Chapter 2

*Methods of in vivo continuous sampling for clinical applications*

Get your facts first, and then you can distort them as much as you please (Mark Twain)
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Introduction

Since the diagnosis of diabetes mellitus in the Middle Ages was performed by tasting the sweetness of the urine, our knowledge of biochemical parameters in health and illness has expanded enormously (Calbreath, 1992). In the 19th Century, the first biochemical techniques for characterizing and analysing compounds in the body fluids became available (Calbreath, 1992). Presently, it is possible to measure sugars or other enzymatically convertible, relatively easy-to-detect body metabolites, and, with the introduction of immunoassays, many other compounds, e.g. hormones and proteins (Gosling, 1990). Measuring analytes in body fluids is routinely used to assist in the medical diagnosis; many clinical decisions are made based on laboratory analysis (Gilbert and Vender, 1996). However, some improvements can still be made. One major improvement would be to have techniques available that would provide concentrations of body fluid constituents continuously and instantaneously (Anderson et al., 1997). To date, samples are often taken batch-wise, and subsequently analysed in a laboratory. The results of such measurements are usually only after several hours available (Gilbert and Vender, 1996), and these analyses are generally rather expensive (Linhares and Kissinger, 1992b). Continuous analysis offers the advantage of providing instantly information of the analyte concentration and facilitating the creation of time-profiles. Methods of continuous analysis avoid the need for frequent sampling, which would be necessary when the concentration of the analyte has to stay within a certain range or when a concentration profile of the analyte over the day is required (Trajanoski et al., 1996). Therefore, one can expect that the quality of patient care would be improved if batch-wise sampling and analysis were replaced by on-line, continuous analysis.

Requirements for accurate on-line analysis

For continuous (bio)chemical monitoring, two methodologically different invasive approaches can be applied (Fischer et al., 1995). The first approach, direct in vivo measurement at the sampling site inside the body (as utilized in many (bio)sensors) has several technical limitations. Among these problems are in vivo calibration and stability (Reach and Wilson, 1992; Carlsson et al., 1996). In addition, the probes are often rather large, thus creating large artefacts in the surrounding tissue (Reach and Wilson, 1992).

The second approach consists in continuous (on-line) sampling, preferably directly coupled to an analysis system. In this overview, we will primarily focus on the current state of the latter approach.

For pathophysiologically relevant continuous measurements following an approach of this kind, two issues have to be addressed: 1) Which sampling technique should be used? and 2) In which tissue or body fluid should the sampling be performed?

This overview will outline two techniques suitable for on-line sampling for clinical practice: microdialysis and ultrafiltration. The merits and limitations of both sampling
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Methods are evaluated. Literature on the application of the microdialysis and ultrafiltration to various sampling sites will be reviewed. Special attention will be given on approaches to estimate the concentration of analytes in blood.

Methods for continuous on-line sampling

At present, most biochemical parameters are measured in (discrete) blood and urine samples. Among these, blood sampling is predominant, because many times changes in the levels of an analyte in blood often occur rapidly after a clinical event, as all tissues are in close contact with the blood circulation (Linhares and Kissinger, 1993b). Furthermore, compared to other body compartments, blood samples are relatively easy to sample and the disturbance of the physiological state of the subject is minimal. Continuous sampling performed rapidly and free of compounds interfering in the detection of the analyte would be ideal for patient monitoring (Linhares and Kissinger, 1992a). Blood is the only body compartment that can be used for continuous, undiluted sampling. However, on-line blood sampling is not performed routinely, because it has some serious drawbacks:

1) Infection hazards (Reach and Wilson, 1992). Placement of a catheter in the bloodstream has to be done and controlled by professionals and special care must be taken to prevent infections. Normally this is only possible in a hospital setting.

2) The subject has to be heparinized to prevent blood clotting both intracorporally as well as in the sampling system. Heparinization also holds certain risks, for example internal bleedings.

3) The internal diameters of the tubings have to be rather large to prevent blood clots blocking the tube (Linhares and Kissinger, 1993b). For this same reason, the flow rate has to be rather large. This means that much blood of the donor is lost in the process of continuous sampling.

