Chapter 11

MICRODIALYSIS AND ULTRAFILTRATION

ELSA M. JANLE AND PETER T. KISSINGER

Bioanalytical Systems, Inc.
West Lafayette, Indiana 47906

I. INTRODUCTION

Microdialysis and ultrafiltration are complementary sampling techniques which have been developed for studying the interstitial space *in vivo* (Janle and Kissinger, 1993). Both of these techniques employ membrane probes that can be implanted in the tissue of interest, and the studies can be conducted in awake moving animals or in human subjects. Because these techniques sample low molecular weight compounds, and because they provide samples that require very little preparation prior to analysis, they hold considerable potential as tools for nutritional research.

Microdialysis and ultrafiltration are separation techniques that involve moving a chemical across a semipermeable membrane. In microdialysis (Fig. 1A), a fluid is pumped through the membrane capillary of a probe. The analyte crosses the membrane by diffusion. The driving force is a concentration gradient. Under ideal conditions, the perfusion fluid is isos-

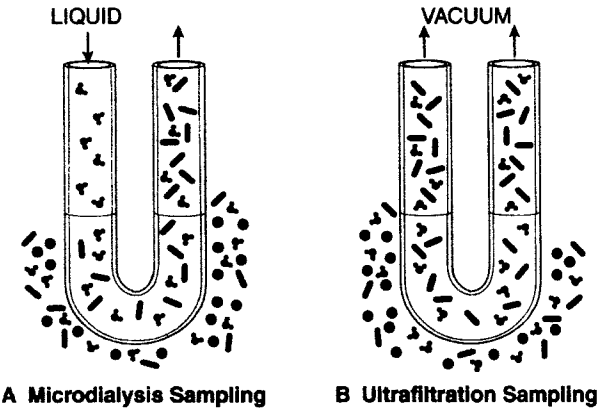

FIG. 1. (A) The dialysis process results from the diffusion of molecules across the membrane wall. (B) In ultrafiltration, small molecules are actively pulled across the membrane.

motic with the tissue, the hydrostatic pressure is minimal, and there is no net water transfer between the perfused fluid and the animal tissue.

In ultrafiltration (Fig. 1B), the driving force is a pressure differential applied across a semipermeable membrane, in this case, in the form of a vacuum. The reduced pressure causes small molecules, here water and solute molecules, that cross the membrane to be pulled out of the probe (Janle-Swain *et al.*, 1987). Large molecules or molecules which are repelled by the membrane are prevented from crossing and are not obtained in the sample.

The use of individual capillary membranes (often called dialysis fibers or hollow fibers) makes it possible to work with the small volumes needed for bioanalytical chemistry. The capillary geometry makes it easy to move microliter samples continuously and provides for a more rapid collection rate.

II. COMPARISON OF MICRODIALYSIS AND ULTRAFILTRATION

Microdialysis and ultrafiltration are complementary techniques. They have many characteristics in common. Both methods sample the extracellular space. Both techniques allow the use of repeated sampling in small animals without withdrawing blood. This often makes it possible for animals to act as their own controls. Single animals can be studied over a longer time or may be used in crossover studies. Because so much more data can be gained from a single animal, it may be possible in many instances to

reduce the number of animals needed for significant data. Both techniques make studies in awake freely moving animals possible. These methods eliminate artifacts resulting from anesthesia and withdrawal of blood (Telting-Diaz *et al.*, 1992). Both techniques provide samples that require very little preparation for analysis. Unlike blood samples, they contain neither cells nor high molecular weight proteins. Microdialysis and ultrafiltration samples are often more stable than blood samples. The analyte has been removed from enzymes that cause degradation (Lunte *et al.*, 1991). There are also notable differences between microdialysis and ultrafiltration. In some cases, one technique may serve as well as the other. However, in other cases, one technique may be preferable.

A. PROBE SIZE AND PHYSICAL CHARACTERISTICS

Ultrafiltration probes (Fig. 2) consist of one or more hollow dialysis fibers connected to a single microbore, nonpermeable outflow tube. The outflow tube is connected to the source of negative pressure, either a roller pump or a Vacutainer. The probes come in various configurations with different numbers of fibers and different fiber lengths. Ultrafiltration probes tend to be larger than microdialysis probes. Typically there are one to three fibers, each 2 to 12 cm in length. The choice of a probe depends on the size of the implantation site and the desired flow rate. With an ultrafiltration probe, membrane surface area (and therefore probe size) affects the flow rate. A subcutaneously implanted probe with three 12-cm fibers would yield a flow rate of 1 μl/min, which would be suitable for subcutaneous implantation in a 200-g rat or any larger animal. For subcutaneous implantation in a mouse, a probe with one or three 2-cm fibers would be appropriate.

