

Recently Kettyle, Horster, Thurau & Valtin (1970) reported a short account of a method for obtaining fluid from kidney portions by ultracentrifugation at 45,000 *g*. This force could well result in the release of intracellular fluids and, although no measurements of solute concentrations were made, the tissue osmolality measured by cryoscopic analysis of the fluid corresponded closely to osmolality calculated by the addition of solute concentrations obtained by whole tissue analysis. This might

possibly indicate that the fluid sample is a compendium of whole tissue fluid. Their method used only one container and all fluids forced out from the tissue remain in contact with the tissue. There are inherent difficulties both in the pipetting off of the minute quantities of fluid (10–20 nl.) from the admixture of oil and papilla, and in obtaining reproducible analysis of solute concentrations. These difficulties are overcome in our method by the use of a much smaller force, the sequential centrifugation, the complete separation of fluid from the tissue, and the use of larger tissue portions to give fluid samples of sufficient volume for the accurate estimation of individual solutes.

Further evidence that the papillary sample from the second centrifugation of our method derives from the interstitium is given by the results obtained in Table 1; the median value of diatrizoate in this sample is only 2·73 times higher than plasma. If the papillary fluid sample was solely derived from the loop of Henle the expected ratio would be of the order of 8 assuming that the papillary fluid sample represents about two-thirds of the maximum concentration gradient at the papillary tip, where the loop of Henle/plasma ratio for inulin is of the order of 12 (Marsh, 1970). The concentration of diatrizoate in interstitial fluids outside the kidney probably approximates to that of plasma; but the concentration of this substance in the renal interstitium is uncertain. Therefore, although the papillary fluid is 2·73 times more active than plasma, this activity could be derived from interstitial fluid.

At present it is not possible to determine the contribution from the loops of Henle and vasa recta to the papillary fluids. Although the method only provides a compendium of fluids from different extracellular sources, the results (Table 3) obtained here indicate that the method will demonstrate the rise in the solute concentrations of the papilla, caused by dehydration of normal Wistar rats. This method cannot demonstrate to what extent the rise is due to changes in total solute or total water in the tissue. However, the magnitude of the observed rise of sodium and urea concentrations is unlikely to be accounted for by change in water content of the tissue alone; there must be an absolute rise in the interstitial content of both solutes with dehydration.

The significant rise in papillary fluid sodium concentration with dehydration has been reported previously for heterozygous Brattleboro rats dehydrated for 48 hours (Lee, Morgan & Williams, 1971*b*). This finding contrasts with that of Valtin (1966), but, since the initial urine osmolalities of his normal rats were about 50 % higher than in our rats, there could have been a difference in hydration of the rats at the beginning of the respective experiments.

The advantage of *in vitro* techniques is that the function of the kidney at

any state of the animal can be determined provided that the kidney is rapidly removed and internal changes minimized. Of the *in vitro* techniques, the present technique of squeezing the papilla is a refinement of whole tissue analysis since it allows the direct measurement of solute concentrations. Also, since collecting duct fluid can be excluded from the papillary fluid sample, and the contribution from intracellular fluid can be minimized, a more accurate estimation of interstitial fluid is obtained.

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EXPLANATION OF PLATES

PLATE 1

This photograph shows two papillae in an upper autoanalyser pot; in the lower pot, the fluid droplet collected after the 1500 *g* centrifugation can be seen under oil. This droplet is about 500 nl. in size. The wire reinforcement was removed from the lower centrifugation, for photographic purposes. Magnification × 3.

PLATE 2

Photograph illustrating the size of a micropipette (lumen approximately 300 nl. in volume) in relation to a papillary fluid droplet. This droplet was 1 μl. in size, and about 700 nl. remains outside the pipette, the lumen of which is completely filled with fluid. The micropipette is sealed into an air-filled glass bulb, inside which pressure adjustments are made. Magnification × 3.