4) Continuous sampling and subsequent biochemical analysis is only useful when the result of the assay is not changed by endogenous physiological processes. Blood cells and enzymes often metabolize compounds and this can only be prevented when special reagents are added (such as citrate).

5) The instruments (such as the Biostator) for continuous sampling of blood, e.g. for analysing the glucose, are rare and very expensive (Ash and Janle-Swain, 1988).

To overcome these drawbacks, alternative body fluids for sampling have been considered. However, most tissues and body fluids do not have enough fluid to be sampled directly, except in case of urine and saliva. As the variations of volume influence the concentration profiles of analytes in urine and saliva, on-line measurements do not reveal relevant information.

Alternative sampling techniques for more general applications and sampling of other body fluids have been developed some decades ago. The first device was a push-pull canulla (Gaddum, 1961; Delgado and Rubinstein, 1964; Gliessman et al., 1986), developed specifically for sampling at places where the amount of fluid is limited. This semi-continuous device was based on adding and subsequent withdrawing of a buffer. The
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probe (the part which is in direct contact with the sampling site) consisted of a double tubular system (Gardner et al., 1993). One tube (push) infuses a physiological buffer into the sampling site. The other tube (pull) withdraws, with the same flow rate, fluid for analysis (see Fig. 2.1A). Although the technique seems rather straightforward, its biochemical application is difficult. At too low flow rates, blockage of the pull cannula is risked, whereas high flow rates can damage the surrounding tissue. Additionally, even small differences in flow rates of the two pumps will cause massive tissue damage (Gardner et al., 1993). The push-pull sampling technique has been used for subcutaneous and brain fluid sampling, but it is not suitable for blood sampling because blood cells and blood platelets block the tubes (Rada et al., 1993). The technique was soon replaced by a more sophisticated technique: microdialysis.

Microdialysis

Microdialysis (MD) is the first universally working continuous sampling technique, a dynamic sampling method based on analyte diffusion across a semi-permeable membrane due to a concentration gradient (Delgado and Rubinstein, 1964; Palmisano et al., 1997). A fluid is pumped with a single pump through a dialysis probe, the semi-permeable membrane. The incoming fluid, the perfusate, is a buffer balanced in pH and ion content with the extracellular fluid in the surrounding tissue. The outgoing fluid, the dialysate, contains body fluid constituents, which have been diffused into the fluid by passive diffusion through the membrane of the probe. The membrane forms the contact area with the body tissue.

MD has many advantages over other sampling techniques. MD allows sampling in the extracellular space of virtually all tissues (Robinson, 1995). Due to the semi-permeable membrane the influx and the outflux of fluid are balanced, preventing tissue damage as in...
the case of the push-pull canullas (Gardner et al., 1993, Robinson, 1995). Due to the cut-off of the semi-permeable membrane of the MD probe, the sample is relatively clean (Deterding et al., 1992; Paez and Hernandez, 1997). Therefore, not only is the chemical analysis less complex (deproteinisation is unnecessary), but the analyte in the dialysate can not be degraded by enzymes. It is an inexpensive, readily applicable, and relatively non-invasive method, causing minor tissue trauma and allowing to define the sampling site clearly (Linhares and Kissinger, 1993b). The introduction of a MD probe creates only moderate pain, comparable to an intramuscular or subcutaneous injection (Müller et al., 1995). It is also possible to infuse compounds into the tissue (Robinson, 1995). Because the focus of this review is sampling, this possibility will not be discussed further in this chapter. Although there are several dialysis probes available, in general they can be schematically represented as in Figs 2.1B-D.

Analyte recovery and its estimation

When MD sampling is performed, the relative recovery of the analyte has to be determined. The relative recovery is defined as the percentage of the concentration found in the dialysate and of that of the original body fluid concentration (Gardner et al., 1993). Sometimes, also the absolute recovery is used to characterize the concentration in the dialysate. The absolute recovery is the total amount of analyte in the dialysate per time interval. In general, the relative recovery is independent of the concentration of the analyte, but depends on:

1) The flow rate and composition of the MD fluid. The higher the flow, the lower the recovery (Linhares and Kissinger, 1993b). In contrast, the absolute recovery will increase at higher flow rates.