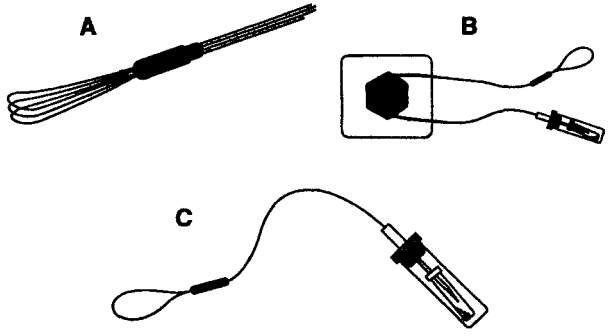

FIG. 2. An ultrafiltration probe consists of hollow dialysis fibers attached to microbore outflow tubing (A). Negative pressure is generated either by using a peristaltic pump (B) or by attaching a needle hub and using a Vacutainer (C).

The one-fiber probe would yield a flow rate of approximately 2 μl/hr; the three-fiber probe would yield 5 to 8 μl/hr.

Microdialysis probes come in several sizes and geometries; they can be much smaller than ultrafiltration probes because the volume of sample collected does not depend on the membrane surface area (Fig. 3A). Therefore, small microdialysis probes are used when precise spatial resolution is desired. When less spatial resolution is needed and higher recoveries are desirable, longer loop-type probes can be used (Fig. 3B).

B. VOLUME CHANGE

In microdialysis, when the perfusion solution is pumped through the fiber capillary, there is ideally no change in volume of the perfusate and no fluid removed from the tissue. This requires a negligible pressure gradient across the membrane. The perfusate must be isosmotic with the tissue. If either the membrane capillary or the outlet capillary is very long, the back pressure at the membrane can become sufficient for it to leak. This can be minimized by using low flow rates and membranes that have relatively low molecular weight cutoffs.

In ultrafiltration, a vacuum is applied to the probe and there is a net volume loss from the tissue sampled. One must keep in mind that the loss includes both water and solutes. Removal of fluid and neurochemicals could affect the phenomena being studied. This makes the use of ultrafiltration unsuitable for some applications, such as study of brain neurochemistry.

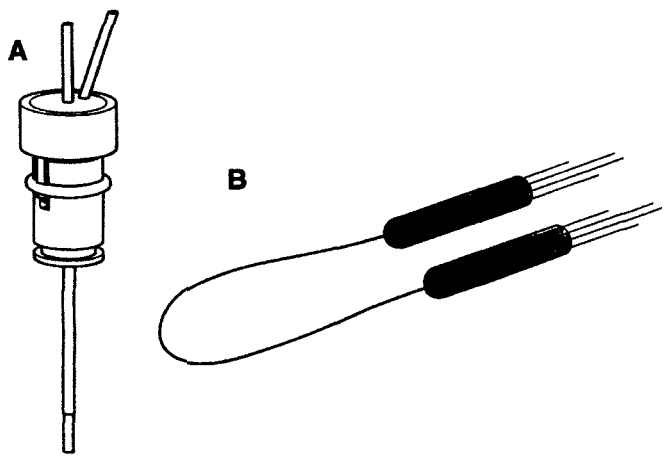

FIG. 3. Microdialysis probes come in various geometries. Some have small membranes (A). Larger loop-type probes (B) have higher recoveries.

Microdialysis, however, can be carried out so that there is no volume change and a minimal amount of analyte is removed.

In other instances, such as the study of metabolites or pharmacokinetics in a subcutaneous location, ultrafiltration may be the method of choice. In this case fluid is readily replenished, and the amount of the chemicals of interest being removed is small with respect to the entire pool.

C. RECOVERY

Recovery is the term used to quantitate the amount of analyte obtained through the probe membrane. Absolute recovery is the total amount of analyte removed from the system via the probe. Relative recovery (expressed as a percentage) is the ratio of analyte concentration in the probe effluent to that in the solution or tissue being sampled.

