

Thesis for doctoral degree (Ph.D.)
2011

Skin Circulation Measured By Fluorescein Flowmetry

Eduardo Patricio Proaño Romero, M.D.



**Karolinska
Institutet**

From the Department of Molecular Medicine and Surgery
Karolinska Institutet, Stockholm, Sweden
Karolinska University Hospital, Solna

Skin Circulation Measured By Fluorescein Flowmetry

By

Eduardo Patricio Proaño Romero, M.D.



**Karolinska
Institutet**

Stockholm 2011

Skin Circulation Measured By Fluorescein Flowmetry

© Eduardo Proaño Romero, 2011

ISBN 9789162825607

Published by Karolinska Institutet. Printed by Printfabriken, Karlskrona, Sweden

A mi madre

*...In judicando ye are a physician.
In curando a surgeon. The patient
asks for cure and not for theory. It
is the doctor who needs the latter.*

*Paracelsus
1493-1541*

ABSTRACT

Fluorescein flowmetry (FF) is an indicator dilution technique using sodium fluorescein as an indicator. If the tissue is illuminated with U-V light or blue light, after a bolus injection of the dye, fluorescence is seen in the tissue, and can be measured either photographically or by the video technique. The development of the fluorescence pattern in the tissue in relation to time can be recorded and fluorescence curves constructed. A fluorescence index is calculated as the ratio between the maximal fluorescence obtained during the first circulatory passage of the dye and the rise time, defined as the time interval between 10 % and 90 % of the maximal fluorescence. This is an expression of the slope of the curve.

The aim of this study was to evaluate the suitability of FF for measurement of skin circulation.

The correlation was studied between FF and other methods regarding assessment of the change in skin circulation between initial measurements at rest and second measurements after provocation of vasodilatation. FF showed a coefficient of correlation (r) to the fast slope of the ^{133}Xe clearance curve of 0.46 ($p < 0.05$), to the slow slope of the ^{133}Xe curve of 0.66 ($p < 0.001$) and to laser Doppler fluxmetry (LDF) of 0.86 ($p < 0.001$).

The coefficient of variation between two measurements of skin circulation with FF in the same healthy individual, with a one-month interval, in an incompletely dilated vascular bed, was 0.46, compared with 0.34 for LDF and 0.03 for skin temperature.

The relationship between weight-bearing pressure and skin circulation in the plantar region was studied in patients with diabetic neuropathy and healthy controls. Gait analysis was performed and skin circulation was assessed with the subject in the supine position, and standing and walking on a podoscope. The skin circulation was arrested at a pressure of 3Ncm^{-2} both in diabetic and control subjects. From $1.0 - 2.0\text{Ncm}^{-2}$ there was no difference in fluorescence index between these groups, but from $2.1 - 3.0\text{Ncm}^{-2}$ there was a successive decline in index in the diabetic subjects, indicating a successive decrease in capillary closure pressure.

FF was used clinically in combination with LDF to determine how different surgical methods influence the circulation of the skin. The effects of the following operations were evaluated: a successful vascular reconstruction in patients with lower limb ischaemia; reduction mammoplasty with a bipedicle vertical dermal flap according to McKissock; and subcutaneous mastectomy and immediate breast reconstruction with implants in patients with breast cancer not suitable for lumpectomy. The influence of the site of skin incision on the circulation in the nipple-areola complex after subcutaneous mastectomy in breast cancer was also studied.

FF cannot be considered to measure blood flow per se in the skin, but it mimics the transport of small solutes both extra and intracellularly. In assessing a change in circulation, FF shows high correlation to other established methods that are considered to measure skin circulation, such as ^{133}Xe clearance and laser Doppler fluxmetry. However, due to the large individual variations, FF is more appropriate for comparisons between groups rather than between individual subjects.

The greatest advantages specific for fluorescein flowmetry are the possibilities of studying heterogeneous circulation and predicting tissue viability in large areas of the skin.

Key words: Skin circulation, Fluorescein Angiography.

CONTENTS

INTRODUCTION

- History
- Anatomy and physiology of the microcirculation system.
- Blood flow measurements, theoretical background
- Sodium fluorescein
 - History
 - Physical and biochemical characteristics
 - Dosage
 - Toxicity

Current methods of studying blood flow in the microcirculation system. An overview.