2) The features of the probe. For example, the surface area of the probe is directly proportional to the recovery (Robinson, 1995).

3) The chemical nature of the MD probe (Hsiao et al., 1990; Linhares and Kissinger, 1993). There are numerous membrane materials, each with different physical and chemical properties. Depending on the analyte, a selection of membrane material has to be made. The membranes normally used are organic polymers (e.g. cellulose, polycarbonate, polysulphone, polyacrylonitrile) which have a cut-off value of 10-30 kDa and hydrophobic properties. The chemical characteristics also determine the biocompatibility (Gaddum, 1961).

4) The nature of the sample. The higher the fluid/tissue ratio of the sampling site, the higher the recovery. When the fluid is in motion, like in blood vessels, the recovery is also higher (Linhares and Kissinger, 1992b).

5) The temperature (Linhares and Kissinger, 1992b). At higher temperature, the diffusion through the membrane will increase, the recovery will therefore be higher.

When the absolute concentration of the analyte in the body compartment is required, the recovery has to be calculated. There are several methods for the quantification of the recovery, enabling calculation of the absolute concentration in the tissue (see also Fig. 2.2).
Fig. 2.2. Recovery estimation approaches. A. Cartoon.
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\[ \text{Rec}_A = \frac{D_A}{c_A} \times 100\% \Leftrightarrow c_A = \frac{D_A}{\text{Rec}_A / 100\%} \]

1. \( \text{Rec}_A = \text{Rec}_{X.A} \Leftrightarrow \frac{D_{X.A}}{c_{X.A}} = \frac{D_A}{c_A} \Leftrightarrow c_A = \frac{c_{X,A}D_A}{D_{X.A}} \)

2. \( D_A = c_A \) if \( t \to \infty \)

3. \( \left[ \frac{D_A}{c_A} \right]_{\text{flow}=1} = \text{Rec}_1 ; \left[ \frac{D_A}{c_A} \right]_{\text{flow}=2} = \text{Rec}_2 ; \left[ \frac{D_A}{c_A} \right]_{\text{flow}=3} = \text{Rec}_3 \Rightarrow c_A = \left[ D_A \right]_{\text{flow}=0} \)

4. if \( P_A = D_A \), then \( P_A = c_A \)

5. \( \text{Rec}_A = (1 - \left( \frac{D_A}{P_A} \right)_{\text{before}}) \times 100\% \)

6. \( 1 - \frac{D_B}{P_B} = \frac{D_A}{c_A} \Leftrightarrow c_A = \frac{D_A}{1 - (D_B / P_B)} \)

7. \( c_A \) known \( \Rightarrow \text{Rec} = \frac{D_A}{c_A} \times 100\% \) known

**Fig. 2.2.** Recovery (Rec) estimation approaches. B. Formulas. See Fig. 2.2A and text for details. 1. In vivo recovery equals to in vitro recovery; 2. Low flow method and stop-flow method; 3. Changing flow rate method; 4. No-net-flux method or equilibrium technique; 5. Retrodialysis method; 6. Internal reference technique; 7. Calibration with an independent method.
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Methods for estimation of the *in vivo* recovery $\text{Rec}_A$ and the subsequent estimation of the *in vivo* concentration $c_A$:

1) *In vivo* recovery equals to *in vitro* recovery (Zetterstrom and Ungerstedt, 1984). Originally, the *in vivo* recovery $\text{Rec}_A$ was estimated by direct usage of the *in vitro* recovery $\text{Rec}_{X,A}$. However, they can not be corrected for the small extracellular volume, and variations in viscosity and tortuosity of the tissue (Sarre et al., 1995).

2) Low flow method (Menacherry et al., 1992; Justice, Jr. 1993; Kaptein et al., 1997) and stop-flow method (Gardner et al., 1993). These methods are based upon the concept that when dialysis fluid $D_A$ and extracellular fluid $c_A$ are in contact with each other for a long period, the recovery will approximate to 100%. When the flow rate is chosen low enough ($<0.5 \text{ µl min}^{-1}$) or alternatively, when the flow is stopped long enough, a nearly 100% recovery may be expected. This assumption can be correct, but can not easily be checked.