Depending on the nature of the experiment, it may be desirable to minimize or maximize these recoveries. If, for example, the concentration of the analyte were low and the experimenter were near the lower end of the sensitivity of the assay, conditions that would maximize the relative recovery may be chosen. However, if the experimenter were studying endogenous substances with sensitive feedback mechanisms, removing significant amounts could alter the physiological parameters and yield artifactual data. In this case, absolute recovery should be minimized.

In microdialysis, the recovery of analyte from the sample depends on a number of factors, including the chemistry of the analyte, temperature, perfusion rate, membrane surface area, membrane characteristics, and the nature of the sample (including its fluid volume percentage and whether it is in motion). Microdialysis is typically done at low perfusion flow rates (0.5 to 2 μl/min). As flow rate increases, relative recovery decreases, but absolute recovery increases. Relative recoveries from small membrane probes are in the 1–20% range. Substantially higher recoveries in the 50–80% range can be obtained with longer loop-type probes.

In ultrafiltration, analyte molecules are basically carried along with the flow of water and electrolytes. The factors determining recovery in ultrafiltration are membrane characteristics, temperature, and chemistry of the analyte. Unlike microdialysis, recovery is not dependent on flow rate, membrane surface area, or probe size. Recovery tends to be higher than for dialysis, since there is no perfusion medium to dilute the collected analyte. Ultrafiltration recovery rates are typically in the 90–100% range. This high recovery rate simplifies determination of *in vivo* analyte concentrations. Table I illustrates some *in vitro* recoveries obtainable with ultrafiltration probes.

TABLE I
ULTRAFILTRATING RECOVERIES

Analyte	% Recovery
Glucose	98
Urea	99
Creatinine	102
Lactate	98
Glutamate	96
Aspartate	97
Asparagine	83
Serine	87
Glutamine	81
Histadine	86
Glycine	97
Sodium	100
Potassium	102

Another factor which may indirectly affect recovery for *in vivo* sampling is the biocompatibility of the probe. The tissue recognizes the probe as a foreign object and may respond by forming a fibrous layer around it. In short-term studies of hours to 2 days, this is not a significant factor. For microdialysis in longer term studies, this fibrous layer would present an additional diffusion barrier and may decrease recovery. In ultrafiltration, since there is bulk flow across the membrane, recovery may not be affected by this barrier; however, the added barrier may affect the volume of fluid which is able to reach the membrane.

1. Determination of in Vivo Concentration

Determination of *in vitro* recovery requires only the measurement of analyte concentration in a probe sample and an unperfused sample and calculation of the percentage difference. In ultrafiltration *in vivo* sampling, because the entire sample comes from the tissue, the analyte concentration of the sample represents the analyte concentration of the tissue.

In vivo microdialysis sampling is not as simple. One cannot use the *in vitro* recovery to calculate an *in vivo* concentration. In many cases, it is not necessary to know the exact *in vivo* concentration; it is sufficient to observe trends. However, if the exact concentration is desired, there are several methods of obtaining an *in vivo* concentration.

a. Difference Method: No Net Flux. The "difference method," developed by Lönnroth and co-workers (1987), is based on the principle that if

the concentrations of analyte in both the perfusate and the tissue are equal, there will be no net transfer of analyte between membrane and tissue. Determining recovery by this method involves perfusing the probe with varying concentrations of the analyte and determining the concentration of the efflux. The difference in concentration between influx and efflux is plotted against perfusate concentration. Where the line crosses the axis (see Fig. 4), the outflow concentration is the same as the inflow concentration, and the perfusate concentration is equal to the tissue concentration. The slope indicates the relative recovery. The problem with this method is that it is time-consuming. If one is measuring a stable analyte, it works well, but if the analyte is changing, the experimenter is chasing a moving target.

b. Extrapolation to Zero Flow. The slower the flow rate of perfusion in microdialysis, the higher the recovery. If the perfusate were not flowing, there would be adequate time to reach equilibrium, and the concentration would be the same inside and outside the probe. Therefore, in the "extrapolation to zero flow" method, the pump is run at progressively slower rates and the concentration of the collected samples is determined. Concentration is plotted against flow rate and extrapolated to find zero flow rate (Jacobson *et al.,* 1985).

