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X-RAY ANALYSIS OF BIOLOGICAL FLUIDS: AN UPDATE

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Abstract

X-ray analysis has been used for some twenty years to quantify elements including Na, K, Cl, Ca, Mg and phosphorous in biological fluid samples. The method consists of accurate pipetting of 50-150 pl samples and appropriate drying to form crystalline deposits. Quantitation is obtained by comparison of x-ray intensities with comparable standard crystalline deposits. Electon microprobe analyses have contributed much to our understanding of renal transport function including physiological controls and pathological influences on the tubular handling of the major elements. New approaches to x-ray analysis of solid tissue will further our knowledge concerning transport properties in the kidney and other tissues.

KEY WORDS:
Microprobe analysis, tubule fluid, micropuncture, sodium, potassium, chloride, calcium, magnesium, phosphorous, sulfur.

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Introduction

X-ray analysis has been used extensively over the last two decades to determine elemental concentrations of very small fluid samples. The purpose of this review is not to set the historical development of the microdroplet technique but to provide an update of applications and achievements of microdroplet analysis to biology over the last half decade. This topic was last reviewed five years ago in Scanning Electron Microscopy by Roinel and de Rouffignac (1982).

The electron microprobe offers the unique advantage of rapid and accurate analysis of ultramicrovolume samples where conventional chemical methods are not possible or feasible (Ingram and Hogben, 1967: Cortney, 1969: Morel and Roinel, 1969; Lechene, 1970; Quinton, 1978a; Rick et al., 1977). Detectability limits for electron microprobe analysis are comparatively poor when considered in terms of the minimum amount of an element detected relative to the total sample in a homogeneous mixture. However, the ultimate sensitivity of electron probe analysis is high considering the actual number of atoms required to produce a detectable signal above background (about 100 ppm or 10^{-16}g). Thus electron microprobe analysis is ideal for analyzing ultrasmall samples of relatively high elemental concentrations. This technique has been extensively applied to investigations of kidney physiology where one routinely collects sample volumes in the nanoliter range from individual nephrons by micropuncture and microperfusion techniques (Morel et al., 1969; Lechene, 1977; Roinel and de Rouffignac, 1982).

Typically, a micropuncture experiment involves collection of nanoliter volume samples by micropipettes from various renal compartments. The small size relative to the large surface area of the sample volumes dictates that all handling be accomplished under an antiwetting phase, usually paraffin oil, with the use of micromanipulators and stereo microscopes. This condition in itself may lead to unforeseen errors in microdroplet analysis. All of the many methods that have been reported for sample preparation involve placing small volumes (10-100 pL) of samples and standards on a support and

either air-drying (Ingram and Hogben, 1967; Cortney, 1969; Quinton, 1978b); or freeze-drying to produce geometrically amorphous deposits comparable to each other (Morel and Roinel, 1969; Lechene, 1970; Rick et al., 1977; Morel et al., 1969; Lechene, 1977; Roinel and de Rouffignac, 1982; Greger et al., 1978). Electron microprobe analysis includes setting the electron beam to the same diameter as the sample and standard deposits and comparing the X-ray intensities. The X-ray intensity is proportional to the number of atoms present in the sample; thus, the absolute concentration of the liquid volumes can be obtained from the standards of known composition. This method can be applied to any aqueous fluid provided adequate standards are prepared.

The wavelength-dispersive spectrometer (WDS) is the most widely used method of X-ray spectral analysis in the microprobe, although the energy dispersive system (EDS) can also be used (Quinton, 1978a and Rick et al., 1977; Van Eekelen et al. 1980). The WDS spectrometer consists of a mechanical assembly that positions a diffracting crystal and gas-proportional detector in such a fashion as to allow selective diffraction by the crystal of characteristic wavelengths (Each element is characterized by its own wavelength spectra). The advantages of WDS are: (1) high line-to-back-ground ratio as a result of the spectrometer's excellent inherent wavelength resolution (which thus yields low elemental detection limits even in the presence of high X-ray background), a particularly important feature in biological fluids containing elements of low X-ray yield such as sodium and magnesium; and (2) unambiguous X-ray line identification, which is important in elemental determination of low concentrations in the presence of very high concentrations of other elements, e.g., phosphorus at 1mM versus chloride at 120mM concentrations, which are commonly encountered in biological fluids. A consequence of these properties is the relatively low counting times required for analysis, a significant consideration in that each routine micropuncture experiment may generate over 100 separate deposits. Although the above ultra-microanalytical procedure appears direct and unaffected, several problems are inherent to the analysis of biological fluids. First, as mentioned above, sample handling requires special techniques owing to the extremely small volume proportional to the large surface area, so that surface contact with air, glass, paraffin oil, and solvents becomes very important with regard to elemental contamination and (more important) elemental loss. Second, the nonuniformity of composition of biological samples may unpredictably alter the background absorption parameters. Third, many of the elements of interest in physiology possess low atomic numbers (e.g., sodium) and may be present in relatively low concentrations (e.g., magnesium). Despite these inherent drawbacks X-ray microanalysis has become a major tool in investigation of kidney physiology.

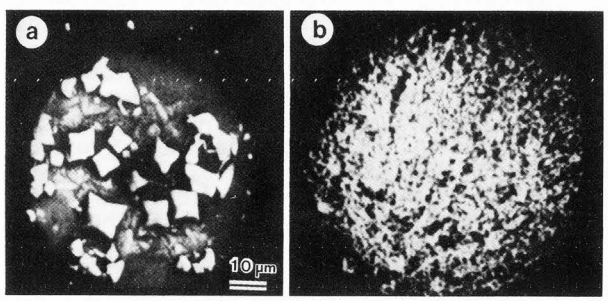

Figure 1. Scanning electron microscope images of deposits obtained from 0.1 nl of a 150 $mmol \cdot^{-1}$ NaCl droplet. (a) Incorrectly freeze-dried and, (b) correctly freeze-dried. From Roinel, (1981).

Practical Details of Microdroplet Analysis

It is not the intent of this review to detail the specifics of sample preparation and electron microanalysis of microdroplets; this has been adequately detailed elsewhere (Morel and Roinel, 1969; Roinel and de Rouffignac, 1982; Greger et al., 1978; Lechene, 1974; Lechene and Warner, 1977; Martoja et al., 1979; Quinton, 1978b). Let us briefly review the procedure, the advantages and some of the hazards in microdroplet analysis. Although the procedure is arduous, it is reasonable and relatively foolproof once established in one's laboratory. However, it is recommended that an investigator spend some time in a laboratory where the technique is ongoing to facilitate assimilation and avoid the many technical pitfalls that may occur.

The general approach to sample preparation for microprobe analysis has not changed over the last half decade. Generally, this consists of depositing 10-150 pl fluid samples on an appropriate support, usually beryllium (Morel and Roinel, 1969; Roinel, 1979; Lechene, 1970). Although other support material has been used (Quinton, 1978a), beryllium is the most convenient as the wetting properties of beryllium are such as to retard spreading of the small droplets. Quantitative pipetting is performed by means of siliconized constriction pipettes (Roinel and de Rouffignac, 1982; Prager et al., 1965) under paraffin oil to avoid evaporation. The mineral oil is then dissolved in m-xylene or other suitable solvent and freeze-dried under vacuum. Ideally, the lyophilization process results in small, uniform crystals (Figure 1). Many times, however, large crystals are formed which prohibit quantitative measurements. Roinel (1975 and 1979) has developed the rehydration technique, a modification of which is routinely used in the author's laboratory. When the lyophilization is complete, the dried samples are allowed to come up to room temperature. A rehydration step is subsequently performed. This second step consists of cooling the support to 40°C on a cooling plate. Condensation, usually from the atmosphere, rehydrates the salt crystals and forms them into droplets which may then be refrozen with an aim to forming small crystals. This series of steps, rehydration and rapid freezing, may be performed again if adequate crystals are not formed.