3) Changing flow rate method (Hegemann et al., 1995). The recovery $\text{Rec}_A$ is calculated by measuring the concentrations at changing flow rates, whereupon the flow rate is extrapolated to zero. Disadvantages of this method are that it is rather time consuming and that it requires a steady-state condition.

4) No-net-flux method (Gardner et al., 1993) or equilibrium technique (Arner and Bolinder, 1991). Perfusates contain a range of pre-set concentrations of the analyte $P_A$. When the concentration of the analyte in the extracellular compartment $c_A$ is higher than in the perfusate, the analyte concentration of the dialysate will increase as compared to the perfusate. At higher concentrations in the perfusate, the analyte concentration in the dialysate will be lower. Interpolation will give the exact extracellular concentration. This method is quite labour intensive and works only for analytes which do not vary during the calibration. Additionally, the method may create an artefact, because the analyte in the perfusate may disturb the physiological extracellular concentration.

5) The retrodialysis method (Müller et al., 1995). This calibration technique is based on the assumption that the diffusion of the analyte through the membrane is equal in both directions. Before the measurement, concentrations around 100 times above normal *in vivo* concentrations are perfused. The recovery can be calculated by measuring the percentage of the fraction of the concentration of this analyte in the perfusate $D_A$ and the perfusate $P_A$ and subtracting this percentage from 100%. This method has two drawbacks. The first is that the local analyte concentration around the probe may increase as a result of this perfusion. The second is that, if the analyte concentration is chosen too low, the endogenous concentration may influence the measured recovery.

6) Internal reference technique (Rosdahl et al., 1993; Sarre et al., 1995; Lonroth and Strindberg, 1995). In this calibration technique, it is assumed that the ratio of the recovery of the analyte and the loss of an internal standard (a kind of an inverse recovery) *in vivo* and *in vitro* is the same. The internal standard (also called *in vivo* marker) is a compound with a “behaviour” similar to the analyte. The advantage of this method is that it can also be applied in non steady-state conditions and that it detects recovery changes during
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sampling. Major problems of this technique are finding a good marker and the need for a dual detection system, detecting the analyte as well as the in vivo marker.

7) Calibration with an independent method (Chen and Steger, 1993).

When the MD sample can be calibrated with an independent technique, the data will be most reliable. For example, discrete blood samples can be analysed and compared with the MD data. However, many MD sampling sites (e.g. subcutaneous tissue) had no alternative methods for sampling.

Of all the afore-mentioned methods, calibration with another technique seems to be the most reliable, but, in practice, only the blood compartment has an optional sampling possibility. An important deficiency of all mentioned methods, with the exception of the internal reference technique, is that they do not account for changes in recovery during the sampling (Sarre et al., 1995). These changes occur often, even within a short period of time, as a result of blockage of the membrane by proteins (e.g. fibrin) or cell structures, or due to pathophysiological changes in the tissue structure (Ash et al., 1992).

In addition to the technical problems of recovery determination, MD may also cause artefacts in the tissue. This is not necessarily due to tissue trauma as a result of insertion of the probe, but rather to drainage of the analyte or of other compounds present in the extracellular fluid (Rosdahl et al., 1993). Drainage is the depletion of analytes from the tissue around the probe as a result of the sampling process, in which the analyte diffuses into the dialysis fluid. Balancing the pH and ion content of the perfusion medium with that of the living tissue is required but is not easily accomplished (Osborne et al., 1991). A MD buffer lacking certain compounds, for example ions present in the endogenous fluid, can influence the physiology at the sampling site. Moreover, drainage of the analyte may not only influence the tissue physiology, but also affects the actual measurement. Rosdahl et al. (1993) calculated that in their experimental set-up the drainage of glucose was in the same order of magnitude as the physiological supply of the analyte in the subcutaneous and muscular interstitial fluid sampling they performed. The best way to counter this phenomenon is to select a flow rate as low as possible and to emulate the extracellular fluid as well as possible (Osborne et al., 1991). The minimal flow rate depends on the recovery of the analyte, the sensitivity of the analysis method and the time resolution required.

