TISSUE OXYGEN TENSIONS UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

(ZUURSTOFMETINGEN IN WEEFSEL ONDER FYSIOLOGISCHE EN PATHOLOGISCHE OMSTANDIGHEDEN)

PROEFSCHRIFT

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To my parents
and Linda

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

For the normal function and survival of an organism, a continuous supply of energy is necessary. Without energy nearly all physiological processes, such as electrolyte pumping, could not take place; in other words, Cannon's 'homeostasis' would not exist. On the cellular level, oxydative phosphorylation produces energy. This process is maintained by an almost continuous supply of oxygen. Therefore oxygen is the decisive vital parameter in (human) life. Disturbance in tissue respiration causes a decrease in cellular function, reversible functional breakdown and, finally, irreversible cell death.

Oxygen transport to tissue occurs in three steps:

1. oxygen uptake in the lung
2. oxygen transport in blood
3. diffusion of oxygen from the capillaries to the tissue and through the tissue to the cells

When pulmonary gas exchange, cardiac output and oxygen transport capacity of the blood are within the normal range, oxygen supply to the tissue is furthermore dependent on the following micro-physiological parameters:

1. Distribution/perfusion ratio of the capillary meshwork
2. Oxygen consumption of the cell
3. Oxygen diffusion parameters from capillaries to tissue, through the tissue and across the cell membrane into the cell

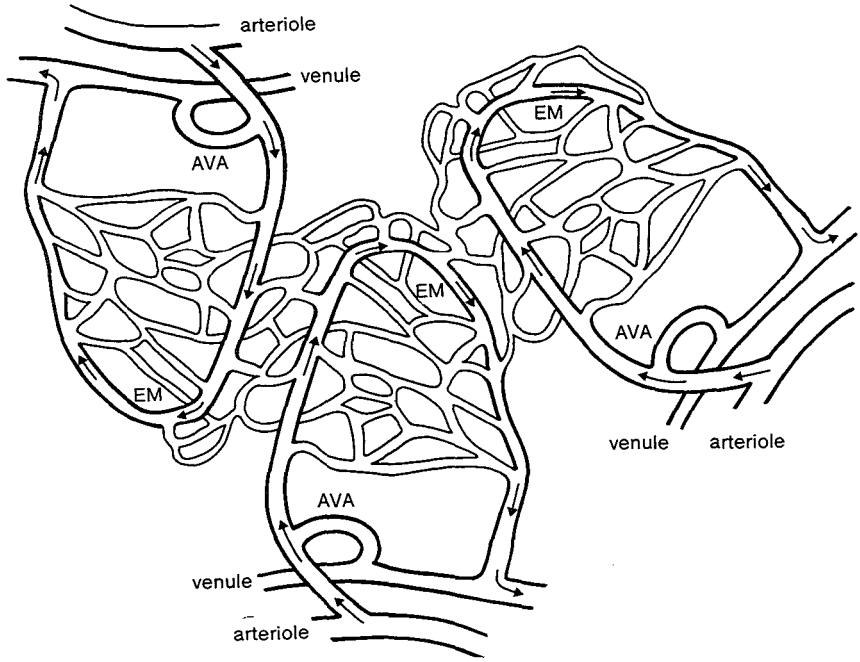

Fig. 1: Two-dimensional schematic drawing of a micro-circulatory unit.
AVA = arteriovenous anastomosis
EM = end of metarteriole

Normally, capillary perfusion and oxygen consumption are in balance and oxygen supply to the cell is continuously autoregulated to its needs. Fig. 1 shows that (micro-)collaterals between capillaries of different arterioles can help to maintain an adequate supply of oxygen to all areas. However, many pathophysiological conditions can severely interfere with this balance, for example: changes in blood PO_2, PCO_2, or pH; acute or chronic occlusion of blood vessels, edema, hypothermia and certain pharmacological drugs. From fig. 1, it can be seen that if, for instance, one of the arterioles is completely blocked, certain parts of the capillary meshwork might not be sufficiently supplied with oxygenated blood, even though the other arterioles might partially compensate. If the distance between this micro-area and the nearest well-oxygenated capillary is too great, the oxygen pressure could fall so low as to cause cell death (fig. 2).

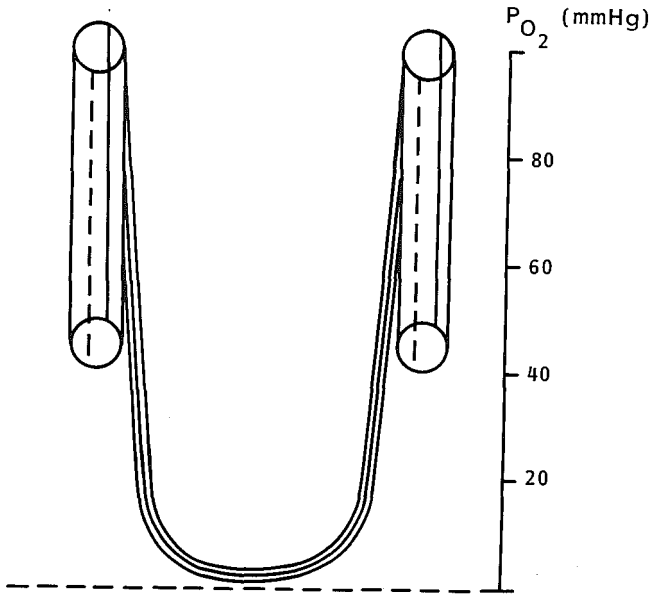

Fig. 2: Oxygen distribution between two capillaries. The tissue PO_2 in those areas which are not in the immediate neighbourhood of a (well-oxygenated) capillary may be very low.

Studies of macrophysiological parameters such as blood gases, blood pressures, blood flow and oxygen consumption of whole organs have been possible for years. In the absence of suitable measuring techniques, many scientists have attempted to explain microphysiology with the aid of theoretical mathematical models. Fick was probably the first to approach the unanswered questions concerning O_2 supply when he stated his diffusion law's in the second half of the last century. Krogh's papers (1904-1918) provided another major step forward, when he discussed the theoretical oxygen partial pressure distribution in a tissue cylinder (fig. 3). These, and other workers, obviously had to make assumptions about the basic conditions in the (for them inaccessible) micro-area in order to develop mathematical models. These assumptions were:

1. Homogenous capillary pattern
2. Homogenous capillary perfusion
3. Homogenous oxygen consumption in the intercapillary tissue
4. Homogenous diffusion coefficient
5. No shunt perfusion
6. No shunt diffusion, or homogenous shunt diffusion.

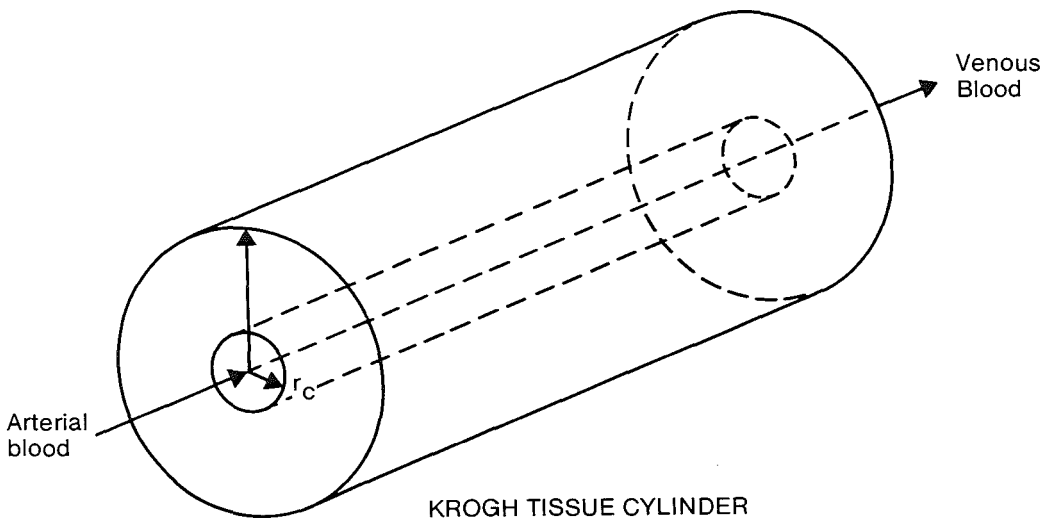

Fig. 3: Krogh's tissue cylinder model. R_c = radius of capillary. This is a two dimensional model, which only considers an average length of the capillary with an average arterial input and venous output.

For years, all calculations concentrated on two-dimensional capillary models. Three-dimensional models were only developed in recent years (Metzger 1969, 1973) (Fig. 4). Bearing in mind that all these computations were based on these assumptions, it is obvious that the information derived through these methods is only an approximation of the actual conditions in the microarea. Indeed, when one microscopically views a capillary network (best represented by fig. 1)

one can see that the capillary flow is a dynamic process, impossible to predict by man or computer.

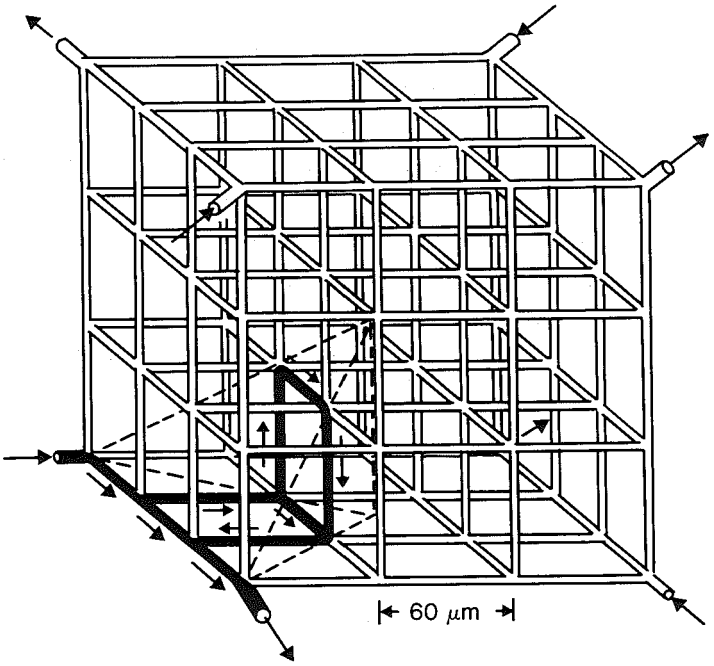

Fig. 4: Metzger's three-dimensional model of a capillary unit. This model is still based on the assumptions mentioned in the text, but takes into account differences in arterial inlet and venous outlet, so thus that one can compute that very low tissue PO_2 values are possible.

Another advance in understanding of oxygen supply to the microarea was the development of microelectrodes for the measurement of tissue oxygen partial pressures. Electrochemical measurements of oxygen with metal electrodes in an electrolyte solution have been performed since the end of the 19th century, when Daneel (1897) observed the reduction of oxygen in a watery solution on a noble metal surface to which a low voltage had been applied. Blinks and Skow (1938) applied this technique for biological purposes and Davies and Brink (1942) developed it for use in mammalian tissue. A renewal of the microelectrode system was made in 1959 by Cater, Silver and Wilson

with the development of a 10-micron platinum-in-glass electrode. Since then many types of electrodes have been produced.

The next chapter explains the theory behind and the fabrication of the microelectrodes that are used in the studies in later chapters. This electrode system, based on the original electrodes made by Erdmann, Krell, Metzger and Nixdorf (1970), are also suitable for simultaneous measurement of oxygen diffusion, tissue perfusion and action potentials (Kunke et al 1972, Erdmann & Krell 1976). For studies in (myocardial) muscle, commercial steel-protected micro-electrodes (Clark Electromedical Instruments) were used. Although these electrodes are thicker, the principles discussed in Chapter 2 remain the same.

In the last fifteen years an enormous number of publications have accumulated on oxygen transport to tissue. In this same period, a relatively small amount of work has taken place on oxygen partial pressure measurements with needle microelectrodes in vivo. This thesis gives a broad view of the implications of oxygen partial pressure measurements in physiological, pathological and even therapeutical conditions in experimental and clinical settings. In Chapter 3 the first example of the application of oxygen partial pressure measurements with needle microelectrodes in physiological conditions is given: a comparison between the oxygen tensions in the pre-ovulatory and non-ovulating ovarium follicle of the rat is shown. Chapter 4 is also basically concerned with physiological conditions, but also mentions the effects of hyperoxia and hypercapnia on the PO_2 in the rabbit cortex. Chapter 5 begins with investigations into normal oxygen partial pressure distributions in the myocardium of the pig. An attempt is made to explain the fluctuations in the readings. The following section shows the effects of acute and chronic ischaemia in the myocardium. After contemplating on the micro-circulatory changes during ischaemia, an experimental trial is presented in chapter 6 where fluorocarbons (oxygen carrying compounds) are used to try and improve the oxygen delivery to the areas of the myocardium where oxygen supply is impaired. Fluorocarbons might also improve oxygen supply and delivery in other tissues. Human extremities are perfused with fluorocarbons and their viability is monitored with, amongst others, oxygen measurements of the(tissue. A special pathophysio-

logical situation is hypothermia, which can be caused by exposure to a cold environment (especially in water), but can also be iatrogenic. Chapter 7 shows the effect of hypothermia on myocardial oxygenation.

AIMS OF THIS THESIS

The aims of this thesis are twofold:

I : to describe a reliable micro-electrode system for determination of oxygen partial pressures in the micro-area of the tissue and to demonstrate that it is applicable in exxerimental and clinical situations.

II: to demonstrate that measuring tissue oxygen partial pressures can increase the understanding of oxygenation in the micro-area and improve our insight in how to act in special clinical situations. This thesis attempts to solve the follgwang problems:

1. What is the normal oxygen pressure in tissues?
2. Is there a relationship between follicular PO_2 and ovulation?
3. What is the effect of infarction on myocardial oxygenation?
4. Do fluorocarbons amprove myocardial oxygenation after infarction?
5. Are fluorocarbons a suitable perfusate in traumatically amputated extremities?
6. What is the effect of hypothermia on tissue oxygenation?

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CHAPTER 2

METHODOLOGY:

ON LINE DETERMINATION OF PO_2, OXYGEN DIFFUSION, TISSUE PERFUSION AND ACTION POTENTIALS WITH A 10 MICRON TIP ELECTRODE SYSTEM

M. Fennema, W. Erdmann

In: Sensor 1985 (5) 8.2.1-17

INTRODUCTION

Cellular O_2 supply is continuously adjusted to meet dynamic needs of cellular metabolism. It is dependent on: arterial PO_2, O_2 transport capacity of the blood, tissue perfusion and oxygen diffusion from the erythrocytes to the cell. Lack of O_2 supply is followed by a breakdown of cellular functions (e.g. action potential generation in neurons). An electrode system has been developed to make it possible to investigate several of these parameters and their interactions simultaneously. The electrode system is based on the polarographic principle.

THE POLAROGRAPHIC PRINCIPLE.

This principle is derived from the phenomenon that a high ohmic layer is formed between an electrolyte solution and a noble metal at the point of contact (Helmholtz Double-layer) which prevents electrons from passing from the metal through the electrolyte to an

indifferent electrode when a voltage is supplied to the metal. This induces an overloading of electrons on the metal surface so that a potential difference is built up to equal that of the supplied voltage. When a suitable voltage is supplied to the noble metal electrode, electrochemical neutral molecules which are in the solution can be reduced (O_2) or oxidised (hydrogen), as soon as they have diffused into the double layer. These molecules therefore serve as electron acceptors or donators. The reduction of O_2 occurs in several steps. The comprehensive formula is as follows (Kolthoff and Lingane, 1952):

$$O_2 + 2H_2O + 2e^- \longrightarrow H_2O_2 + 2OH^-$$
$$H_2O_2 + 2e^- \longrightarrow 2OH^-$$
$$2OH^- + 2H^+ \longrightarrow 2H_2O$$

Oxidisation occurs as follows :

$$2H_2 + O_2 \longrightarrow 2OH^- + 2H^+$$
$$2H^+ + 2OH^- \longrightarrow 2H_2O_2 + 2e^-$$

The electrons consumed or supplied by this chemical process are compensated by a current flowing to or from the electrode surface. The indifferent electrode must not be polarisable; this means that there has to be a transmitter surface between the metal and the electrolyte, consisting of the metal in ionic form and an ion out of the solution. This can be done by chloridising silver, so that silver chloride acts as a transmitter layer. There are also other indifferent electrodes e.g. Calomel-mercury. The current measured in the circuit is dependent on the voltage supplied to the electrodes, on the amount of substance to be reduced or oxidised and on the kind of metal used. When the voltage current curve for O_2 is registered (fig. 1), nearly stable levels of current can be observed between -500 and -1100 mV (gold); the so-called plateau for gold. The plateau has a range of 350 mV for platinum and 600 mV for gold. When the applied voltage is further increased, the current increases again because now other molecules or ions in the solution take over acceptor function.

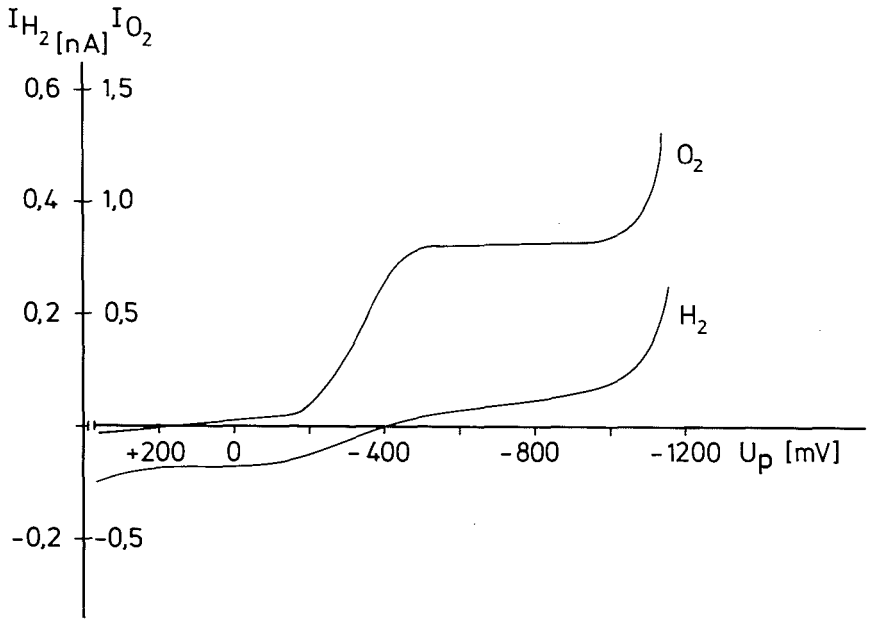

Fig. 1: O_2 and H_2 polarograms of a 10 micron gold electrode. The O_2 curve has a broad plateau between -500 and -1100 mV, the H_2 curve between +200 and -100 mV, and a secondary less characteristic one between -600 and -1000 mV. As the O_2 voltage current curve cuts the base line at about +200 to +100 mV, this would be the voltage range suitable for H_2-clearance measurements.

OXYGEN CONCENTRATION VERSUS PARTIAL PRESSURE MEASUREMENTS.

In the range of the plateau, a further increase in voltage does not induce an increase in current because all the electrons which are at the surface are immediately reduced. Thus, the current is only dependent on O_2 diffusion rate to the surface of the electrode (diffusion limited current). This diffusion rate is proportional to the O_2 concentration in the solution. This means that the measured current is proportional to the O_2 concentration in the solution. In medical research, as well as in clinical medicine, O_2 concentration is

of minor importance because it changes from micron to micron, depending on the highly variable O_2 solubility coefficient. The only comparable value in blood and tissue is the O_2 partial pressure, which according to the gas laws equilibrates in different mediums and is independent of the solubility coefficient dependent O_2 concentration:

O_2-concentration = $PO_2 \times \alpha O_2$

α = solubility coefficient

PO_2 = O_2 partial pressure

In order to change the concentration measuring system described above into a partial pressure measuring system, a constant medium has to be maintained on the electrode surface, which equilibrates with the partial pressure of the surrounding mediums. Therefore, a recessed electrolyte-filled chamber covers the noble metal surface, separated from the surroundings by an O_2 permeable membrane. The membrane material must also prevent proteins diffusing to the metal surface, as well as preventing contamination of the electrolyte solution, which would result in a change in its solubility coefficient.

OXYGEN DIFFUSION AND CONDUCTIVITY MEASUREMENTS.

When starting a pO_2 measurement with a bare electrode, a primarily high current is observed with a rapid exponential decrease (fig. 2). the time course of the diffusion limiting current can be divided into two parts:

1. an initial time dependent change of the diffusion limiting current with a steep decrease from a maximal value directly after closing the circuit.
2. after 30 msec., the diffusion limiting current approaches a constant final value, the stationary part of the curve.

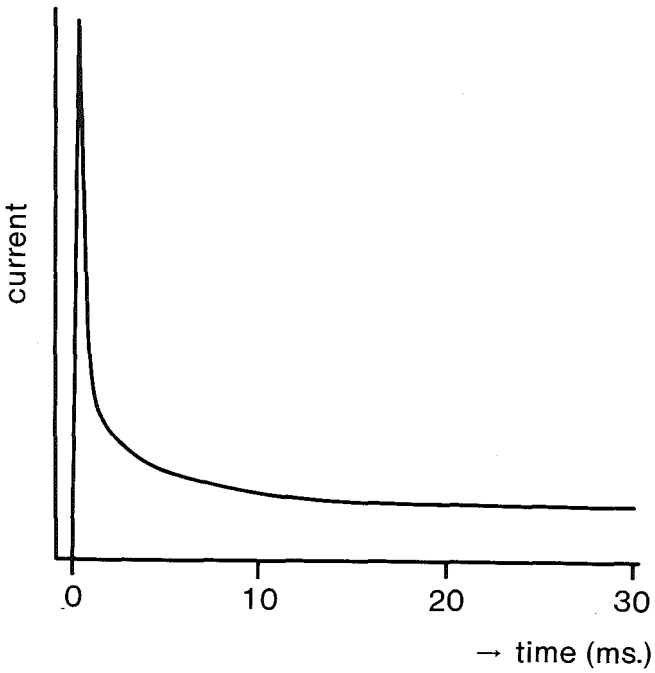

Fig. 2: Time course of diffusion limited current after closing the electric circuit for O_2 polarography.

Before measuring, the O_2 concentration is constant, also at the surface of the electrode. When the electric circuit is closed, all O_2 molecules directly adjacent to the electrode surface are reduced so that a maximal current is produced (together with the current necessary to load the electrode capacitance when closing the circuit). Due to this instant reduction of the molecules at the surface of the electrode, a steep concentration difference between the surrounding medium and the area adjacent to the electrode develops. O_2 molecules in the immediate area diffuse into the area and are reduced as soon as they reach the electrode surface. This means that an O_2 gradient field is building up around the electrode. Thus, in the course of time it takes the O_2 molecules longer to diffuse to the electrode surface; in other words the reduction rate is decreasing. This process continues until the oxygen gradient field has reached its final dimensions which are also determined by convection in the medium. At this point as many O_2 molecules will diffuse to the surface as are consumed there,

marking the second stable part of the current/time curve where the diffusion limited current remains constant. The time dependent change of the diffusion gradient during the first part of the curve is defined solely by the O_2 diffusion coefficient (DO_2). According to Fick's second law, the diffusion time is reciprocally proportional to DO_2. This is independent of the area geometrics (Thews, 1960).

A hypothetical case of O_2 diffusion through a membrane: it is assumed that the O_2 concentration at one side of the membrane remains constant, and that inside and behind the membrane there are no O_2 molecules. All O_2 molecules arriving behind the membrane are immediately counted and removed, thus keeping the O_2 molecule concentration constantly at zero. At the start of O_2 diffusion, molecules are diffusing into and through the membrane. The number of molecules arriving per time unit behind the membrane is now dependent on two membrane factors:

1. on the resistance with which the membrane material is opposing the diffusing O_2 molecules
2. on the number of O_2 molecules remaining in the membrane.

Instead of using resistance we may talk of the reciprocal value which is conductivity; in this case represented by the diffusion conductivity coefficient (KO_2). The number of molecules remaining inside the membrane is defined by the O_2 solubility coefficient (αO_2). When the membrane is saturated with O_2, the diffusion is only dependent on KO_2 in the membrane, and the surrounding PO_2, according to Thews (1960):

$$D = \frac{K}{60x\,\alpha}$$

The diffusion coefficient is directly proportional to KO_2 (diffusion conductivity coefficient) and inversely proportional to αO_2 (solubility coefficient).

Assuming that by means of the electrode and the connecting measuring system, O_2 molecules can be counted and eliminated, we can conclude that the primarily rapidly decreasing current is defined by DO_2 (as it is independent from αO_2 and KO_2) and that the final stable value is defined by KO_2 and PO_2 in the medium (Erdmann and Krell,

1976). Changes of DO_2 in a medium can only be recognised according to the change of the first part of the current/time curve. Changes in KO_2 correspond with changes in the stationary part of the final value, assuming that PO_2 and all other influences such as temperature, pH, convection etc. are kept constant. On the basis of these considerations, it is possible to evaluate at least the relative values of diffusion defining parameters. If a continuous measurement of the actual PO_2 is performed by membrane covered electrodes, it is possible to measure the DO_2, KO_2 and αO_2 with a second bare electrode.

Determination of DO_2.

As described above, the relationship between diffusion coefficient and diffusion time is reciprocal. The hyperbol function is taken for mathematical analysis of the time course of the adjustment curve:

$$1)\ Y = \frac{a}{X} + b$$

where X = time passed
Y = current values registered

b can be calculated according to:

$$b = \frac{X_1Y_1 - X_2Y_2}{X_1 - X_2}$$

a can be calculated with known b according to:

$$a = (Y-\bar{b})X$$

For the calculation of the unknown diffusion coefficient of a medium, $\bar{a}$ is measured in physiological NaCl with known $DO_2 = 2{,}0.10^{-5}$ ($cm.sec^{-1}$) (Goldstick, 1966). Then $\bar{a}$ of the medium with unknown DO_2 is measured and DO_2 is calculated according to:

$$2)\ DO_2 = \frac{2{,}0.10^{-5}}{a_1} \cdot a_2$$

a_1 = mean value of n constants of a in physiological NaCl
a_2 = mean value of n constants of a in analysed medium

Determination of KO_2.

Bare and membrane covered electrode tips are calibrated in a glycerine concentration with known $KO_2 = KO_{2(1)}$ (Gmelin, 1958). The output voltages of the two amplifiers are adjusted to the same value. The measurement is repeated in a second glycerine concentration with $KO_{2(2)}$ and the difference between the output voltages ($V_{d(1)}$) of the two electrodes is noted. This difference is proportional to the difference of KO_2 in the two solutions The KO_2 ($KO_{2(3)}$) of the medium to be analysed is calculated as follows:

$$3) \quad KO_{2(3)} = KO_{2(1)} + \frac{KO_{2(1)} - KO_{2(2)}}{V_{d(1)}} \cdot V_{d(2)}$$

$V_{d(1)}$ = difference of output voltages in second glycerine solution
$V_{d(2)}$ = difference of output voltage in analysed medium

CONSTRUCTION OF MICROELECTRODES.

Fabrication of gold/glass wire.

Gold is chosen for electrode material as it is known that gold is better suited for O_2 measurements than platinum. To simplify electrode production a newly developed method, similar to the Taylor method (1924), is used for constructing glass-covered gold wires (Nixdorf, 1967). They give a good basis for electrode construction and provide the possibility to construct 3-dimensional multi-electrodes. A glass tube is filled with gold dust and heated until the glass is soft and the metal has melted; then it is pulled out into a thin fibre. The feed ratio of the glass/metal combination, in relation to the drawing velocity of the fibre determines the diameter of the wire. This may be smaller than 1 micron. With this method the production of thin gold wire insulated by glass is simple, inexpensive and reproducible. The glass-gold wire is cut into pieces of 1 - 2 cm. These gold wire pieces are then put into a plastic tube cut at a 45^{o} angle. The intermediate space is filled out with a quick hardening plastic (Technovit 4071-d, Kulzer & Co., Germany). The electrode tips are then ground in three steps: Rough and fine sandpaper and finally leather polish. After the grinding process, the plastic block with the electrode tips in it is

put into acetone for a few hours to dissolve the plastic. The electrode tips are now ready; they have the same length, they have an oblique tip and are well ground with a defined gold surface.

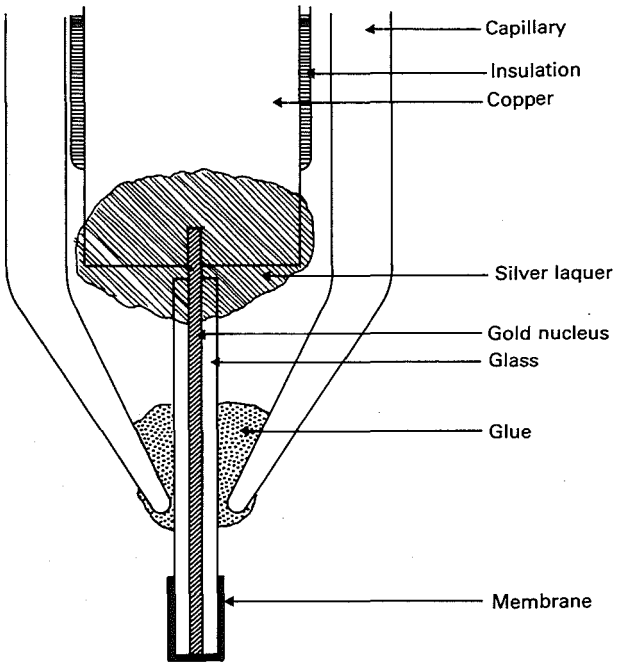

Fig. 3: Oxygen microelectrode.