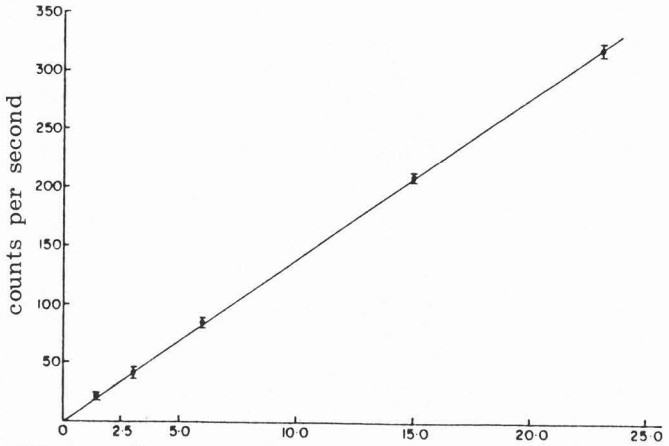

Figure 2a. Phosphorus concentration mg%

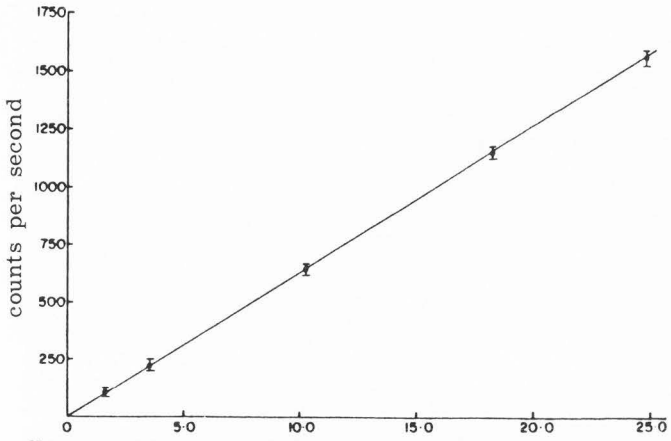

Figure 2b. Calcium concentration meq/litre

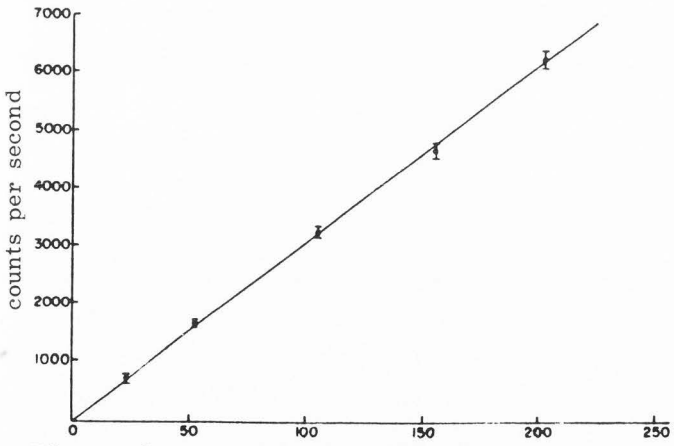

Figure 2c. Sodium concentration meq/litre

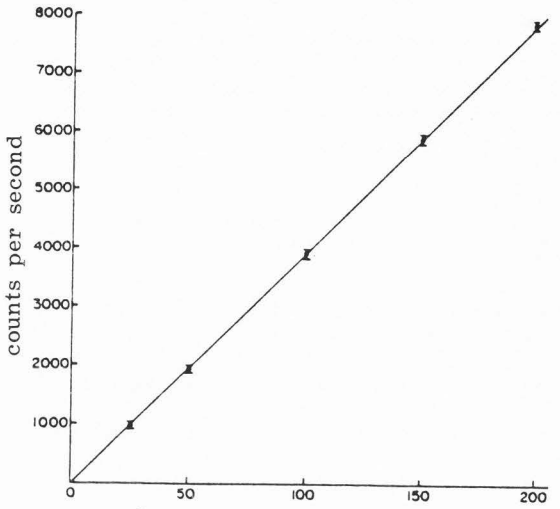

Figure 2d. Chloride concentration meq/litre

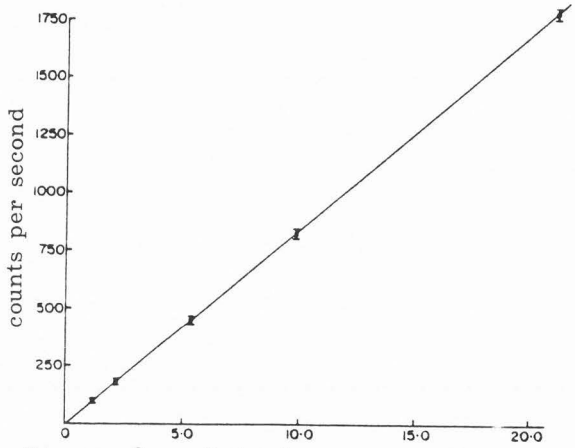

Figure 2e. Potassium concentration meq/litre

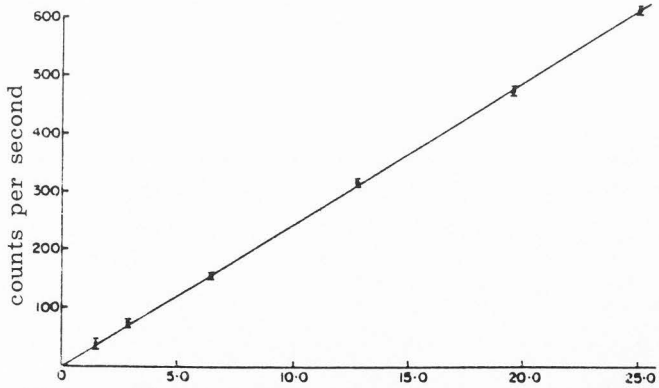

Figure 2f. Magnesium concentration meq/litre

Figure 2. Representative calibration curves for Na, Cl, Ca, Mg, P, and K. Samples were typically 100 pl in volume , which produced 55 μm diameter deposits. The standard deposits were probed with an accelerating voltage of 15 kV, beam current 400 nA; for 10 seconds.

Beryllium supports with crystalline deposits may be stored indefinitely in a dry atmosphere before probing. The individual deposits resulting from the pipetted fluids are analyzed by standard electron microprobe techniques. This has been facilitated by computer controlled automated stages and computer derived calibration curves of standards and unknowns (Roinel et al., 1973 Lechene, 1974). Figure 2 illustrates some typical standard curves generated by the use of electron microprobe analysis. All standard

curves are forced through background off deposit; this does not make a large difference as most curves pass through the origin. As long as the crystalline deposits for the unknowns are similar to those of the standards a good proportionality exists between the x-ray intensities emitted from unknowns and standards and absolute concentrations can be readily calculated. The errors resulting from mass-thickness and matrix effects have been detailed elsewhere (Roinel and de Rouffignac, 1982).