In 1987, the first report was published on a sampling technique alternative to MD (Janle-Swain et al., 1987): Ultrafiltration. This alternative not only avoids the difficult and time consuming recovery calculations of MD, but prevents drainage as well.

Ultrafiltration

Ultrafiltration (UF) is a technique in which endogenous fluid is withdrawn from the sampling site driven by underpressure (Janle-Swain et al., 1987). Lust like MD, the UF probe consists of a semi-permeable membrane that excludes large molecules (e.g. proteins), whereas small analytes (e.g. glucose) enter the probe together with the water and salts
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(Fig. 2.3). UF was first described by Janle-Swain et al. (1987) for batch-wise sampling in subcutaneous tissue. Approximately 40-50 µl h\(^{-1}\) (0.01 µl mm\(^{-1}\) probe min\(^{-1}\)) (Ash and Janle-Swain, 1988; Ash et al., 1992) can be sampled with this technique. In 20-minute time intervals, they did not observe any significant difference in the acetaminophen concentration measured with two probes placed contralaterally in a single subject (Linhares and Kissinger, 1993a).

Recently, Moscone et al. (1996) and Kaptein et al. (1997) have used UF for on-line sampling (see also chapter 3 and 4). The underpressure was created with a small, disposable syringe and the flow rate was regulated with a flow restriction tube, a tube with a very small internal diameter. Accordingly, a constant flow rate of 100-300 nl min\(^{-1}\) for a 4 cm probe was obtained, and sampling was performed up to 24 hours. Sampling was performed in subcutaneous tissue as well as intravenously in the rat, and the sample was analysed on the glucose and lactate concentration.

![Fig. 2.3. Schematic representation of microdialysis (A) and ultrafiltration (B).](image)

UF provides “recoveries” above 95 % for low molecular weight molecules (Linhares and Kissinger, 1993a), because there is no dilution factor. A small correction factor, by which the tissue concentration appears to be smaller than the measured concentration, must be included for the osmotic pressure due to the exclusion of restricted compounds (e.g. proteins) (Linhares and Kissinger, 1992b). Another correction factor should be included, because in particular larger molecules are hindered to pass the membrane of the UF probe, leading to an underestimation of the analyte concentration (Linhares and Kissinger, 1992b; Scheiderheinze and Hogan, 1996). In theory, both correction factors can be determined in vitro (Linhares and Kissinger, 1993a) and it is hypothesized that it will not be changed by partial blocking of the probe surface.

Reflection upon MD and UF

MD and UF have many similarities: Both techniques can use disposable material, and create relatively clean, on-line samples. The partial purification of the sample allows direct
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analysis for example with HPLC-systems. They also share the drawback that hydrophobic compounds (such as steroids) cannot be measured accurately, because they do not diffuse through the membrane easily and/or adsorb to the tubing material. Moreover, these compounds are in vivo mainly bound to (serum) proteins.

Larger molecules are barely or not at all diffusing through the membrane in case of MD (Ungerstedt, 1991). In contrast, for UF, large molecules can enter, though the “recovery” is below 100% (Scheiderheinze and Hogan, 1996).

The necessity of an additional fluid in MD sampling may lead to changes in the surrounding tissue. Sterility is more difficult to achieve and to maintain, because of the addition of extra fluid. Furthermore, the probe configuration is more complex. However, little is known about the effect of having a probe implemented and the withdrawal of fluid when UF sampling is performed. Janle-Swain et al. (1987) have demonstrated a zone of fibrin tissue around the probe after three months of sampling with an UF probe. In case of MD, such a fibrin layer would not allow to recover the analyte. The influence of this on UF sampling with low flow rates has not yet been investigated in detail, though it might be expected that it may change some of the analyte concentrations because of metabolic activities of the tissue (Ash et al., 1992). Examination using electron microscopy should also be performed in order to gain insight into the direct effects of the probe on the tissue.