- Television microscopy
- Fluorescence videomicroscopy
- Capillary filtration techniques
- Electromagnetic flowmetry
- Transcutaneous oxygen tension
- Radioactive washout techniques
- Laser Doppler fluxmetry
- Fluorescein angiography
- Fluorescein flowmetry

AIMS OF THE PRESENT STUDY

MATERIALS AND METHODS

- Fluorescein flowmetry
- Laser Doppler fluxmetry
- Xenon clearance
- Skin temperature measurements
- Provocation of vasodilatation with alcohol and external heat box
- Weight-bearing pressure and gait analysis
- Podoscope

STATISTICS

RESULTS

GENERAL DISCUSSION

CONCLUSIONS

ACKNOWLEDGEMENTS

REFERENCES

STUDIES I-VII

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following studies, which will be referred to by their Roman numerals:

- I. Correlation between the uptake of sodium fluorescein in the tissue and $^{133}\text{Xenon}$ clearance and laser Doppler fluxmetry in measuring changes in skin circulation.
Proaño E, Svensson L, Perbeck L.
Int J Microcirc. 17:22-28, 1997.
- II. Effect of exposure to heat and intake of ethanol on the skin circulation and temperature in ischaemic limbs.
Proaño E, Perbeck L.
Clinical Physiology 14, 305-310, 1994.
- III. Changes in skin blood flow in ischaemic limbs after vascular reconstruction measured by fluorescein flowmetry and laser Doppler flowmetry.
Proaño E, Perbeck L.
Clinical Physiology 13:599-609, 1993.
- IV. The effect of weight-bearing pressure on the plantar circulation in diabetes mellitus.
Proaño E, Määttänen H, Perbeck L, Solders G, Turan I.
Diabetic Medicine 9:722-729, 1992.
- V. Skin circulation in the nipple after reduction mammoplasty with a bipedicle vertical dermal flap.
Perbeck L, Proaño E and Määttänen H.
Scand J Plastic Reconstr Hand Surg. 25:41-45, 1991.
- VI. The circulation in the nipple-areola complex following subcutaneous mastectomy in breast cancer.
Perbeck L, Proaño E, Westerberg L.
Scand J Plastic Reconstr Hand Surg. 26, 217-221, 1992.
- VII. Influence of the site of skin incision on the circulation in the nipple-areola complex after subcutaneous mastectomy in breast cancer.
Proaño E and Perbeck L.
Scand J Plastic Reconstr Hand Surg. 30: 195-200, 1996.

INTRODUCTION

History

In 1628, in Frankfurt, the English physician William Harvey (1578-1657), published his book "Exercitatio anatomica de motu cordis et sanguinis in animalibus", a study of the anatomy of the heart and blood flow in animals, in which he described the phenomenon of the circulation of the blood and was the first to mention the existence of the microcirculation, which he deduced from anatomical and physiological studies at the university of Padua. Thirty-three years later, Marcello Malphighi, an Italian anatomist (1628-1694), confirmed Harvey's deductions when he described the presence of the capillaries as a result of direct microscopic observations in "the pulmonis observationis"(1661). In 1882, Ehrlich used sodium fluorescein to investigate the secretion of the aqueous humour in the rabbit's eye.

In 1920 Danzer and Hooker made determinations of blood pressure in man using a microcapillary tonometer. The first direct measurements of the blood pressure in human capillaries were performed by Carrier and Rehberg in 1923 by cannulating capillary loops in the nailfold, using glassmicropipettes. By connecting a manometer to the micropipettes, Landis et al (1930) found it possible to measure the pressure on both sides of the capillary loops. These techniques have been developed in recent years and new techniques for measuring both pressure and flow have been elaborated by the use of sophisticated instruments of visualisation and recording of data, e.g. television microscopy (Zimmer and Demis, 1964). Intaglietta et al. described a cross-relation method in 1975 for measuring capillary erythrocyte velocity.

Lund and Lund introduced dynamic fluorescein angiography in 1972, and this was later followed by the video technique. By determining the appearance time of injected sodium fluorescein and the time of maximal fluorescence in a determined area, a relative value of flow velocity can be obtained. Perbeck et al (1985) further developed the method by measuring the change in the fluorescence pattern in relation to time during the first circulatory passage of sodium fluorescein. They named the method fluorescein flowmetry. In the intestine, this measures a relative capillary blood flow expressed in arbitrary units, (density units/s); here the extraction of the dye is 100 per cent, there is no back flux and no recirculation of the indicator.