Fixing the electrode tips to the conducting wire.

This is achieved with the use of a stereomicroscope. A gold-glass wire is stuck on a piece of adhesive tape. About 1 mm of glass is tweaked off the unground side of the gold core with the aid of tweezers. Then a conducting wire is fixed to a microdrive and brought into contact with the free gold end of the electrode tip. A drop of silver lacquer (Auromal 37 M, Doduco-Ng., Pforzheim, Germany) Fixes both wires together in a few seconds, giving better conductivity than soldering (see fig. 3). Finally the electrical contact is checked by connecting the electrode system to a 4 V battery and measuring the current flow.

Insertion of electrode system into guide capillaries.

For the final preparation of the electrodes, glass capillaries of 1 mm inner diameter are pulled out in a electrode puller by means of a

heated platinum sling (own design). The conical tip is carefully broken to a length of 0.5 - 1.5 cm, depending on the length of the gold-glass wire. The diameter of the conical tip can be regulated by the ratio of melting heat to pulling power. The glass capillaries are fixed under the microscope and gold-glass wires are carefully inserted under optical control. This procedure is easy because of the flexibility and stability of the gold-glass wire combination. If the wire bends, a short withdrawal is sufficient. The wire is inserted until it sticks out of the capillary at the desired length. A drop of waterproof glue is applied to both ends of the glass capillary. Capillary action sucks the glue up, ensuring a perfect seal. The electrodes are placed in the open air for about 3 hrs to harden the glue.

For production of the recessed chamber, the gold-glass conducting wire system is connected to a 9 V battery and introduced into a beaker of HCl. This is placed on a loudspeaker which vibrates at a controlled rate. A horizontally adjusted microscope permits checking of the chamber size. When ready, the electrodes are dipped into a solution of 3 molar KCl for the electrolyte in the measuring chamber and then into a special plastic such as Primal Ac+35. Primal produces very thin membranes down to 1 to 2 micron and adheres tightly to the surface of the electrode. The membrane characteristics of Primal are well suited to the stirring effect, do not affect response time too much and save recalibrating the values in different mediums (Krell 1973, Med Diss. Mainz).

Construction of PO_2 multi-microelectrodes.

One-tip microelectrodes are the basis for the production of multi-microelectrodes. A glass capillary is drawn out to a diameter which will suit as total diameter of the multi-microelectrode. Then a gold-glass wire with the conducting wire is inserted into the capillary and fixed as described above for one-tip electrodes. Six more electrode tips are fixed equidistantly to the outside by silver lacquer to form a hexagon. The electrode tips are left free and as long as the central gold-glass wire (fig. 4). By moving the multi-microelectrode into the tissue in defined steps, a 3-dimensional PO_2, tissue perfusion and action potential registration in a tissue cylinder can be performed.

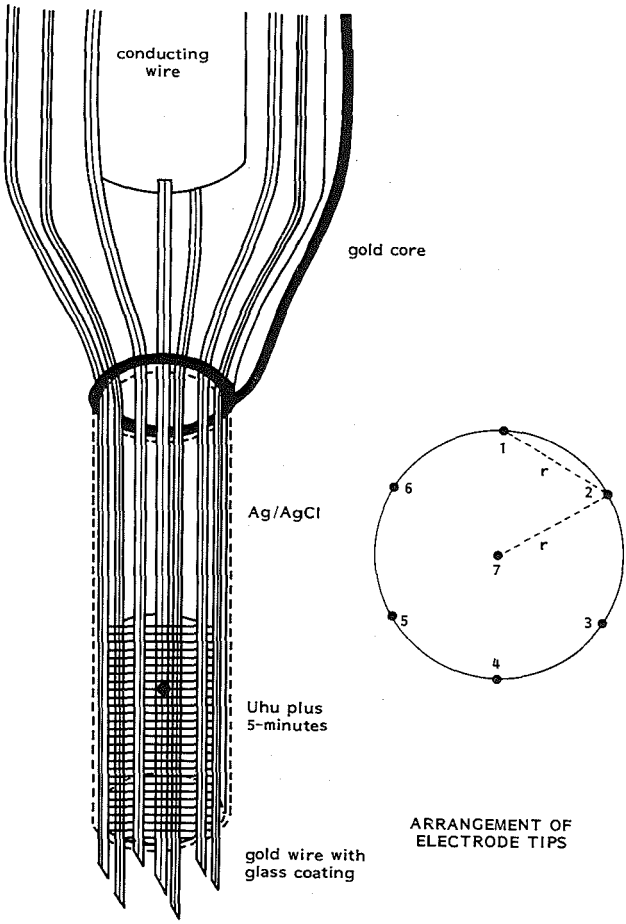

Fig. 4: Diagram of multi-microelectrodes.

Indifferent electrode.

For indifferent electrodes, Ag/AgCl electrodes can be used. For this purpose, a thin layer of the above described silver lacquer is applied to the outside of the electrode. In multi-electrodes, the lacquer used for fixing the electrodes to the capillary can be used. Sometimes a separate reference electrode is necessary (eg. when using custom-made myocardial electrodes). In this case, a silver wire electrolytically covered with AgCl can be inserted in the nearby tissue. As AgCl tends to form chemical complexes with protein, the bare AgCl electrode cannot be used for highly accurate measurements.

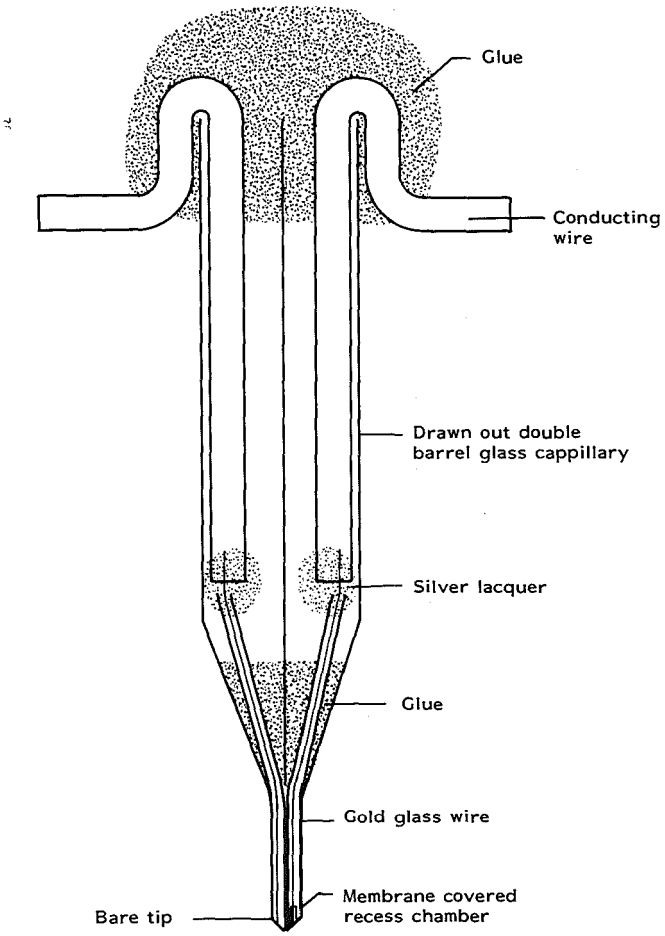

Fig. 5: Double-barrelled microelectrode for PO_2, O_2 concentration, KO_2 and O_2-solubility determination.

For this purpose the Ag/AgCl electrode is modified: a silver wire combined with AgCl is inserted into a glass capillary and one side is fixed with glue. This capillary is then filled with a 1 M KCl solution. The other side of the capillary is then closed with agar-agar in 1 M KCl. This way contact is prevented between tissue protein and AgCl while a reliable electrolyte bridge is ensured.

Construction of double-barrelled microelectrode for PO_2, O_2 concentration, DO_2, KO_2 and O_2 solubility determination. (fig. 5).

The measuring device consists of a double-barrelled O_2 microelectrode: a bare O_2 concentration measuring electrode and a membrane covered recessed tip PO_2 measuring electrode. The PO_2 electrode is calibrated in the polarographic circuit in 25% albumin at 150 mm Hg and in 0.2 M KCl at 150 mm Hg. Only electrodes which yield the same current in both solutions, proving absolute partial pressure measurements, are used for the studies.

Design of electronic equipment.

The PO_2 amplifying circuit.

The current measured by microelectrodes can only be registered through extremely high amplification. For this purpose, integrating amplifiers with field effect transistor input are used (10^{11} ohms input impedance, type 100902 or 1421, Philbrick/Niexus, Massachusetts, USA). These operational amplifiers (OA's) are characterised by maximal amplification (open feedback circuit) of 10^5; this means 100 microvolts at the input of the amplifiers is enough to obtain maximal output voltage (10 V). Because of the extremely high input impedance of the OA the current runs over the resistor in the feedback circuit. As the potential difference at the input of the amplifier is only 100 microvolts during maximal amplification, negative input of the amplifier merely is on mass potential. The potential drop of 100 microvolts is extremely small compared with the polarisation voltage of 800 millivolts; this means that the polarisation voltage is almost completely supplied to the electrodes. The voltage drop over the resistor in the feedback circuit is identical to the output voltage of the amplifier. As I_e runs over the resistor R, U_a will be according to ohm's law: $U_a = I_e.R$. The current running over the amplifier thus produces at the output of the amplifier a voltage which is directly proportional to the current. This voltage can be used to drive registrating apparatus. To obtain different amplification factors, changeable resistors from 10 megohm to 1 gigohm are brought into the feedback circuit.

Additional circuit for measurement of O_2 concentration and diffusion parameters.

A second polarographic circuit is connected to the bare electrode tip and O_2 concentration is registered. A circuit breaker is introduced which intermittently disconnects the bare electrode from the polarisation voltage while shorting it to the reference electrode. After re-application of the polarisation voltage a diffusion determined curve is registered from which DO_2 is calculated as previously described.

Equipment for combined O_2 and H_2-measurement.

It is also possible to measure hydrogen clearance with the same electrode tip. The time course of hydrogen clearance is used to determine tissue perfusion. For this purpose, the polarisation voltage must be rapidly changed from -800 mV for PO_2 measurements to +100 mV for PH_2 measurements. The demands on the amplification circuit are: rapid reproducible change of polarisation voltage within milliseconds and sensitivity for current measurements in the range of 10^{-11} to 10^{-6} amperes.

General circuit.

The polorisation voltage of -800 mV and +100 mV is supplied by 2 batteries over 2 voltage dividers. The desired polorisation voltage can be separately adjusted by means of 2 linear working 10 kilohm potential meters. When changing over from one polorisation voltage to the other, the measuring circuit is short-circuited for 10 to 100 msec so that capacitive components in the circuit are unloaded, ensuring rapid adjustment. The feedback circuit of the amplifying oa consist of 3 different resistors to change the amplification factor. A capacitor of 100 pf eliminates noise amplification.

Specified circuit for H_2 - O_2 polarography.

The very simply constructed electronic circuit described above is much more complicated when changing over from 3 batteries to one battery supplying the OA's and the polorisation voltages. This means that additional separating amplifiers have to be introduced to prevent interference between working and polorisation voltages. Furthermore, it is sometimes desirable to be able to register the voltage/current curve (polarogram) of the microelectrodes for H_2 and O_2. The elec-

tronic circuit is shown in fig. 6.

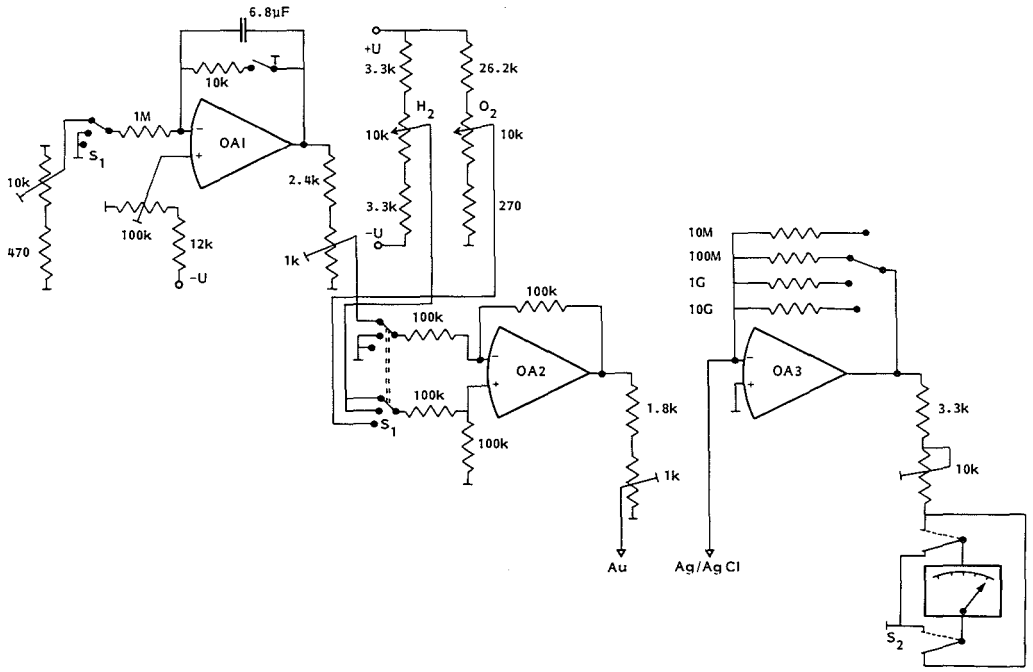

Fig. 6: Diagram of a specified H_2-O_2 measuring system.

Polarisation voltage is produced by the output of an OA (OA2). For the measuring circuit, a current to voltage converting amplifier, OA3, with a high input impedance of 10^{12} is used. The range of current measurement is changed by switching the feedback resistor. The polorisation voltage can be changed by means of different potentiometers. an integrator is included for automatic registrations of O_2- and H_2-polarograms. The integration time can be changed by introducing different resistors between battery and integrator OA1. The voltage range of integration is given by the position of the two potentiometers described above.

Electronic equipment for simultaneous action potential measurements and PO_2-H_2-polarography.

Sometimes it is necessary to obtain a functional relationship between the regional PO_2 value, tissue perfusion and one of the most important physiological parameters in the brain tissue: the local action potentials. The electronic circuit has been adapted so that

simultaneous registration of the action potentials and either tissue PO_2 or H_2 clearance is possible. In this case, two opposing requirements must be fulfilled: AP measurements have to be performed using an amplifier with a high input impedance, while PO_2 measurements require a current amplifier with a low input impedance. At first resistors (R_v) of up to 10^6 ohms were connected in series with the electrode (R_{el}; $R_v < R_{el}$), but their resistance was too low; one was essentially short circuiting the input of the dc amplifier, making the input signal almost unmeasurable. Later, resistors (R_v: R_{9-12}) of 10^7-10^10 ohms ($R_v > R_{el}$) were used. Under these circumstances one decreases sensitivity as well as adding non-linearity. Since the voltage between the electrodes (U_{el}) now changes in response to R_{el}. U_{el} becomes a function of R_{el} and R_v according to the formula $U_{el} = U_a . R_{el} . (R_{el} + R_v)$ (Erdmann, Kunke & Krell, 1973).

To obtain a constant polarisation voltage and to overcome the described difficulties, a feedback circuit is introduced. In the new system (fig. 7), the polarisation voltage is measured and regulated by an amplifier (OA1). The input of OA1 is connected via potentiometer R_1 to the negative pole of the power supply. R_1 is adjusted to the desired polarisation voltage. The output potential of OA1 corresponds to the difference between the desired polarisation voltage and the actual value. As the output potential should compensate for changes in polarisation voltage, the output value must be inverted (OA2). The output of OA2 is connected to the electrode in series with resistor R_v (in this case R_{10}).

Dynamic properties of the feedback circuit: The DC voltage across R_{el} is kept constant because OA2 only responds to very slow changes in potential. Therefore the input voltage to OA4 is a function of R_{el}, which is proportional to PO_2. OA4 is a leaky integrater and the output voltage is proportional to PO_2. OA1 and OA3 respond to rapid changes in potential and measure action potentials.

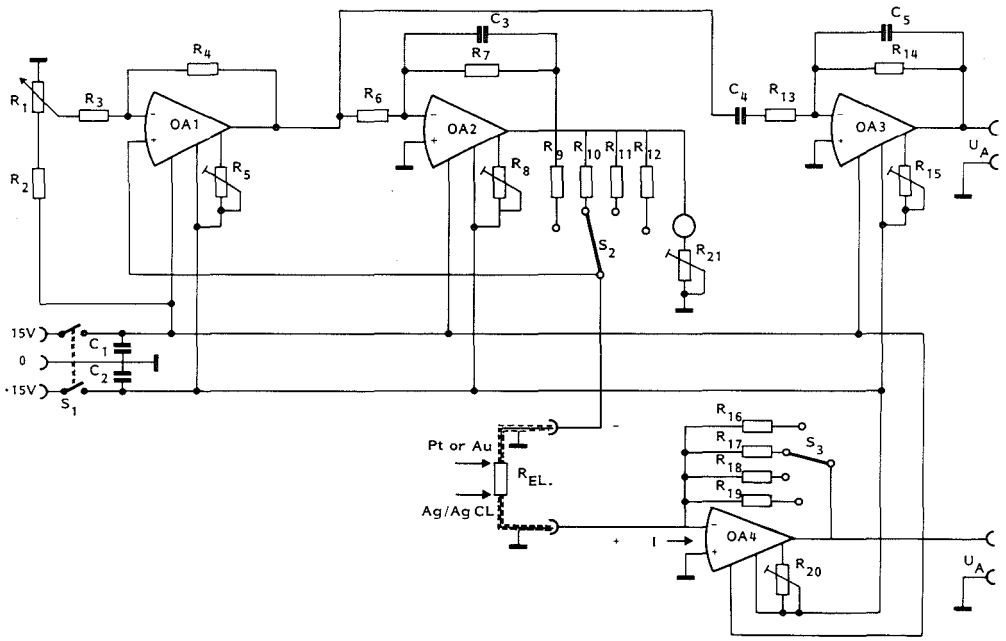

Fig. 7: Electric feedback circuit maintaining a constant polarisation voltage at a desired value. This voltage is measured by operational amplifier OA1. OA2: inverting amplifier; OA3: for AC amplification; OA4: O_2/H_2 amplifier. List of major resistance and capacitance values:

R_1 = 500 ohm	R_{13} = 10 k	C_3 = 100 nf
R_2 = 4.7 k	R_{14} = 1.2 M	C_4 = 220 nf
R_3 = 10 k	R_{16} = 10 M	C_5 = 8 pf
R_4 = 50 k	R_{17} = 80 M	
R_6 = 10 k	R_{18} = 680 M	
R_7 = 1 M	R_{19} = 1 G	
R_{10} = 100 M		

Accuracy of the polarisation voltage: Only a minimal potential difference of 2 mV at the input of OA1 is necessary for regulating the polarisation voltage to a constant value, because the open-loop

circuit amplifies 500 times. This means that the accuracy is within 0.2%.

AP measurements: Action potentials are coupled with the electrode system by capacitance of the plastic membrane and the electrical double layer at the electrode tip. OA1 has an amplification factor of 5; a secondary amplification (100) of the AP signal is obtained by OA3. Frequency linearisation is possible by varying capacitance C_4 and C_5. OA2 is a DC amplifier. The bandwidth is limited to about 5 Hz by means of a feedback capacity.

THE PROBLEMS OF SHIELDING WHEN WORKING WITH 10 MICRON ELECTRODES.

The signal to noise ratio can be very low because AP signals are sometimes far below 100 microvolts and PO_2-dependant currents in the picoampere range. Capacitive filtering cannot be used because the action potential signals would also be filtered out. Thus shielding is necessary. This must comply with several requirements:

a. Measurements must be performed in a closed metal cage.
b. Connection cables must be well shielded.
c. As registration instruments are supplied by line voltage, disturbances may occur. Another source of interference may come from registration instruments themselves (e.g. spikes from electromagnetic equipment). A disconnection should therefore be introduced between the shielded measuring circuit and the registrating circuit.

Shielding procedure:

The experiments are performed in a closed chamber of 5 mm steal with a second internal chamber of 2 mm copper; or in a highly shielded pre-fabricated chamber by Siemans (Faraday cage). All cables in the system are shielded. In this way outside disturbances are suppressed (fig. 8). However, disturbances are still caused by the registration system, through the output circuit of the amplifier, into the highly sensitive pre-amplifying system. These disturbances can only be suppressed by a total disconnection of the amplifying system and recording system. This absolute galvanic isolation can be achieved using an infra-red telemetric transmission of the information (fig. 9).

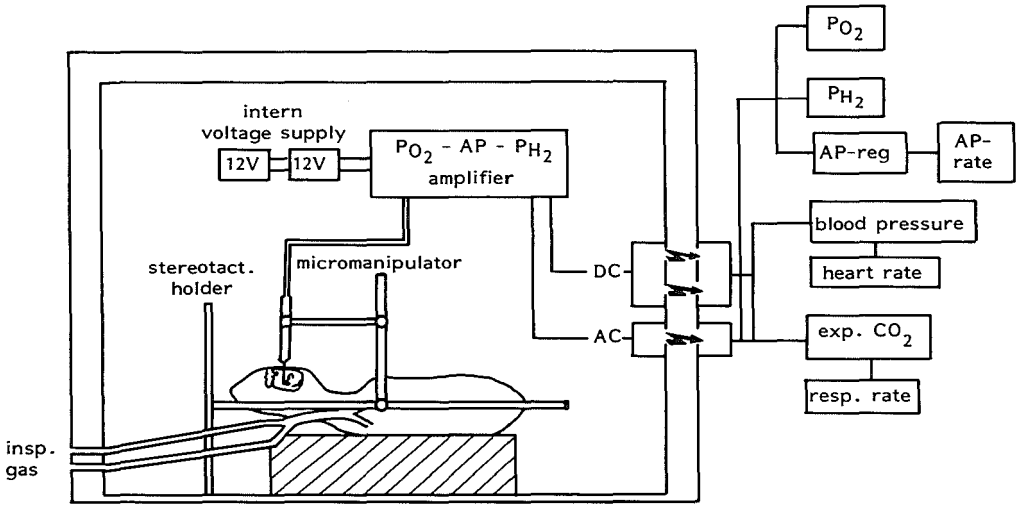

Fig. 8: Experimental procedure in highly shielded cage. The animal is fixed in a stereotactic holder inside a chamber of 2 mm copper surrounded by a chamber of 5 mm steel. The electrode is driven in the tissue by means of a micromanipulator. The signals are outputed via LED's so that no galvanic connection exists between the inside and outside of the cage.

Electronic equipment for AP and PO_2 - DO_2 transmission:

The voltage output of the amplifier drives a light-emitting diode (LED; gallium-arsenide diode). The light-modulated signal is received by a phototransistor, which is connected to another amplifier. High output voltage of the amplifying system causes high light intensity of the emitting diode and produces a higher current in the phototransistor (Through emitter and collector). The voltage drop in the emitter circuit of the phototransistor is amplified by the succeeding amplifier mentioned above. The output of this amplifier supplies the recording system.

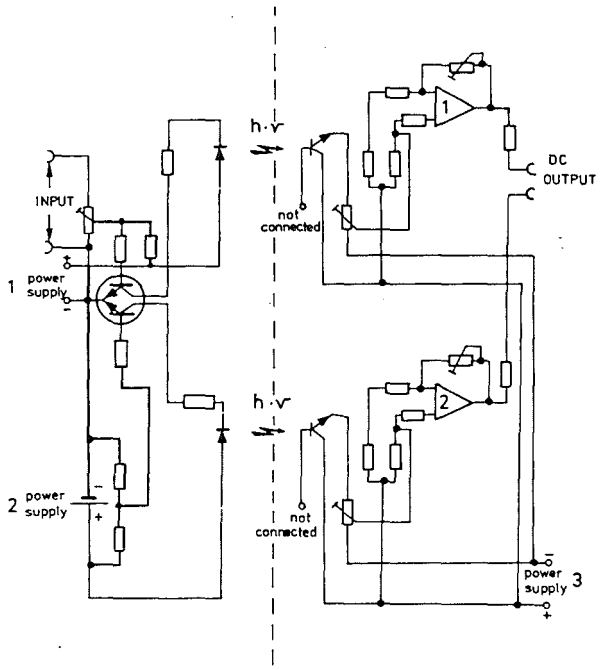

Fig. 9: Diagram of a symmetric two-channel electronic system for DC transmission with automatic temperature correction.

The LED is driven by the output of the pre-amplifying system, which means by the battery inside the cage. On the other hand, the photo-transistor and corresponding amplifier are supplied by their own battery, so that the pre-amplifying and recording system are fully disconnected. The active parts of these circuits are sensitive to temperature changes. So for PO_2/H_2 transmission, temperature stabilisation is achieved with a symmetric two-channel opto-electronic system driven by a double transistor. Both channels consist of exactly the same parts, implying that they have the same temperature error. One channel reproduces the information while the other shows a comparison (indifferent channel). As the output signal is picked up as the difference between the output of both channels, temperature error is compensated. To prevent loss of energy by diverging light, a glass fibre bundle can be used. That way, considerable distances can be bridged, even around corners.

Problems relating to the continuous registration of cardiovascular and respiratory parameters must also be taken into consideration. Two demands have to be fulfilled: (a) the electronic circuit must be DC supplied, working on batteries inside the cage; (b) all parameters must also be transmitted via light bridge as described above.

RECORDING EQUIPMENT.

All commonly used recorders for either DC or AC registration as well as oscilloscopes are applicable. As the output of the OA is always secured against accidental short circuiting by 1-10 kilohm resistors, the input impedence of the recorder should be at least 10 times higher (preferably 1 megaohm) in order to avoid voltage dividing problems.

CONCLUSION.

The described system has been routinely used in our laboratory to investigate basic physiological problems that were, until now, inaccesssible. The combined measurement of various micro-physiological parameters offers new insight into processes within the cell itself. This in turn has significant implications for clinical knowledge and, ultimately clinical treatment regimes.*

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GENERAL REMARKS ON PO_2 ELECTRODE CHARACTERISTICS

The previous section explains the theory behind PO_2 measurements, and gives details of the fabrication of the microelectrodes and the electronic circuits. In order to use the electrodes the reliability of the electrode system must also be tested. Therefore certain factors must be taken into consideration, including: linearity, calibration, drift, temperature and response time.

LINEARITY

This was checked by bubbling 100% N_2, 2% O_2, 5% O_2 and air through a bubble chamber into which the electrode was inserted. It could thus be shown that in all electrodes there was a positive linear relationship between the PO_2 and the readout of the amplifier or the deflection of the recorder. This linearity remains true even if there is a drift in the absolute measurements in the course of time. The drift itself, in these in vitro studies, also proved to be linear in the course of time. As described in the previous section, only those electrodes were used which produced the same results in saline as in protein solutions when equilibrated to the same PO_2.

CALIBRATION PROCEDURE

Before each experiment the electodes were inserted into a bubble chamber containing a saline solution kept at 37^{o} C. Gases of a known PO_2 were bubbled through the chamber and, after equilibration, the measurements recorded. The in vivo measurements could be then compared to the calibration points to calculate the actual PO_2.

DRIFT

There are two kinds of drift:

Baseline drift

If PO_2 is measured in 100% N_2, the current measured should be nil. This is only so, however, if the electrodes are allowed to stabilise for 1 to 2 hours (depending on the type of electrode) after polarisation. After this period of time the current measured with 0% O_2 is indiscriminantly low, thus this baseline drift can be discarded.

Measurement drift

The electrodes were also calibrated at the end of each experiment. Often a drift in the measured current was observed. It was therefore necessary to calculate the drift per time unit and correct all measurements. If the drift was more than 7.5% per hour, the electrode was discarded.

TEMPERATURE

Due to the physical qualities of the noble metal, the current produced by the microelectrodes is proportional to the temperature. In vitro it could be shown that this current is linear to the temperature between 20° C and 38° C at different PO_2's. With the aid of a Philips P2000 computer all measurements were corrected for temperature and drift.

RESPONSE TIME

The response time of the electrode system is difficult to measure because of the quick response of the microelectrodes. The response can only be demonstrated on an oscillograph in the form of the adjustment current as demonstrated in fig. 2 (previous section). In this figure it can easily be seen that adjustment to a terminal new PO_2 value takes less than 30 ms. To reach within 10% of this value takes less

than 10 ms. This is the case in all electrodes. Direct measurement of the response time is not possible (or necessary) as changes in PO_2 from one partial pressure environment to another takes longer than the response time itself.

CONCLUSION

Now that it is possible to fabricate a reliable electrode system that will produce realistic and reproducible results, it is possible to use this system in vivo. This is shown in the following chapters.

CHAPTER 3

OXYGEN TENSION IN THE PRE-OVULATORY AND NON-OVULATING FOLLICLE OF THE RAT

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INTRODUCTION

In 1978 Edwards and Steptoe were the first to fertilise an oocyte in vitro. Succesful reimplantation of the embryo subsequently led to the birth of the first 'test tube' baby. This procedure has been repeated many times and since 1982 has become routine in this institute (Zeilmaker et al, 1984). In view of these developments, it has become more and more important to understand the mechanisms determining ovulation, so that oocytes can be incubated under optimal conditions.