A problem that was encountered when on-line UF was performed in abdominal (subcutaneous) tissue in man was that the probe also held a resistance (Tiessen et al., 1997), thereby decreasing the flow rate. When analysis is based on absolute amounts of an analyte per time interval, the concentration is then difficult to estimate.

In conclusion, a general decision as to whether MD or UF should be used cannot be made. All parameters involved in the planned analysis have to be considered before choosing the sampling technique. Therefore, not only the sampling site, but also the (expected) recovery, the sensitivity of the analysis method and the conditions in which the measurements will be accomplished (e.g. in a hospital or at home) have to be taken into account. When, for example, a 24-hour profile of an analyte of an ambulant patient is to be made, UF holds a lower infection risk, and no additional fluid has to be carried, so if the applied flow rate does not cause hindering for passing the membrane of the probe, this is the method to be chosen. However, when the sampling site has a limited fluid production, MD is a better alternative.

To prevent drainage and improve recovery in MD, studies have been made with MD with a low flow rate (100-300 nl min$^{-1}$) in subcutaneous tissue of rats. In comparison with UF it showed a recovery for MD at these flow rates of 100% (Kaptein et al., 1998). Also, there was no flow restriction over the probe for UF sampling. This means that MD at low flow rates might be an alternative for UF when the sampling sites do not have sufficient fluid, whereas in other cases, if enough fluid is available, UF may be the best choice.
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Places for sampling

A very important issue in obtaining useful data is the decision in which tissue or which body fluid the sampling should be performed. The outcome of this decision is influenced by:

1) Safety considerations. This includes risk evaluation of infections, inflammation and blood clotting.

2) Occurrence of the analyte at the sampling site and fast change of the analyte after the event of interest. For example, protein markers for acute myocardial infarction are not expected to diffuse to subcutaneous tissue, whereas small molecules like glucose do. On the other hand, monitoring of these protein markers might well be performed in heart muscle.

3) Knowledge of the concentration of the analyte at the sampling site. Clinical decisions of biochemical parameters can only be made when the “behaviour” of the analyte at the particular sampling site is known.

As stated in the introduction, at present most clinically relevant analytes are measured in discrete blood samples and estimation of the concentration of an analyte in blood is preferable. For such measurements there are four places of sampling, as described in literature: intravenous, subcutaneous, transcutaneous and in the peritoneal cavity (Reach and Wilson, 1992). Of these four, the peritoneal cavity is not an attractive option. It is difficult to reach, carries a large risk of damaging the probe due to the peristaltic movement of the intestines, and the probe will be covered by, among other things, fibroblasts (Woodward, 1982). The intravenous, subcutaneous and transcutaneous samplings, however, all have useful applications and will be discussed below. Also, some examples of sampling in other organs, e.g. the brain, liver or kidney, are discussed, as such applications reveal important information for specific cases.

Intravenous

Both MD and UF can be used for continuous sampling of the blood compartment. The properties of the membrane prevent blood cells and large compounds (e.g. proteins) to enter the sampling tube (Paez and Hernandez, 1997). MD sampling in the blood compartment has been used to study pharmacokinetics (Telting-Diaz et al., 1992; Ekstrom et al., 1995) and endogenous compounds such as glucose (Rada et al., 1993; Chen and Steger, 1993; Paez et al., 1996). The apparently controversial findings in the recovery with the intravenous (i.v.) probes is quite remarkable. Chen and Steger (1993) found that the recovery of glucose in heparinized rats remained stable, whereas in unheparinized rats the recovery dropped from 28% to 1% within 24 hours. On the other hand, Rada et al. (1993) and Stenken et al. (1993) stated that for the analytes they measured (glucose, epinephrine), the in vivo recovery was equivalent to the in vitro recovery, and remained stable during the seven days of measurements. However, most current reports show that the in vivo and in vitro recovery differ (Sarre et al., 1995). In
humans, glucose measurements with i.v. MD have been performed in the cubical vein of healthy volunteers (Paez and Hernandez, 1997). Until present, intravenous UF sampling has only been described once. Kaptein et al. (Chapter 3) measured glucose in the jugular vein of rats with a sample flow rate of 100 nl min$^{-1}$.