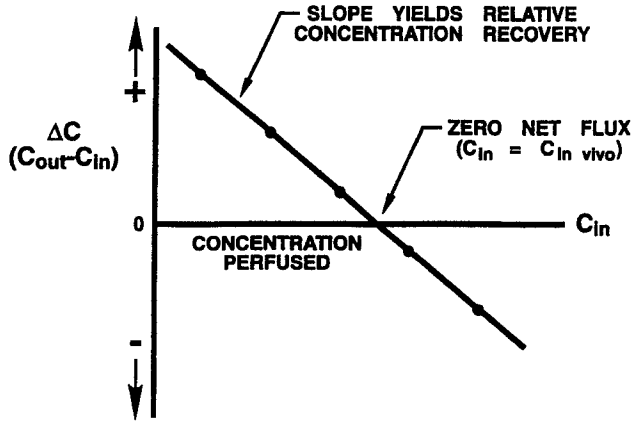

FIG. 4. The difference (or "no net flux") method of determining *in vivo* recovery involves perfusing the probe with varying concentrations of the analyte. The difference in concentration between influx and efflux is plotted against perfusate concentration. Where the line crosses the axis, outflow and inflow concentrations are the same, and the perfusate concentration is equal to the tissue concentration. The slope indicates relative recovery.

c. Retrograde Dialysis. The "retrograde dialysis" method assumes that the diffusion of a calibrator into the tissue will be the same as the diffusion of an analyte out of the tissue (Wang *et al.,* 1993). Therefore, if one were to put a calibrating compound in the perfusate and measure its loss, the *in vivo* recovery of the analyte could be estimated. If the analyte of interest were an endogenous compound, one could choose a structurally similar compound for retrograde dialysis. For an exogenous compound, one could use the compound itself to calibrate recovery. For example, if one were studying the pharmacokinetics of a drug, a preliminary study could be done in which the drug was placed in the perfusate and its loss from the perfusate was measured. This would be used as the *in vivo* recovery. After an appropriate wash-out time to eliminate any drug that might have been absorbed during the preliminary test, the drug could then be administered by the conventional route, and the pharmacokinetics could be studied.

D. SAMPLE COLLECTION

The fact that ultrafiltration and microdialysis probes allow for repeated studies in the same animal with minimum trauma to the animal makes long-term studies possible. Microdialysis probes can be used for weeks. Ultrafiltration is especially useful for long-term studies, since ultrafiltration probes are very durable and will last for months with no decrease in recovery. In these long-term studies, there is minimal tissue reaction to the probe. A small fibrous sheath forms around the probe fibers in about a week, but then remains stable. This fibrous sheath may contribute to a decrease in flow rate during the first week in some species. Ultrafiltration probes have been used for up to 6 months in dogs (Janle *et al.,* 1991), 55 days in mice (Janle *et al.,* 1992), and 1 month in a human clinical study (Ash *et al.,* 1992).

In larger animals, the ultrafiltration technique permits complete freedom of movement by using the Vacutainer collection method. Equipment vests can be worn by medium-sized animals, such as dogs. With larger animals, such as horses, collection vessels can be attached to the mane or taped to the leg, depending on the site of probe insertion.

Both microdialysis and ultrafiltration can be automated. The microdialysis pump can be combined with a syringe selector and computer-controlled to perfuse with different solutions on a timed schedule. The samples can be collected in a computer-controlled fraction collector at preset intervals or injected directly into an HPLC analyzer. Ultrafiltration sample collection can also be automated with the use of a pump and a fraction collector.

III. EXAMPLES OF STUDIES USING MICRODIALYSIS AND ULTRAFILTRATION

The literature on studies utilizing microdialysis is extensive. Ultrafiltration is a newer technique, so fewer studies have been documented. Several examples of each technique will be discussed to indicate the range of possibilities for application of these *in vivo* sampling techniques.

A. ULTRAFILTRATION

1. Subcutaneous Glucose in Diabetics

In this study, long-fiber ultrafiltration probes were implanted subcutaneously in diabetic dogs. Since the three fibers (12 cm long each) were looped, the actual implanted length was 6 cm. An attached hub and Vacutainer were used to generate a vacuum. Each dog wore an equipment vest with a Vacutainer placed in the pocket. The implantation site was chosen such that the exit site was immediately under the equipment pocket. To obtain a sample, the Vacutainer was changed. The dogs were allowed complete freedom of movement. Glucose was monitored daily using the probe. Once a week, for as long as the probe functioned, correlation studies between blood and ultrafiltrate glucose were conducted. Glucose levels were manipulated with food and insulin. Blood and ultrafiltrate samples were taken at hourly intervals. Figure 5 shows that even after 9 weeks of implantation,