With modern electron probes, the precision of microdroplet analysis is principally determined during the pipetting and sample determination stage. In most hands, the precision is in the order of 5-10%, which is considered adequate for most quantitative measurements. The sensitivity of the approach is not good but is adequate for the abundant elements found in biological fluids, including Na, K, Cl, Mg, Ca, S and phosphorus. The minimum of detectable limits obtained by WDS analysis for the abundant elements have been reported to be in the order of 0.04 to 0.10 $mmol \cdot l^{-1}$, with a beam voltage of 15 kV, a beam current intensity of 300 nA and a reasonable counting time of 20-100 sec (Roinel and de Rouffignac, 1982, Greger et al., 1978; Roinel, 1975). These values are in accord with our practical experience. Optimal sensitivity of microdroplet analysis is highly dependent on adequacy of standard and specimen preparation. It is important that the sample areas and matrix densities be comparable; normally we try to form deposits of 55 um diameter from a 100 pl sample. The minimum detectable level increases with larger volume deposits, which is not practically improved by longer counting times. Figure 3 shows the mimimum detectable level as a factor of analytical time. A normal micropuncture experiment may generate 10-50 samples. Accordingly, counting times of 10-30 sec are necessary for reasonable analysis.

As alluded to above, a number of procedures or "tricks" have been developed to avoid some of the pitfalls involved with microdroplet analysis; most are in the sample preparation. Details of these procedures are available in the literature (Quinton, 1978b, Roinel and de Rouffignac, 1982, Greger et al., 1978; Roinel, 1975 and 1981; Lechene and Warner, 1979; Leroy and Roinel, 1982; Muhlert et al., 1982; Warner and Lechene, 1980). A few approaches are highlighted which may, in our experience, facilitate microdroplet analysis. The first concerns the handling and pipetting of ultrasmall volumes. Manipulation of nanoliter volumes presents the inherent problem of large surface area contact relative to minute volume size; thus, any surface such as glass or oil may greatly alter the composition. All glass surfaces used should be well coated with antiwetting solutions. We have observed significant losses of calcium and ^{45}Ca in our in vitro perfusion pipettes amounting to 10% of the total calcium concentration over 10 min. We routinely siliconize all glass pipettes and plates prior to use. The second major surface is the paraffin oil. Paraffin or mineral oil must be hydrated prior to use in order to avoid dehydration of the small samples (Warner and Lechene, 1980). Additionally, the necessity to use paraffin oil

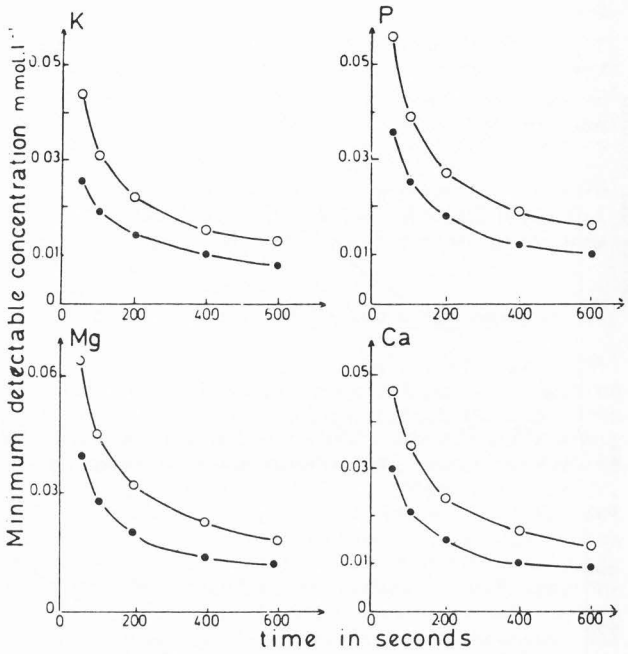

Figure 3. Minimum detectable concentrations as a function of analysis time for the elements K, P, Mg, and Ca. Beam accelerating voltage, 13 kV; beam current , 300 nA; sample diameter, 80 μm; sample volume, 0.12 nl. Minimum detectable concentration was calculated according to Roinel and de Rouffignac, (1982). Practical counting times are in the order of 10-30 sec for studies involving a large number of samples. It is evident that longer counting times cannot compensate for poorly prepared samples.

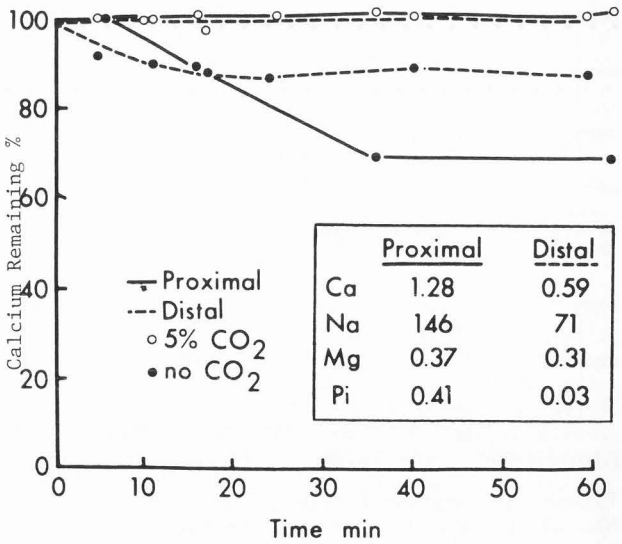

Figure 4. Calcium disappearance from tubule fluid samples collected by micropuncture from proximal and distal nephrons of the dog. Volumes of tubule fluid samples were 33 and 18 nl, repectively. Samples placed in paraffin oil which was pre-equilibrated with 5% CO_2 preserved their calcium content. See text for details. From Mulhert et al., (1982).

leads to specific loss of divalent cations. Figure 4 illustrates this effect in biological samples obtained by micropuncture from the dog kidney. The present studies confirm our past experiences in handling samples of tubule fluid so as to retain the calcium composition (Muhlert et al., 1982; Quamme and Dirks, 1986). This is done by acidifying the aqueous samples to pH values below 7.0. This may be conveniently accomplished by equilibrating the paraffin oil with 100% CO_2 prior to contact with the alkaline aqueous samples, provided the buffer capacity of the samples is rather low, as it normally is for tubule fluid. Presumably the oil and samples are equally equilibrated, thereby reducing the pH of the moderately buffered samples. Calcium is lost almost instantaneously from aqueous samples and to an extent dependent on the hydrogen ion concentration of the sample; certainly it is significant at pH values above 7.0. Magnesium and phosphate are also lost from microsamples following placement under paraffin oil, but only with pH values greater than 7.6. This phenomenon also occurs with radioactive isotopes of these elements, which are often used to reflect absolute concentration.

The mechanism of calcium loss is unknown. A number of observations suggest that it is the paraffin oil interface that combines with calcium and results in the apparent disappearance from the small sample (Muhlert et al., 1982). First, in our experiments, all glass surfaces were carefully siliconized, a procedure that minimizes any loss onto the glass surface. In addition, we have in the past used disposable plastic surfaces, with results that were similar to those with the siliconized glass. Second, the disappearance of calcium is dependent on the sample-to-oil surface contact rather than the sample-to-glass contact. Moreover, the calcium was completely recovered from alkaline solutions by simple acidification of the samples. This suggests to us a pH-sensitive anionic paraffin oil-aqueous interface that is able to complex calcium and perhaps, to a lesser extent, magnesium.

Silicone oils have been used as substitutes for paraffin oil but, unfortunately, they may take up water to as much as 20% of their volume (Muhlert et al., 1982). Prior hydration may prevent the initial dehydration of the samples, but evaporation from the surface of the silicone oil is quickly translated into water abstraction from the samples. We have observed significant water loss from microsamples after 5-10 min of exposure to "hydrated" silicone oil. In addition, it would appear that the amount of calcium lost in these samples is similar to the amount that was observed in paraffin oils.