As shown in figure 1, ovulation commences after a sharp increase in the luteinising hormone (LH) and follicle stimulating hormone (FSH). Pincus (1935), after demonstrating in vitro oocyte maturation in the absence of hormonal changes, concluded that the hormonal changes that take place only have an indirect influence on the oocyte. Zeilmaker et al (1972) showed that, in vitro, oocytes incubated in a high oxygen concentration (90%) matured, while those incubated in the absence of oxygen did not. This in vivo investigation was performed to determine the relationship between oxygenation of the follicle and ovulation.

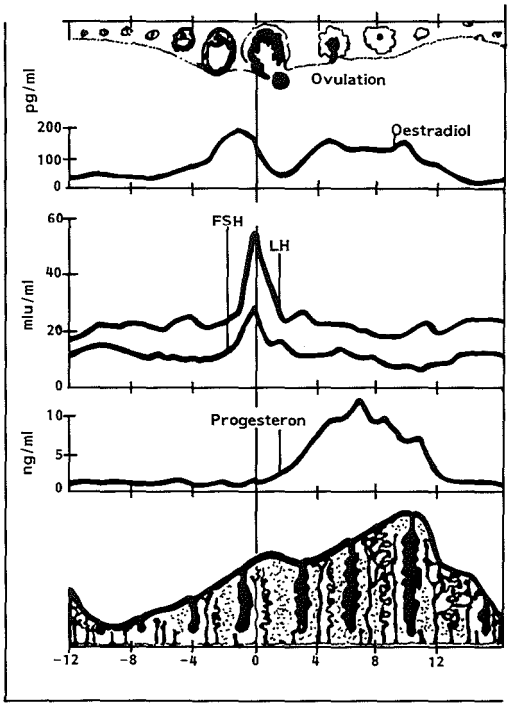

Fig. 1: Monthly changes in the human ovarium tissue (top) and the uterus (bottom) are induced by changes in gonadotropic hormone concentrations in the blood. The surge of secretion of luteinising hormone (LH) and the follicle stimulating hormone (FSH) leads to ovulation: the oocyte is released after the first meiotic division. Degeneration of the corpus luteum causes menstruation.

MATERIAL AND METHODS

Two groups of RxU rats were compared:

- A non-ovulating group of proestrus rats, where ovulation was inhibited by phenobarbitone (35 $mg.kg^{-1}$). This was always given at 13.00 hrs to ensure that experiments were always performed in the same phase in the oestrus cycle.

- An untreated pre-ovulatory group.

The measurements always took place between 16.00 and 18.00 hrs so that all the rats would be secreting the pre-ovulatory LH-surge. This was confirmed by examination of vaginal smear. Under tribromoethanol anaesthesia, the abdomen was opened, and an ovary exposed. An O_2 microelectrode was then introduced into an ovarian follicle using a micromanipulator and stereo-microscope (Leitz).

Polarographic techniques were employed to measure oxygen partial pressure (PO_2). When a noble metal electrode is negatively polarised, oxygen is reduced, causing a current to flow through the measuring circuit. At -800 mV, this current is directly proportional to the oxygen concentration, or the partial pressure if measured in a medium with a constant O_2-solubility coefficient, as described by Erdmann et al (1970). Briefly, these electrodes are made as follows (fig. 2): a glass-covered gold wire of approximately 10 microns diameter is glued with silver lacquer to a copper wire and this combination is inserted into a tapered glass capillary (2 mm diameter) so that the gold protrudes from the tapered end by about 0.5 mm. The gold wire is glued into the capillary, while the copper wire is also glued to the capillary so that it cannot be pulled out. The tip of the gold electrode is covered with an oxygen permeable plastic membrane (Rhoflex) so that the electrode measures in a medium with a constant O_2-solubility coefficient. This ensures correct partial pressure measurements. This membrane also prevents protein poisoning and minimises convection disturbances. A detailed description of the fabrication of the electrodes and electronic circuitry involved is described elsewhere* (Fennema and Erdmann 1985).

An hour before each series of measurements, a microelectrode was connected to the polarisation voltage, using a Ag/AgCl wire as reference electrode. This system was connected to a Knick nano-amperemeter which amplified the current measured so that the readings could be recorded on a Ricka Denki recorder model KA 60. Because the currents measured are so small (10^{-8} to 10^{-12} amperes), the measurements were performed in a Faraday cage.

*see chapter 2

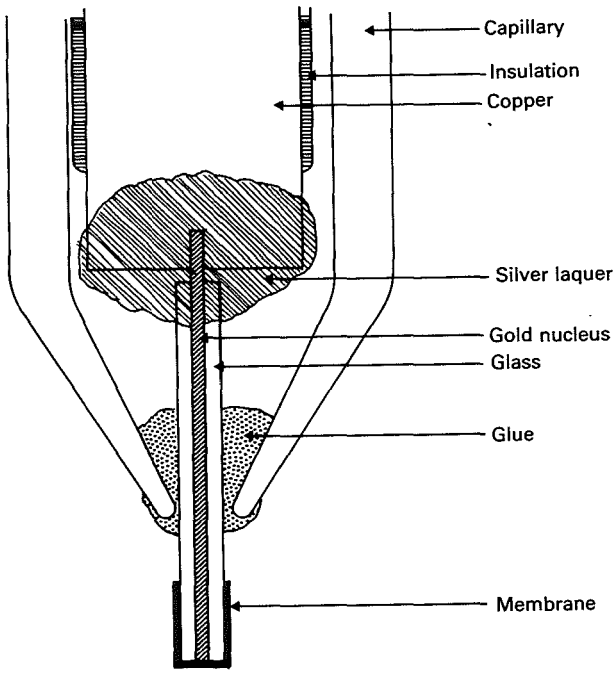

Fig. 2: Oxygen microelectrode.
This electrode consists of a gold wire of 1-5 microns, with a glass layer of the same thickness. The gold is connected to a conducting copper wire with silver lacquer. This wire is connected to the polarisation voltage (-800 mV). The gold and copper is protected by a glass capillary.

After stabilisation, the electrode was calibrated in a 37° C saline solution, through which gases containing 2 or 5% oxygen in nitrogen could be bubbled for equilibration. The electrodes were recalibrated after the experiment, and the results discarded if the drift was more than 7.5% per hour. After discarding measurements that did not meet the above mentioned criterea, there remained 41 different follicle oxygen tension measurements in the non-ovulating group, and 68 in the pre-ovulatory group*. Statistical analysis, consisting of unpaired

*12 and 16 rats resp.

Student-t tests and the Kolmogorow-Smirnow tests for distribution differences, were performed using a Commodore-64 microcomputer. Significance was accepted at a p value of less than 0.05.

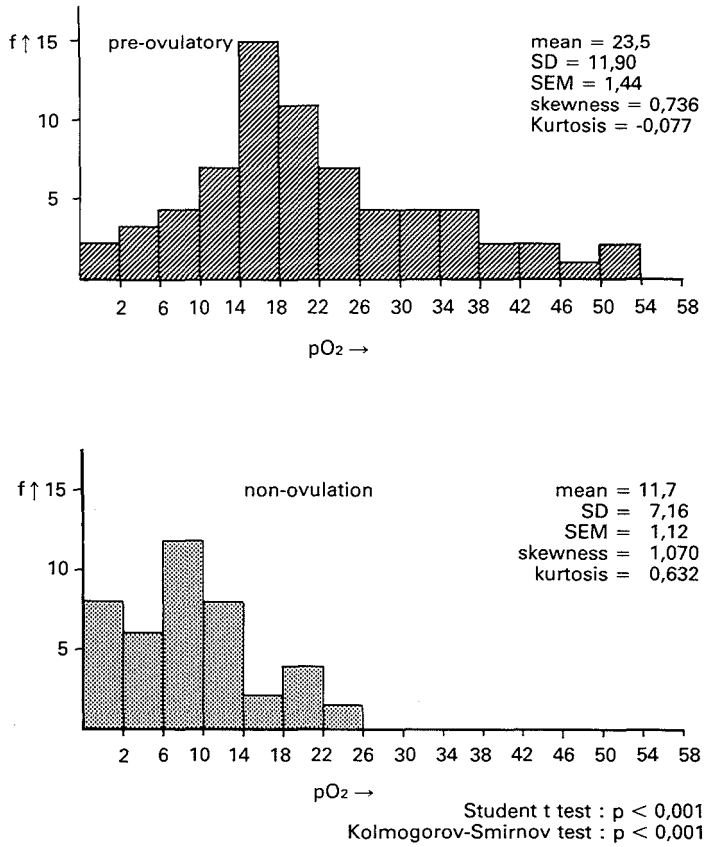

Fig. 3: Histograms of oxygen tension in the rat follicles.

RESULTS

The mean PO_2 in the non-ovulating ovarian follicles was 11.7 mm Hg (±1.12 SEM). This was lower than the oxygen tension in the ovulatory follicles, which showed values of 23.5 mm Hg (±1.44 SEM). These results were highly significant on an unpaired Student-t test ($p < 0.001$). As shown in figure 3, there was a significant difference in

PO_2 distribution between the two groups (Kolmogorow-Smirnow test, $p < 0.001$).

DISCUSSION

This investigation demonstrated that oxygen partial pressure in the ovarian follicle increased just before maturation of the oocyte. Zeilmaker et al (1974) have postulated that oxygen may be the limiting factor in the resumption of the first meiotic division in the pre-ovulatory oocyte. The PO_2 is probably critical, as Whiten (1969) has shown that incubation in a too high concentrations of O_2 is probably detrimental to oocyte and zygote development. Although some investigations have been performed (Zeilmaker et al, 1972, 1974), more biochemical information is needed to determine the relationship between LH increase, oxygen partial pressure increase, metabolism and ovulation. It is possible that a change in oxidation/reduction potentials in the follicular fluid effects the oocyte. Direct or indirect effect of LH might increase the oxygen permeability of the follicular wall and this would cause the PO_2 to increase. More ATP could be produced, which might enable continuous maturation of the oocyte.

More investigations are needed to establish the optimum PO_2 for oocyte and embryo (or zygote) incubation. The onset of ovulation and the time of reimplantation can then be manipulated to coincide with ideal uterine environment.

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CHAPTER 4

TISSUE OXYGEN TENSION IN THE CEREBRAL CORTEX OF THE RABBIT

INTRODUCTION

Oxygen supply to the tissue micro-area is dependent not only on cardiac minute volume, pulmonary gas exchange and the distribution of circulatory blood volume, but also on:

- capillary perfusion,
- oxygen diffusion through erythrocyte, plasma, capillary wall and interstitium,
- oxygen consumption of the cells in the surrounding area.

Conditions such as bleeding, shock, ischaemia and drug administration, as well as anaesthetic procedures, may markedly affect one or more of these factors. Thus simple external factors can affect many internal mechanisms with a resulting decrease or increase in tissue oxygen tension (PO_2). These changes may lead to a disturbance in the regulation of the supply/demand ratio, sometimes with severe adverse effects.

It is known that autoregulative mechanisms exist to maintain balance in the internal environment. Chen et al. (1978) and Erdmann (1978) have hypothesised that an autoregulative mechanism exists in the cell membrane which enables it to maintain an intracellular PO_2 at a (low) value of 5-6 mm Hg. Their studies showed that the relationship between intra- and intercellular PO_2 is not static, thus making prediction of PO_2 profiles by computer models difficult (Chapter 1).

This study was performed to test the reliability of the glass-gold microelectrodes in vivo, to assess the cerebral PO_2 profile under normal conditions of anaesthesia and to investigate the effects of

hyperoxia and hypercapnia on cerebral tissue PO_2 and its autoregulation.

MATERIAL AND METHODS

A pilot study was performed in 7 adult albino New Zealand rabbits (± 2.5 kg). For measurement of cortical PO_2, single-barrelled glass-protected gold microelectrodes (as described in Chapter 2) were used. Because such electrodes break with any lateral mechanical movement, the animal was treated to preclude all possible movement. This was achieved in the following manner:

A few days before the experiment the rabbit was subjected to induction of anaesthesia with halothane and operated on under strict sterile conditions. After exposing the frontal portion of the skull, a bolt was fixed to the skull by means of dental cement. The grip of the cement was strengthened by four small bolts, screwed into the skull around the large bolt head. The animal was then allowed to recuperate for three days. For the actual experiment (4th day), induction of anaesthesia was performed with thiopental (30 mg/kg i.v.) and the rabbit was intubated. Ventilation was maintained with 30% oxygen and 70% nitrous oxide using a Keuskamp infant ventilator, set to a frequency of 40 per minute. Muscle relaxation was achieved with administration of pancuronium (2 mg/hour) via an ear vein. The head of the rabbit was firmly attached to a modified stereotactic holder (own design) by means of the 'unicorn' bolt and the electrode attached to a micromanipulator as a fixed part of the stereotactic holder; thus movement of the rabbit's head, in relation to the electrode, was impossible.

Two small holes were drilled into the skull to facilitate EEG recordings. Arterial pressure was monitored by means of a catheter inserted in one of the femoral arteries. To check adequate ventilation, end expiratory CO_2 was measured by capnography at the entrance of the intubation tube. The tubing leading to the capnograph was ± 2 m long with a total volume of ± 2 ml, accounting for a time delay in registration of about 3 seconds when using a suction rate of 40 ml/min.

A burr hole was drilled above the right visual cortex. After removal of the dura mater, a thin layer of paraffine was applied to the cortex, supplemented by a layer of agar-agar to prevent O_2 leakage and minimise movement of the cortex due to ventilation. The electrode was calibrated and inserted into the cortex with a micromanipulator under visual guidance of a stereo-microscope. The reference electrode was inserted into a muscle exposed by the surgical wound. All parameters were registered and recorded on a mingograph recorder (Siemens).

The experiments were performed in a Faraday cage, while the ventilatory and registration apparatus were outside the cage to eliminate feedback noise and electrical interference (see Chapter 2). This meant that the length of tubing from the ventilator to the animal was about 2 m. Consequently, there was an enlarged system volume and a rather long latency period following changes in inspiratory gas concentrations.

RESULTS AND DISCUSSION

On insertion of the oxygen electrode into the cortex, with the aid of the micromanipulator, a PO_2-profile can be produced (fig. 1). This profile can be reasonably well reproduced by withdrawing and re-inserting the electrode. Changes in the PO_2 profile can, in part, be attributed to damage caused by the needle electrode. Another cause of differences in the profile are due to the fact that PO_2 is in a state of constant change, though these oscillations are relatively small providing that there are no changes in other parameters. With an FiO_2 of 0.3, the average PO_2 is about 6 mm Hg (fig. 2). Nearly all measurements are below 25 mm Hg; measurements above 50 mm Hg are rare, although they have been observed. These higher measurements are probably due to the proximity of the electrode to an arteriole (fig. 1). Although PO_2's vary in the broad range, local cerebral PO_2's in different regions and different animals showed the same characteristic changes when subjected to the same changes in some general physiological parameter.

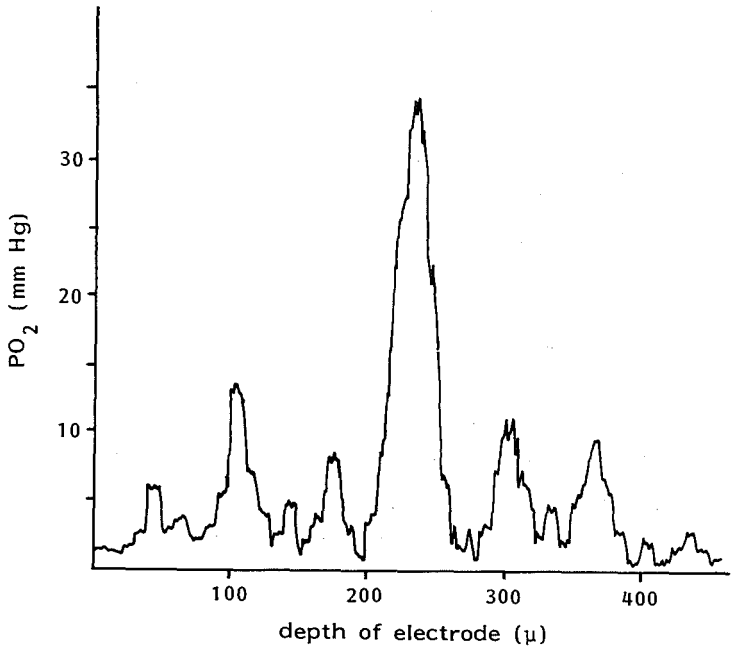

Fig. 1: Typical example of a PO_2 profile in the cortex of a rabbit. Note the high sensitivity of the electrode.

It should be noted that these electrodes can measure values less than 1 mm Hg. In these studies measurements of even lower values were not necessary as mitochondrial activity ceases at 0.4 mm Hg (Chance et al., 1957).

Figure 3 shows the typical pattern of cerebral tissue PO_2 following a change from an FiO_2 of 0.3 to 1.0. The arterial pressure and expiratory CO_2 remain constant. Whilst still ventilated with 30% O_2, the tissue PO_2 in this animal was $\pm$ 23 mm Hg. This high value is probably due to the electrode being close to a well-oxygenated arteriole. Measurements in a poorly-oxygenated area will produce less marked effects of hyperoxia. The period of more than one minute delay after the increase in inspiratory O_2 is partly due to the enlarged system volume of the tubing system. In addition, there is a time lag before the increased FiO_2 actually has an effect on the capillary and

tissue PO_2. After a preliminary almost two-fold increase in PO_2, which remained constant for about 10 seconds, the PO_2 gradually decreased to approximately 120% its original value. This effect is probably induced by autoregulatory vasoconstriction. Guyton (1981) suggests that this is possibly due to a direct relationship between tissue PO_2 and pre-capillary sphincter tension; an increase in PO_2 leads to an increase in the sphincter smooth muscle O_2, causing an increase in contraction and a decrease in blood flow. This in turn will lead to a decrease in tissue PO_2. The reverse effect occurs during hypoxia. Figure 3 shows an oscillatory drop in PO_2 as the autoregulatory mechanism attempts to maintain equilibrium. These oscillations were often observed in this study and other experiments (Chapter 5).

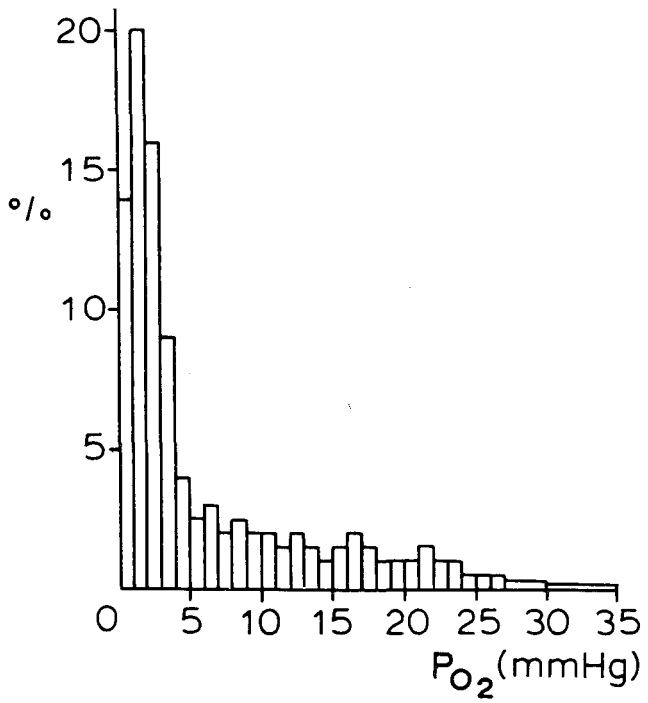

Fig. 2: Frequency distribution of cortical PO_2's in a rabbit.

Figure 4 shows the typical pattern of cerebral PO_2 when 12% CO_2 is added to the inspiratory gas mixture. This effect can be seen in the rapid increase to 16% in end expiratory CO_2, with a time delay of $\pm$ 10 seconds. The arterial pressure remains constant. PO_2 values increase

slowly to nearly three times their original value and show little tendency to compensate. This increase in PO_2 is due to a direct effect of CO_2 and H^+ on blood vessels, leading to vasodilation and a consequent increase in blood flow and tissue PO_2. In normal circumstances this increase in blood flow would eliminate CO_2, diminishing the vasodilation stimulus so that PO_2 would decrease. In this study CO_2 was kept artificially high, so that maximum vasodilation was maintained. It should be noted that in non-ventilated animals (or humans) a high arterial PCO_2 leads to hyperventilation, with an increase in tital volume, causing a transient rise in expiratory CO_2. Inspiratory CO_2 values of higher than 9% cause depression of respiration, rather than a further increase. This can ultimately lead to death.

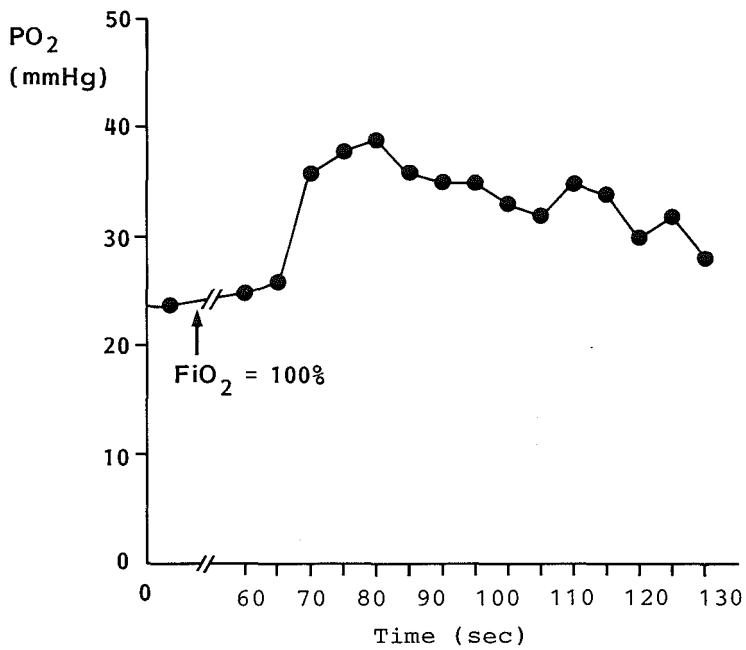

Fig. 3: An example of change in cerebral PO_2 in response to a change in FiO_2 from 0.3 to 1.0, in a rabbit.

This experiment demonstrates that, as is generally accepted, CO_2 stimulates an increase in blood flow. This effect is stronger than the effect of hypoxia. A level of 12% inspiratory CO_2 will lead to an increase in cerebral tissue PO_2. During the relatively short period of observation ($\pm$ 5 min) PO_2 values remained high; no compensation for the vasodilatory effect of CO_2 was observed.

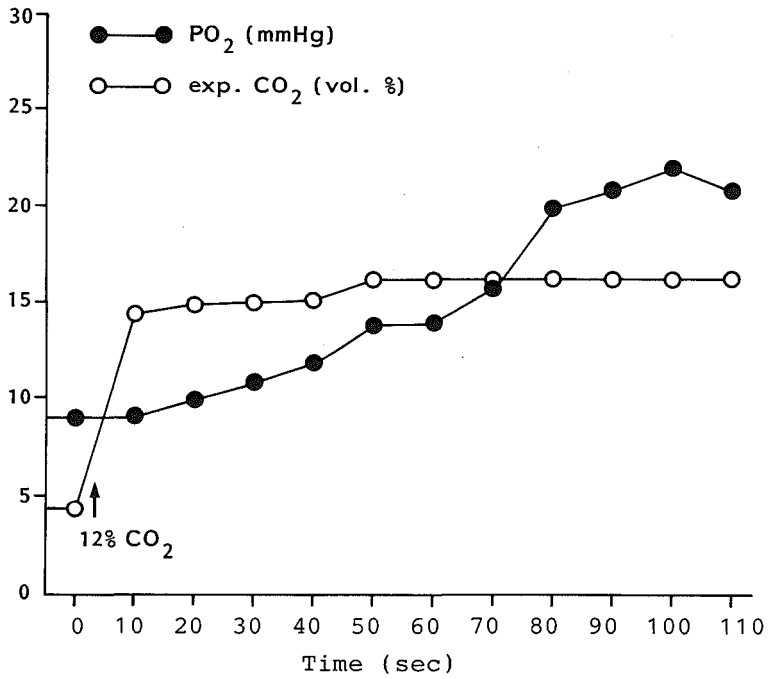

Fig. 4: An example of cerebral tissue PO_2 in a rabbit in relation to end expiratory CO_2.

The above results can be explained by relatively simple microphysiological mechanisms. These mechanisms provide an explanation for the changes in cerebral intercellular PO_2's. Erdmann et al. (1979) and Clark et al. (1978) showed that intracellular PO_2 can be regulated by changes in the tissue O_2 diffusion coefficient and that the changes in O_2 diffusion can have significant effects. More investigations using the technology described in Chapter 2 are needed to help explain the mechanisms involved in the causes of cell death (and survival) in critical situations.

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CHAPTER 5

OXYGENATION OF THE MYOCARDIUM

INTRODUCTION

Acute myocardial infarction continues to be a major cause of morbidity and mortality in spite of considerable advances in the treatment of this condition over the last decade. Currently, much effort is directed towards investigation of methods aiming to produce an increased salvage rate of the ischaemic myocardium. Many deaths still occur in the immediate post-infarction period. These are usually the result of fatal myocardial arrythmias occuring within two hours of the beginning of the ischaemic period (Corr and Sobel 1979) and are due to electrophysiological disturbances caused by hypoxia and the accumulation of noxious metabolites as a consequence of reduced tissue perfusion. In view of the vital importance of oxygen for the normal functioning of physiological processes it would seem logical to judge the severity of myocardial infarction by measuring changes in intramyocardial oxygen tension (PmO_2). Most of the studies on PmO_2 that have been performed in the beating heart have been carried out in dogs (Moss 1968, van der Laarse 1978, Reves et al. 1978, Rude et al. 1982, Rude et al. 1984, Schuchhardt 1985). This species is known to have a number of microscopic collateral vessels, in contrast to humans or pigs that have few such structures (Berne and Rubio 1979). As far as we are aware, no studies have been performed to determine the distribution of PmO_2 values in the myocardium of the pig. Nor have investigations been made on minute-to-minute changes in PmO_2 distribution during conditions of acute myocardial ischaemia. In this chapter, results based on PmO_2 measurements in pigs under physiological and pathological conditions will be presented.

MATERIAL AND METHODS

Juvenile Yorkshire pigs (± 25 kg) were anaesthetised with an intraperitoneal injection of sodium thiopentone at a dose of 30 $mg.kg^{-1}$ bodyweight. After the trachea had been intubated with a 7.5 mm Rusch cuffed endotracheal red rubber tube, anaesthesia was maintained with a mixture of nitrous oxide in oxygen (2:1) and 1-1.5% halothane. The animals were artificially ventilated using a Minivent ventilator, which was adjusted to maintain the arterial carbon dioxide partial pressure in the range of 35-45 mm Hg (4.7-6.0 kPa). This ventilator is especially suitable for use in the electrically noise-free atmosphere of the Faraday cage. Muscular relaxation was maintained by continuous infusion of pancuronium bromide at a rate of 0.5 $mg.kg^{-1}.hour^{-1}$ using a Braun Melsungen Perfusor pump.

A six French gauge BP 6 angiographic catheter (William Clark, Europe) was introduced into the femoral artery through an incision in the right inguinal region and a seven French gauge KMA thermodilution Swan Ganz catheter was introduced into the left femoral vein. The latter was advanced under pressure monitoring into a position in the pulmonary artery in which pulmonary capillary wedge pressure could be obtained. Thermodilution cardiac output measurements were performed using a KMA cardiac output computer Model 3500 E. Into the other femoral vein another Cook BP 6 angiographic catheter was passed into the inferior vena cava in order to obtain central venous pressure measurements. All the above pressure lines were connected to Gould Statham P23DC pressure transducers and the output amplified using a 12 channel Grass 7D polygraph. Via the right carotid artery, a Millar microtip pressure transducer PC470 was introduced into the left ventricle to measure left intraventricular pressure and dP/dt. The thorax was opened via a midline thoracotomy and the pericardium was opened. The heart was supported in a pericardial sling and an electromagnetic flow probe was mounted around the left circumflex coronary artery and connected to a Transflow 601 flowmeter (Skalar). A permanent recording was made using a Grass ink writing oscillograph. This data was fed into a Philips P2000 microcomputer so that the following parameters could be calculated: left and right ventricular minute work, systemic and pulmonary vascular resistance.

Mixed venous and arterial blood gas estimations were performed at regular intervals using a Radiometer ABL 1 acid/base laboratory. Arterial and mixed venous haemoglobin estimations and oxyhaemoglobin saturations were measured using a Radiometer OSM2 Hemoximeter.

Because the PO_2 measurements took place in the myocardium, it was impossible to use the glass-protected microelectrodes. Therefore we used steel-protected microelectrodes (Clark Electromedical Instruments). These electrodes, 200 microns in diameter, were embedded in a thin flexible plastic plate $\pm$ 5 cm x 2 cm, which was sewn onto the epicardium. The distance between electrodes was 1 cm and in position they protruded 3 mm into the left ventricular wall. With these electrodes it was also possible to measure local myocardial activity, using Grass AC EEG amplifiers. As described in Chapter 2, special adjustments to the electronic circuitry are necessary in cerebral action potential measurement, but this is not the case for measurements of myocardial activity because the ECG voltages are much higher and of a lower frequency than in EEG measurements.

