SUMMARY AND FINAL COMMENTS

This thesis describes experimental and clinical studies which were performed to investigate the possibilities of microdialysis sampling and continuous flow analysis for continuous chemical monitoring. Chapter 1 gives a brief overview on the use of biosensors that are currently available for in vivo chemical monitoring in clinical medicine and a review of the use, features and principles of in vivo microdialysis sampling. The main advantage of microdialysis over solid state biosensors is that it allows continuous sampling of almost any substance of small molecular weight and may be combined with manifold analytical techniques for ex-vivo chemical detection without the risk of exposing the subject studied to toxic substances. A disadvantage that microdialysis has in common with biosensors, is that it is invasive and the measurement is based on the consumption of the substance and therefore dependent on diffusion of the substance through the tissue probed.

Chapter 2 describes the main analytical characteristics of continuous flow analysis with enzymatic fluorescence detection for on-line measurement of the lactate, ethanol, and glucose concentration in dialysate fluid and the in vitro testing of the two types of probes used in this thesis; a transversal probe used for invasive sampling, and a newly developed probe used for transcutaneous sampling. Continuous flow analysis with fluorescence detection is quite sensitive. The detection limit was minimally 0.1 µmol/l for glucose. The fluorescence response was linear for glucose and ethanol, but levelled off for lactate. The sensitivity of the system shows a slight decrease with time, requiring that the system had to be calibrated with one or more standards before and after monitoring during long term experiments. The time resolution of the system was dependent on the experimental set up and the dead space of tubing between probe and detector and was minimally about 2 minutes. In vitro testing showed that the dialysate concentrations were highly linearly related to the medium concentrations of lactate, ethanol and glucose, for both types of probes. It was also shown that the recovery of a substance is dependent on the dialysis flow rate, the size of dialysis membrane of the probe and characteristics of the test medium such as its temperature and whether the medium was stirred or quiescent.

Chapter 3 describes continuous monitoring of the dialysate lactate concentration in rat muscle tissue, during electric stimulation, ischemia, and exercise. We also determined the extracellular lactate concentration by mathematical extrapolation. The results indicate that the dialysate lactate concentration is influenced by both the intracellular lactate production and the plasma lactate concentration. The extracellular lactate concentration was about twice the plasma lactate concentration, probably due to cellular metabolism, which was confirmed by the observation that the dialysate lactate concentration decreased after blocking the glycolysis with iodoacetate. At a perfusion flow rate of 8 µl/min, the in vivo recovery of lactate varied between the experiments (CV ± 42%). After selective electrical stimulation of the gastrocnemius muscle to tetanic contractions up to 2 minutes, the dialysate lactate concentrations were only moderately elevated as compared to the higher expected intracellular lactate concentrations. During 15 to 30 minutes swimming, which includes vigorous exercise of many muscles, the dialysate lactate concentration from a probe placed in the tibialis anterior muscle increased by a factor of 6 to 8 indicating an increased blood lactate concentration.

Chapter 4 describes the continuous monitoring of ethanol, as a model substance, in freely moving rats with subcutaneous microdialysis sampling after an oral ethanol dose of 1 g ethanol per kg body weight. The dialysate ethanol concentrations reflected the plasma ethanol concentration after peak values were reached 5 and 15 minutes after oral dosing in plasma
and dialysate, respectively. This indicates that equilibration of ethanol between plasma and dialysate is delayed during the relatively short absorption phase. The recovery of ethanol showed a moderate variation between the different experiments (CV 18%). After a single point calibration plasma ethanol could be estimated from the dialysate values, and closely monitored the measured plasma ethanol concentration. The calibration required analysis of the plasma ethanol concentration of one blood sample collected during the early phase of ethanol elimination.

Chapter 5 and 6 describe the attempts to monitor the plasma lactate concentration with subcutaneous microdialysis probes in rats during intra-peritoneally induced Escherichia coli sepsis and in a group of patients admitted to the intensive care unit with shock. Although there was a significant correlation between the plasma and dialysate lactate concentration in both studies, the dialysate lactate did not accurately monitor the plasma lactate concentration. In the rat study the dialysate lactate concentrations levelled off at the end of the experiment, possibly due to a reduction of the subcutaneous adipose tissue blood flow in the late hypodynamic phase of shock. In the patient study which included patients with a wide range of pathology the estimated values of the plasma lactate concentrations calculated from dialysate lactate concentrations were insufficiently accurate to be clinically acceptable despite a significant linear correlation between the plasma and estimated plasma lactate concentration. It was found in the majority of patients that the recovery of lactate (dialysate/plasma ratio) was inversely related to the plasma lactate concentration. This complicates a practical approach for a calibration of dialysate values to estimated plasma values.

The construction and the use of a newly developed microdialysis probe for transcutaneous monitoring blood glucose and ethanol in human volunteers is described in chapter 7. This aluminum probe is heated to 42 °C and contains a 0.4 μl flow cell covered by a cellulose membrane. The probe was attached to the human skin which was stripped first with cellophane tape to remove the greater part of the stratum corneum in order to reduce the barrier function of the skin. We found a linear relationship between the blood and dialysate concentration for glucose and ethanol following an oral glucose test and after an oral ethanol dose, respectively. However, for both substances there was a great variation in the in vivo recovery between the various subjects (CV 101 % for ethanol and CV 49 % for glucose), possibly due to differences of the skin permeability and the quite uncontrollable effect of skin stripping. The transcutaneous recovery was greater for ethanol than for glucose due to the lipophilic character of ethanol. Arterialization of the blood flow of the skin by heating the probe, however, may not be obligatory for transcutaneous glucose monitoring, because the in vivo recovery of glucose in fasting persons increased relatively equally much as the in vitro recovery of glucose when the probe temperature was varied from 32 to 42 °C. Although the use of lower probe temperatures will allow sampling for longer periods without the risk of skin burns heating of the skin, however, may still be preferred to stabilize the skin temperature and diffusion.