Despite their physiological advantages, i.v. MD and UF are not yet used routinely, obviously due to the risks of having an artificial object in the blood stream and the relative unfamiliarity of the technique. The technique is still rather invasive. Subcutaneous sampling is, therefore, suggested as an alternative for i.v. sampling.

**Subcutaneous**

Subcutaneous (s.c.) monitoring has been performed for two reasons: to study local effects and to estimate analyte concentrations in blood. Subcutaneous tissue fluid is believed to reflect the blood characteristics of many analytes relatively well (Poitout et al., 1993; Thome Duret et al., 1996). The implantation of a sampling or measurement device in sc. tissue is relatively easy, and the risks for infection and body-reactions are mild when compared to full-blood contact (Arner and Bolinder, 1991). Subcutaneous implantation of an UF or MD probe is quite ‘patient-friendly’, because in most cases it can be performed without anaesthetic or complicated surgical procedures (Ash et al., 1993). The recovery in s.c. tissue is generally lower than in blood (Bolinder et al., 1983; 1989), and larger molecules, such as proteins, rarely penetrate into subcutaneous tissue. However, long term effects such as blocking of the sampling probe by proteins or encapsulation by collagen may occur (Reach and Wilson, 1992).

Most research with MD sampling in humans has been performed in sc tissue. In particular, glucose has been studied intensively, for the monitoring of diabetic patients and to set up an insuline administration schedule. Literature on subcutaneous glucose is all but conclusive as to whether it represents an accurate reflection of blood concentrations. Clinical decisions on the outcome of sc tissue concentrations are therefore controversial. Several reports suggest that the kinetics of glucose in blood and s.c. tissue are similar (e.g. Bolinder et al., 1989; Reach and Wilson, 1992), because blood capillaries are rarely more than 20 µm away from any single functioning cell (Linhares and Kissinger, 1993b). However, after close inspection of the available data (Pickup et al., 1989; Ertefai and Gough, 1989; Lonnroth, 1996; Sternberg et al., 1996) and the preliminary results of our studies (Tiessen et al., 1997; Kaptein et al., 1997) we are prompted to believe that the subcutaneous tissue fluids form a distinct body compartment, and that analyte concentrations exhibit different time profiles than in case of blood. From a physiological viewpoint, this can be explained by the fact that the cells in the subcutaneous tissue might change the concentration of glucose, e.g. metabolism of glucose by subcutaneous fibroblasts (Ash et al., 1992). Obviously, the relation of s.c. tissue concentrations and plasma water concentrations are different for every analyte studied and may vary from subject to subject.
Besides estimation of the blood compound concentration, it is also possible to perform measurements in subcutaneous tissue to study local metabolism and kinetics (Arner and Bolinder, 1991). For example, chemotherapy and local drug deliveries require low systemic and high local concentrations. If the chemicals are perfused in subcutaneous tissue, such measurement in this tissue may provide very useful information. Hegemann et al. (1995) studied local effects of the nicotine distribution in the dermis of healthy volunteers. They observed an increase in blood flow around the probe for up to one hour, and noticed that the maximum levels may depend on the location of the probe. When the probe is in close contact to blood vessels, it might (partially) sample blood compounds directly, either because of damage to blood vessels or because of the short diffusion distance (Linhares and Kissinger, 1993b).

**Transcutaneous**

An even more controversial way of estimating blood concentrations is measuring transcutaneously. For example, sweat can be collected continuously. Non-invasive sampling of sweat or analyte on the surface of the skin can be performed with MD (Korf et al., 1993; De Boer et al., 1994). However, the recovery is low (approx. 2%) and the concentration of analytes is strongly influenced by the volume of sweat produced. In addition, many analytes do not, or only in very small amounts, diffuse through the skin. In particular, large molecules (e.g. proteins), hardly penetrate the skin.

An interesting application of transcutaneous sampling has been described by de Boer et al. (1994). They measured glucose in neonates with a transcutaneous microdialysis probe. Transcutaneous measurements seem to be restricted to cases when the skin is very thin, for example, the transcutaneous samples of the neonates resembled the glucose concentration in blood almost perfectly.