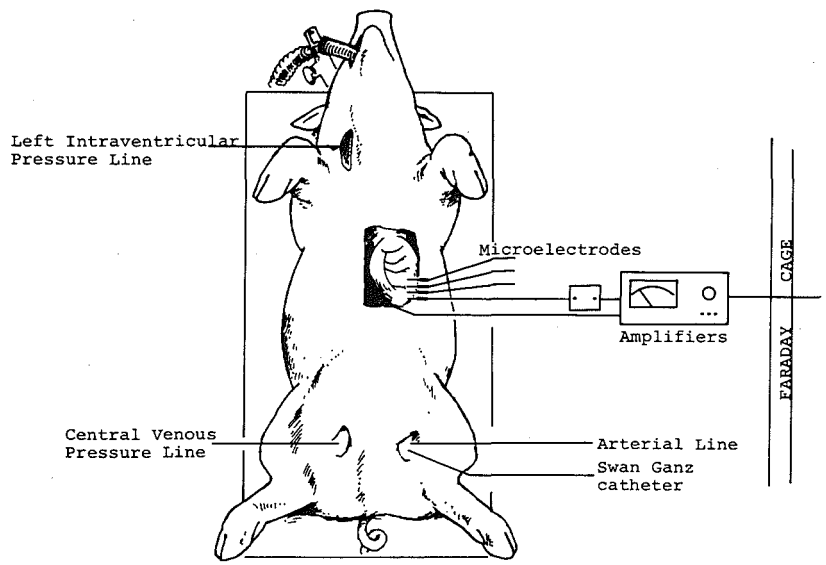

Fig. 1: Experimental arrangement of the animal in the electrically noise-free Faraday cage.

The electrode plate was sewn onto the epicardium in such a way that if the left anterior descending artery (LAD) was occluded at two-thirds its length two electrodes would be in the infarcted area (with one in deep ischaemia and one in the border area) and two in the non-ischaemic area (with one in the border area). Expertise in the correct placement of the plate was obtained in a pilot study of 10 pigs. Once this technique had been mastered, a follow-up study in 18 pigs was performed. The electrical output from the electrodes was amplified by DC amplifiers (CRW, Rotterdam) situated inside the Faraday cage and on-line PmO_2 was displayed on a Rikadenki recorder model KA 60 and the Grass oscillograph. Measurements were recorded after allowing time for stabilisation. The experimental arrangement is demonstrated diagramatically in figure 1.

All the measured and derived parameters were stored on floppy discs for statistical analysis. Statistical significance was assessed using paired Student's t tests. PmO_2 distributions were compared using the Kolmogorov-Smirnov test.

The following four sections attempt to explain oxygenation under normal circumstances (or as close as possible under experimental conditions). The remaining two sections describe the effects of ischaemia. Results on experiments attempting to improve myocardial oxygenation under ischaemic conditions are presented in Chapter 6.

During the pilot study it became apparent that oxygen pressure values measured at the tip of the microelectrodes varied considerably between individual electrodes. This depended on the distance of the electrode from a blood vessel and the state of oxygenation of that blood vessel (see chapters 1 and 4). Also, the oxygen values measured with each electrode were not constant in time but showed, in all studies, two patterns of change which will be described in the following two sections.

Results

Almost invariably, variations in oxygen tension coinciding with heart beat were observed, the amplitude of which varied between individual electrodes. The degree of variation depended on the oxygenation state of the heart. In general, the higher the PmO_2 measurement the greater the variation in the PmO_2 throughout the heart cycle.

Individual electrodes displayed a constant variation in PmO_2 depending on the phase of the heart cycle. Figure 2 shows that, in this electrode, maximum intramyocardial oxygen tension coincided with ventricular diastole - when the left ventricular pressure was at its lowest.

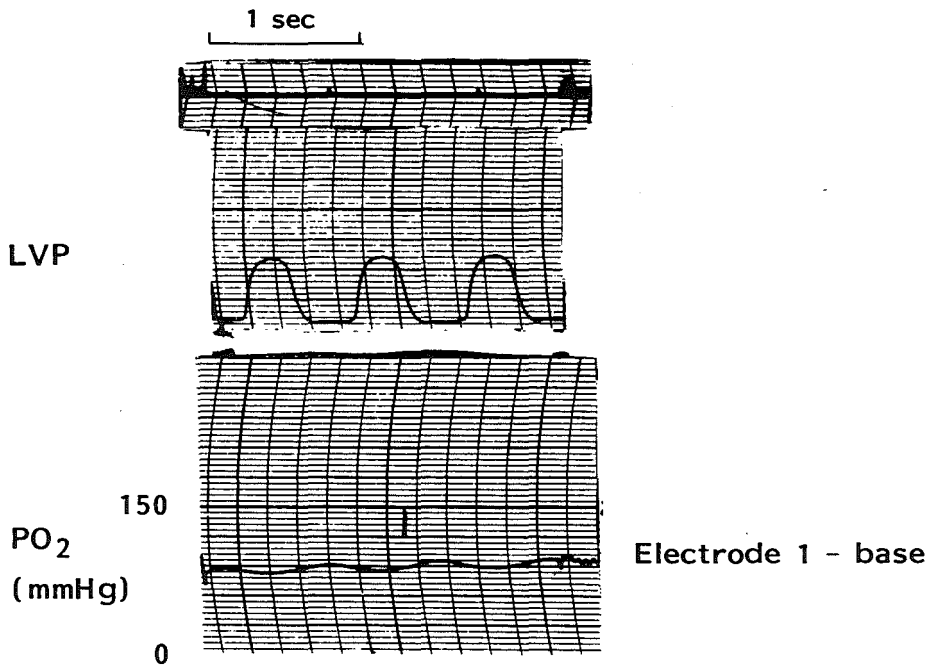

Fig. 2: Tracings demonstrating a rise in myocardial oxygen tension during ventricular diastole.

Figure 3 shows that maximal coronary flow coincides with the dicrotic notch of the arterial pressure. This notch is caused by the closing of the aortic valve, which implies that maximal coronary flow occurs during diastole (Marston et al, 1959). In electrodes 1 and 3 maximum PO_2 values were reached about 200 msec after aortic valve closure (during diastole) so that there seems to be a postive correlation between coronary flow and PmO_2. In figure 4 this pattern is repeated in electrodes 3 and 4, but peak oxygen values in electrodes 1 and 2 were reached during ventricular systole. This figure also shows that the amplitude of PO_2 variation is closely related to the mean myocardial O_2 tension at that electrode. These differences in both the timing of peak values and the degree of amplitude of PO_2 were a common feature in all studies.

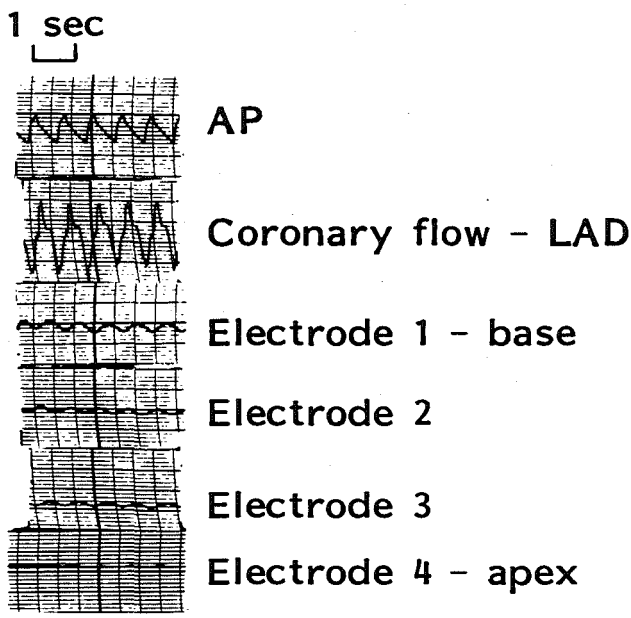

Fig. 3: An example of the relationship between myocardial oxygen tension and coronary artery flow.

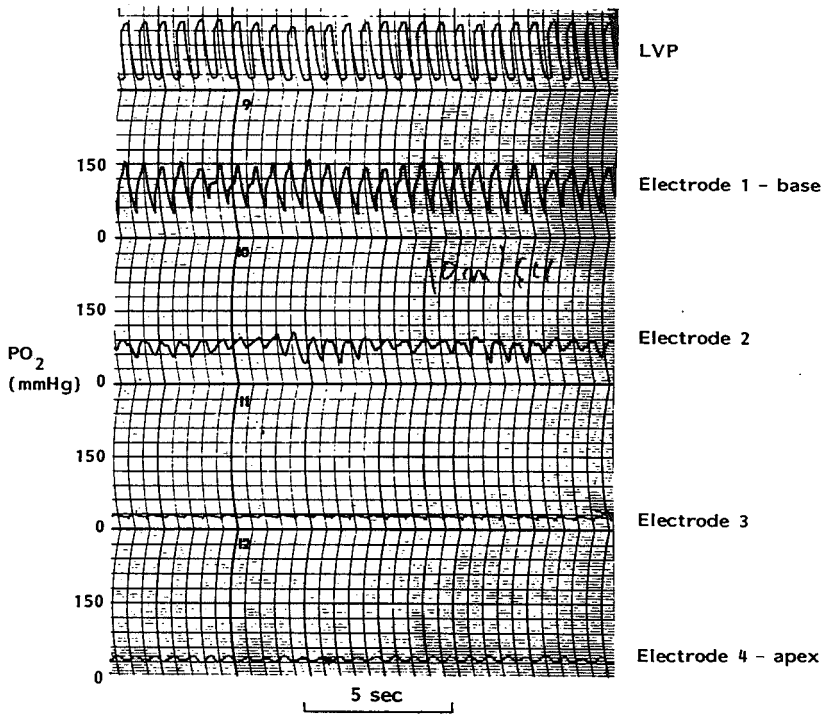

Fig. 4: An example of the relationship between myocardial oxygen tension and intraventricular pressure.

Discussion

The beat-to-beat period of the pig's heart cycle is about 600 msec. That means that 300 msec is the maximal phasic difference in PO_2 that can be measured between two electrodes. Chapter 2 demonstrates that the response time of the electrode system is at the most 30 msec, so that even if the response time per electrode differs slightly, this could never explain the phasic difference in PO_2 measurements. Therefore there must be a physiological explanation for the phasic difference in cardiac swings.

As mentioned previously, coronary artery blood flow occurs predominantly during diastole in the period immediately following

closure of the aortic valve. These investigations have shown, however, that this observation does not necessarily imply arrival of oxygen to the tissues during diastole.

The blood supply of the myocardium is modified by extravascular compression of the coronary vessels as they course through the myocardium, and Gregg (1963) has shown that this may cause a small back flow in the artery at the beginning of systole. This effect is demonstrated in figure 3. Whereas flow in the main coronary arteries of the left ventricle is greater in diastole than in systole, the reverse is true for the right ventricle, in which flow during systole is not greatly opposed by the much smaller intra-mural tension generated in this chamber.

The above statements are true for the major coronary arteries, though they do not apply in the case of microcirculation. Tillmans et al (1974), using high-speed cinematography with transillumination of the left ventricle, have studied flow in arterioles, capillaries and venules in turtle and dog hearts. They demonstrated that there is a transition of flow pattern from predominantly diastolic flow to predominantly systolic flow at the region of the arteriolar-capillary junction. Hence, it is reasonable to conclude that the maximum oxygenation measured with microelectrodes will vary, depending on where they are positioned. For example, when the microelectrode is situated near an arteriole, maximum oxygenation will occur in diastole; when the microelectrode is placed in the vicinity of a capillary or venule, however, maximum oxygenation will occur in systole.

This condition is further complicated by the spread of contraction through the ventricles. This spread begins in the intraventricular septum and at the atrial ventricular region. Spread through the ventricular walls passes from endocardium to epicardium. Harris (1941) has shown that the last portion of the ventricle to be activated is the apex. Hence, in some parts of the ventricle, blood will be squeezed onwards by extra-vascular compression during systole, slightly earlier than in other parts of the ventricle. This causes further variation in the precise timing of the arrival of maximal oxygen flux in the myocardium during systole.

RESPIRATORY SWINGS

In addition to variation in PmO_2 during the cardiac cycle, variations in oxygen pressure measurements coinciding with different phases of the respiratory cycle were also observed: during inspiration there was always a downward deflection of PmO_2. These respiratory swings were sometimes very large and, superimposed on the cardiac swings, caused variations of up to 10% in measured PO_2. Figure 5 shows examples of these swings in two different electrodes.

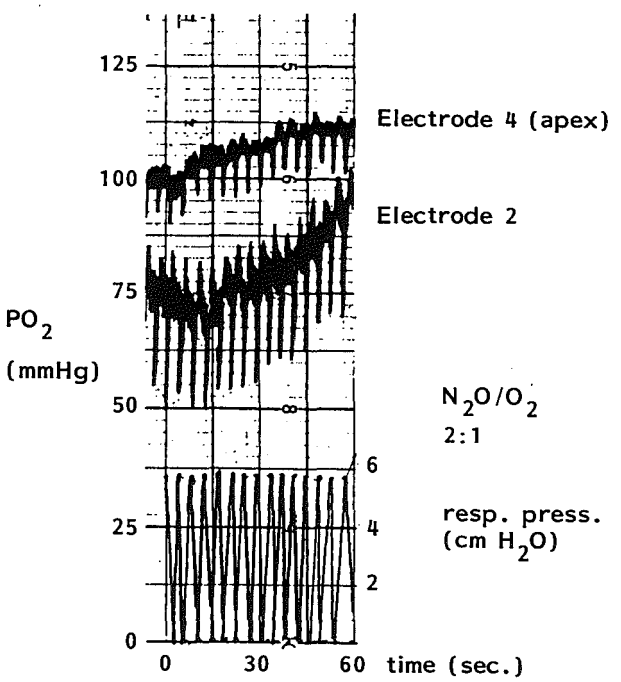

Fig. 5: Examples of variation in myocardial oxygen tension in relation to the respiratory cycle

In Figure 5 the large downward deflections of PO_2 coincide with inspiration and, hence, increased intravascular pulmonary pressure (Fig. 6) caused by intermittent positive pressure ventilation (IPPV). These decreases in PmO_2 also coincide with a rise in CVP, PAP and AP. The following factors are involved in causing these changes: inflation of the lungs leads to an increase in blood flow to the left atrium,

this in turn increases left ventricular fibre length and thus increases the force of contraction, leading to increased cardiac work load concomitant with a rise in interstitial static pressure. Coronary blood flow decreases and myocardial oxygen consumption increases leading to a fall in oxygen concentration in the myocardial tissue (change in the supply/demand ratio).

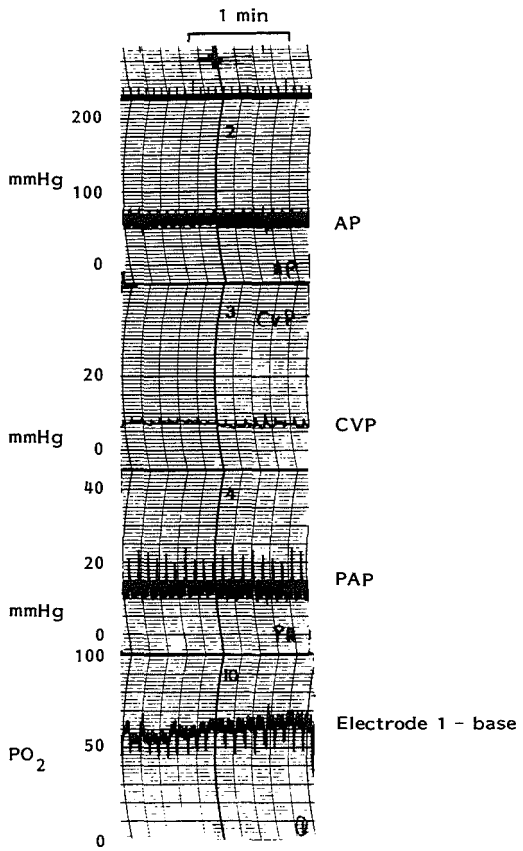

Fig. 6: An example of the relationship between myocardial oxygen tension and various cardiovascular parameters.

ELECTRICAL ACTIVITY

Generally, the amplitude of electrical activity measured at the tip of the electrode was in proportion to measured PmO_2. There appeared to

be a critical PmO_2 below which the amplitude of electrical activity was very low and only electrical background noise remained. These effects are shown in Fig. 7, which demonstrates that electrical activity markedly declined when, in this example, PmO_2 measurements were below 30 mm Hg. The critical PmO_2 level, however varied greatly depending on the area being measured.

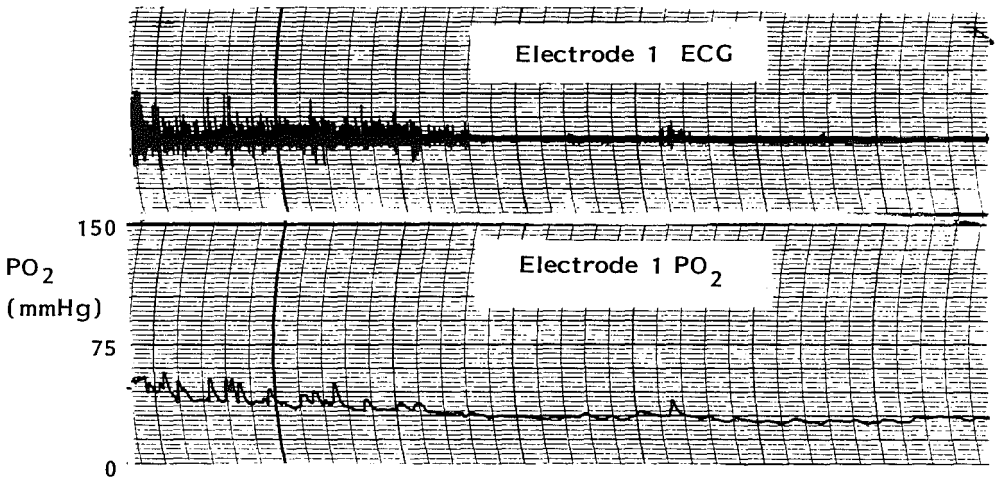

Fig. 7: An example of decreases in local action potentials concomitant with decreases in local myocardial oxygen tension.

CHANGES IN THE INSPIRATORY PERCENTAGE OF OXYGEN (FiO2)

An increased percentage of oxygen in O_2:N_2O ratio of the inspired gas mixture led to changes in PmO_2. A delay of up to 10 minutes was observed, depending on the length of the tubing in the ventilatory system. Paradoxically, changes in PO_2 measurements showed not only an increase but, in some electrodes, a decrease. Thus, the range of PO_2 values increased, as did the average value. This, and the fact that there was already a wide spread in PmO_2's at an FiO_2 of 0.3 (see next section) means that there is a poor correlation between arterial PO_2 and myocardial PO_2.

Figure 8 shows a histogram of 58 PmO_2 measurements in 18 pigs ventilated with 100% O_2. The mean PmO_2 was 112.7 mm Hg (± 8.48 SEM) with a median value of 99.2 mm Hg. More than 75% of the values lay between 40 and 140 mm Hg.

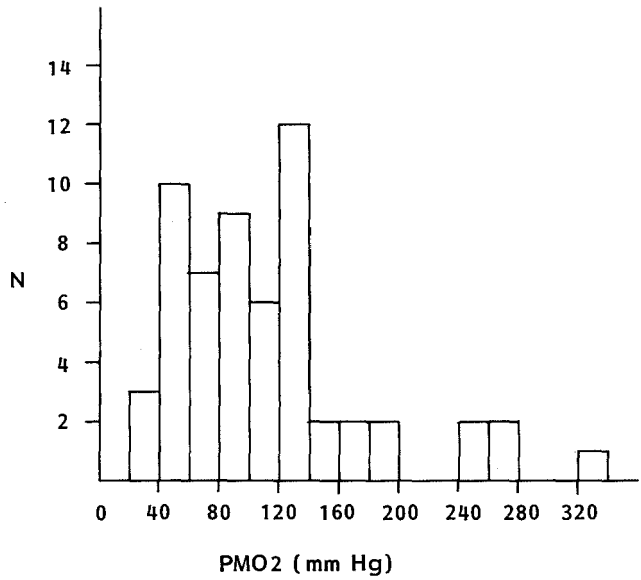

Fig. 8: Distribution of myocardial oxygen tensions (PmO_2) in 18 pigs ventilated with 0,5% halothane in oxygen.

TEMPORARY OCCLUSION OF THE LEFT ANTERIOR DESCENDING CORONARY ARTERY

As mentioned before, a pilot study in 10 pigs was performed to try and predict the proper placement of the microelectrodes to register PmO_2 changes during LAD occlusion.

Results

Under normal conditions, with an FiO_2 of 0.3 and before LAD occlusion, PO_2 measurements displayed a broad range of values, mostly between 20 and 120 mm Hg (Figs 9 and 10).

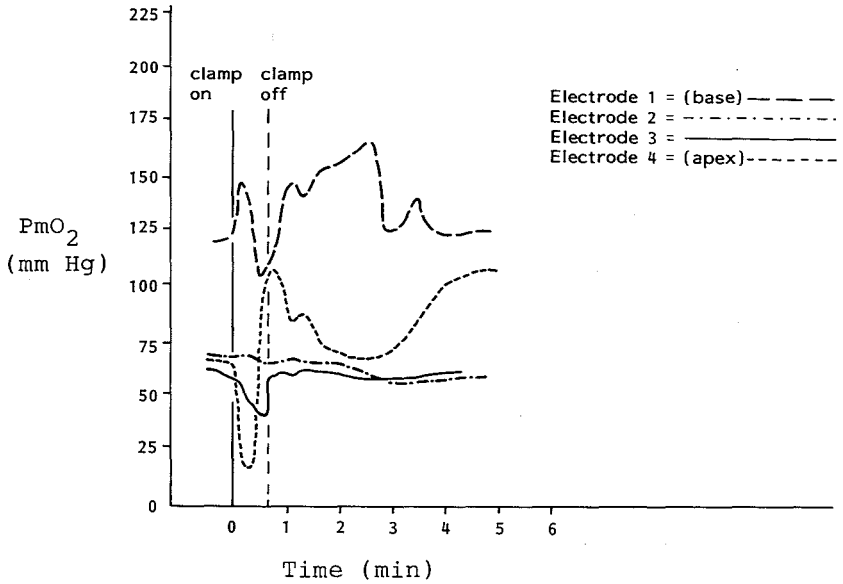

Fig. 9: An example of myocardial PO_2 (PmO_2) responses to LAD occlusion.

Following occlusion, the PmO_2 responses varied greatly depending on the position of the electrode in the capillary network and on the degree of ischaemia, collateral or steal perfusion and reactive hyperaemia. This is clearly demonstrated in Figs 9 and 10. In strongly affected areas, tissue PO_2 decreased and remained low throughout the occlusion period. This effect occurred after some delay (Fig. 10, electrode 3) or immediately following occlusion (Fig. 9, electrode 3). Some tissue PO_2 measurements dropped to values below 1 mm Hg. In other areas, PO_2 values fell drastically following occlusion, then recovered and even displayed hyperoxic supply (Fig. 9, electrode 4). Conversely, tissue PO_2 values increased following occlusion in some areas, followed by a decline in values (Fig. 9, electrodes 1 and 2; Fig. 10, electrodes 1,2 and 4). This secondary decrease in PO_2, following a hyperoxic phase, can even drop to below the original value (Fig. 9, electrodes 1 and 2; Fig. 10, electrode 2). In other cases, PO_2 decreased but measurements remain higher than the original value (Fig. 10, electrodes 1 and 4).

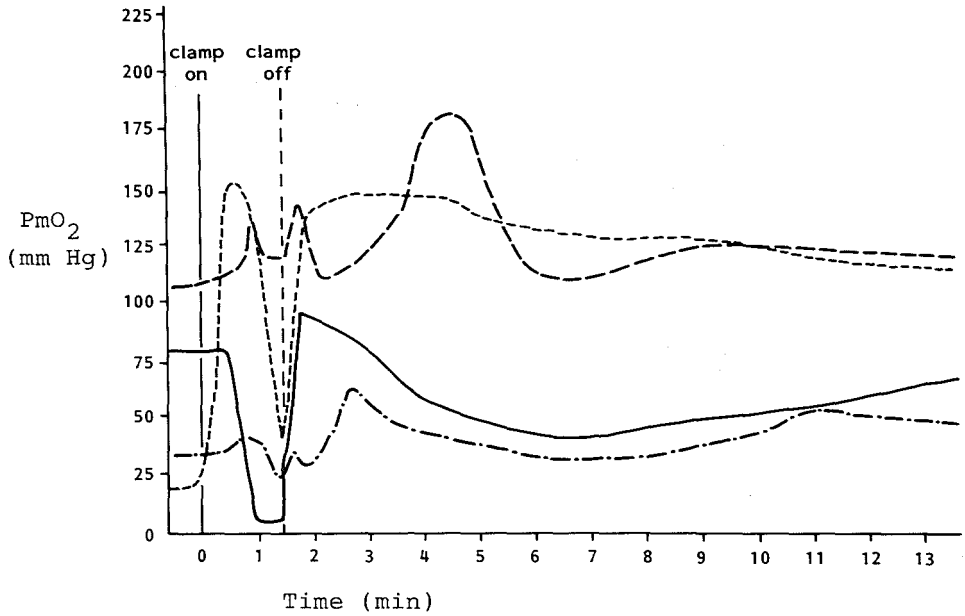

Fig. 10: An example of myocardial PO_2 (PmO_2) responses to LAD occlusion. (Electrode numbers are the same as in Fig. 9).

At termination of the occlusion period, tissue PO_2 rapidly increased to hyperaemic values in those areas where tissue PO_2 was low at the end of the occlusion phase (Fig. 9, electrodes 1; Fig. 10, electrodes 2,3 and 4). Electrodes displaying hyperoxic values at termination of occlusion phase showed an immediate fall in PO_2 (Fig. 9, electrode 4) to the original value. The post-occlusion period is followed by an oscillatory phase of tissue PO_2 values. Often, a considerable length of time ($\pm$ 5 mins) is required before PO_2 returns to pre-clamping values.

Discussion

Reves et al. (1978) have presented results of cross-clamping the LAD in dogs using 300 micron oxygen electrodes and have described a myocardial 'steal' effect in which PO_2, in normal areas of the

myocardium, decreased. They attributed this effect to the shunting of blood via collaterals into hypoxic border areas in which auto-regulation had resulted in decreased coronary vascular resistance.

The biphasic nature of the curves in Figs 9 and 10 indicate that the initial decreased oxygen supply due to impaired myocardial circulation is, in some instances, rapidly compensated by auto-regulation and opening of the collateral supply channels. Many authors have postulated the existance of these channels. Stam and de Jong (1977) produced embolisation of the coronary arteries of isolated perfused rat hearts using 20-80 micron diameter microspheres. Coronary blood flow decreased rapidly following microsphere injection but then increased to achieve a constant rate ten minutes later. At this point blood flows were significantly higher than that observed two minutes after embolisation.

Berne and Rubio (1969) considered adenosine to be the only substance that could account for the entire process of coronary dilation observed in myocardial ischaemia. Stam and de Jong (1977) noted that no rise in coronary flow following micro-embolisation occurred if the perfusate contained adenosine, a breakdown product of ATP, produced by de-phosphorylation of AMP. De Jong and Goldstein (1974), in pig studies, investigated the effects of ischaemic hypoxia on the release of inosine, hypoxanthine and lactate from the myocardium. They found highly significant increases in the level of these substances over a six minute period following 72% occlusion of the LAD.

Local release of ATP breakdown products may be of sufficient rate and magnitude to account for the extremely rapid increases in PmO_2 that were observed. Indeed, Thompson et al. (1980) observed cyclical changes in the adenosine level during the cardiac cycle, with increases occurring during each systolic contraction of the myo-cardium. It may equally well be argued that these changes are caused by local haemodynamic changes in the microcirculation leading to increased local perfusion in certain areas.

PERMANENT LAD OCCLUSION

Once the pilot study mentioned in the previous section was completed, it became possible to predict whether an electrode would be in an ischaemic or non-ischaemic area following LAD occlusion. A follow-up study in 18 pigs was performed to enable statistical analysis of the effects of LAD occlusion on different areas of the myocardium. As the analysis was based on averages, the rapid changes occurring within a minute of occlusion were averaged out. This section is primarily concerned with the 'long-term' effects of LAD occlusion on the ischaemic and non-ischaemic myocardium.

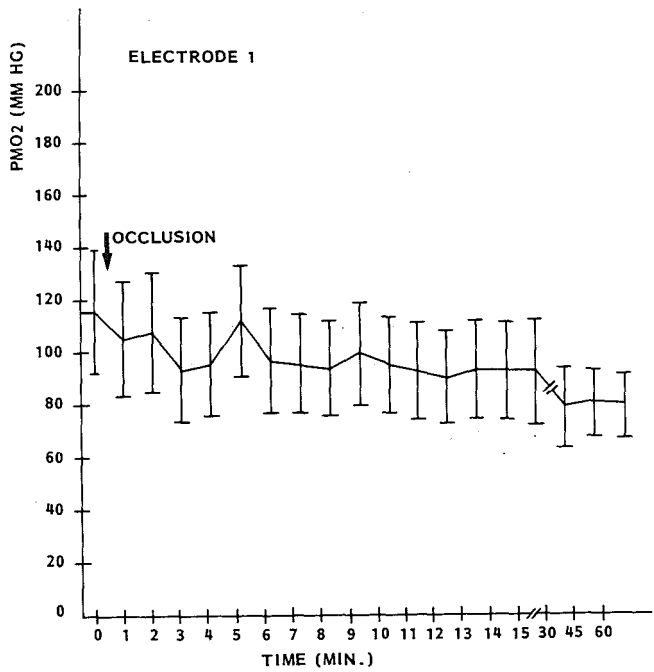

Fig. 11: The effect of LAD occlusion on PmO_2 in the normal myocardial tissue, situated furthest from the ischaemic area (n=13).