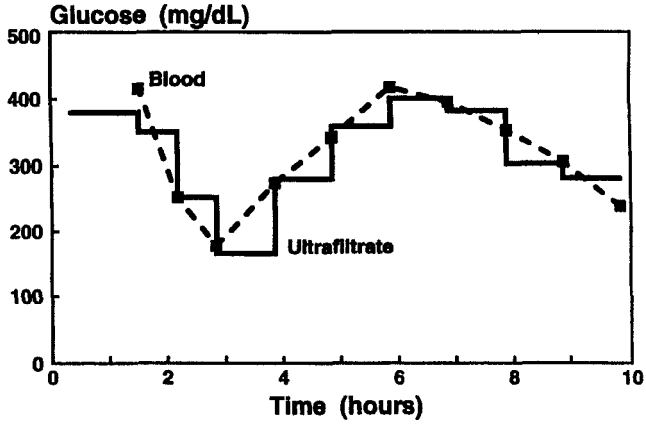

FIG. 5. Subcutaneous ultrafiltrate glucose tracks plasma glucose in a diabetic dog, 9 weeks after probe implantation.

ultrafiltrate glucose tracked blood glucose very closely (Janle *et al.*, 1992). Regression analysis on all the blood and ultrafiltrate data in this 3-month study yielded $r = 0.91$ and $P = 0.000$ (Fig. 6). The ultrafiltrate probe has been used in a 1-month clinical trial in human diabetics to monitor glucose (Ash *et al.*, 1992).

Being able to monitor glucose without obtaining blood samples has opened up the possibility of doing diabetes research on small rodents which previously could be done only on larger animals.

In one study (Janle *et al.*, 1992), diabetes was induced in mice by the injection of 50 mg/kg of streptozotocin for 5 consecutive days. The mice were individually monitored for up to 55 days postinjection. In this particular study, only daily average glucose concentrations were needed to follow the progress of the disease and to quantify the metabolic derangement, so small ultrafiltrate probes were used and samples were collected once a day. It could be seen from this monitoring procedure that the development of diabetes in different animals, given the same initial dose of the drug, varied considerably (Fig. 7). Using ultrafiltrate data, the progress of the diabetes and the total exposure to hyperglycemia could be quantitated and correlated with other sequelae of the disease.

2. *Subcutaneous Urea and Creatinine*

Blood plasma and ultrafiltrate concentrations of urea and creatinine have been studied with subcutaneous ultrafiltrate probes in diabetic dogs. The dogs had varying degrees of kidney complications secondary to the diabetes,

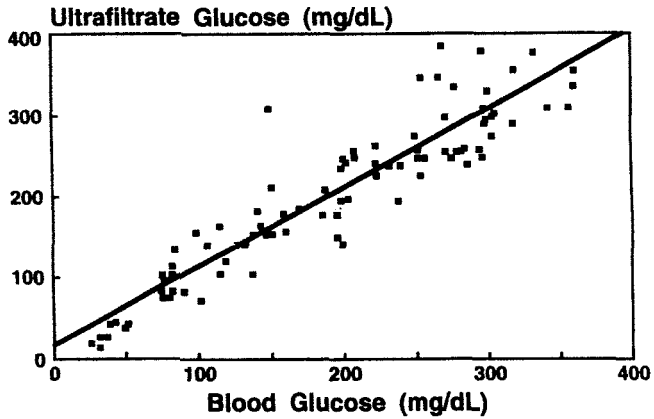

FIG. 6. Regression analysis on all blood and ultrafiltrate glucose in a 3-month study in diabetic dogs yielded $r = 0.91$ and $P = 0.000$.

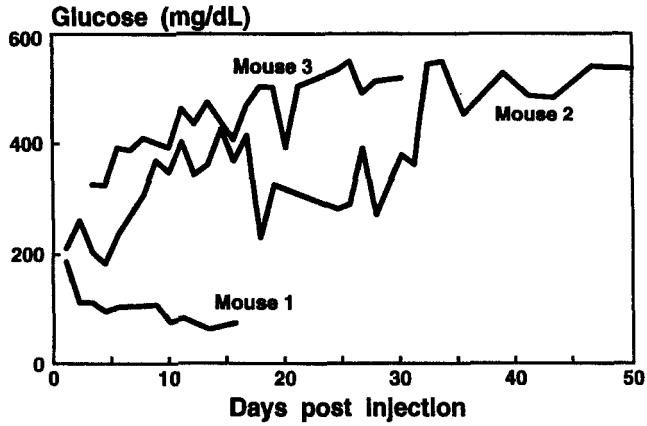

FIG. 7. Mice treated with injections of 50 mg/kg of the diabetogenic drug streptozotocin for 5 successive days. Average daily glucose levels were monitored using ultrafiltrate probes.

and urea levels ranged from 10 to 50. Animals were studied in the fasting condition and after a meal. Ultrafiltrate levels of both urea and creatinine tracked blood levels well. The correlation of blood and ultrafiltrate was $r^2 = 0.90$ for urea and $r^2 = 0.97$ for creatinine (Janle and Ash, 1994).