In chapter 9 the relationship between the transcutaneous dialysate and blood glucose concentration was investigated more thoroughly in a group of newborn infants admitted to the neonatal intensive care unit who required intravenous glucose feeding. Skin stripping was standardized by measurement of the transepidermal water loss (TEWL), but the in vivo recovery of glucose still varied greatly between the different infants (CV 123%), probably due to skin characteristics other than TEWL. In each infant, the blood glucose concentration was correlated to the dialysate glucose concentration during manipulation of the blood glucose concentration by changing the rate of the intravenous glucose feeding. A multiple point regression analysis was needed to calibrate the dialysate glucose concentration to
between plasma and
recovery of ethanol
(18%). After a single
values, and closely
required analysis of
early phase of
concentration with
Escherichia coli
with shock. Although
lactate concentration in
end of the experiment,
flow in the late
ments with a wide range
ions calculated from
lactate concentration.
lactate/plasma ratio)
complicates a practical

Microdialysis probe for
testers is described in
ul flow cell covered
which was stripped first
in order to reduce
between the blood and
ose test and after an
a great variation in
ol and CV 49 % for
quite uncontrollable
on than for glucose
flow of the skin by
lactate monitoring,
tively equally much
32 to 42 °C.
gger periods without
stabilize the skin
and blood glucose
infants admitted to
Skin stripping was
21 %), but the in vivo
123 %), probably
concentration
concentration of the blood
ending. A multiple
concentration to
estimated blood values and to yield an acceptable level of accuracy. A single point calibration
method resulted in a considerably lower accuracy of the estimated blood glucose

Chapter 8 describes our attempts to use subcutaneous and transcutaneous microdialysis
for monitoring the plasma lactate concentration in human volunteers during bicycle exercise.
While the lactate concentration in the dialysate from both types of probes increased during
exercise they cannot be used for monitoring plasma lactate. The increase of the subcutaneous
dialysate lactate concentration underestimated the increase in plasma lactate concentration,
possibly due to lactate production by subcutaneous adipose tissue which may have a relatively
greater influence during the resting condition or due to diffusion limitation during the fast
increase of the plasma lactate concentration. The increase of the transcutaneous dialysate
lactate concentration during exercise relatively exceeded those in plasma by a factor of 6.
This was mainly due to exercise induced sweat lactate which was demonstrated by blocking
the sweat glands with atropine.

In conclusion, these studies showed that continuous in vivo chemical monitoring is
possible with microdialysis sampling, but quantitative interpretation of the results may be
quite difficult, and varied between the different tissues probed and substances measured.
During in vivo use of microdialysis sampling, the physiological behavior of the substance
sampled and its interactions with the tissue probed are more important factors than the
sampling characteristics of microdialysis itself.

The interaction of microdialysis sampling with the interstitial fluid can be considered to
consist of three processes. In the first place, the microdialysis probe drains substances by
diffusion due to the removal of the dialysate. Second, the metabolic activity of the cells of
the tissue where the probe is placed may add or consume substances from the extracellular
fluid. Third, the blood vessels of the micro-circulation may also add or remove substances
from the extracellular fluid. Thus, although the chemical composition of the dialysate
originates from the interstitial fluid, it will also be influenced by intracellular processes,
substrate exchange with the micro-circulation and diffusion characteristics of the substance
through the extracellular fluid.

The results of monitoring the plasma concentration of an extracellularly located substance
such as glucose (chapter 7 and 9) were quite good and indicate that microdialysis sampling
may be used for monitoring blood glucose. The same applies to ethanol (chapters 4 and 7)
which is known to distribute well throughout aqueous and lipid soluble compartments of the
body allowing both subcutaneous and transcutaneous monitoring. For lactate (chapters 3, 5,
6 and 8) the results were more complex. Lactate is produced predominantly intracellularly
in each of the tissues probed in this thesis (striated muscle, subcutaneous adipose, and skin
tissue). Therefore, the dialysate lactate concentrations, although significantly correlated with
the plasma concentration are also influenced by intracellular processes and tissue blood flow.

While it is to be expected that transcutaneous sampling may be used for sampling
extracellular substances other than glucose and ethanol, it has to be realized that these
possibilities will be limited by the diffusion barrier of the skin. Due to the relatively long
diffusion distance the dialysate concentrations were much lower than with subcutaneous
microdialysis. Transcutaneous microdialysis sampling may possibly be improved by using
t openphoresis, penetration enhancers or by increasing the dialysis surface of the probe.

For the clinical application of microdialysis for in vivo chemical monitoring it is
important to take into account of the influence of physiological processes like tissue blood
flow blood, microvascular exchange, and substrate metabolism, for each substance and tissue
separately.
Following the work presented in this thesis it remains unclear whether microdialysis sampling is clinically applicable and therefore further research is needed. Reliable and quick methods for in vivo calibration need to be developed. Probes and detection techniques should meet clinical requirements such as robustness and ease of use. This challenge requires a multidisciplinary approach by chemists and medical physiologists.