The animals were ventilated with oxygen (99.5%) and halothane (0.5%). This was necessary because after one hour of ischaemia the pigs were subjected to another protocol requiring a high arterial PO_2

(see Chapter 6). A period of at least 30 minutes was allowed for stabilisation before measurements were commenced and, after baseline PmO_2's had been obtained, the LAD was tied. On-line PmO_2 values, corrected for temperature and drift, were obtained each minute from 0-15 minutes and then every 15 minutes up to 1 hour.

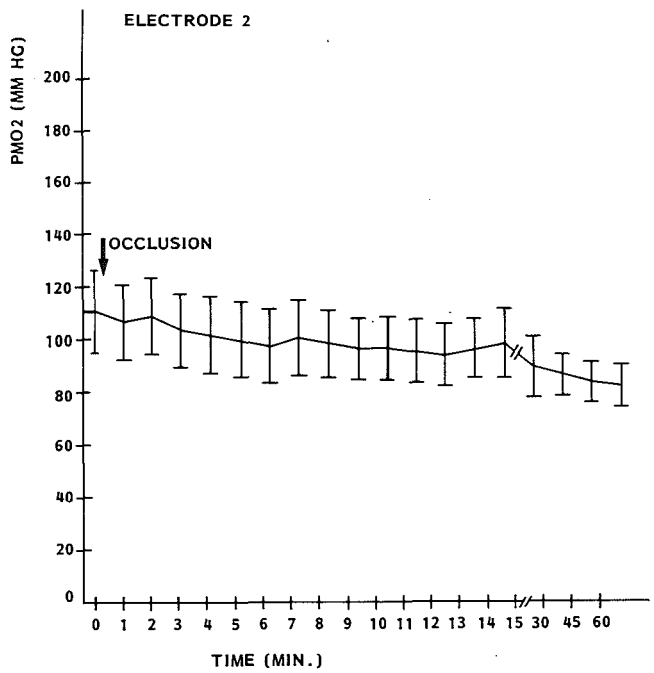

Fig. 12: The effect of LAD occlusion on PmO_2 in normal myocardial tissue bordering the ischaemic area (n=14).

Results

18 experiments were performed. After discarding electrodes with a drift of more than 7.5%/hour or other technical faults, two to four electrodes per experiment could be used for statistical analysis. A total of 58 electrodes yielded satisfactory PmO_2 measurements. The pre-occlusion values are presented in histogram form in figure 8.

Figures 11-14 show changes in PmO_2 following LAD occlusion in four separate areas, each averaged over eighteen animals. The electrodes were situated so that electrode 4 was expected to be situated in the most ischaemic area (referred to as the ischaemic deep electrode) and electrode 1 at the opposite end of the plate in the non-ischaemic area (the non-ischaemic deep electrode). Electrodes 2 and 3 were in the border areas (the non-ischaemic and ischaemic border electrodes). Before occlusion, there was no significant difference between the 4 areas (Student's t grouped test).

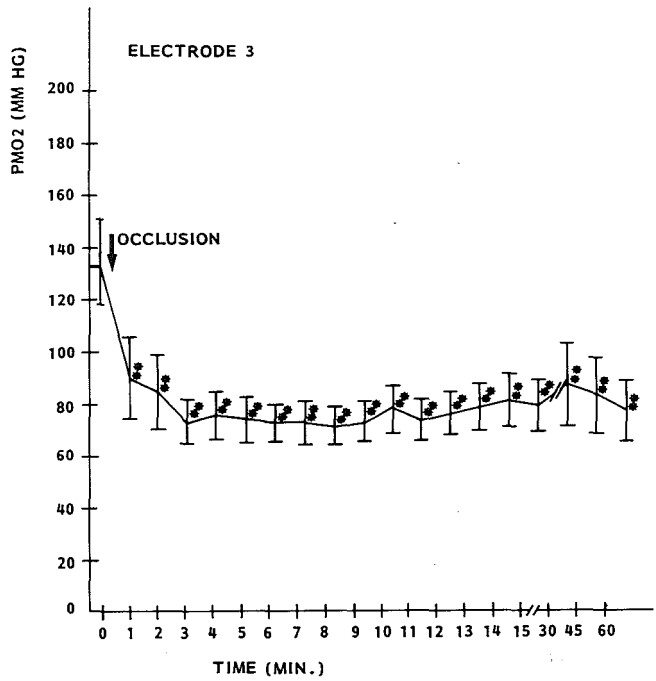

Fig. 13: The effect of LAD occlusion on PmO_2 in the ischaemic border area (n=16). Significance from pre-ischaemia: **=p<0.001.

Following LAD occlusion, immediate and significant decreases in PmO_2 were observed in the ischaemic deep area (Student's t paired test). By four minutes post-occlusion these decreases had abated but at the expense of the adjacent ischaemic border area. This area

continued to show highly significant decreases in PmO_2 throughout the first hour of post-occlusion. No significant decreases were seen in the non-ischaemic border or non-ischaemic deep areas.

Following occlusion, the distribution of PmO_2 values in the 18 pigs was compared with the pre-ischaemic values for each area. The distributions were compared using the Kolmigorov-Smirnov test and a p value of 0.01 was taken to indicate significance. Only in the ischaemic border area were significant changes in PmO_2 distribution patterns observed when compared to pre-occlusion values. These changes were seen at minute 3 and minutes 5-13 after LAD occlusion. Figure 15 clearly shows the shift in distribution towards lower PmO_2 values at 8 minutes of ischaemia. This is the point at which mean percentage decreases in PmO_2 were maximal.

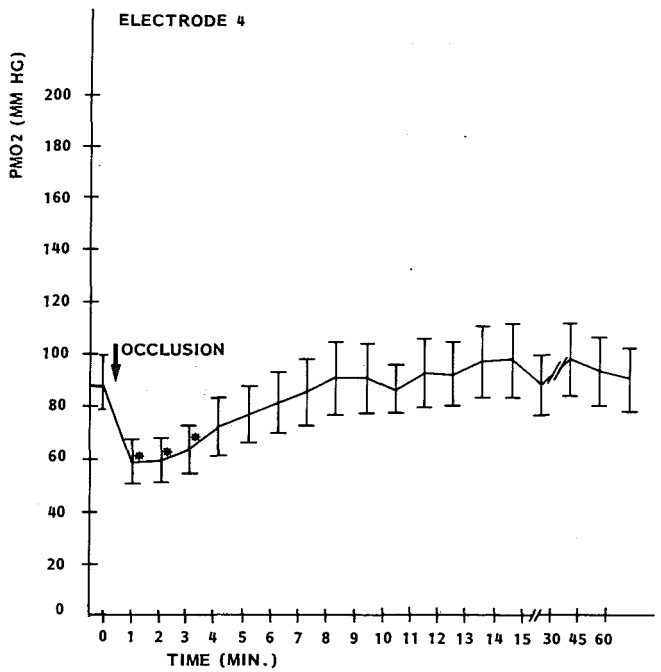

Fig. 14: The effect of LAD occlusion on PmO_2 in the deep ischaemic area (n=15). Significance from pre-ischaemia: *=p<0.01.

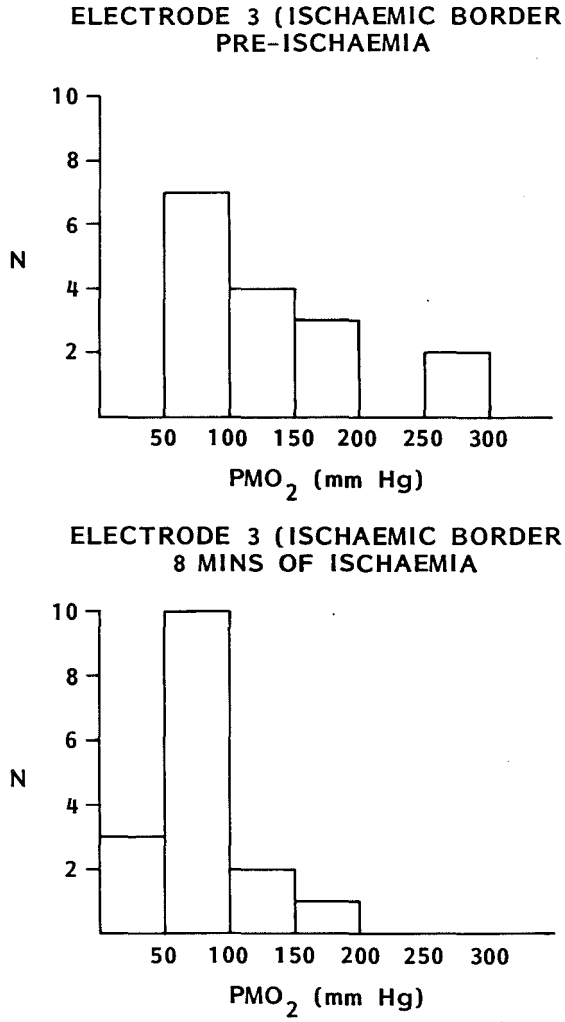

Fig. 15: Distribution of myocardial oxygen tension (PmO_2) in the ischaemic border area before and after onset of ischaemia (n=16).

Discussion

Reves et al. (1978) have demonstrated the presence of a myocardial steal effect in dogs. They were able to demonstrate steal from both border and normal areas but made no attempt at statistical evaluation

of its significance. Studies on PO_2 in both ischaemic and border areas in pigs have been carried out by Walfridsson et al (1985). They measured epicardial oxygen tension (PeO_2) in animals in which the PaO_2 was maintained between 75 and 105 mm Hg. They reported mean resting values of 55.5 mm Hg in the epicardium. LAD occlusion resulted in marked decreases in PeO_2 and significant persistent distribution changes in the ischaemic area. In the border area, significant distribution changes were seen 5 minutes after occlusion, but these had recovered by 30 minutes post-occlusion. Our results suggest that PeO_2 changes are not typical for conditions in the myocardium. It would seem that myocardial steal occurs, with blood flow shifting during occlusion from the border area to the severely ischaemic area.

Our experiments took place under hyperoxia (99.5% O_2). That would mean that the oxygen supply/demand ratio would be favourable. It is quite possible that when ischaemia occurs under normoxic conditions the steal effect would not be so efficient.

GENERAL DISCUSSION

Oxygen tensions in the myocardium (PmO_2) have been estimated in a variety of species over the last few years. Some investigators (Rude et al. 1982, 1984) used relatively large mass spectrometer probes embedded in the myocardium. Others used epicardial surface electrodes to study the distribution of partial pressures of oxygen over much smaller areas than can be achieved with the aid of mass spectrometry (Kessler et al. 1983, Walfridsson et al 1985). The latter methods are relatively non-invasive, enabling many measurements in a short period of time to rapidly produce PO_2 histogram distributions of the underlying epicardium. It is debatable however, as to whether the values so obtained truly reflect the PO_2 values of the underlying myocardium. Studies demonstrating the occurrence of lower PO_2 values in the sub-endocardial myocardium than in the sub-epicardial tissues (Moss 1968, v.d. Laarse 1978) increase this doubt.

Studies on PmO_2 have also been carried out using polarographic methods and employing needle electrodes. These methods are, of course, considerably more invasive than the use of epicardial electrodes and

are particularly susceptible to movement artifacts. Movement of fluid about the tip, or movement of the tip of the electrode (the stirring effect) will enhance current flow in the polarographic circuit and lead to abnormally high PO_2 values. It is also inevitable that a certain amount of tissue damage will be caused by the introduction of the needle and that this will interfere with microcirculatory flow in the vicinity of the tip of the electrode. However, it has been shown (v.d. Laarse 1978) that probes as large as 2 mm in diameter may cause only minimal mechanical damage of the cells around the insertion channel. Lund (1985) has, however, maintained that both needle electrodes and mass spectrometer probes may, by compression of surrounding tissues, cause PmO_2 readings that are too low.

Another question concerns the effect that the halothane, present in the inspired gas mixture, may have on the accuracy of the PmO_2 measurements. Severinghaus (1971) showed that a number of anaesthetic gases, including halothane, can be detected with polarography. The presence of substantial quantities of halothane tends, in some instances, to give a somewhat high reading for PO_2. It was concluded however, that in view of the low concentrations associated with clinical anaesthesia, errors would be relatively small in most cases. In the experiments presented in this thesis, the inhaled concentration of halothane was kept constant for at least half an hour before measurements of PO_2 were commenced and it was thereby hoped that a constant low level of halothane would be maintained in myocardial tissue. Thus, though any error occurring would be constant, changes in PmO_2 would be of no significance. Indeed, the presence of halothane error would tend to decrease the percentage changes of PmO_2 readouts. It is, thus, possible that the calculated percentage changes in myocardial PO_2 may in fact be <u>less</u> than the true changes.

As far as we are aware very few measurements of PmO_2 have been carried out during hyperoxia - none at all using pigs. Results obtained from dogs have been extremely variable. V.d. Laarse (1978) has reported small but significant increases in PmO_2 in dogs when the arterial oxygen tension (PaO_2) was increased from 93 to 220 mm Hg. In our experiments, the mean PaO_2 was much higher and amounted to 359 mm Hg. Feola et al (1979), using smaller electrodes than those used in our series, reported a mean PmO_2 of 14 mm Hg ($\pm$ 3 SEM) in dogs

breathing a gas mixture containing 50% O_2. Schuchhardt (1985) has contended that a PmO_2 value of more than 100 mm Hg is very rarely seen and, using polarographic needle electrodes, has measured mean values of 46 mm Hg in dogs with a mean PaO_2 of 440 mm Hg. Rude et al (1984) using mass spectrometer probes has measured myocardial tensions in dogs breathing 100% O_2 following infusion of dextran or fluorocarbon emulsions. In the former, PmO_2 was 66 mm Hg ($\pm$ 18 SEM) at a mean PaO_2 of 490 mm Hg, while in the latter the mean value of PmO_2 was 127 mm Hg at a mean PaO_2 of 546 mm Hg.

In conclusion, we have demonstrated that considerable variation in PmO_2 may be measured throughout the cardiac cycle and that these are a net result of the supply/demand situation in the tissues. Oxygenation of the myocardium in the normally functioning heart is not uniform but varies considerably over a relatively short distance - this, we suggest, is governed by the relationship to different vascular structures. Rapid and seemingly unpredictable changes may occur in myocardial PO_2 as a result of occlusion of terminal branches of the coronary arteries, but this chapter tries to show that there is an autoregulatory mechanism at the micro-level which attempts to compensate for the threatening hypoxia.

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CHAPTER 6

MICROCIRCULATION AND FLUOROCARBONS

INTRODUCTION

Chapter 5 discussed myocardial microcirculation, using oxygen tissue tension as a major parameter. The changes in PmO_2 during acute ischaemia are shown and the effects of intercapillary collaterals are explained. It is apparent that the level of collateral blood flow is not sufficient to compensate for myocardial infarction. Due to redistribution of the blood flow, portions of the micro-area become hypoxic and this can lead to necrosis. In this situation, blood flow might be improved by decreasing the viscosity of the perfusion fluid. This can be achieved by diluting the blood with, for instance, dextran. Unfortunately, dextran has a low oxygen carrying capacity (Zander and Makowski, 1982) being almost the same as plasma ($\pm$ 0.3 ml O_2 per 100 mm Hg and 100 ml plasma). Later in this chapter, it will be shown that dextran actually slightly increases hypoxia due to haemodilution which leads to a reduction in O_2 transport capacity. If haemodilution is performed with a preparation that not only reduces viscosity but also has an oxygen transporting capacity significantly higher than that of plasma, oxygenation of ischaemic and/or hypoxic tissue could be improved.

Fluorocarbons, also known as perfluorochemicals or perfluorocarbons, are almost completely inert chemical substances with a high solubility for oxygen. This property was dramatically demonstrated by Clark and Gollan (1966) in mice surviving total immersion in these liquids. As fluorocarbons are almost completely insoluble in water (or blood), they must be emulsified before use. One example of these products is marketed under the trade name of Fluosol-DA (Green Cross Corporation, Osaka, Japan). The composition of Fluosol-DA 20% (weight per volume %) is shown in Table I.

Table I: Composition of Fluosol-DA 20% (weight per volume percent). (after Naito and Yokoyama, 1978)

Perfluorodecalin	14.0	KCL	0.034
Perfluorotripropylamine	6.0	$MgCl_2$	0.020
Pluronic F-68	2.7	$CaCl_2$	0.028
Yolk phospholipids	0.4	$NaHCO_3$	0.210
Glycerol	0.8	Glucose	0.180
Hydroxyethyl starch	3.0	NaCl	0.600

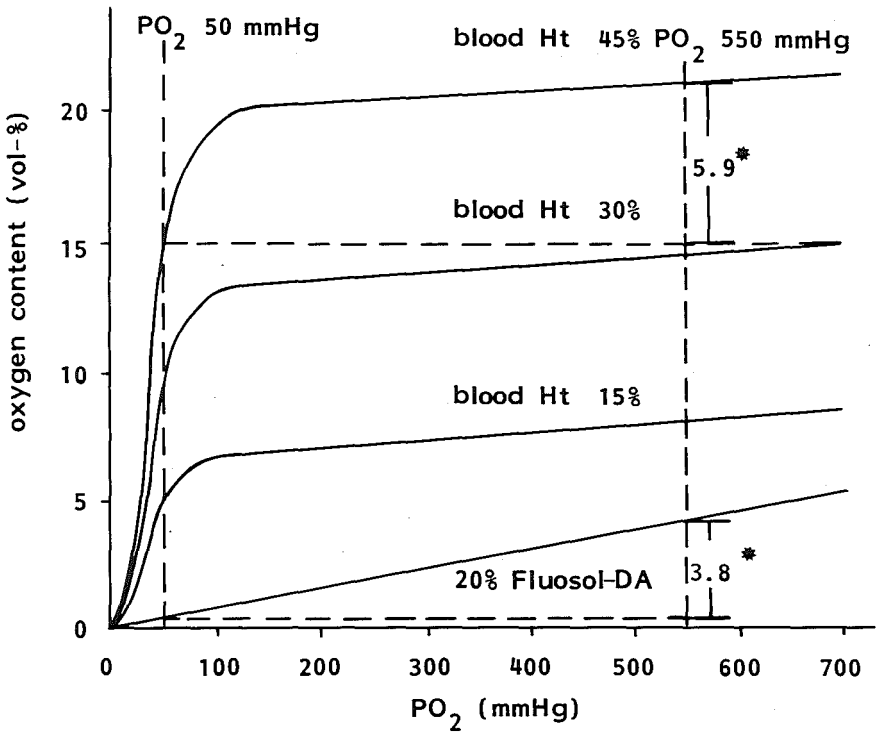

Fig. 1: Oxygen content of Fluosol-DA emulsion and blood at various haematocrits (after Grote et al., 1985). The amount of oxygen released between PO_2 values of 550 and 50 mm Hg are indicated by asterisks.

As shown in figure 1, Fluosol-DA 20% (the product used in our investigations) can carry about 0.75 ml O_2 in 100 ml per 100 mm Hg (Grote et al 1985). This capacity is very low in comparison to the content in normal human blood under similar conditions. Hence, in practice, it is necessary to breathe as high a percentage of oxygen as possible. At an arterial PO_2 of 550 mm Hg, being about the maximal achievable with an FiO_2 of 1.0, Fluosol-DA 20% contains about 4.1 ml O_2 per 100 ml emulsion. Normal blood contains about five times that amount of oxygen but, because of the proportional relationship between PO_2 and oxygen content in fluorocarbons, the delivery of O_2 to tissue is nearly the same with Fluosol-DA 20% as with blood (Fig. 1).

Fluorocarbon emulsions have a very low viscosity, about 0.55 times that of blood. Furthermore, whereas the viscosity of blood increases markedly under the low-flow shear rates existing in the microcirculation, the viscosity of fluorocarbon emulsions is almost independent of shear rate. As can be seen in Fig. 2, Fluosol-DA 20% is only about 0.34 times as viscous as blood under these conditions.

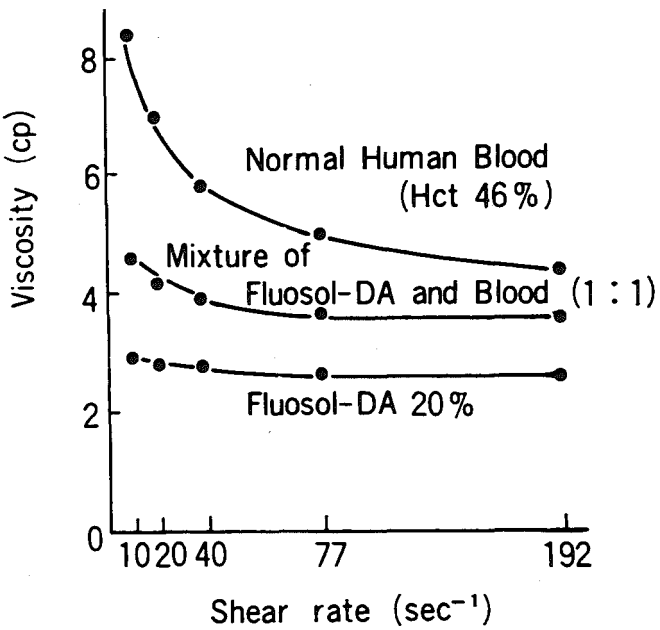

Fig. 2: Viscosity of Fluosol-DA, whole human blood and their mixture (1:1) at various shear rates, at a temperature of 37° C (after Naito and Yokoyama, 1978).

The low viscosity, together with the low particle size of the emulsion, should ensure good penetration into the microcirculation. Fluosol, and blood mixed with Fluosol, should pass more easily through capillary beds that are supplied from an extended perfusion pathway under conditions of, for example, vascular insufficiency or tissue infarction. These emulsions, by bypassing and reoxygenating impacted red blood cells, should be particularly suitable for oxygenation of the micro-area under conditions such as myocardial infarction, or re-establishment of circulation following prolonged cessation of perfusion. The investigations described in this chapter tend to confirm these propositions.

THE EFFECTS OF HAEMODILUTION ON OXYGEN TENSION IN THE ACUTELY ISCHAEMIC MYOCARDIUM.

Material and methods

The same experimental procedure was followed as described in Chapter 5. In our experience, fluorocarbons tend to interfere with the measurement of oxyhaemoglobin saturations performed with a Radiometer OSM2 Hemoximeter (Faithfull et al., 1986). Therefore the oxy-haemoglobin saturation in blood samples containing fluorocarbons were calculated using the computer subroutine described by Kelman (1966). This subroutine, together with mixed venous and arterial blood-gas estimations allowed arterial and mixed venous oxygen content (including the relative and absolute contributions from haemoglobin and simple solubility) to be calculated on a Philips P2000 microcomputer. If fluorocarbons were present, their contribution to the above parameters could also be displayed. In addition, total and fractioned oxygen tissue flux and consumption were calculated.

The pigs were ventilated with 100% oxygen (and 0.5% halothane). After stabilisation of the haemodynamics, the distal third of the LAD was occluded. The changes in PmO_2 are presented in Chapter 5. The animals were randomly divided into three groups:

- a control group of five animals receiving no treatment. Following five hours of myocardial ischaemia the pigs were sacrificed by

ventilation with 100% nitrous oxide.

- a second group of five animals in which bleeding was commenced after one hour of vascular occlusion. 20 ml of blood per kg bodyweight was removed and then immediately replaced with the same volume of Fluosol-DA 20%.
- a third group of five pigs in which 5% dextran 40 (400000 molecular weight) in 0.9% saline was used for haemodilution instead of Fluosol.

The haemodilution procedures were completed within one hour. After haemodilution there was no significant difference in haematocrit between the dextran and Fluosol groups. After five hours of infarction (about three hours of haemodilution) all pigs were sacrificed and the hearts removed for pathological examination.

Results

The measured and calculated systemic cardiovascular parameters for the three groups are presented in Tables II, III and IV respectively. In Table V, the calculated values for whole body oxygen flux (arterial oxygen content times cardiac output) are presented for the three groups of animals.

As can be seen in Table II, in which results of the control group are presented, the degree of myocardial infarction was not severe enough to cause statistically significant changes in any of the presented variables. The procedure did, however, produce a macro- and microscopically visible infarctive area in the myocardium.

Haemodilution with Fluosol-DA 20% was commenced after 1 hour of ischaemia. At this point no significant changes had occurred (Table III). Haemodilution was complete by 2 hours post-occlusion. At this stage cardiac output was significantly higher in the group as a whole than was the case in the control group. At 3 hours, mean arterial pressure and systemic vascular resistance were also significantly lower than in the control group. However, the changes seen did not reach values that were significantly different from those observed prior to LAD occlusion.

Table II: Non-treatment (control) group: Changes in systemic cardiovascular variables following left anterior descending coronary artery (LAD) occlusion (n=5).

	Pre-LAD occlusion	1 hour	2 hours	3 hours	4 hours	5 hours
HR	112 ±7.4	122 ±8.5	127 ±9.6	129 ±8.7	128 ±8.6	132 ±9.5
AP	10.9 ±0.47	11.0 ±0.63	10.7 ±1.10	12.1 ±0.54	11.4 ±0.76	12.2 ±0.77
CO	2.1 ±0.22	2.1 ±0.20	2.0 ±0.26	2.3 ±0.25	2.3 ±0.32	2.4 ±0.40
LVW	22 ±2.5	22 ±2.6	19 ±3.8	25 ±3.2	25 ±4.2	25 ±3.7
SVR	302.9 ±43.18	320.2 ±48.25	293.9 ±70.12	304.2 ±38.18	297.9 ±51.29	338.8 ±60.43

Results are expressed as mean ± SEM. Times are given from occlusion. No significant differences from pre-LAD occlusion values are present.

HR = Heart rate (beats/min)
AP = Mean arterial pressure (kPa)
CO = Cardiac output (l/min)
LVW = Left ventricular work (J/min)
SVR = Systemic vascular resistance (kPa.sec/l)

Table III: Haemodilution with Fluosol-DA 20%: Changes in systemic cardiovascular variables following left anterior descending coronary artery (LAD) occlusion (n=5).

	Pre-LAD occlusion	1 hour	2 hours	3 hours	4 hours	5 hours
HR	102 ±5.1	99 ±1.3	113 ±3.7	108 ±4.1	118 ±7.1	122 ±5.3
AP	9.5 ±0.93	9.7 ±0.64	9.7 ±0.48	9.5# ±0.37	9.8 ±0.70	9.9 ±0.87
CO	2.6 ±0.31	2.8 ±0.24	3.5# ±0.42	2.7 ±0.36	2.5 ±0.22	2.5 ±0.09
LVW	20 ±2.4	24 ±2.5	27 ±3.0	22 ±2.4	21 ±2.6	21 ±2.6
SVR	211.3 ±46.00	197.9 ±31.19	153.3 ±30.10	173.4# ±15.20	220.0 ±32.07	206.5 ±19.80

Haemodilution was performed between 1 and 2 hours after LAD occlusion. Times are given from occlusion. Results are expressed as mean ± SEM. No significant differences from pre-occlusion values are present. Significant difference from the control group: #=$p<0.05$

HR = Heart rate (beats/min)
AP = Mean arterial pressure (kPa)
CO = Cardiac output (l/min)
LVW = Left ventricular work (J/min)
SVR = Systemic vascular resistance (kPa.sec/l)

Table IV: Haemodilution with dextran: Changes in systemic cardiovascular variables following left anterior descending coronary artery (LAD) occlusion (n=5).

	Pre-LAD occlusion	1 hour	2 hours	3 hours	4 hours	5 hours
HR	91 ±6.0	90 ±5.2	98 ±4.1	106 ±7.4	109 ±7.2	111 ±7.7
AP	10.4 ±0.13	10.7 ±0.77	10.7 ±0.69	9.5$^{\#}$ ±0.80	9.2 ±0.62	9.9$^{\#}$ ±0.44
CO	2.2 ±0.20	2.0 ±0.24	2.8 ±0.22	2.7 ±0.22	2.4 ±0.25	2.5 ±0.25
LVW	21 ±3.6	20 ±4.0	25 ±2.6	23 ±3.7	21 ±3.1	25 ±2.5
SVR	251.2 ±16.30	289.9 ±28.16	195.5** ±24.52	182.4$^{***\#}$ ±18.89	200.7** ±24.22	216.7 ±28.57

Haemodilution was performed between 1 and 2 hours after LAD occlusion. Times are given from occlusion. Results are expressed as mean ± SEM. Significant difference from pre-occlusion values: **=p<0.01, ***=p<0.001. Significant difference from the control group: #=p<0.05

HR = Heart rate (beats/min)
AP = Mean arterial pressure (kPa)
CO = Cardiac output (l/min)
LVW = Left ventricular work (J/min)
SVR = Systemic vascular resistance (kPa.sec/l)

Haemodilution with 5% dextran 40 (Table IV) produced very significant decreases in systemic vascular resistance in comparison to pre-occlusion values. Mean arterial pressure was lower than that obtained in the control group at 1 and 3 hours after the completion of haemodilution. In the dextran group there was no significant change in CO, therefore, because of the drop in oxygen content due to haemodilution, very significant decreases in whole body oxygen flux occurred (Table V). No decrease in oxygen flux occurred in the Fluosol group and it should be noted that at all times after haemodilution the oxygen flux in the Fluosol group was significantly higher than that of the dextran group. Although there was no significant difference in oxygen flux between the Fluosol group and the control group, the oxygen extraction coefficient in the Fluosol group was significantly higher than that of the control group at the end of haemodilution.

Table V: Changes in whole body oxygen flux (ml/min) following left anterior decending coronary artery (LAD) occlusion (n=5).