A further problem in microdroplet analysis, this one related to probing, is the loss of chloride and other halides during the duration of counting (Leroy and Roinel, 1982). Exposure of the target to harsh counting conditions leads to loss of chloride from the deposit, usually greater from the standard deposits than from biological fluids. Figure 5 illustrates that samples can be successfully analyzed for chloride provided the beam current is decreased to 50-100 nA and the counting time decreased to 5 sec. Even with these changes, caution has to be used in determining

Fig. 5

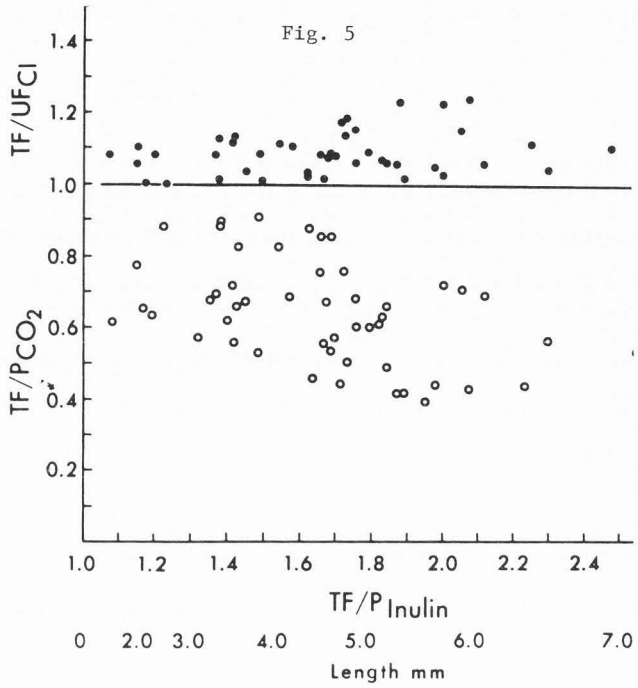

Figure 5. Relationship of tubule fluid chloride and tubule fluid bicarbonate concentration along superficial proximal tubule of the dog. $(TF/UF)_{Cl}$ is ratio of tubule fluid to ultrafilterable chloride, and $(TF/P)CO_2$ concentration. The dog, like other mammals, acidifies the proximal tubule fluid, resulting in bicarbonate absorption and elevation of chloride concentration along the length of the proximal tubule. Chloride concentrations were determined by microdroplet analysis with a WDS electron microprobe. From Wong and Quamme, (1981).

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In summary, the techniques of sample preparation and probe analysis in microdroplet measurements have not altered drastically over the last 5-6 years. With the development of better instrumentation and automation the method is reasonably straightforward. As suggested above, the difficulties are in sample preparation; the results are only as good as the sample deposits. It is our advice to neophytes interested in this method to obtain instruction from established laboratories.

Contributions of Microdroplet Analysis to Biology

Microdroplet analysis lends itself particularly to the determination of electrolyte composition of tubule fluid samples collected by means of micropuncture techniques. Tubule fluid is an ultrafiltrate of plasma and as such is practically devoid of proteins. Accordingly, pipetting accurate volumes is facilitated and elements of interest are present in reasonable concentrations, Na, Cl, K, Ca, Mg and phosphate, may be quantitatively determined. Micropuncture is the technique of inserting small pipettes into biological tissue in order to gather chemical and physical information (Quamme and Dirks, 1986). As the name implies, these are performed at micro-level, usually requiring stereotaxic micro-manipulators and stereoscopic microscopes. The major contribution of micropuncture has been in our current understanding of the functions of the accessible renal nephrons or tubules. The flow rate in nephrons is relatively rapid, 30 to 50 nl/min, but the total volume at any given time is minute. For example, the proximal tubule, with a radius of 13 ™m and a length of 10 mm, contains 5 nl of tubule fluid. Smaller volumes are contained in the superficial distal segments, about 0.2 to 0.3 nl. Thus, collection rates may be long and the fluid volumes collected tiny. This has given impetus to efforts in developing ultra-microanalytical approaches to chemically define the composition of the tubule fluid and thereby describe segmental functions of the nephron. X-ray microanalysis is ideally suited for this job.

A number of important observations have been made over the last half decade concerning tubular handling of electrolytes which were dependent on the use of microdroplet analysis. De Rouffignac and colleagues, in a series of elegant studies, demonstrated the importance of cAMP-mediated hormones in electrolyte reabsorption in the loop of Henle (de Rouffignac et al., 1983; de Rouffignac and Elalouf, 1983 and 1988; Elalouf et al., 1983, 1984a and 1984b; Bailly et al., 1984). Hormones such as antidiuretic hormone, calcitonin, glucagon and parathyroid hormone act through adenyl cyclase to enhance NaCl transport in the thick ascending limb segments of the loop. Atrial natriuretic peptide, on the other hand, diminishes sodium transport within the tubule segments distal to the loop of Henle (Sonnenberg et al., 1982); the action of which appears to be independent of hemodynamic alterations (Roy, 1986; Peterson et al., 1987). Sawyer and Beyenbach (1985) demonstrated that the mechanisms for NaCl secretion in the proximal tubule of shark renal tubules was similar to the absorptive NaCl transport located in mammalian thick ascending limb (Sawyer and Beyenbach, 1985).

Microdroplet analysis has been used to investigate chloride transport in proximal and distal tubules of the dog (Wong and Quamme, 1981). As with other mammals, bicarbonate is preferentially transported in the proximal tubule, resulting in an elevation of luminal chloride concentration (Wong and Quamme, 1981; Wong, et al., 1986a and 1986b). Cellular adaptation during experimental chronic renal failure leads to enhanced sodium bicarbonate reabsorption and diminished sodium chloride transport (Wong et al., 1982 and 1984).

The mechanism of potassium handling in the proximal tubule remains controversial (Jamison et al., 1982). Free-flow micropuncture studies in rats and dogs have shown that the TF/PK+ ratio remains near unity or slightly below 1.0 along the accessible convoluted tubule (Roy et al., 1982a). However, Le Grimellec and colleagues have reported that the ultrafiltration of potassium across the glomerular membrane may be in the order of 80-90%, accordingly, the tubule fluid-to-ultrafilterable potassium (TF/UF_K) value may be greater than unity (Le Grimellec, 1975). No clear association of TF/UFK+ with TF/P inulin has been demonstrated in the rat or dog (Le Grimellec et al., 1975; Wong et al., 1979). However, in a number of animal species, including the chinchilla (Weisser et al., 1970), the desert rodent, Psammomys (de Rouffignac et al., 1973), and Perognathus penicillatus (Braun et al., 1981), and the rabbit (Wong, et al., 1986b) the TF/UF_K value is clearly above unity. This is particularly evident in the Perognathus and the rabbit where the TF/UF_K rises along the proximal tubule to reach 1.4 at the end of the accessible portion. From this data, Jamison and colleagues have taken the view that potassium absorption is passive and driven by a diffusional flux down a concentration gradient that develops as a consequence of water absorption (Jamison et al., 1982).

Recent experiments have demonstrated that potassium is recycled in the renal medulla (Arrascue et al., 1981; Dobyan et al., 1979; Roy et al., 1982a, Roy 1985). Jamison and colleagues have proposed that potassium is reabsorbed from the medullary collecting duct, trapped by counter-current exchange in the medullary interstitium, and secreted into the juxtamedullary pars recta and thin descending limb. Work et al. (1982) and Wasserstein and Agus (1983) have shown that potassium is secreted into the pars recta; and Stokes (1982b) has identified the collecting duct segment in the outer medulla as a potential site for potassium reabsorption. Potassium transport in the ascending thin and thick segments in the medulla is likely to occur in a net reabsorptive direction (Jamison et al., 1982).