3. *Distribution of Endogenous Substances in Blood, Subcutaneous Tissue, and Muscle*

The horse has been used as a model to study the distribution of intravenously infused amino acids and calcium from blood to the subcutaneous and muscle interstitial fluids. Subcutaneous ultrafiltrate levels of both amino acids and calcium were higher than blood concentrations under basal conditions and after infusion. After infusion of amino acids, subcutaneous ultrafiltrate concentrations tracked the rise and fall of plasma concentrations, but ultrafiltrate concentrations were greater than plasma concentrations at all time periods. Similarly, subcutaneous calcium ultrafiltrate concentrations tracked plasma concentrations after intravenous infusion of calcium. Subcutaneous concentrations were also slightly higher than plasma concentrations. Muscle concentrations of calcium were considerably higher than either plasma or subcutaneous concentrations, both in the basal state and after infusion. Calcium concentrations remained elevated for a longer time in muscle than in plasma or in subcutaneous tissue (Sojka *et al.*, 1995; Spehar *et al.*, 1995). These studies demonstrate the potential of using ultrafiltration to study distribution of nutrients in different tissues.

B. MICRODIALYSIS

1. Metabolism in Human Adipose Tissue

Microdialysis has been used to study metabolism in adipose tissue *in situ*. Steady-state concentrations of glycerol, glucose, pyruvate, lactate, and adenosine have been determined (Arner and Bolinder, 1991). The concentration of a metabolite is determined by two factors. One factor is the amount of metabolite delivered to the site by the vascular system; the other is the amount produced or consumed by the tissue. If production or consumption of a metabolite is small, the concentration measured is more representative of whole body concentrations. If, however, the production or consumption of the tissue is extensive, concentrations will be indicative of tissue metabolism. Steady-state glucose levels were found to be similar in blood and adipose tissue, whereas glycerol levels were higher than in blood. This reflected the local production of glycerol by fat cells. Glycerol concentrations after glucose ingestion fall to lower levels in adipose tissue than in blood and rise more after exercise.

Microdialysis has also been used to demonstrate differences in metabolic activity of adipose tissue in different locations during exercise. Microdialysis can also be used to investigate differences in metabolism in different locations. For example, glycerol levels have been found to rise higher in abdominal adipose tissue than in the femoral or gluteal. Microdialysis can also be used to investigate the intricacies of hormonal control on tissue metabolism. Hagström-Toft and co-workers (1992) demonstrated the decrease of adipose tissue glucose during insulin infusion despite the maintenance of normoglycemia. Pyruvate and lactate concentrations increased and glycerol decreased to a greater extent in adipose tissue than in blood. Using microdialysis, beta adrenoreceptor stimulation was shown to oppose the action of insulin on glucose uptake and lipolysis but to have synergistic effects with insulin on nonoxidative glucose metabolism.

2. Effects of Ischemia on Kidney Metabolism

Ischemia may have profound effects on tissue metabolism. On the other hand, different tissues may respond differently (or to a different degree) to the same amount of ischemia. Most methods of studying metabolic changes due to ischemia require termination of the animal, and thus do not allow metabolic and survival studies in the same animal. With microdialysis, both survival and metabolic studies can be conducted.

Eklund and co-workers (1991) studied the effect of ischemia on two different areas of the rat kidney—the cortex and the medulla. The ischemia

was induced by clamping the renal pedicle. It was found that the metabolites lactic acid, inosine, and hypoxanthine increased as a consequence of anaerobic metabolism. The increase in lactate was greater in the medulla, indicating that the medulla had a greater capacity for anaerobic metabolism than the cortex.

IV. SUMMARY

Microdialysis and ultrafiltration are complementary sampling techniques that facilitate acquisition of data in awake, freely moving animals. Because the necessity of blood removal is eliminated, sampling frequency is not limited by animal size. The samples obtained by these techniques usually require no processing for analysis.

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