	Pre-LAD occlusion	1 hour	2 hours	3 hours	4 hours	5 hours
Group I (control)	336 ±44.2	316 ±44.2	336 ±54.1	382 ±41.5	340 ±54.6	389 ±42.3
Group II (Fluosol)	359 ±36.4	399 ±30.2	428 ±34.3	374 ±30.4	334 ±20.4	329 ±24.7
Group III (Dextran)	306 ±28.8	280 ±31.6	240** ±23.0	233***# ±25.4	223** ±32.8	235**# ±28.1

Haemodilution was performed between 1 and 2 hours after LAD occlusion. Times are given from occlusion. Results are expressed as mean ± SEM. Significant difference from pre-occlusion values: **=p<0.01, ***=p<0.001. Significant difference from the control group: #=p<0.05

Following LAD occlusion, marked and abrupt changes in PmO_2 were observed. This was interpreted as being due to complicated interactions taking place and was the end result of impairment of blood supply caused by vascular occlusion, dilation of vessels in the 'border' areas and a concomitant diversion of blood to collateral capillary circulatory pathways (described in detail in Chapter 5). This experiment aimed at improving PmO_2 in the most hypoxic area, which was not necessarily the area in the middle of infarction. Analysis of measurements in each animal was therefore confined to the electrode indicating the greatest percentage decrease in PmO_2 during the first hour of occlusion. This electrode was then assumed to be in the 'most hypoxic area' of the infarct.

Table VI: Changes in myocardial oxygen tension (mm Hg) in the most hypoxic area of the myocardium following left anterior decending coronary artery (LAD) occlusion (n=5).

	Pre-LAD occlusion	1 hour	2 hours	3 hours	4 hours	5 hours
Group I (control)	140.2 ±34.92	51.1* ±11.18	44.7* ±9.12	41.4* ±8.84	38.4* ±10.24	37.4* ±8.37
Group II (Fluosol)	146.3 ±32.60	76.4* ±17.80	79.1 ±23.37	81.8 ±19.07	81.9# ±14.12	131.8# ±31.19
Group III (Dextran)	141.6 ±50.34	73.6* ±22.70	68.8* ±28.73	68.0* ±32.26	60.7* ±28.67	52.8* ±25.53

Haemodilution was performed between 1 and 2 hours after LAD occlusion. Times are given from occlusion. Results are expressed as mean ± SEM. Significant difference from pre-occlusion values: *=$p<0.05$. Significant difference from the control group: #=$p<0.05$

The values obtained from the electrodes defined as being in the most hypoxic area are presented in Table VI. Significant decreases in PmO_2 were observed in all groups at one hour after LAD occlusion; there were no significant intergroup differences at this point. Mean PmO_2 values in the control group continued to decline slowly from 51.1 to 37.4 mm Hg (26.8%) between 1 and 5 hours post-occlusion. The PmO_2 values in the dextran group showed a slightly steeper decline from 73.6 to 52.8 mm Hg (28.3%) in the same period, while in the Fluosol group the PmO_2 values showed a mean increase from 76.4 to 131.8 mm Hg (72.5%). At 4 and 5 hours post-occlusion the Fluosol group values were significantly higher than those in the control group. These percentage changes in PmO_2 as compared to 1 hour LAD occlusion are shown in Fig. 3. After 5 hours of ischaemia, mean PmO_2 in the Fluosol group had recovered to 90.1% (± 19.6 SEM) of the pre-occlusion values, whereas PmO_2 in the dextran and control groups were still significantly reduced to 37.3% and 26.7% respectively.

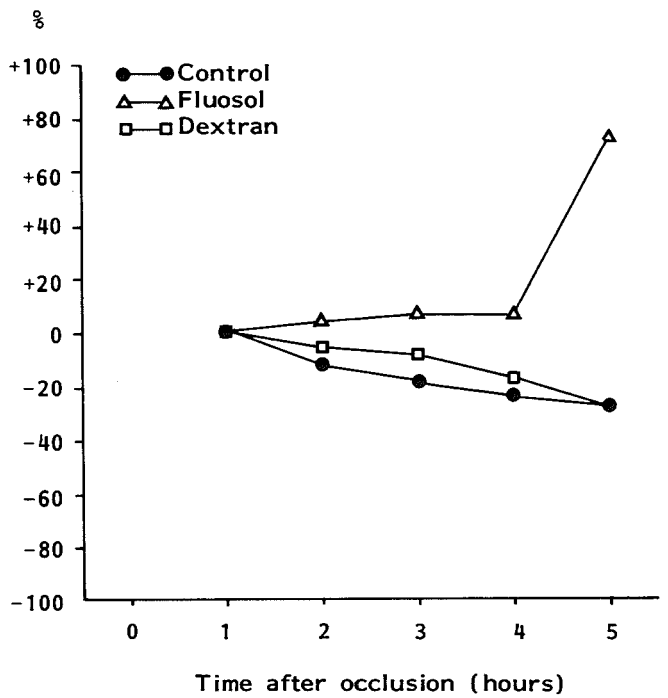

Fig. 3: Percentage changes in PmO_2 in the most ischaemic area of infarction. 1 hour after infarction all PmO_2's are considered as 100%; mean changes from these values have been plotted.

Pathalogical examination showed areas of ischaemic change in all three groups. No significant difference in the degree of ischaemia could be seen between the groups. A reason for this could be that the period of time between infarction and death is relatively short (5 hours) for pathological examination.

Discussion

This study shows that Fluosol at least prevents a further decrease in myocardial oxygenation after an infarct. In fact, it actually improves oxygenation. The delay in apparent improvement could be due to several factors. Firstly, there is a delay of two hours between infarction and completion of haemodilution. Secondly, it takes time for the Fluosol molecules to penetrate the ischaemic area. Once the small blood vessels and erythrocytes in this area have been re-oxygenated, blood flow will increase so that a further improvement of oxygenation can take place (see general discussion at the end of this chapter).

Haemodilution with Ringer's lactate solution has been employed by Yoshikawa et al. (1973) to study the effect of low haematocrit on canine myocardial ischaemia. Following extreme haemodilution (haematocrit of 6%) they observed that ischaemia, assessed by ECG ST segment elevations, did not increase. Brazier et al. (1974) performed similar work with dextran. They postulated that the maintenance of myocardial oxygenation was due to an increase in coronary blood flow secondary to a decrease in viscosity. Cohn et al. (1975) showed that haemodilution, in dogs, with dextran 40 or 70 could improve the surface manifestations of ischaemic myocardial injury, assessed by ST elevation, during temporary (30 min) LAD occlusion. Following permanent ligation of the LAD, administration of dextran 70 improved blood flow to non-ischaemic areas, but no increase in flow was observed in the areas of infarction, in spite of improvement of ST elevation in this area. Tucker et al. (1980), following a study of infarct size treated with dextran haemodilution, concluded that infarct size was not increased by a 50% reduction in red cell mass.

Moores et al. (1983) have shown that 50% exchange with stroma-free haemoglobin (SFH) solutions has no effect on myocardial blood flow

during exercise in pigs. In dogs suffering from myocardial ischaemia, Biro and Beresford-Kroeger (1980) demonstrated that there was a greater increase in myocardial collateral blood flow with SFH haemodilution than with dextran haemodilution; oxygen delivery was also better in the SFH group. The superiority of SFH over dextran has been confirmed by Feola et al. (1979) and is, of course, due to the oxygen carrying capabilities of the haemoglobin. Unfortunately some problems with this solution still exists, such as its short half-life and low P50 values; these problems are being investigated.

Kessler et al. (1983) have reported near normal epicardial PO_2 histograms in dogs breathing 30% oxygen at a haematocrit of only 8% and a 'fluorocrit' of 16%. Biro (1983), also working in dogs, used microspheres and histological staining to assess the volume of myocardial infarction and the effect of subsequent haemodilution with dextran or Fluosol-DA 20%. A marginal decrease in infarcted muscle was found in the Fluosol group, though a marked improvement in blood flow to the ischaemic area was observed. Nunn et al (1983), assessing the ratio of the area of necrosis to the area at risk in myocardial infarctions, found significantly less necrosis in dogs treated 1 hour post-occlusion with Fluosol-DA 20% haemodilution than those treated with saline or left untreated.

This study has demonstrated that haemodilution itself does not actually improve myocardial oxygenation, whereas re-oxygenation of areas of the ischaemic myocardium is possible following haemodilution treatment using fluorocarbon emulsions. This has been proved possible in pigs - animals known to possess only minimal functional collaterals in comparison with dogs (Eckstein, 1954), and are also considered to be more suitable (from the point of view of clinical parallels) for cardiovascular investigations (Moores et al., 1983).

PROPHYLACTIC TREATMENT AGAINST MYOCARDIAL ISCHAEMIA WITH FLUOROCARBONS.

Material and methods

The previous section demonstrated that treatment with fluoro-

carbons after myocardial infarction improves myocardial PO_2. The subsequent study was to investigate whether prophylactic haemodilution with fluorocarbons could provide protection against myocardial ischaemia.

Table VII: Haemodilution with Fluosol-DA 20% before left anterior descending coronary artery (LAD) occlusion: Changes in systemic cardiovascular variables following LAD occlusion (n=5).

	Pre-LAD occlusion	1 hour	2 hours	3 hours
HR	141 ±12.9	142 ±15.6	148 ±20.5	152 ±18.2
AP	9.7 ±0.72	9.4 ±0.56	9.8 ±0.77	9.7# ±0.53
CO	3.3# ±0.55	3.3# ±0.36	3.1# ±0.36	2.9 ±0.44
LVW	34 ±6.7	26 ±4.0	27 ±3.8	27 ±4.8
SVR	138# ±15.9	165# ±17.1	162 ±15.5	182 ±26.2

Haemodilution was performed 30 min. before LAD occlusion. Times are given from occlusion. Results are expressed as mean ± SEM. No significant differences from pre-occlusion values are present. Significant difference from the control group: #=$p<0.05$.

HR = Heart rate (beats/min)
AP = Mean arterial pressure (kPa)
CO = Cardiac output (l/min)
LVW = Left ventricular work (J/min)
SVR = Systemic vascular resistance (kPa.sec/l)

The experimental setup was the same as described in the previous study, except that five pigs were haemodiluted by bleeding 20 ml/kg bodyweight and replacing the withdrawn blood with Fluosol-DA 20% 30 minutes before the distal third of the LAD was occluded. These pigs were compared to the control group of 5 non-treated animals.

Results

After prophylactic treatment with Fluosol (and before LAD occlusion), cardiac output was significantly higher and systemic vascular resistance significantly lower in the Fluosol group. This corresponds to the changes in the group treated with Fluosol after occlusion. LAD occlusion caused no significant changes in cardiovascular parameters for the 3 hours during which measurements were made (Table VII).

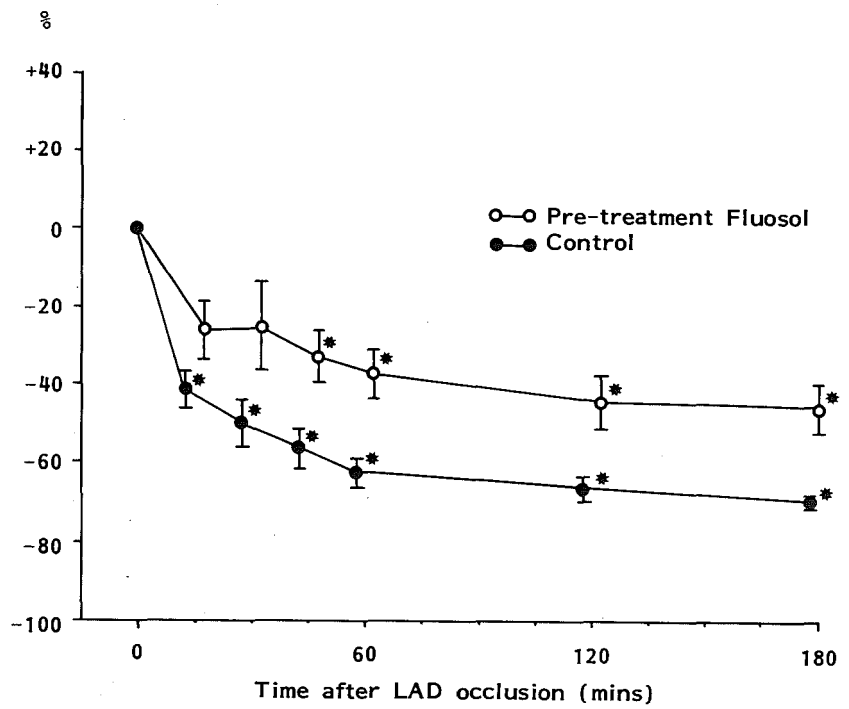

Fig. 4: Percentage change in PmO_2 in the most ischaemic area of myocardial infarction in pigs (mean ± SEM). Significant changes (in measured values) following occlusion: *=$p<0.05$

Initial analysis of myocardial oxygen tension was performed by the same method as described in the previous sub-chapter; i.e. results were analysed from those microelectrodes showing the greatest percentage decrease in PmO_2 over the first hour of occlusion in each animal. Figure 4 shows percentage change, from such electrodes, for control and Fluosol groups. Mean PmO_2 significantly decreased after 15 minutes of ischaemia in the control group, whilst this effect occurred after 45 minutes in the Fluosol group.

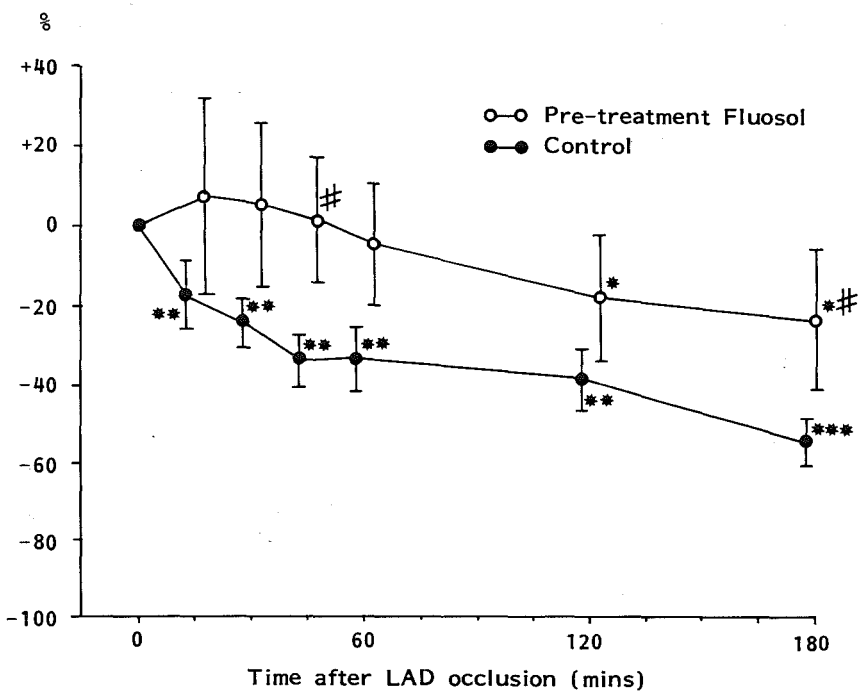

Fig. 5: Percentage change in myocardial oxygen tensions in all electrodes (mean ± SEM) following LAD occlusion in pigs. Significant changes (in measured values) following occlusion: *=p<0.05, **=p<0.01, ***=p<0.001. Significant difference from the control group: #=p<0.05

Percentage changes for all electrodes are shown in figure 5. Even though the mean PmO_2 in this figure also represents non-ischaemic tissue, the mean PmO_2 was significantly lower after 15 minutes in the

control group. This was not seen untill two hours after LAD occlusion in the Fluosol pre-treated group; indeed the mean PmO_2 actually rose in the first 45 minutes following occlusion. Mean PmO_2 in the Fluosol group was significantly higher than in the control group at 45 minutes and at the end of the experiments.

Discussion

Studies on myocardial protection using fluorocarbons have, as far as we know, only been carried out in dogs, a species that may not give clinically relevant results. Glogar et al. (1981) haemodiluted dogs to a haematocrit of 25% with a 15 volume percent emulsion of fluorocarbons and then produced myocardial infarction. They demonstrated that the diameter of the infarcted area decreased, as opposed to a group of animals diluted to similar haematocrits with Ringer's Lactate solution.

The present study indicates that prophylactic haemodilution with fluorocarbons can help to maintain myocardial oxygen tensions in the presence of ischaemia. The question as to whether prophylactic treatment with fluorocarbons is more effective than haemodilution with fluorocarbons following infarction is difficult to evaluate due to the difference in the length of time that fluorocarbons circulate in the body during the two experimental protocols. Comparison of percentage changes in the most ischaemic area after three hours of ischaemia reveals that the mean PmO_2 in the group treated 30 minutes prior to LAD occlusion decreases by about the same percentage as the group treated with fluorocarbons between 1 and 2 hours after ischaemia i.e. 44% (not significantly different from the control group). One definite advantage of pre-treatment with Fluosol is its protective function against hypoxia occuring within the crucial first hour of infarction. During this period the physician has the opportunity to instigate other courses of action. Whether this is a feasible therapy in the case of high-risk patients still has to be investigated.

Material and Methods

In five patients who had undergone traumatic amputations, the amputated part was perfused with Fluosol-DA 20% for between 16 and 46 hours:

Patient A: a 42-year-old man whose left lower leg was severed in a car accident. Due to extensive crush injury, replantation was not possible without shortening the leg by about 20 cm.

Patient B: a 50-year-old right-handed man who was admitted to hospital after an accident involving a circular saw. The first and third digits of his left hand were injured and the second digit was subtotally amputated. The two damaged digits were surgically repaired, but the amputated finger could not be replanted due to poor blood outflow of both digital arteries proximal to the injury.

Patient C: a 49-year-old mechanic who suffered total amputation of his left thumb at the metacarpophalangeal joint. Arterial outflow was very poor probably due to the fact that the patient was a heavy smoker. Soft tissue and bone crush was extensive over a long distance, making replantation impossible.

Patient D: a 28-year-old left-handed carpenter who severed digits 2,3,4 and 5 of his left hand with a rotary saw. Replantation surgery was performed in which digit 3 was replanted on ray 2, digit 4 on ray 3, and digit 5 on ray 5. Digit 2 was preserved by perfusion with Fluosol. The reason for this decision was based on experience with similar cases in which replantation of the final digit almost invariably produced poor results, due to the longer period of ischaemia. Digit 2 was chosen for this treatment as it had been amputated at the interphalangeal joint and thus had the worst prognosis for viability

Patient E: a 32-year-old right-handed tulip bulb grower who amputated his left thumb with a circular saw, just proximal to the

interphalangeal joint. At the time of admission, surgeons of the replantation team were engaged in another emergency operation. Consequently an attempt was made to preserve the thumb by perfusion with Fluosol.

The main supplying artery of the extremity or digit was cannulated, and perfusion was started with Fluosol-DA 20%. The perfusate, cooled to 5°C, was oxygenated by means of a bubble oxygenator using a gas mixture of 95% oxygen and 5% carbon dioxide. A pulsatile pump was used and the mean arterial pressure was maintained at about 90 mm Hg as measured by an aneroid pressure gauge. The perfusate was allowed to drain spontaneously from veins and venules at the cut surface and drip into the oxygenator (Fig. 6).

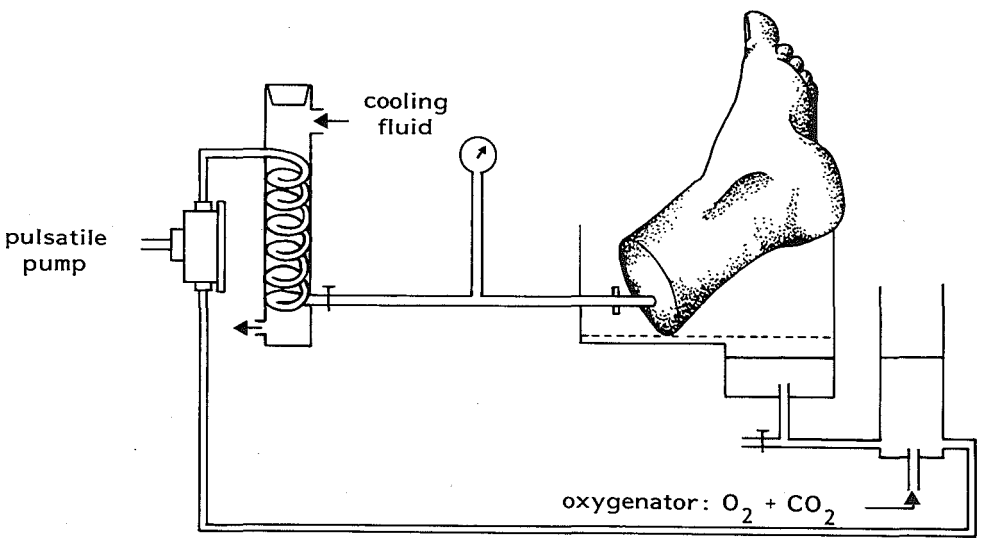

Fig. 6: Experimental setup for perfusing amputated extremities.

Microcirculatory oxygenation was monitored by placing steel-protected gold microelectrodes at various intramuscular and subcutaneous sites. Biochemical measurements of K^+, lactate and glucose from the total perfusate were performed at regular intervals of 4 or 6 hours. Gas tensions and acid-base status were also monitored at the

same intervals. Muscle biopsies were taken from the foot of patient A and examined histologically. The levels of ATP and its breakdown products were measured in several muscle specimens. At the end of the experiments, sections were taken of muscle, artery and vein for electron microscopic examination.

Results

Clinical Appearance

During perfusion with Fluosol-DA 20%, oedema gradually developed and was clinically manifest after 8 hours of perfusion. The weight of the perfused digits increased by ± 2.5 g (10% increase). At the end of the perfusion period, when Fluosol was replaced by blood to simulate replantation, the colour of the extremity and digits became pink, with normal capillary refill and blood outflow from the veins. In Patient D, the digit was replanted after 24 hours (8 hours cold ischaemia and 16 hours perfusion). This digit survived for 3 days but unfortunately, together with another digit, had to be removed. Patient E achieved completely successful replantation of a perfused thumb 22 hours after amputation (3 hours warm ischaemia, 2 hours cold ischaemia and 17 hours perfusion).

Histology

Light microscopy. In the foot of Patient A there were no gross alterations between the histology of tissue before perfusion and after 46 hours of perfusion. The only changes observed was an increased space between the muscle fibres, probably caused by oedema. In sections of the digits of the other patients, oedema was present mainly in the vicinity of the blood vessels.

Electron microscopy. The only pathological finding was the absence of vascular endothelium. After one month, a biopsy was taken of the replanted thumb in Patient E. No abnormal findings were detected in this tissue.

K^+, Glucose, Lactate and ATP Levels

Both glucose and K^+ levels showed a slight but constant rise during perfusion. Lactate levels, however, fell during the first 12 hours of perfusion, but later rose again and remained stable until the end of perfusion. This may have been due to the decrease in fluorocarbon content in the perfusate. In the whole-body circulation, the half-life of Fluosol-DA in man has been estimated to be between 7.5 and 22 hours, this being dose dependant (Yokoyama et al. 1981). The primary route of excretion is via the lungs and it is possible that during perfusion and bubble oxygenation the products will have a relatively short half-life. Levels of ATP and ATP breakdown products in muscle specimens were very low, which corresponds with the findings of Hicks et al. (1980) in their perfusion experiments.

Oxygen Measurements

A typical recording is shown in Figure 7. Within 30 minutes of perfusion, tissue PO_2 levels in the digit began to rise. There was usually an 'overshoot' of PO_2, which then fell to lower values. This could be caused by hyperaemia followed by vasoconstriction and indicates viability of the extremity.

Figure 8 demonstrates this 'overshoot' effect during perfusion of Patient A's amputated foot. The average PO_2 was about 60 mm Hg for several hours, but after 32 hours oxygenation began to deteriorate and the pH of the perfusate fell below 7.20. Following exchange of fluorocarbons, oxygenation was possible for a further 12 hours, with an average PO_2 of about 40 mm Hg.

The ability of fluorocarbons to penetrate the microcirculation is well demonstrated in Fig. 9. After 20 hours perfusion of this finger, during which time all three electrodes indicated reasonable levels of PO_2 (about 30 mm Hg), the perfusate was exchanged for compatible blood. Measured PO_2 values then fell rapidly to zero and remained at that level.

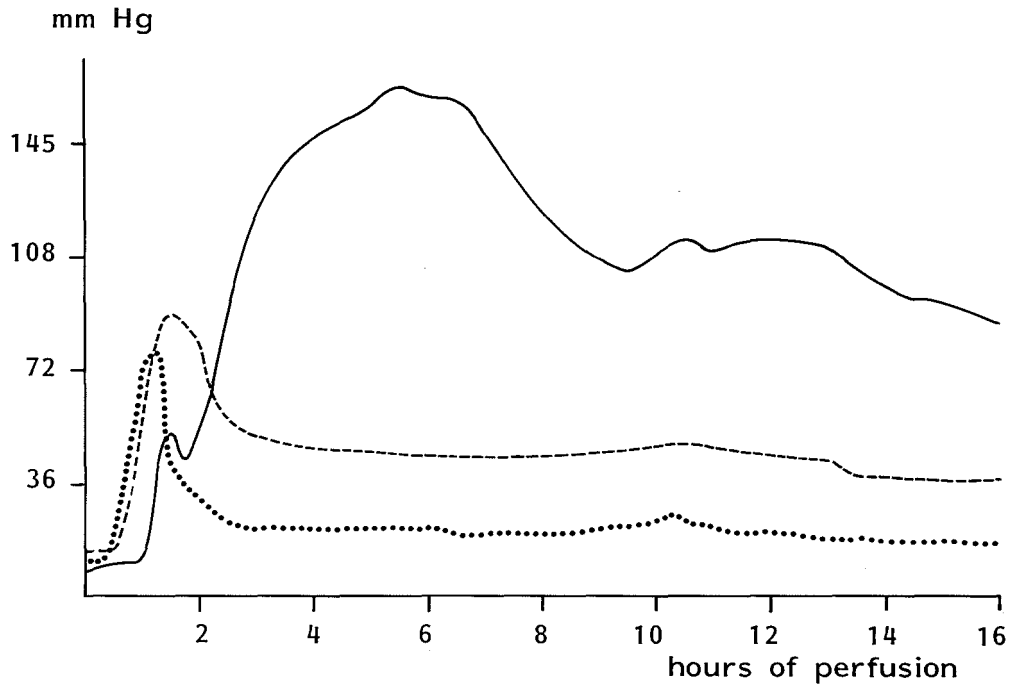

Fig. 7: Tissue oxygen tensions during Fluosol perfusion of an isolated digit.

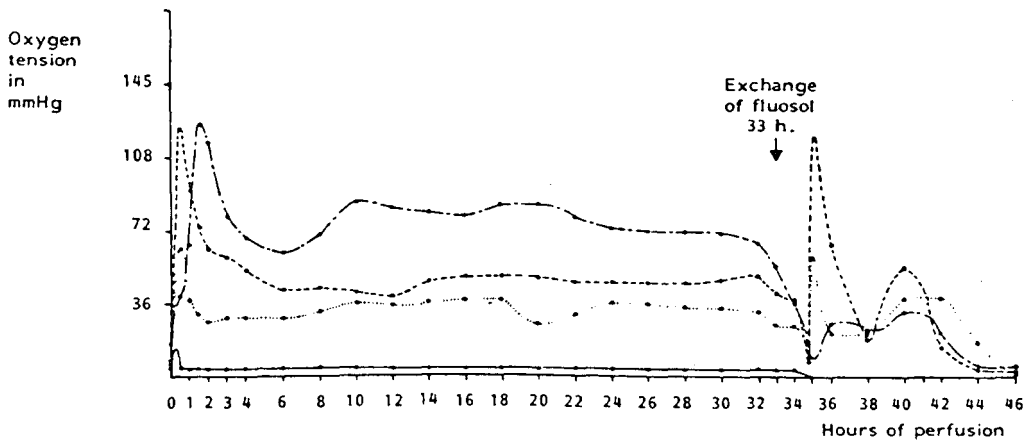

Fig. 8: Tissue oxygen tensions during Fluosol perfusion of a traumatically amputated foot.

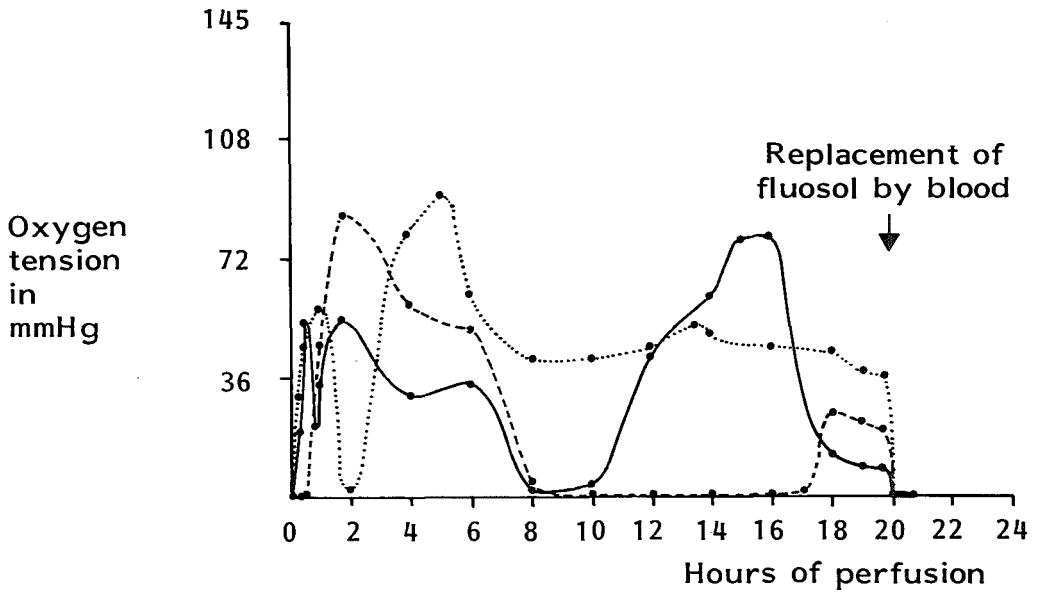

Fig. 9: Failure to re-perfuse with blood following Fluorosol perfusion of an isolated digit.