The functional consequences of medullary potassium recycling are unclear. Stokes (1981 and 1982a) recently demonstrated that elevating the potassium concentration in bath and luminal fluid caused a reversible reduction in voltage of the medullary thick ascending limb perfused in vitro.

When the potassium concentration in the bath alone was raised, net potassium transport was reversed from reabsorption to secretion, sodium reabsorption was reduced 40%, and chloride reabsorption was virtually abolished. When the potassium concentration of the perfusate alone was increased, potassium reabsorption increased 20-fold but potassium delivery out of the segment was still elevated, chloride reabsorption was slightly reduced, and sodium reabsorption was inhibited 90%. Based on these findings, Stokes (1982a) proposed that potassium recycling would produce a diuresis, natriuresis, and accelerate kaluresis. Inhibition of active NaCl reabsorption by the thick ascending limb, which provides the driving force for concentrating the renal medulla, would reduce the medullary osmotic gradient. The consequent decrease in water extraction from the descending limb would increase the flow rate of fluid to the ascending limb. Since the ascending limb has a very low osmotic water permeability, the increase in flow rate would be transmitted to the distal tubule. The increased fluid flow rate would promote potassium secretion in the distal tubule, and the increased delivery of sodium would stimulate potassium secretion in the cortical collecting tubule. In sum, the foregoing effects on water, sodium, and potassium reabsorption would all act to accelerate urinary potassium excretion. The results of Sufit and Jamison (1983) support this notion.

Previous studies of de Rouffignac and colleagues have demonstrated that 1-desamino-8-D-arginine vasopressin (dDAVP), a synthetic analogue of antidiuretic hormone, strongly stimulates K secretion in the distal tubule accessible to micropuncture (Elalouf et al., 1984b). These results were obtained from hormone-deprived rats (de Rouffignac et al., 1983; Elalouf et al., 1984a), that is, from rats depleted in antidiuretic hormone (ADH), parathyroid hormone (PTH), calcitonin, and glucagon, all four of which stimulate the adenylate cyclase system of the distal tubule. Further, they showed in these animals that ADH stimulated medullary potassium recycling by juxtamedullary nephrons (Elalouf et al., 1985), whereas calcitonin inhibited medullary K recycling (Elalouf et al., 1986b). Roy et al. (1982b) reported the effects of acute metabolic acidosis and alkalosis on potassium recycling.

One area of renal physiology where x-ray microanalysis has played an essential role is in the elucidation of magnesium handling by the nephron (Quamme, 1986). There is no other adequate means of measuring magnesium concentration in micro-samples. Although no single homeostatic control has been demonstrated for magnesium, the cellular availability of this cation is closely regulated by the gastrointestinal tract, kidney and bone. The excretory side of magnesium balance involves appropriate changes in renal magnesium handling. Present evidence suggests that the renal handling of magnesium is normally a filtration-reabsorption process. Experimental support for secretion within the descending limb of Henle's loop remains unconvincing (Quamme and Smith, 1984; Roy et al., 1982a). Renal magnesium reabsorption has distinctive features when compared with that of sodium and calcium. The concentration of magnesium in the proximal tubule rises 1.5 times greater than the glomerular filtrate (Morel et al., 1969). Some 20-30% of the filtered magnesium is reabsorbed in the proximal tubule compared to the fractional absorption of sodium or calcium of 50-60%. Although the fractional reabsorption of magnesium is only half that of sodium, it changes in parallel with that of sodium in response to changes in extracellular fluid volume (Sutton et al., 1983). The major portion of filtered magnesium (some 65%) is reabsorbed in the loop of Henle, mainly in the thick ascending limb (de Rouffignac et al., 1973). Recent evidence suggests that magnesium reabsorption in the ascending limb may be voltage-dependent and secondary to active sodium chloride reabsorption (Shareghi and Agus, 1982). Evidence also suggests an important competition between magnesium and calcium for transport at the basolateral surface of the ascending limb cell (Quamme and Dirks, 1980; Wong, et al., 1983). The loop of Henle appears to be the major nephron site where magnesium reabsorption is controlled (de Rouffignac et al., 1984). The principal factors which alter magnesium reabsorption in the loop include cAMP-mediated hormones such as parathyroid hormone, antidiuretic hormone, calcitonin, glucagon (de Rouffignac et al., 1983; Bailly and Amiel, 1982; Bailly et al., 1984; Elalouf et al., 1983, 1984a and 1986a) and changes in plasma magnesium and calcium concentration and the loop diuretics (Quamme, 1986). About 10% of the filtered magnesium is delivered into the distal nephron where only a small fraction of the filtered magnesium is reabsorbed and the transport capacity is readily exceeded with increased magnesium delivery. A number of hormones, including calcitonin, glucagon, antidiuretic hormone and parathyroid hormone enhance magnesium absorption in the distal tubule (Elalouf et al., 1983 and 1984b; Bailly et al., 1985; Bengele et al., 1981). Metabolic acidosis impairs magnesium reabsorption in the loop and distal tubule (Wong et al., 1986c; Roy et al., 1982b; Shapiro et al., 1987). A number of drugs have been shown to alter magnesium handling: these include antibiotics such as gentamycin, antineoplastic agents such as cisplatin and immunological suppressive drugs such as cyclosporin (Quamme, 1986; Mavichak et al., 1985). The cellular alterations of these diverse drugs leading to renal magnesium wasting are not well understood.

Microdroplet analysis has been successfully used to determine phosphate concentration in tubule fluid. The principal factors which sensitively control renal phosphate handling are parathyroid hormone, availability of phosphate, and acid-base influences (Mizgala and Quamme, 1985). In a series of microperfusion experiments, Quamme et al. demonstrated the importance of intraluminal pH and the actions of parathyroid hormone (Quamme and Wong, 1984) and adaptations to dietary phosphate intake (Quamme et al., 1985). Further, they showed that acid-base balance may modulate tubular phosphate transport independent of intraluminal pH and phosphate concentration.

These changes depend on the chronicity of exposure, as chronic metabolic acidosis inhibits transport, whereas acute acidosis is without effect, and the alterations are independent but integral to the effects of parathyroid hormone and the intrinsic adaptation to dietary phosphate availability (Quamme, 1985). Pastoriza-Munoz et al. (1983) demonstrated that acute phosphate deprivation enhances reabsorption in the proximal tubule, although the phosphaturic effect of parathyroid hormone in this segment is not abolished. Resistance to the inhibitory effect of PTH on phosphate reabsorption in some portion of the loop of Henle and possibly also in the distal convolution accounts for the absence of a significant phosphaturic effect of the hormone in acutely phosphate-deprived rats. Prolongation of phosphate deprivation results in unresponsiveness to parathyroid hormone extending to the proximal tubule (Pastoriza-Munoz et al., 1983). Wong et al. reported the effects of phosphate infusions on tubular phosphate and calcium handling with the use of microdroplet analysis (Wong et al., 1985).

Microdroplet analysis has not been limited to micropuncture studies of mammals. Strange et al. (1982) demonstrated that the rectal salt gland of saltwater mosquito larvae secretes a hyperosmotic fluid and is a major site of Na, K, Mg, Cl and HCO_3^- excretion and regulation. They also showed that HCO_3^- secretion in the anterior segment of the rectal salt gland of Aedes dorsalis is mediated by 1:1 exchange of luminal Cl^- from serosal HCO_3^- (Strange et al., 1984). They further provided evidence that the anterior segment of the rectal salt gland was an important regulator of pH control as well as salt balance.