**Sampling in other organs**

Besides the previously mentioned body compartments, there are other organs which have been the subject of sampling, as described in many (animal) studies. Most of these organs are not expected to become routinely available for sampling for clinical diagnosis in humans, because of ethical and experimental considerations (Hamberger et al., 1991). However, in some particular cases, there might be a need to perform sampling in other tissues.
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Brain

MD has predominantly been applied in the study of brain chemistry of experimental animals in vivo (Justice, Jr. 1993). MD is the first method allowing continuous sampling of the hitherto relatively inaccessible neural compartment in vivo (Robinson, 1995).

One of the first reports describing microdialysis was written by Ungerstedt and Pycock (1974). They implanted a hollow dialysis fiber in the brain of a rat and applied a constant perfusion. The MD technique has been further optimised and characterized over the years. Over 2000 studies have been performed since then, studying neurotransmitters (Rosdahl et al., 1993), drugs (Stahle, 1992), metabolites (Stjernstrom et al., 1993), allergic mechanisms (Petersen et al., 1992) etc. in animals in vivo.

An example of intracerebral microdialysis in human subjects was given by Kanthan et al. (1995). They reported a study about the glutamate release during ischemia. Because of ethical and experimental reasons, this type of application is not likely to develop significantly. The same can be said about sampling in lumbar subarchnoid space in humans (Persson et al., 1993). Such methods are only applicable under very special circumstances, e.g. after surgery, when important medical information can be obtained by this technique.

Muscle

Müller et al. (1995) demonstrated time profiles of paracetamol and gentamicin from MD probes in muscle, comparing the data to serum samples. They did not find significant differences in case of simultaneously measuring in the muscle on the left and right side of the body. This was especially true for gentamicin, a compound that responds differently in the blood and the muscle compartment, so that differences at the sampling site might be expected, because in this case, the distance of the probe from a blood vessel is very important. However, possible differences may have gone unnoticed, because of the poor time resolution (20 minutes).

Langeman et al. (1996) inserted a probe into the interventricular septum of the heart during an aorta-coronary bypass operation. They claim that the MD in this tissue is feasible, although this would require an additional effort from the surgeon.

Other organs

In case of specific organ malfunctions or physiology, sampling in other organs can provide important information. For example, chemotherapy and local drug deliveries can be checked locally instead of systemically. This enables one to adjust the concentrations so that they are high locally and low in blood. Ekstrom et al. (1995) have reported successful MD in muscle, kidney and liver. These organs are very different in structure, morphology, enzymatic activity and uniformity. Additionally, sampling has been performed in the uterus of the rat, the ovary, the pineal, the pituitary gland (Robinson, 1995), the adrenal gland (Jarry et al., 1989) and the corpus luteum (Maas et al., 1992).
Concluding remarks

The MD sampling technique holds the potential to become a powerful new technique, for both research and clinical applications. On-line, continuous in vivo sampling can be performed, not only in blood, but also in other body compartments. For some analytes, monitoring the concentration in the subcutaneous extracellular space, or even with transcutaneous sampling, can estimate the blood concentration. Insertion of sampling probes in these areas is less invasive than in blood vessels, and reduces the risks. However, in many cases it is not possible to predict concentrations in blood by these measurements. For example, in some cases the analyte profile might be significantly delayed, and in other cases it is impossible for the analyte to diffuse to the s.c. tissue because of its size or polarity, and thus to be present in the s.c. fluid. Therefore, a decision on the best sampling site has to be made after careful consideration of all parameters involved in that particular application.

A definite general choice between MD or UF sampling in all conditions can not be made. The amount of fluid present at the sampling site, the risks of infection and the sensitivity and required sample size of the subsequent analysis method, are the factors which determine the choice between these two methods.

This overview clearly demonstrates the potential of on-line sampling with UF or MD. Implementation of on-line sampling in clinical practice for diagnosis of illness and studiest in the pathophysiology, will lead to new insights and new findings.

References

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Chapter 2


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