Discussion

Although cases of extremely long periods of ischaemia prior to re-plantation have been reported (May, 1981), these tend to be incidental successes that are difficult to repeat. The successful replantation of larger amputated parts (a complete hand or foot) with a period of cold ischaemia greater than 24 hours has also been reported (Lapchinsky 1960). However, this often results in a hazardous postoperative period and the necessity for re-amputation. In these cases, moreover, the ultimate level of function is generally poor as impaired oxygenation ultimately results in muscle fibrosis.

The use of suitable perfusion fluids in order to avoid irreversible tissue damage due to ischaemia has been investigated by several authors (Harashina and Buncke 1975, Hayhurst et al. 1974, Hicks et al. 1980, Maurer at al. 1982). Basically these papers report that low temperature of the perfusate is the most important factor, due to the

fact that this decreases metabolism. In addition, perfusion can help to wash out (toxic) breakdown products. Perfusion with fluorocarbons has the added advantage of increasing oxygen supply. Fluorocarbons in replantation surgery was initiated by Asai et al. (1981), who perfused an amputated left forearm for half an hour prior to replantation. No significant problems occurred following replantation.

In our studies, the anoxic period was drastically shortened in comparison to those of the above-mentioned authors. No significant changes occurred in the microscopic sections, probably due to continuous delivery of oxygen to the tissues. Electron-microscopic tests showing the lack of endothelium suggests, however, that the vessels were indeed damaged. This may be due to the period of warm ischaemia prior to perfusion (Morrison et al. 1976), or it might be a consequence of oxygen radical toxicity. The fact that replantation is possible after 24 hours of perfusion supports the idea of neo-intima formation (Smith et al. 1980).

Perfusion of extremities with fluorocarbons at low temperatures will both increase the oxygen carrying capacity of the perfusate and decrease oxygen consumption of the extremity. At 15°C the oxygen carrying capacity of Fluosol-DA 20% is increased by approximately 20% (Naito and Yokoyama, 1978), whereas oxygen consumption is decreased by approximately 85% (Benazon, 1974). It may, therefore, be possible to perfuse with fluorocarbons equilibrated with room air instead of 95% O_2 which may prevent the disappearance of vascular endothelium.

The benefits of fluorocarbon perfusion can be summarised as follows:

1. Storage of digits with minimal tissue damage is possible, e.g. in patients with multiple digit replants, in situations where the replantation team is occupied, or in multi-traumatised patients in whom life-threatening conditions must be treated first.
2. Patient and extemities transportation over longer distances or periods of time might be feasible.
3. Perfusion might provide useful data (PO_2 levels, good inflow and outflow) that indicate the condition of an amputated extremity. This information may then influence decisions as to whether replantation surgery is indicated, especially in doubtful cases.

In conclusion, the above work has demonstrated that extracorporal perfusion with fluorocarbons can maintain oxygenation and viability of isolated extremities for periods of up to 48 hours.

GENERAL DISCUSSION

In order to understand the mechanism whereby improvement in oxygenation is effected by fluorocarbons, it is necessary to consider some of the properties of normal blood and of the Fluosol-DA 20% used in these studies. Blood is a non-Newtonian fluid: a liquid whose viscosity changes with shear rate; at low flows its viscosity can be 10 to 100 times that at high flows. This situation will tend to impede flow in the microcirculation. Two important factors tend to improve the circulation through capillaries:

1. **'Plasma skimming'** takes place, in which the smaller vessels receive proportionately more plasma and less erythrocytes than are present in the general circulation. This decrease in haematocrit leads to a lower viscosity in the smallest vessels.
2. The **'Fahraeus-Lindquist phenomenon'**, whereby erythrocytes can flex to such an extent that they can pass through vessels of considerably smaller diameter than themselves. This is so effective that the blood then behaves like a Newtonian fluid, with a viscosity that does not differ from pure plasma (Goslinga, 1982). This phenomenon only holds good to a certain extent and, at critical diameters of the capillaries, the so-called 'inversion phenomenon' occurs, whereby further decreases in capillary diameter results in a sudden sharp increase in viscosity.

Under certain conditions, the inversion phenomenon occurs at a greater vascular diameter than normal. These conditions include platelet aggregation and acidosis, in which vascular flexibility is decreased (Schmid-Schoenbein et al. 1973). In an ischaemic area, considerable decreases in pH may be found and Cohn et al. (1974) have used epicardial pH measurements to map the extent of experimental myocardial ischaemia. They found a statistically significant correlation between ST segment elevation and pH decreases with a mean surface pH of 7.23 in the maximal ischaemic area. According to

Schmid-Schoenbein et al. (1973), this would be enough to reduce pliability of the erythrocytes. Khuri et al. (1979) measured even lower pH values in the myocardium following 3 hours of LAD occlusion in the dog. Hence, blood viscosity will increase markedly under conditions of ischaemic hypoxia and impaction of erythrocytes will occur.

It is well established that whole body blood viscosity is increased in patients with myocardial infarction (Dintenfass, 1964; Ditzel et al. 1968). Jan et al. (1975) have shown that the early changes in blood viscosity are due to a combination of high haematocrit and increases in plasma viscosity and red cell aggregation - factors which will impede collateral capillary flow to ischaemic areas. Biro (1983) has shown that viscosity is markedly raised 1 hour after LAD occlusion. When fluorocarbon or dextran haemodilution is performed, viscosity is considerably lowered in comparison with the control animals.

The particles of emulsified fluorocarbons in Fluosol are very small. The mean diameter is 0.118 microns and more than 90% of the particles are less than 0.2 microns in diameter (Naita and Yokoyama, 1978). The mean diameter of erythrocytes is very large in comparison. It is probable that a combination of low viscosity and small emulsion particle size confers a unique ability for penetrating deep into collateral capillary pathways and for bypassing stiffened erythrocytes. It is also possible that reoxygenation of these impacted red cells occurs with subsequent further improvement in penetration of the ischaemic area. In this way the viscious circle of stagnant acidosis can be broken.

The clinical implications of the above findings are numerous and diverse. Fluorocarbons relieves us of the problem of bloodtype incompatibilities, although, as in all plasma expanders, adverse clinical reactions can occur (Tremper et al. 1984). The use of Fluorocarbons instead of blood products can help to reduce the number of cases of hepatitis B and A.I.D.S. There are many more possible applications of fluorocarbons including the treatment of Jehovah's Witnesses needing blood transfusion (Lapin, 1983), the treatment of solid tumours (Teicher and Rose, 1984) and intraperitoneal perfusion

with fluorocarbons in pulmonary insufficiency (Klein et al. 1986). Faithfull (1987) has reviewed these applications in more detail. The importance of fluorocarbons in maintaining and improving microcirculatory oxygenation should not be underestimated and, in the future, even more possibilities may be discovered.

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CHAPTER 7

CHANGES IN INTRAMYOCARDIAL OXYGEN TENSION DURING HYPOTHERMIA IN PIGS.

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INTRODUCTION

Hypothermia is firmly established as a therapeutic measure in open-heart surgery. The effects of hypothermia on the body were noted as early as 1798 when James Currie of Liverpool treated patients with fever by cooling them to 32^{o} C. Observations on the effect of body cooling on animals were made during the 19th and early 20th century by Claude Bernard. Smith and Fay (1940) demonstrated the analgesic effects of hypothermia in the cancer patient. Bigelow et al. (1950) cooled dogs by surface immersion in cold water to below 25^{o} C and maintained circulatory arrest for 15 minutes. The introduction of cardiopulmonary bypass in the 1950's enabled rapid safe cooling of the patient; hypothermia was improved for clinical use during cardiac surgery by Swan (1984).

Many workers have reported on the metabolic changes occurring during hypothermia (Lloyd and Mitchell 1974, Prakash et al. 1978, Becker et al. 1981, White 1981, Wong 1983, Swain et al. 1984) but, as far as we are aware, no studies have been made on the effects of hypothermia on myocardial oxygen tension (PmO_2). Experiments were

therefore performed in pigs to investigate any changes in PmO_2 that occur under whole body hypothermia. This paper reports our preliminary findings.

MATERIAL AND METHODS

In eight juvenile Yorkshire pigs (± 25 kg), anaesthesia was induced with intraperitoneal sodium thiopentone (30 $mg.kg^{-1}$) and maintained on mixtures of nitrous oxide and oxygen (3:2). Muscular relaxation was maintained by continuous infusion of pancuronium bromide (0.5 $mg.kg^{-1}.hr^{-1}$) using a Braun Melsungen perfuser. The animals were intubated and ventilated using a Servo ventilator, the minute volume of which was adjusted to produce an end tidal carbon dioxide percentage of between 4 and 5 volumes percent.

A six French gauge BP6 angiographic catheter (William Cook, Europe) was introduced into the femoral artery, through an incision in the right inguinal region, and a seven French gauge KMA thermodilution Swan Ganz catheter was introduced into the left femoral vein. The latter was advanced under pressure monitoring into the pulmonary artery so that pulmonary capillary wedge pressure could be obtained. Thermodilution cardiac output measurements were performed using a KMA cardiac output computer Model 3500E. Via the other femoral vein, another Cook BP6 catheter was passed into the inferior vena cava in order to obtain central venous pressure measurements. All the above pressure lines were connected to Gould Statham P23D pressure transducers. Via the right carotid artery, a Millar microtip pressure transducer PC470 was introduced into the left ventricle for ventricular pressure and dP/dt measurements. ECG signals were obtained from needle electrodes.

A midline thoracotomy was performed and the pericardium was reflected. An electromagnetic flow probe was mounted around the left circumflex coronary artery and connected to a transflow 601 flowmeter (Skalar). All the above signals were fed into a 12 channel Grass 7D polygraph and recorded on paper. After offline input of the cardiovascular variables into a Philips P2000 microcomputer, left and right ventricular minute work and systemic and pulmonary vascular resistance could be calculated.

Temperatures from the nasopharynx, rectum and tympanic membrane were measured by the use of thermistor probes and an Ellab Instruments electrical thermometer system type TE 5. In addition, a needle thermister was placed subcutaneously in the thoracotomy wound. The pulmonary arterial temperature was displayed on the KMA cardiac output computer.

Mixed venous and arterial blood gas estimations were performed at regular intervals using a Radiometer ABL 1 Acid/base laboratory. Arterial and mixed venous haemoglobin estimations and oxyhaemoglobin saturations were measured using a Radiometer OSM2 Hemoximeter. The above mentioned measurements allowed the following parameters to be calculated on the Philips P2000 microcomputer: arterial and mixed venous oxygen contents and whole body flux and consumption. Statistical significance was assessed on a Commodore 64 microcomputer using paired Student's t tests and the nul hypothesis was rejected at $p<0.05$.

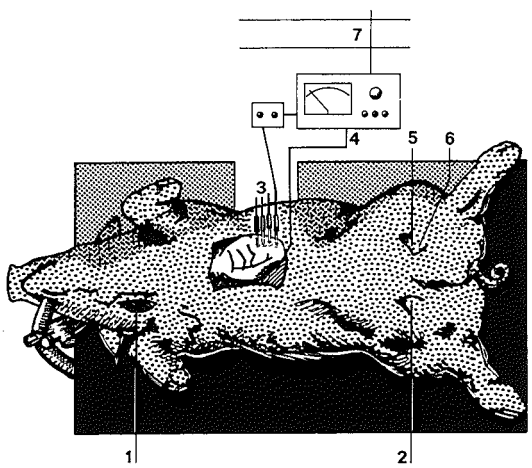

Fig. 1: Experimental setup.
1= Intraventricular line
2= Central venous line 3= Microelectrodes
4= Amplifier 5= Arterial line
6= Swan Ganz TD catheter 7= Faraday cage

Measurement of PmO_2 was accomplished with the use of four steel-protected gold microelectrodes (Clark Electromedical Instruments) and a Ag/AgCl reference electrode. These electrodes, 200 microns in diameter, were embedded in a thin flexible plastic plate $\pm$5 cm x 2 cm, which was sewn onto the epicardium of the left ventricle. The distance between the electrodes was 1 cm and in position they protruded 3 mm into the left ventricular wall to ensure contact with the myocardium. When the gold electrodes are negatively polarised, oxygen is reduced causing a current to flow through the amplifier (Knick nanoamperemeter). At a voltage of -800 mV, this current is directly proportional to the O_2 concentration. To ensure partial pressure measurement, the electrode tips are covered with a plastic membrane, which also prevents protein poisoning and minimises convection dependency (Erdmann et al. 1979, Fennema and Erdmann 1985). Because the currents are so small (1-20 nanoamperes) measurements were performed in an isolated Faraday cage. The amplified signals were registered on a Rickadenki recorder model KA 60.

About 2 hours after polarisation, the electrodes were calibrated in a 22^{o} C saline solution, through which gases of known O_2 partial pressure could be bubbled for equilibration. After the experiments, the electrodes were again calibrated to ensure reliability and to estimate any drift that had taken place. Results were discarded from any electrode having a drift of more than 7.5% per hour. Due to the physical qualities of the noble metal, the current produced by the microelectrodes is proportional to the temperature, so that the PmO_2 measurements had to be corrected for temperature and drift with the use of the Philips P2000 computer.

After allowing time for stabilisation of the preparation, cooling was commenced using a Thermo-Rite heat exchanger and mattress. The temperature was reduced at a rate of 2^{o} C per hour until a temperature of approximately 30^{o} C was achieved. The pig was then rewarmed, at about the same rate, until the temperature was stable at 37-38^{o} C. Our research group, and other workers (Rahn and Reeves 1982, Wong 1983, Swan 1984), consider that the blood gas values should not be corrected for temperature and uncorrected arterial carbon dioxide tensions should be maintained between 35 and 45 mm Hg. For this reason ventilation was not adjusted during hypothermia.

RESULTS

The tympanic temperature, an indication of the brain temperature (Swan 1984), was constantly about 0.2^{o} C lower than the temperature measured in the pulmonary artery which was approximately 0.1^{o} higher than the rectal temperature. The temperature of the nasopharynx was always lower than all the temperatures mentioned above and this discrepancy increased during rewarming. The thoracic wall subcutaneous temperature was 2 to 4 degrees lower than the blood temperature. For these reasons the temperature at the tip of the Swan-Ganz catheter was used as a marker for core temperature (Reinhold et al 1982).

Table I shows the changes occurring in measured and derived cardiovascular values during induction and recovery from hypothermia. During induction of hypothermia, significant decreases in cardiac output (CO) and left ventricular work (LVW) were seen, accompanied by a significant increase in systemic vascular resistance (SVR) (fig. 2). During the initial decrease in temperature, both arterial pressure (AP) and dP/dt showed significant increases, returning to normal values at 31^{o} C. In general these changes were reversed during rewarming, but SVR and AP remained high at the end of rewarming. In general, these changes were also reflected in the pulmonary circulation.

As previously mentioned, the ventilation was not adjusted during the experiment, and therefore end expiratory CO_2 concentration decreased during hypothermia ($p<0.001$). The arterial PCO_2, bicarbonate and pH also showed significant decreases, but remained within the normal range (Table II). After rewarming, the bicarbonate concentration was still lower than control levels ($p<0.01$). The haemoglobin concentrations increased ($P<0.05$), whilst the oxyhaemoglobin saturations remained constant throughout the whole experiment. None of the above mentioned changes presented problems.

Table I: Cardiovascular changes during hypothermia and rewarming

	37°C	35°C	33°C	31°C	+33°C	+35°C	+37°C
AP	107 ±4.9	114 ±7.4	120* ±6.7	113 ±7.5	123* ±7.5	124* ±6.6	117 ±6.1
PCWP	12 ±1.4	12 ±1.9	12 ±1.8	11 ±2.3	13 ±2.3	13 ±3.4	13 ±3.5
CO	2.53 ±0.32	2.35 ±0.33	2.22 ±0.29	1.61** ±0.27	1.72 ±0.22	1.94 ±0.34	1.78 ±0.48
CF	39 ±2.7	41 ±2.0	41 ±2.1	38 ±3.8	49 ±5.8	48 ±6.4	53 ±9.0
dP/dt	4.23 ±0.27	4.90 ±0.28	5.88* ±0.54	5.25* ±0.32	5.92 ±0.45	6.56 ±0.50	5.92 ±0.37
HR	131 ±10.1	145 ±7.9	120 ±20.4	124 ±2.9	135 ±11.7	153 ±7.7	151 ±13.5
LVW	3.14 ±0.52	3.33 ±0.53	3.32 ±0.44	2.34* ±0.32	2.66 ±0.44	3.00 ±0.58	2.66 ±0.82
SVR	3500 ±370 (8)	4310 ±917 (8)	4520 ±722 (8)	5850* ±1100 (7)	5430* ±717 (5)	5110 ±816 (5)	5580* ±826 (5)

Results are expressed as mean ± SEM. Significance on paired Student t-test compared to control (37° C): *=p<0.05, **=p<0.01. The number of observations are in parenthesis at the bottom of the Table.

AP = Arterial Pressure (mm Hg)

PCWP= Pulmonary Capillary Wedge Pressure (mm Hg)

CO = Cardiac Output (l/min)

CF = Left Circumflex Coronary Flow (ml/min)

HR = Heart Rate (beats/min)

LVW = Left Ventricular Work (kg.m/min)

SVR = Systemic Vascular Resistance (Dynes.sec/cm^5)

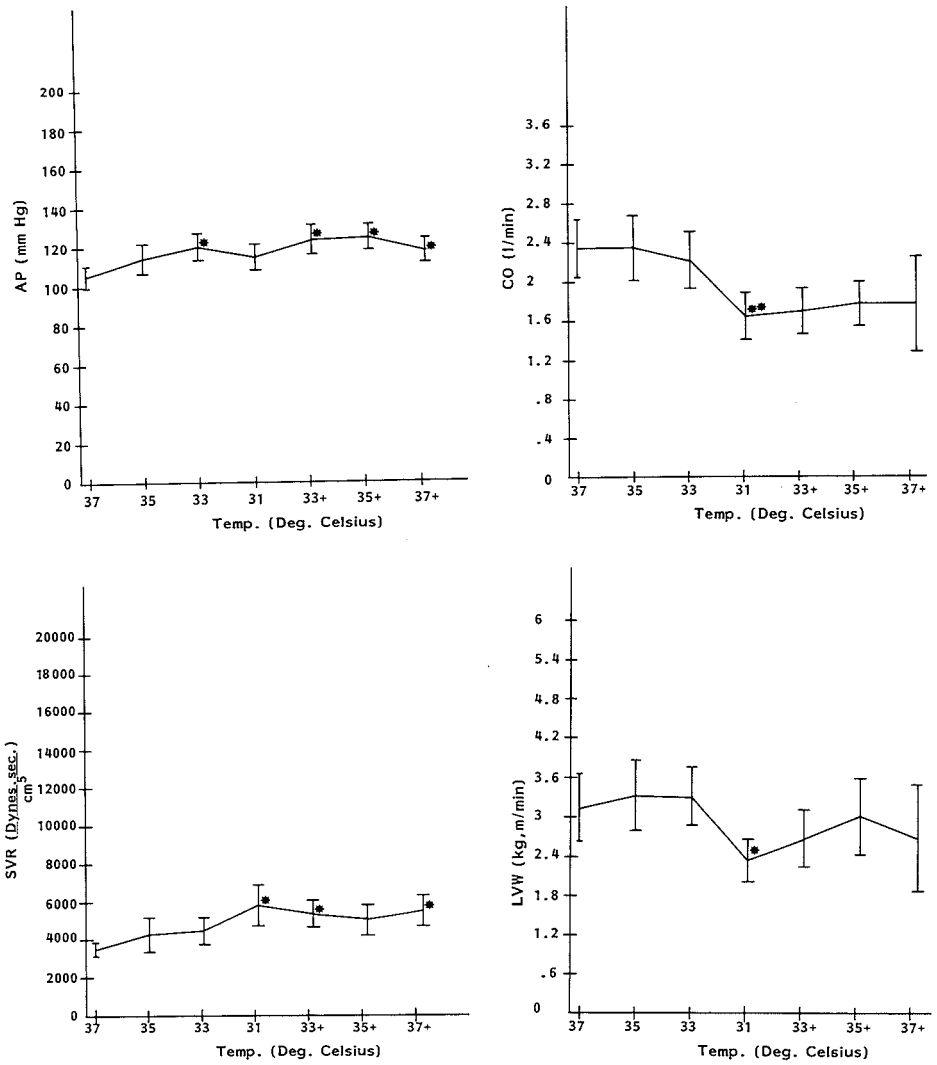

Fig. 2: Changes in arterial pressure (AP), cardiac output (CO), systemic vascular resistance (SVR) and left ventricular work (LVW) during hypothermia and rewarming. Significance on a paired Student's t test: *=$p<0.05$, **=$p<0.01$

Table II: Acid-base, haemoglobin and oxygenation status during hypothermia and rewarming.

	37°C	35°C	33C	31°C	+33°	+35°	+37°C
Hb	10.93 ±0.31	12.18* ±0.33	12.40* ±0.62	12.35* ±0.58	12.66 ±0.68	13.10 ±0.87	12.34 ±0.75
Art. Sat.	97.9 ±0.3	94.1 ±2.7	95.0 ±2.0	97.4 ±1.0	94.8 ±2.7	91.3 ±4.3	93.6 ±2.7
$PaCO_2$	34.2 ±1.4	34.7 ±0.6	33.2 ±1.1	31.1 ±1.3	33.6 ±1.5	34.6 ±0.9	37.2 ±2.8
H^+	32.4 ±0.96	35.4* ±1.23	37.3* ±1.60	35.3 ±1.36	39.4** ±1.43	38.9 ±1.82	39.7 ±2.07
HCO_3^-	26.1 ±0.8	24.1* ±0.7	21.9*** ±0.8	21.6*** ±0.6	21.0** ±1.1	21.9* ±1.3	21.7* ±1.3
Exp. CO_2	4.5 ±0.27	4.1* ±0.19	3.5** ±0.22	2.9*** ±0.16	3.8* ±0.24	4.1 ±0.21	4.7 ±0.24
PaO_2	153 ±7.6	142 ±10.3	141 ±11.5	141 ±16.1	155 ±13.3	153 ±15.1	130 ±7.2
PvO_2	53 ±1.7	41** ±1.7	41 ±1.4	38 ±2.9	45 ±1.2	42 ±3.2	44 ±1.1
CaO_2	15.1 ±0.62	16.1 ±0.53	16.0 ±0.44	16.4** ±0.61	16.7 ±0.51	16.8 ±0.87	16.0 ±0.45
CvO_2	9.0 ±0.28	10.6* ±0.64	11.0* ±0.95	9.4 ±0.83	9.7 ±1.28	9.2 ±1.30	7.5 ±1.14

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Table II continued.

	37°C	35°C	33C	31°C	+33°	+35°	+37°C
(a-v) D_{O_2}	6.1 ±0.65	5.6 ±0.83	5.0 ±0.84	6.9 ±1.15	7.0 ±0.88	7.6 ±1.16	8.5 ±0.91
Q_{O_2}	380.2 ±51.3	368.5 ±46.4	366.3 ±50.1	261.5* ±38.5	293.4 ±52.8	323.4 ±55.3	291.4 ±89.6
V_{O_2}	148.0 ±22.2 (8)	112.5 ±7.9 (8)	100.6 ±6.4 (8)	102.1 ±15.1 (7)	114.5 ±7.3 (5)	132.5 ±6.3 (5)	137.5 ±26.4 (5)
PmO_2	87.3 ±8.6 (15)	74.7* ±8.2 (15)	69.4* ±9.2 (15)	54.9** ±10.7 (11)	85.1* ±12.1 (9)	93.8 ±10.9 (9)	96.3 ±13.2 (9)

Results are expressed as mean ± SEM. Significance on paired t-test compared to 37° C: *=p<0.01, **=p<0.01, ***=p<0.001. The number of observations are in parenthesis.

Hb = Haemoglobin (g/100 ml)
Art.Sat.= Arterial oxygen saturation (%)
$PaCO_2$ = Arterial carbon dioxide oxygenation (mm Hg)
H^+ = Hydrogen concentration (pH; nmol/l)
HCO_3^- = Bicarbonate (mmol/l)
Exp. CO_2= Expiratory carbon dioxide (vol. %)
PaO_2 = Arterial oxygenation (mm Hg)
PvO_2 = Mixed venous oxygenation (mm Hg)
CaO_2 = Arterial oxygen content (ml/100 ml)
CvO_2 = Mixed venous oxygen content (ml/100 ml)
(a-v)DO_2= Oxygen content difference (ml/100 ml)
QO_2 = Oxygen flux (ml/min)
VO_2 = Oxygen consumption (ml/min)
PmO_2 = Myocardial oxygenation (mm Hg)

Table II shows the changes in some of the parameters that determine the oxygenation status of the body. Due to an increase in haemoglobin concentration, increases were seen in both arterial and mixed venous oxygen content. Nevertheless, due to significant decreases in cardiac output, whole body oxygen flux was significantly decreased. Decreases in oxygen consumption did not reach significance. From the 8 pigs, 15 microelectrode measurements fell within our requirements. Intramyocardial oxygen tension steadily declined from a normothermic mean value of 87.3 mm Hg ($\pm$8.61 SEM) to a mean value of 54,9 mm Hg ($\pm$10.72 SEM) at 31° C ($p<0.01$) as shown in fig. 3. All the above mentioned values returned to their normal values after rewarming.

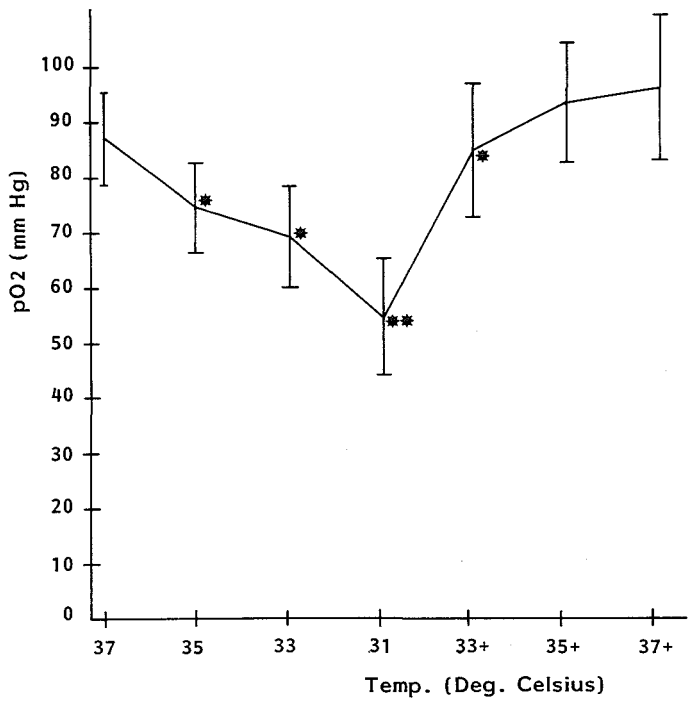

Fig. 3: Myocardial oxygen tensions during hypothermia and rewarming. the readings have been corrected for temperature and drift. Significance on paired Student's t test compared to 37° C: *=$p<0.05$; **=$p<0.01$.

DISCUSSION

In this investigation, higher PmO_2's were measured than by other workers (Lund 1986). Moss (1968), using bare tipped electrodes, measured very low PmO_2's of 18 mm Hg $\pm$2.3 SEM in dogs that were ventilated with room air. Because these electrodes were not protected, progressive protein poisoning would cause lower measurements. As also shown by Moss, electrode needle movement within the myocardium would cause higher PmO_2 measurements, due to the stirring effect. Using a polarisation voltage of -660 mV, v.d. Laarse (1978) measured canine PmO_2's of 25,8 mm Hg $\pm$0.4 SEM.

As far as we know, no studies have been performed in which porcine PmO_2 has been measured. However, Walfridson et al (1986) have measured a mean of 45 mm Hg in the pig epicardium using an MDO surface electrode. The PaO_2 in their study was kept at 100 mm Hg whereas in the present investigation the pigs were ventilated with 40% oxygen so that arterial PO_2 was 153 mm Hg ($\pm$7.6 SEM). Hence it would seem that the PmO_2 is higher in pigs than in dogs. Even though the absolute measurements obtained with our electrodes seem to be rather high, the relative changes obtained in the past have always been realistic (Faithfull et al 1985, Smith et al 1985).

Intramyocardial oxygen tension greatly decreases under hypothermia. One might assume that this could be explained by a decrease in oxygen flux to the myocardium and consequent relative excess of consumption over flux. There was a significant decrease in whole body oxygen flux, but it is debatable whether this was the case in the myocardium, because the coronary flow barely changed. It is known that plasma viscosity may be increased by hypothermia (Somer 1966). This, in combination with the haemoconcentration that was observed in the present study, would increase whole blood viscosity and effect the microcirculatory distribution of blood flow. Erythrocyte stiffening also occurs under hypothermia (Barnikol and Burkhard 1984) and this would also disturb the myocardial microcirculation and decrease oxygen availability to the myocardium.