We have spent some time reviewing the contributions of microdroplet analysis to micropuncture (Warner and Lechene, 1980) and the contributions of its use; now we would like to speculate on some of the new approaches currently being developed which may have future application in this area. First, a solid tissue punch has been developed which, when used in conjunction with the electron microprobe, may provide information on total luminal, intracellular and interstitial elemental concentrations. Electron microprobe analysis of solid tissue samples obtained with the use of a large micropuncture punch or tissue slices have been used to determine total elemental composition. Potentially, this approach offers a very sensitive method of determining elemental concentrations within the tubule lumen, cell and interstitium of any region within the kidney (Bulger et al., 1981; Dörge et al., 1975 and 1978; Beck et al., 1980; Saubermann et al., 1981a and 1981b). This approach, if successfully applied, may eliminate micropuncture. Unfortunately, to date it has been fraught with difficulties; principal among them is fixation of electrolytes during the tissue sampling and preparation. Ionic constituents diffuse very rapidly during these procedures. Another problem is the selection of appropriate standards for accurate, reliable quantitation. Nevertheless, with proper care this approach may prove beneficial to the micropuncture field.

The use of x-ray analysis in tissue and single cell composition is reviewed elsewhere in this paper. However, to provide an example as to what is being done along these lines in nephrology, Beck et al. have recently reported some interesting results (Beck et al., 1984; Mason et al., 1981). It is known that the osmolality within the kidney increases from the corticomedullary border to the tip of the papilla. Thus, the renal cells are exposed to very different osmolalities. Beck and colleagues determined the element concentrations in various intra- and extracellular compartments of the tip of the rat renal papilla during antidiuresis using electron microprobe analysis (Beck et al., 1984). To quantify the microprobe data obtained in various extra- and intracellular compartments, an internal standard was introduced by applying a thin layer of albumin solution of known electrolyte composition to the papillary surface. This was achieved either by dipping the excised papilla in the albumin solution or by opening the renal pelvis and dripping the standard onto the papilla. Urinary concentrations (means±SEM) were: urea, 1509±116; potassium, 268±32; sodium, 62±19 $mmoles \cdot l^{-1}$; and osmolality, 2548±141 $mOsm \cdot kg^{-1}$. Electrolyte concentrations in the interstitial space were: sodium, 437±19; chloride, 438±20; and potassium, 35±2 $mmoles \cdot kg^{-1}$ wet wt. The vasa recta plasma exhibited almost identical element concentrations. The values in the papillary collecting duct cells were: sodium, 28±1; chloride, 76±3; potassium, 135±3; and phosphorus, 316±7 $mmoles \cdot kg^{-1}$ wet wt. Similar concentrations were observed in the papillary epithelial cells. In interstitial cells potassium and phosphorus concentrations were virtually identical to those of the collecting duct cells, whereas sodium and chloride concentrations were higher by about 30 mmoles. kg^{-1} wet wt. The elemental composition of the various papillary cells is, thus, not substantially different from that of proximal tubular cells. This finding demonstrates that cellular accumulation of electrolytes is not the regulatory mechanism by which papillary cells adapt osmotically to their high environmental osmolality and sodium chloride concentration. These are very interesting observations and time will tell if this approach will eliminate micropuncture techniques.

In summary, microdroplet analysis continues to provide valuable information to investigators interested in electrolyte and membrane physiology. The major difficulty in applying microdroplet analysis to biological fluids is in the preparation of sample deposits. Although the sample preparation is rather difficult, it has been adequately detailed in the literature and is available to interested investigators. It is envisioned that this approach will continue to be an important tool in our technical armametarium for the next decade.

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References

Arrascue JF, Dobyan DC, Jamison RL. (1981). Potassium recycling in the renal medulla: effects of acute potassium chloride administration to rats fed a potassium-free diet. Kidney Int. 20, 348-352.

Bailly C, Amiel C. (1982). Effect of glucagon on magnesium renal reabsorption in the rat. Pflügers Arch. 392, 360-365.

Bailly C, Roinel N, Amiel C. (1984). PTH-like glucagon stimulation of Ca and Mg reabsorption in Henle's loop of the rat. Am. J. Physiol. 246 (Renal Fluid Electrolyte Physiol. 15), F205-F212.

Bailly C, Roinel N, Amiel C, Coureau C, Couette C, Legendre Y, Malorey P. (1985) Stimulation by glucagon and PTH of Ca and Mg reabsorption in the superficial distal tubule of the rat kidney. Pflügers Arch. 403, 2-34.

Beck F, Bauer R, Bauer U, Mason J, Dörge A, Rick R, Thurau K. (1980). Electron microprobe analysis of intracellular elements in the rat kidney. Kidney Int. 17, 756-763.

Beck F, Dörge A, Rick R, Thurau K. (1984). Intra- and extracellular elements concentrations of rat renal papilla in antidiuresis. Kidney Int. 25, 397-403.

Bengele HH, Alexander EA, Lechene CP. (1981). Calcium and magnesium transport along the inner medullary collecting duct of the rat. Am. J. Physiol. 239, 24-29.

Braun EJ, Roy DR, Jamison RL. (1981). Micropuncture study of the superficial nephron of Perognathus penicillatus. Am. J. Physiol. 241 (Renal Fluid Electrolyte Physiol. 10), F612-F617.

Bulger RE, Beeuwkes III R, Saubermann AJ. (1981). Application of scanning electron microscopy to x-ray analysis of frozen-hydrated sections: III. Elemental content of cells in the rat renal papillary tip. J. Cell. Biol. 88, 274-280.

Cortney M. (1969). Renal tubular transfer of water and electrolytes in adrenalectomized rats. Am. J. Physiol. 216, 3, 589-598.

De Rouffignac C, Corman B, Roinel N. (1983). Stimulation by antidiuretic hormone of electrolyte tubular reabsorption in rat kidney. Am. J. Physiol. 244 (Renal Fluid Electrolyte Physiol 13), F156-F164.

De Rouffignac C, Elalouf JM., Roinel N, Bailly C and Amiel C. (1984) Simlarity of the effects of antidiuretic hormone, parathyroid hormone, calcitonin, and glucagon on rat kidney. Nephrology 1, 340-357.

De Rouffignac C, Elalouf JM. (1983). Effect of salmon calcitonin on the renal concentrating mechanism. Am. J. Physiol. 245 (Renal Fluid Electrolyte Physiol 14), F506-F511.

De Rouffignac C, Morel F, Moss N, Roinel N. (1973). Micropuncture study of water and electrolyte movements along the loop of Henle in Psammomys with special reference to magnesium, calcium and phosphorus. Pflügers Archiv. 344, 309-326.

Dobyan DC, Lacy FB, Jamison RL. (1979). Suppression of potassium recycling in the renal medulla by short-term potassium deprivation. Kidney Int. 16, 704-709.

Dörge A, Rick R, Gehring K, Mason J, Thurau K. (1975). Preparation and applicability of freeze-dried sections in the microprobe analysis of biological soft tissue. J. Microsc. Biol. Cell 22, 205-214.

Dörge A, Rick R, Gehring K, Thurau K. (1978). Preparation of freeze- dried cryosections for quantitative x-ray microanalysis of electrolytes in biological soft tissues. Pflügers Arch. 373, 85-97.

Elalouf JM, Roinel N, de Rouffignac C. (1983). Stimulation by human calcitonin of electrolyte transport in distal tubules of rat kidney. Pflügers Arch. 399, 111-118.

Elalouf JM, Roinel N, de Rouffignac C. (1984a). ADH-like effects of calcitonin on electrolyte transport by Henle's loop of rat kidney. Am. J. Physiol. 246 (Renal Fluid Electrolyte Physiol. 15), F213-F220.

Elalouf JM, Roinel N, de Rouffignac C. (1984b). Effects of antidiuretic hormone on electrolyte reabsorption and secretion in distal tubules of rat kidney. Pflügers Arch. 901, 167-173.

Elalouf JM, Roinel N, de Rouffignac C. (1985). Effects of dDAVP on rat juxtamedullary nephrons: stimulation of medullary K recycling. Am. J. Physiol. 249 (Renal Fluid Electrolyte Physiol. 18), F291-F298.

Elalouf JM, Sari DC, de Rouffignac C. (1986a). Additive effects of glucagon and vasopressin on renal Mg reabsorption and urine concentrating ability in the rat. Pflügers Arch. 407, 566-579.

Elalouf JM, Roinel N, de Rouffignac C. (1986b). Effects of human calcitonin on water and electrolyte movements in rat juxtamedullary nephrons: inhibition of medullary K recycling. Pflügers Arch. 406, 502-508.

Greger GF, Lang FC, Knox FG, Lechene CP. (1978). Analysis of tubule fluid. In Methods in Pharmacology, M Martinez-Maldonado (ed), Plenum Press, New York, vol. 4B, 105-140.

Ingram MJ, Hogben CA. (1967). Electrolyte analysis of biological fluids with the electron microprobe . Anal. Biochem. 18, 54-57.

Jamison RL, Work J, Schafer JA. (1982). New pathways for potassium transport in the kidney. Am. J. Physiol. 242 (Renal Fluid Electrolyte Physiol. 11), F297-F312.

Lechene CP. (1970). The use of the electron microprobe to analyse very minute amounts of liquid samples. Proceedings 5th Conference of the Microbeam Analysis Society, New York, P Lublin (ed), San Francisco Press, San Francisco, 32A-32C.

Lechene CP. (1974). Electron probe microanalysis of picoliter liquid samples. In Microprobe Analysis as Applied to Cells and Tissues. T Hall, P Echlin, R Kaufmann (eds). Academic Press, London, 351-367.

Lechene CP. (1977). Electron probe microanalysis: its present, its future. Am. J. Physiol. 232 (5), F391-396.

Lechene CP, Warner RR. (1977). Ultramicroanalysis: x-ray spectrometry by electron probe excitation. Ann. Rev. Biophys. Bioeng. 6, 57-85.

Lechene CP, Warner RR. (1979). Electron probe analysis of liquid droplets. In Microbeam Analysis in Biology, C Lechene, R Warner (eds), Academic Press, New York, 279-296.

Le Grimellec C. (1975). Micropuncture study along the proximal convoluted tubule. Electrolyte reabsorption in first convolutions. Pflügers Arch. 354, 113-150.

Le Grimellec C, Poujeol P, de Rouffignac C. (1975). ^{3}H-inulin and electrolyte concentrations in Bowman's capsule in rat kidney. Comparison with artificial ultrafiltrate. Pflügers Arch. 354, 117-131.

Leroy A, Roinel N. (1982). Radiation damage to lyophilized mineral solutions during electron probe analysis: quantitative study of chlorine loss as a function of beam current-density and sample mass-thickness. J. Micros (London), 131, 1, 97-106.

Martoja R, Ballan-Dufrancais C, Roinel N. (1979). Echantillons biologiques: preparation et analyse. In Microanalyse et microscopie electronique a balayage, F Maurice, L Meny, R Tixier (eds), les Editions de Physique, Orsay, 361-380.

Mason J, Beck F, Dörge A, Rick R, Thurau K. (1981). Intracellular electrolyte composition following renal ischemia. Kidney Int. 20, 61-70.

Mavichak V, Wong NLM. Quamme GA, Magil AB, Sutton RAL, Dirks JH. (1985). Studies on the pathogenesis of cisplatin-induced hypomagnesemia in rats. Kidney Int. 28, 914-921.

Mizgala CL, Quamme GA. (1985). Renal handling of phosphate. Physiol. Rev. 65, 431-466.

Morel F, Roinel N. (1969). Application de la microsonde electronique a l'analyse elementaire quantitative d'echantillons liquides d'un volume inferieur a 10^{-9} l. J. Chim. Phys. Phys. Chim. 66, 1084-1091.

Morel F, Roinel N, Le Grimellec C. (1969). Electron probe analysis of tubular fluid composition. Nephron 6, 350-364.

Muhlert M., Julita M, Quamme G. (1982). Disappearance of calcium and other electrolytes from microvolume samples. Am. J. Physiol. 242, F202-F206.

Pastoriza-Munoz E, Mishler DR, Lechene C. (1983). Effect of phosphate deprivation on phosphate reabsorption in rat nephron: role of PTH. Am. J. Physiol. 244 (Renal Fluid Electrolyte Physiol. 13), F140-F149.

Peterson LN, de Rouffignac C, Sonnenberg H, Levine DZ. (1987). Thick ascending limb response to dDAVP and atrial natriuretic factor in vivo. Am. J. Physiol. 252 (Renal Fluid Electrolyte Physiol. 21), F374-F381.

Prager DJ, Bowman RL, Vurek GG. (1965). Constant-volume, self-filling nanoliter pipette: constriction and calibration. Science 147, 606-608.

Quamme GA. (1985). Effects of metabolic acidosis, alkalosis, and dietary hydrogen ion uptake on phosphate transport in the proximal convoluted tubule. Am. J. Physiol. 249 (Renal Fluid Electrolyte Physiol. 18), F769- F779.

Quamme GA. (1986). Renal handling of magnesium: drug and hormone interactions. Magnesium 5, 248-272.

Quamme GA, Dirks JH. (1980). Effect of intraluminal and contraluminal magnesium on magnesium and calcium transfer in the rat nephron. Am. J. Physiol. 238, 187-198.

Quamme GA, Dirks JH. (1986). Micropuncture techniques. Kidney Int. 30, 152-165.

Quamme GA, Mizgala CL, Wong NLM, Whiting SJ. (1985). Effects of intraluminal pH and dietary phosphate on phosphate transport in the proximal convoluted tubule. Am. J. Physiol. 249 (Renal Fluid Electrolyte Physiol. 18), F759-F768.

Quamme GA, Smith C. (1984). Magnesium transport in isolated proximal straight tubule of the rabbit. Am. J. Physiol. 246, F544-F550.

Quamme GA, Wong NLM. (1984). Phosphate transport in the proximal convoluted tubule: effect of intraluminal pH. Am. J. Physiol. 246 (Renal Fluid Electrolyte Physiol. 15), F323-F333.

Quinton PM. (1978a). Techniques for microprobe analysis of fluids (sweat, saliva, urine) with an energy-dispersive x-ray spectrometer on a scanning microscope. Am. J. Physiol. 234, F235-F259.

Quinton PM. (1978b). Ultramicroanalysis of biological fluids with energy dispersive x-ray spectrometry. Micron 9, 57-69.

Rick R, Horster M, Dörge A, Thurau K. (1977). Determination of electrolytes in small biological fluid samples using energy-dispersive x-ray microanalysis. Pflügers Arch. 369, 95-98.

Rick R, Beck FX, Dörge A, Thurau K. (1987). Cl transport in the frog cornea: an electron microprobe analysis. J. Membr. Biol. 95, 229-240.

Roinel N, Richard JP, Robin G, Morel F. (1973). An automatic electron microprobe sample-feeder for the quantitative analysis of dried $1-^{-10}$L volume samples. J. Microsc. (Paris) 18, 285-290.