In conclusion it has been demonstrated that the main cardiovascular changes occurring during hypothermia in the pig may be

attributed to a progressive decrease in cardiac output in conjunction with an increase in peripheral resistance. PmO_2's are reversably decreased under hypothermia and the possibility of microcirculatory mismatch of supply and demand at low temperatures should be born in mind.

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CHAPTER 8

GENERAL CONCLUSIONS

The fabrication of the micro-electrode system is described in the beginning of this thesis. Here it is shown that if the electrodes are calibrated carefully and certain precautions are taken (e.g. temperature correction, electrical noise-free environment), accurate measurements of tissue oxygenation can be achieved. Under normal conditions tissue PO_2 shows a broad range of values from as low as 1 mm Hg to up to 100 mm Hg, depending on in which organ the measurement takes place and where the electrode tip is positioned in relation to the capillary network. Therefore, a single momentary measurement is of little use as the exact morphological position of the electrode cannot be determined. However, PO_2 measurements have an informative value if trend analysis is performed with one, or preferably more, electrodes. This is demonstrated particularly well in chapter 6 where the viability of amputated extremities perfused with flurocarbons is monitored with the aid of PO_2 microelectrode measurements. On the other hand multiple momentary measurements can be useful for answering scientific questions when statistical analysis is applied, as is shown in chapter 3 when measuring follicular PO_2's.

This thesis covers a large area in (patho)physiology with, as common denominator, micro-circulation and oxygenation. Basically this thesis shows the necessity to consider tissue oxygen tensions in medical science. This is supported with the aid of PO_2 micro-electrode measurements.

In conclusion, I would like to summarise which new insights have been achieved by studying the questions outlined in the aims of this thesis:

1. There is a wide range of PO_2's, the so called PO_2-profile, so that there is no normal PO_2 in tissues. Factors that influence tissue PO_2 are: PaO_2, O_2 transport of the blood, tissue perfusion, distance from capillary, O_2 diffusion and O_2 consumption. The absolute PO_2 is not important, but the relative changes are, as cells show an environmental adaption to local PO_2. An intact auto-regulation is necessary to ensure adequate oxygenation.
2. At least in the rat, an increase in follicular PO_2 is associated with ovulation. This could mean that it is important to consider environmental PO_2 during in vitro fertilisation.
3. An important consequence of infarction in the pig, is myocardial oxygen steal from the border area to the ischaemic area. Considering that the porcine heart is similar to that of the human, this effect might also be the case in man.
4. Fluorocarbons can effectively reduce ischaemia in the infarcted myocardium, whether given before or after infarction.
5. Fluorocarbons are a suitable perfusate in traumatically amputated extremities, especially in situations where immediate replantation is not possible. Tissue PO_2 measurements are needed for deciding whether sufficient revitilisation has taken place before proceeding with replantation.
6. Even though oxygen consumption decreases under hypothermia, myocardial PO_2 also decreases, so that one must still consider an oxyen supply/demand mismatch.

CHAPTER 1 INTRODUCTION

For the normal function and survival of an organism, a continuous supply of energy, and therefore oxygen, is necessary. Oxygen transport to tissue occurs in three steps:

1. Oxygen uptake in the lung.
2. Oxygen transport in blood.
3. Diffusion of oxygen from the capillaries to the tissue, and through the tissue to the cells.

When pulmonary gas exchange, cardiac output and oxygen transport capacity of the blood are within the normal range, oxygen supply to the tissue is dependent on the following microphysiological parameters:

1. Distribution/perfusion ratio of the capillary meshwork.
2. Oxygen consumption of the cell.
3. Oxygen diffusion parameters from the capillaries to the tissue, through the tissue and across the cell membrane into the cell.

Normally capillary perfusion and oxygen consumption are in balance and oxygen supply to the cell is continuously autoregulated to its needs. However, many pathophysiological conditions can severely interfere with this balance.

Studies of macrophysiological parameters such as blood gases, blood pressures, blood flow and O_2 consumption of whole organs have been possible for years. In the absence of suitable measuring techniques, many scientist have tried to explain microphysiology with the aid of theoretical mathmatical formulae. These workers obviously had to make assumptions for the basic conditions in the, for them, inaccesible micro-area. It would, therefore, seem obvious that the information derived through these methods is only an approximation of the actual conditions in the microarea.

Another step forward to the understanding of the oxygen supply to the microarea was the development of microelectrodes for the measurement of tissue oxygen partial pressures. Chapter 2 explains the fabrication of these electrodes. The next chapters prove the importance of this technique in explaining microphysiology in pathophysiological conditions.

CHAPTER 2 METHODOLOGY - ON LINE DETERMINATION OF PO_2, OXYGEN DIFFUSION, TISSUE PERFUSION AND ACTION POTENTIALS WITH A 10 MICRON TIP ELECTRODE SYSTEM.

Electrodes for PO_2 measurements are fabricated as follows: A gold wire is drawn out in glass to a diameter of 5 microns and cut into adequate pieces for electrode production. The bare gold surface is ground obliquely and covered with a membrane so that it can serve as a polarographic PO_2 sensor.

PO_2 measurements are performed as current measurements to the negatively charged (-800 mV) noble metal with an Ag/AgCl electrode serving as an indifferent anode. Perfusion measurements can be performed intermittently via H_2-clearance with the same electrode system, but with a polarisation voltage of +200 mV.

The electronic circuit can be adjusted to achieve simultaneous amplification of the low PO_2 dependent DC current and the high frequency AC potentials of neuronal activity.

For oxygen diffusion (DO_2) measurements, a second blank gold/glass wire is inserted, in addition to a membrane covered sensor, into a double-barrelled drawn-out glass capillary. O_2 diffusion can be calculated from the current/time curve when the bare electrode is polarised. By comparing the difference in measurements between the bare and membrane covered electrode, it is possible to calculate the oxygen conductivity coefficient (KO_2). Since one can determine DO_2 and KO_2, it is now possible to calculate the solubility coefficient (α) for oxygen in the tissue according to:

$$\alpha O2 = \frac{KO2}{DO2x60}$$

To avoid noise disturbance, the measurements are performed in a double shielded Faraday cage. If necessary, the pre-amplified (PO_2) values can be transmitted by light across the interspace of the two walls.

CHAPTER 3 OXYGEN TENSIONS IN THE PRE-OVULATORY AND NON-OVULATING OVARIUM FOLLICLE OF THE RAT.

To test the hypothesis that ovulation might be accompanied by an increase in the PO_2 of the ovarian follicle, PO_2 was measured with microelectrodes of 10 microns diameter. Two groups were compared:

1. A non-ovulating group of proestrous rats, where ovulation was inhibited with phenobarbital (35 mg/kg BW).
2. An untreated pre-ovulatory group.

The mean PO_2 in follicles not exposed to the LH surge was 11.7 mm Hg $\pm$ 1.12 SEM, as opposed to 23.5 mm HG $\pm$ 1.44 SEM in the ovulatory control follicles ($p<0.001$; Student's t test).

These observations lend support to the hypothesis that oxygen may be the limiting factor in the resumption of the first meiotic division in the pre-ovulatory oocyte.

CHAPTER 4 TISSUE OXYGENATION IN THE CEREBRAL CORTEX OF THE RABBIT.

Clinical events such as bleeding, shock, ischaemia and drug administration can influence oxygen transport to tissue decisively. This simple external factors can effect many internal mechanisms with a resulting decrease or increase in tissue PO_2, sometimes with severe adverse effects.

This study was performed to assess the cerebral PO_2 profile under normal conditions of anaesthesia and investigate the effects of hyperoxia and hypercapnia on cerebral tissue PO_2.

When inserting an oxygen electrode into the cortex with the aid of a micromanipulator, one can produce a PO_2-profile. This profile can be reasonably well reproduced by withdrawing and re-inserting the electrode. With an FiO_2 of 0.3, the overall average PO_2 is about 6 mm Hg. Nearly all measurements are below 25 mm Hg and measurements above 50 mm Hg are very rare.

When the FiO_2 is increased to 1.0, tissue PO_2 increases, then gradually decreases to approximately 120% it's original value. This is probably due to vasoconstriction.

When 12% CO_2 is added to the inspiratory gas mixture the PO_2 rises slowly to nearly three times it's original value and shows little tendancy to compensate. This rise in PO_2 is due to the direct effect of CO_2 and H^+ on the blood vessels, causing vasodilation, and therefore an increase in blood flow and tissue PO_2. Normally this increase in blood flow would wash out the CO_2, decreasing the vasodilation stimulus so that PO_2 would drop. In this case CO_2 is kept artificially high, so that maximum vasodilation is maintained.

CHAPTER 5 OXYGENATION OF THE MYOCARDIUM

Aspects on oxygenation of the myocardium are discussed in relation to myocardial contraction, coronary flow, ventilation and ischaemia. These theories are supported by tissue measurements in the pig, using steel protected microelectrodes, 200 microns in diameter. The electrodes were embedded in a thin flexible plastic plate which was sewn onto the epicardium. The distance between the electrodes was 1 cm and they protruded 3 mm into the left ventricular wall. The plate was placed in such a way that when occluding a branch of the LAD, 2 electrodes would be in the ischaemic area, and 2 in the non-ischaemic area. The most important findings are:

Cardiac swings

Oxygen tension swings coincide with heart beat, but there is a phasic difference between the areas measured. There are several explanations, the most important being that during myocardial

contraction blood flow in the arterioles decreases, while it increases in capillaries and venules. Also, the degree of contraction varies in different areas of the myocardium, depending on the progress of the depolarisation wave over the heart.

Respiratory Swings

During intermittent positive pressure ventilation, inspiration causes an increase in left ventricle blood volume which in turn causes both a decrease in coronary blood flow and an increase in cardiac work. This change in the oxygen supply/demand ratio causes the myocardial tissue oxygen tension to drop.

Electrical activity

There appears to be a critical PmO_2 below which myocardial electrical activity ceases. This threshold varies in different areas of the myocardium.

Changes in FiO_2

When FiO_2 is increased, the average PO_2 increases although, paradoxically, in some areas the PO_2 decreases.

Temporary occlusion of the LAD

In short term occlusion ($\pm$30 sec.) some areas show a decrease in PO_2, but some show an increase. Often there is an overshoot in the opposite direction. The biphasic nature of the oxygen curves indicate that the initial decreased oxygen supply is in some cases rapidly compensated for by autoregulation.

Permanent occlusion of the LAD

The results of an experiment to investigate the long term effects of occlusion of the distal third of the LAD are discussed. Following occlusion, immediate highly significant decreases in PmO_2 were seen in the most ischaemic area. 4 minutes post-occlusion these had recovered at the expense of the adjacent ischaemic border area which continued

to indicate highly significant decreases of PmO_2 throughout the first hour post-occlusion, These changes were interpreted as indicating a high degree of myocardial steal.

CHAPTER 6 MICROCIRCULATION AND FLUOROCARBONS

Fluorocarbons are chemical substances with a high solubility for oxygen. The emulsified product available to us can carry about 0.75 ml O_2 in 100 ml per 100 mm Hg, and releases O_2 more readily than haemoglobin. Furthermore, the fluorocarbon emulsion has a low viscosity which, as opposed to blood, is almost independent of the low flow shear rate existing in the microcirculation. This, together with the small particle size, helps to ensure better penetration in the microcirculation to improve oxygenation after, for instance, myocardial infarction or limb amputation.

The effects of haemodilution on oxygen tension in the acutely ischaemic myocardium

The pigs mentioned in the previous chapter were divided into three groups. In group I no treatment was given and in group II and III 20% haemodilution was performed with fluorocarbons or dextran respectively after one hour of LAD occlusion. After haemodilution PmO_2 in the fluorocarbon group II (in contrast to groups I and III) was no longer significantly lower than pre-occlusion values. After 5 hours of occlusion mean PmO_2 in this group had returned to 90.1% of the pre-occlusion values whereas in groups I and III it was 26.7% and 37.3% respectively. This study demonstrates that haemodilution itself does not improve myocardial oxygenation, whereas re-oxygenation of the areas of the ischaemic myocardium is possible following haemodilution treatment using fluorocarbon emulsions.

Prophylactic treatment against myocardial ischaemia with fluorocarbons.

In this case 5 pigs were haemodiluted with fluorocarbons 30 minutes before the distal third of the LAD was occluded. Mean PmO_2 was always (sometimes significantly) higher than the non-treated control animals.

This supports the theory that prophylactic administration of fluorocarbons might help protect the myocardium in the vital first hours of infarction.

Limb Preservation in replantation surgery

Five patients are presented in whom a traumatically amputated extremity or digit was perfused with oxygenated fluorocarbons. Perfusion lasted between 16 and 46 hours. Besides tissue PO_2 measurements, K^+, lactate, glucose and ATP levels were measured and electron microscope sections of vessels were investigated. All these parameters suggest that the tissue was sufficiently provided with oxygen and viable after many hours of perfusion. Conclusive evidence of the viability of a perfused digit was given by its successful replantation after 17 hours of fluorocarbon perfusion.

CHAPTER 7 CHANGES IN INTRAMYOCARDIAL OXYGEN TENSIONS DURING HYPOTHERMIA IN PIGS

In 8 juvenile pigs, 4 PO_2 microelectrodes were introduced into the myocardium as described before. After stabilisation, the pigs were cooled down to 30^{o} C and then rewarmed. Although there were changes in cardiovascular and acid-base parameters, these did not present any problems. Due to a significant decrease in cardiac output during cooling, whole body oxygen flux was significantly decreased. This was probably not the case in the myocardium because coronary flow hardly changed. There was, however, a significant decrease in intramyocardial PO_2, probably due to an increase in viscosity which effects the microcirculatory distribution of blood flow. Therefore, even though the animal recovers after rewarming, microcirculatory mismatch of supply and demand at low temperatures should be born in mind.

HOOFDSTUK 1 INLEIDING

Het normale functioneren en de overleving van een organisme is afhankelijk van een continu voorraad van energie, dus ook van zuurstof. Zuurstofvervoer naar weefsel neemt in drie stappen plaats:

1. Zuurstofopname in de longen
2. Zuurstofvervoer in het bloed
3. Diffusie van zuurstof van de capillairen naar het weefsel en door het weefsel naar de cellen

Als pulmonaire gasuitwisseling, cardiac output en de zuurstof dragende capaciteit van het bloed binnen het normale bereik blijft, is de zuurstoftoevoer naar het weefsel afhankelijk van de volgende microfysiologische parameters:

1. Verdeling/perfusie verhouding van het capillaire netwerk.
2. Zuurstofconsumptie van de cel.
3. Zuurstofdiffusie parameters van de capillairen naar het weefsel, door het weefsel heen en door het celmembraan de cel in.

Gewoonlijk zijn capillaire perfusie en zuurstofconsumptie in evenwicht. De zuurstoftoevoer naar de cel is d.m.v. autoregulatie aangepast aan de behoefte. Maar vele pathofysiologische omstandigheden kunnen dit evenwicht ernstig verstoren.

Het bestuderen van macrofysiologische parameters zoals bloedgassen, bloeddrukken, bloedstroomsnelheid en zuurstofconsumptie van organen is al vele jaren mogelijk. In de afwezigheid van toepasbare meettechnieken hebben vele wetenschappers microfysiologie proberen te verklaren m.b.v. wiskundige modellen. Daardoor moesten zij bepaalde simplificaties aannemen voor de basis condities van de, voor hun, onbereikbare micro-gebieden. Het moet dus duidelijk zijn dat de gegevens die op deze manier gevonden worden, alleen maar een benadering van de werkelijkheid zijn.

Een grote stap vooruit in het begrip van de zuurstoftoevoer naar het micro-gebied was de ontwikkeling van de microelectodes voor het meten van de zuurstof partiële druk. Hoofstuk 2 legt uit hoe deze electrodes gemaakt worden. De volgende hoofdstukken bewijzen het belang van die techniek in het verklaren van microfysiologie in pathofysiologische omstandigheden.

HOOFDSTUK 2 FABRICAGE VAN ZUURSTOF MICROELECTRODES.

Een electrode systeem is ontwikkeld om het mogelijk te maken diverse parameters en hun interacties gelijktijdig te onderzoeken, nl. partiële zuurstof druk (PO_2), zuurstofdiffusie, weefselperfusie en actie potentialen. Een gouden draad wordt in glas uitgetrokken tot een diameter van ±5 micron. De naakte gouden punt wordt schuin afgeslepen en dan met een membraan gedekt zodat het geschikt is voor polarografische PO_2 metingen.

PO_2 metingen worden uitgevoerd als electrische stroom metingen tussen het edelmetaal met een spanning van -800 mV ten opzichte van een indifferente Ag/AgCl electrode. Perfusiemetingen worden uitgevoerd met dezelfde electrode maar met een polarisatiespanning van +200 mV.

Het electronisch cicuit kan aangepast worden zodat gelijktijdig meten van laag PO_2 afhankelijk DC stroom en hoog frequent AC potentialen van neuronen mogelijk is.

Voor zuurstofdiffusie (DO_2) metingen wordt een naakte goud-glas draad samen met een membraan beschermde draad in een dubbel-loops glas capillair gebracht. Zuurstofdiffusie kan worden berekend uit de stroom/tijd curve als het naakte metaal onder spanning wordt gezet. D.m.v. het verschil in stroom uitslag tussen het naakte en de beschermde electrodes is het mogelijk om de zuurstof conductiviteits coëfficient (KO_2) uit te rekenen. M.b.v, deze parameters is het nu mogelijk om de zuurstofoplosbaarheids coëfficient (αO_2) uit te rekenen:

$$\alpha O2 = \frac{KO_2}{DO_2 \times 60}$$

Om electrische ruis uit te sluiten, vinden de metingen in een Faraday kooi plaats. Indien nodig worden gegevens via licht-transmissie buiten de kooi gebracht.

HOOFDSTUK 3 PO_2 METINGEN IN HET OVARIUM FOLLIKEL VAN DE RAT

Om de hypothese te toetsen dat ovulatie samenhangt met een toename van PO_2 in het ovarium follikel, werd de zuurstofspanning gemeten m.b.v. 10-micron naald electrodes. Twee groepen werden vergeleken:

1. Een niet-ovulerende groep ratten in pro-oestrus, bereikt d.m.v remming met fenobarbitol (35 mg/kg).
2. Een onbehandelde pre-ovulatoire groep.

Onder tribromo-ethanol anasthesie werd de ovarium blootgesteld en de electrode in een follikel geïntroduceerd. De gemiddelde PO_2 van de follikels niet onderheven aan een LH piek was 11,7 mm Hg, significant lager dan 23,5 mm Hg in de pre-ovulerende controle groep.

Deze waarnemingen ondersteunen de hypothese dat zuurstof de beperkende factor is om de eerste meiotische verdeling in de pre-ovulatoire oöcyte te laten continueren.

HOOFDSTUK 4 WEEFSEL OXYGENATIE IN DE CEREBRALE CORTEX VAN DE KONIJN.

Klinische toestanden zoals bijv. haemorrhagieën, shock, ischaemie en toediening van geneesmiddellen kunnen zuurstoftoevoer naar weefsels ingrijpend beinvloeden. Dit betekent dat een externe factor diverse interne mechanismes kan beinvloeden, met het uiteindelijke resultaat van of een toename, of een afname van weefsel PO_2, soms met ernstige consequenties. Met dit onderzoek werd de cerebrale PO_2 profiel onder normale omstandigheiden, en onder omstandigheiden van hyperoxie en hypercapnie onderzocht.

Als een PO_2 electrode m.b.v. een micromanipulator in de cortex wordt ingebracht, kan men een PO_2 profiel produceren. Dit profiel is redelijk herhaalbaar als de electrode teruggetrokken wordt, en dan weer ingebracht. Bij een FiO_2 van 0,3 is de gemiddelde PO_2 6 mm Hg. Bijna alle metingen zijn onder de 25 mm Hg, en metingen boven de 50 mm Hg zijn zeldzaam.

Als de FiO_2 wordt verhoogd tot 1,0 stijgt weefsel PO_2, en dan daalt het langzaam tot ongeveer 120% van de oorspronkelijke waarde. Dit is waarschijnlijk een gevolg van vasoconstrictie.

Als 12% CO_2 wordt toegevoegd aan de inademingsgassen, neemt weefsel PO_2 tot bijna drie keer zijn oorspronkelijke waarde en neigt niet tot dalen. Dit komt door het directe vasodilatoire effect van CO_2 en H^+ zodat de bloed doorstroming toeneemt. Normaal gesproken zou de CO_2 uitgewassen worden zodat vasodilitatie af zou kunnen nemen, maar hier wordt CO_2 kunstmatig hoog gehouden.

HOOFSTUK 5 ZUURSTOFTOEVOER NAAR HET MYOCARD

Aspecten van oxygenatie van het myocard worden besproken in relatie tot contractie van het hart, doorstromen van de coronaire vaten, ademhaling en ischaemie. Deze theorieën werden ondersteund met weefsel metingen in het varken, gebruik makende van staal-beschermde micro-electrodes van 200 micron diameter. De electrodes waren gefixeerd in een dunne flexibele plastic plaat en gehecht op de epicard. De afstand tussen de electrodes was 1 cm en zij staken 3 mm in de myocard van de linker ventrikel. Er werd naar gestreefd om de plaat op zo een manier te bevestigen dat als een deel van de voorste ramus inter-ventricularis onderbonden werd, 2 electrodes in het ischaemisch, en 2 in het niet ischaemisch gebied zich zouden bevinden. De voornaamste bevindingen waren:

Cardiale schommelingen

De myocardiale PO_2 (PmO_2) toont schommelingen met dezelfde frequentie als de hartfrequentie, maar vaak met een faseverschil tussen de electrodes. Er zijn diverse verklaringen, met als belangerijkste het

feit dat gedurende myocardcontractie, bloeddoorstroming in de arterioles afneemt, terwijl het toennemt in capillairen en venules. De graad van contractie varieert over het myocard, afhankelijk van de vordering van de depolarisatie golf over het hart.

Ademhalingsschommelingen

Met intermitterend positief druk ventilatie (IPPV) is er een toename in de linker ventrikel bloed volume. Dit veroorzaakt een afname in de coronair flow en een toename in hart arbeid. Deze wijziging in de zuurstof vraag/aanbod verhouding bewerstelligt een afname van PmO_2.

Electrische activiteit

Er is blijkbaar een kritisch PmO_2, waaronder myocard electrische activiteit ophoudt. Deze drempel varieert in verschillende gebieden van het myocard.

Veranderingen in FiO_2

Als FiO_2 verhoogd wordt, neemt de gemiddelde PO_2 toe alhoewel in sommige gebieden het PO_2 juist afneemt.

Kortdurende coronaire onderbinding

Tijdens een kortdurende occlusie (±30 sec.) is er niet alleen een afname van PmO_2, maar vaak ook een toename. Het bifasisch karakter van de zuurstofcurves geven aan dat de oorspronkelijke afname in zuurstoftoevoer soms snel wordt gecompenseerd door autoregulatie.

Langdurig coronaire onderbinding

Gelijk na de occlusie werd er een hoog significant daling in PmO_2 in het meest 'ischaemisch' gebied waargenomen. Vier minuten na occlusie was deze weer hersteld ten koste van de ernaast liggende 'ischaemisch rand' gebied. Dit gebied bleef significant lager PmO_2 meten gedurende het eerste uur van ischaemie. Dit werd geïnterpreteerd als een hoge graad van 'lenen van' zuurstof van het 'randgebied'.

HOOFDSTUK 6 MICROCIRCULATIE EN FLUOROCARBONEN

Fluorocarbonen zijn chemische stoffen met een hoge oplosbaarheids-coëfficient voor zuurstof. Het door ons toepasbaar middel is een emulsie die ongeveer 0,75 ml O_2 in 100 ml per 100 mm Hg draagt. Het geeft zuurstof makkelijker af dan haemoglobine. Verder heeft de fluorocarbon emulsie een laag viscositeit die, i.t.t bloed, haast niet toeneemt in de microcirculatie. Dit, samen met zijn kleine afmeting, helpt om een betere penetratie van de microcirculatie te bewerkstelligen zodat oxygenatie verbeterd kan worden bij, bijvoorbeeld, myocard infact of extremiteit amputatie.

De effecten van bloedverdunning m.b.v. fluorocarbonen of dextraan in het acuut ischaemisch myocard

De eerder genoemde varkens werden verdeeld in 3 groepen van 5 dieren. Groep I was een controle groep. Na afname van 20 ml/kg bloed kreeg groep II fluorocarbonen en groep III dextraan. Hierdoor was er in groep II (i.t.t. groepen I en III) geen significant verschil meer in PmO_2 in vergelijking met voor occlusie. Na 5 uur van occlusie was de gemiddelde PmO_2 in deze groep 90,1% van voor occlusie. In groep I (controle) was dat 26,7%, en in groep III 37,3%. Deze studie bewijst dat haemodilutie niet voldoende is om myocard oxygenatie te verbeteren, maar dat re-oxygenatie wel mogelijk is m.b.v. haemodilutie met fluorocarbonen emulsies.

Prophylactisch behandeling tegen myocard ischaemie met fluorocarbonen

Vijf varkens werden 30 minuten voor occlusie van de distale derde van de LAD verdund met fluorocarbonen. PmO_2 was altijd hoger (soms significant) dan in de controle groep. Dit ondersteunt de theorie dat prophylactisch behandeling met fluorocarbonen het myocard beschermt in de vitale eerste uren na een infarct.

Preservatie van extremiteiten in reimplantatie chirurgie

Vijf patienten worden voorgedragen waarbij een extremiteit die traumatisch geamputeerd was, geperfundeerd werd met geoxygeneerde fluorocarbonen. Perfusie varieerde van 16 tot 46 uren. Microcirculatoire oxygenatie werd gecontrolleerd met 200 micron zuurstofelectrodes. K^+, lactaat, glucose en ATP werden ook gemeten, en electron microscopisch onderzoek van bloedvaten werden verricht. Alle bovengenoemde parameters gaven aan dat de weefsels voldoende met zuurstof waren voorzien en vitaal waren na vele uren van perfusie. Onomstotelijk bewijs van de vitaliteit van een geperfundeerde vinger werd gegeven na een gelukte reimplantatie na 17 uur van perfusie met fluorocarbonen.

HOOFDSTUK 7 HYPOTHERMIE EN PO_2 METINGEN

In acht jonge varkens werden zuurstofelectrodes in het myocard geplaatst zoals hiervoor beschreven. Na stabilisatie werden de varkens afgekoeld tot 30° C en daarna weer verwarmd. Alhoewel er wel veranderingen in cardiovasculaire en zuur/base parameters waar te nemen waren, veroorzaakte die geen moeilijkheden. De zuurstofflux daalde met 32%. Dit nam waarschijnlijk niet plaats in de myocard omdat de coronair flow niet noemenswaardig veranderde. Maar PmO_2 daalde wel met 37%, dit waarschijnlijk onder andere t.g.v een toename in viscositeit die een negatief effect heeft op microcirculatoire bloed doorstroming. Men moet dus realiseren dat, alhoewel het dier herstelt na verwarming, het mogelijk is dat er een negatieve verandering heeft plaatsgevonden in de zuurstof vraag/aanbod verhouding van de microcirculatie.

GLOSSARY OF ABBREVIATION

$\bar{a}$	Average calculated a
AC	Alternating current
AgCl	Silver Chloride
AP	Systemic arterial pressure
AP	Action potentials
Art Sat	Saturation of haemoglobin with oxygen in arterial blood
Art PCO_2	Partial pressure of carbon dioxide in arterial blood.
ATP	Adenosine Triphosphate
$(a-v)DO_2$	Arterial and mixed venous difference in oxygen content
αO_2	Oxygen solubility coefficient
C	Capacitor
CaO_2	Arterial oxygen content
CF	Coronary flow
CO	Cardiac output
CO_2	Carbon Dioxide
CvO_2	Mixed venous oxygen content
CVP	Central venous pressure
DC	Direct current
dP/dt	Change in ventricular pressure over change in time
DO_2	Oxygen diffusion coefficient
e^-	Electron
el	Electrodes
ECG	Electrocardiaogram
EEG	Electroencephalogram
exp. CO_2	Expiratory carbon dioxide
f	Frequency
FiO_2	Inspiratory percentage of oxygen
FSH	Follicle stimulating hormone
G	Giga-ohm
H^+	Hydrogen ion or proton
Hb	Haemoglobin concentration
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate
HR	Heart rate
Ht	Haemotocrit
IPPV	Intermittent positive pressure ventilation

k	Kilo-ohm
KO_2	Oxygen conductivity coefficient
LAD	Left anterior descending coronary artery
LED	Light emmitting diode
LH	Lutienising hormone
LVW	Left ventricular work
M	Mole
M	Mega-ohm
n	Population
N_2O	Nitrous Oxide
OA	Operational amplifier
OH^-	Hydroxide radical
O_2	Oxygen
p	Statistical probability of chance
PaO_2	Partial pressure of oxygen in the arterial blood
PAP	Pulmonary arterial pressure
PCO_2	Partial pressure of carbon dioxide
PCWP	Pulmonary capillary wedge pressure
PeO_2	Partial pressure of oxygen in the epicardium
pH	Concentration of hydrogen ions in a solution
PH_2	Partial pressure of hydrogen
PmO_2	Partial pressure of oxygen in the myocardium
PO_2	Partial pressure of oxygen
PvO_2	Partial pressure of oxygen in mixed venous blood
QO_2	Oxygen flux
r	Radius
R	Resistor
SD	Standard deviation
SEM	Standard deviation of the mean
SVR	Systemic vascular resistance
U	Voltage
U_p	Polarisation voltage
VO_2	Oxygen consumption

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CURRICULUM VITAE

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