Roinel N. (1975). Electron microprobe quantitative analysis of lyophilized 10^{-10}L. volume samples. J. Microsc. (Paris), 22, 261-268.

Roinel N. (1979). Electron probe analysis of biological fluids. A review of preparative techniques, characteristics and pitfalls. Uppsala Journal of Medical Sciences, 26, n°63.

Roinel N. (1981). Electron probe analysis of microdroplets: factors affecting the proportionality between measured x-ray intensity and concentration. J. Microsc. (London) 123, 3, 311-321.

Roinel N, de Rouffignac C. (1982). X-ray analysis of biological fluids: contribution of microdroplet technique to biology. Scanning Electron Microsc. 1982; III:1155-1171.

Roy DR. (1985). Effect of calcitonin on fractional delivery of sodium and solute to the juxtamedullary end-descending limb of the rat. Can. J. Physiol. Pharmacol. 63, 89-95.

Roy D. (1986). Effect of synthetic ANP on renal and loop of Henle functions in the young rat. Am. J. Physiol. 251 (Renal Fluid Electrolyte Physiol. 20), F220-F225.

Roy D, Blouch KL, Jamison RL. (1982a). Effects of acute acid-base disturbances on K^{+} delivery to the juxtamedullary end-descending limb. Am. J. Physiol. 243 (Renal Fluid Electrolyte Physiol. 12), F188-F196.

Roy DR, Blouch KL, Jamison RL. (1982b). Effects of acute acid-base disturbances on renal tubule reabsorption of magnesium in the rat. Am. J. Physiol. 243 (Renal Fluid Electrolyte Physiol. 12), F197-F203.

Saubermann AJ, Echlin P, Peters PD, Beeuwkes III R. (1981a). Application of scanning electron microscopy to x-ray analysis of frozen-hydrated sections: I. Specimen handling techniques. J. Cell Biol. 88, 257-267.

Saubermann AJ, Beeuwkes III R, Peters PD. (1981b). Application of scanning electron microscopy to x-ray analysis of frozen-hydrated sections: II. analysis of standard solutions and artificial electrolyte gradients. J. Cell Biol. 88, 268-273.

Sawyer DB, Beyenbach KW. (1985). Mechanism of fluid secretion in isolated shark renal proximal tubules. Am. J. Physiol. 249 (Renal Fluid Electrolyte Physiol. 18), F884-F890.

Shapiro RJ, Yong CKK, Quamme GA. (1987). Influence of chronic dietary acid on renal tubular handling of magnesium. Pflügers Arch. 409, 492-498.

Shareghi GR, Agus ZS. (1982). Magnesium transport in the cortical thick ascending limb of Henle's loop of the rabbit. J. Clin. Invest. 69, 759-769.

Sonnenberg H, Cupples WA, DeBold AJ, Veress AT. (1982). Intrarenal localization of the natriuretic effect of cardiac atrial extract. Can. J. Physiol. Pharmacol. 60, 1149-1195.

Stokes JB. (1981). Potassium secretion by cortical collecting tubule: relation to sodium reabsorption, luminal sodium concentration, and transepithelial voltage. Am. J. Physiol. 241 (Renal Fluid Electrolyte Physiol. 10), F395-F482.

Stokes J. (1982a). Consequences of potassium recycling in the renal medulla. Effects on ion transport by the medullary thick ascending limb of Henle's loop. J. Clin. Invest. 70, 219-229.

Stokes JB. (1982b). Na and K transport across the cortical and outer medullary collecting tubule of the rabbit: evidence for diffusion across the outer medullary portion. Am. J. Physiol. 242 (Renal Fluid Electrolyte Physiol. 11), F514-F520.

Strange K, Phillips JE, Quamme GA. (1982). Active HCO_3^--secretion in the rectal salt gland of a mosquito larva inhabiting $NaHCO_3$-CO_3 lakes. J. Exp. Biol. 101, 171-186.

Strange K, Phillips JE, Quamme GA. (1984). Mechanisms of Cl^-- HCO_3^{2-} exchange. Am. J. Physiol. 246, R735-R740.

Sufit CR, Jamison RL. (1983). Effect of acute potassium load on reabsorption in Henle's loop in the rat. Am. J. Physiol. 245 (Renal Fluid Electrolyte Physiol. 14), F569-F576.

Sutton RAL, Wong NLM, Quamme GA, Dirks JH. (1983). Renal tubular calcium transport: effects of changes in filtered calcium load. Am. J. Physiol. 245 (Renal Fluid Electrolyte Physiol. 14), F515-F520.

Van Eekelen CAG, Boekstein A, Stols ALH, Stathouders AM. (1980). X-ray microanalysis of picoliter microdroplets: improvement of the method for quantitative x-ray analysis of samples of biological fluids. Micron 11, 137-145.

Warner RR, Lechene CP. (1980). Details of the liquid droplet technique. In Microbeam Analysis, D Wittry (ed), San Francisco Press, San Francisco, 120-125.

Wasserstein AG, Agus ZS. (1983). Potassium secretion in the rabbit proximal straight tubule. Am. J. Physiol. 245 (Renal Fluid Electrolyte Physiol. 14), F167-F174.

Weisser R, Lacy FB, Weber H, Jamison RL. (1970). Renal function in the chinchilla. Am. J. Physiol. 219, 1706-1713.

Wong NLM, Quamme GA, Dirks JH, Sutton RAL. (1982). Divalent ion transport in dogs with experimental chronic renal failure. Can. J. Physiol. Pharmacol. 60, 1296-1302.

Wong NLM, Dirks JH, Quamme GA. (1983). Tubular maximum reabsorptive capacity for magnesium in the dog. Am. J. Physiol. 244, 78-83.

Wong NLM, Quamme GA. (1981). Tubular handling of bicarbonate and chloride in the dog. Am. J. Physiol. 241 (Renal Fluid Electrolyte Physiol. 10), F219-F223.

Wong N, Quamme GA, Dirks JH. (1984). Tubular handling of bicarbonate in dogs with experimental renal failure. Kidney Int. 25, 912-918.

Wong NLM, Quamme GA, Sutton RAL, Dirks JH. (1979). Effects of mannitol on water and electrolyte transport in the dog kidney. J. Lab. Clin. Med. 94, 683-692.

Wong NLM, Quamme GA, Dirks JH, Sutton RAL. (1985). Effects of phosphate and calcium infusion on renal phosphate transport in the dog. Renal Physiol. 8, 30-37.

Wong NLM, Reitzik M, Quamme GA. (1986a). Tubular electrolyte handling in the primate. Renal Physiol. 9, 29-37.

Wong NLM, Whiting SJ, Mizgala CL, Quamme GA. (1986b). Electrolyte handling by the superficial nephron of the rabbit. Am. J. Physiol. 250 (Renal Fluid Electrolyte Physiol. 19), F590-F596.

Wong NLM, Quamme GA, Dirks JH (1986c). Effects of acid-base disturbances on renal handling of magnesium. Clin. Sci. 70,277-284.

Work J, Troutman SL, Schafer JA. (1982). Transport of potassium in the rabbit parts recta. Am. J. Physiol. 242 (Renal Fluid Electrolyte Physiol. 11), F226-F237.

Discussion with Reviewers

N. Rionel: You are right to insist upon the possibility of elemental loss during storage of droplets under water-saturated paraffin oil. Subsequent to your earlier observations (Mulher et al. 1982), have you performed further experiments in this respect? At Saclay we obtained some data which will be published in the near future.

Author: No, we have not recently performed similar experiments to follow up these observations.