Anatomy and physiology of the microcirculation system.

The circulation is a system consisting essentially of a pump, i.e. the heart, and arteries, veins and capillaries, through which blood circulates in a continuous circuit. The microcirculation is an integrated microsystem including arterioles, capillaries and venules, with flow of fluids and exchange of nutrients and waste to and from the cells. In the organism, each tissue has its own characteristic micro vascular system, and that in the skin is illustrated in Figure 1.

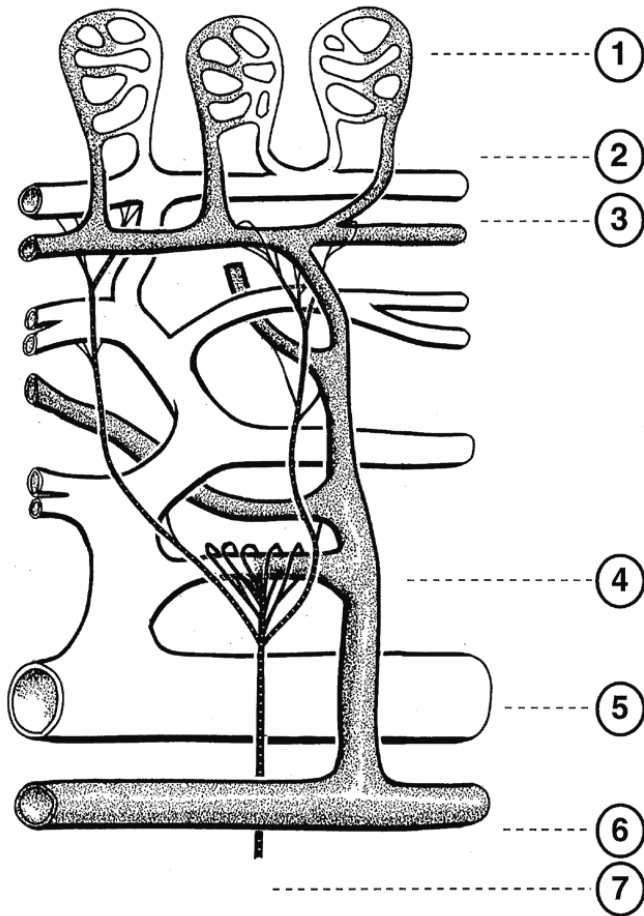


Figure 1. Schematic representation of the microcirculation system in the skin. 1/ Capillaries 2/ Venules 3/ Arterioles 4/ Arteriovenous shunts 5/ Veins 6/ Arteries 7/ Sympathetic nerve fibres. (Björn Löfgren)

The blood enters the microcirculation system through an arteriole (50-80 μm in diameter), which then divides into a number of metarterioles which lead the blood into the capillaries (7-20 μm). The capillaries may either connect directly with a venule (preferential channels) or terminate in a venule (true capillaries) by branching. When the blood has passed through the capillaries, it leaves the system, by way of venules, through the veins (Guyton, 1986).

The arterioles have a strong muscular coat and the metarterioles are coated with sparse but highly active smooth muscle fibres. The venules also have a coat of smooth muscle, but this is much less extensive than that of the arterioles. The capillary wall is composed of a thin, generally unicellular layer of endothelial cells and is surrounded by a thin basement membrane on the outside. The total thickness of the membrane is about 0.5 μm . The capillary wall is perforated by minute channels which connect the interior of the microcirculation system with the exterior and it is through these channels that fluids, nutrients and wastes flow to and from the interstitial spaces.

These channels, or pores, have a diameter of about 80-90 Angstroms. The blood flows at an intermittent rate through the microcirculatory system on account of the intermittent contraction of the metarterioles and precapillary shunts. The most important factor in determining the number of contraction periods and the degree of contraction and therefore the blood flow is the availability of oxygen to the tissues. The lower the concentration of oxygen in the tissues the greater the number of contraction periods and the greater the blood flow, which in turn will increase the oxygen delivery to the tissues. The system is auto regulated by the availability of oxygen to the tissues and their oxygen demand. The gradient of hydrostatic pressure between the arteriole and the venule in the microcirculation system is another factor which allows blood to flow through the system (Guyton, 1986).

Substances: fluids, nutrients and wastes are transported from the interior of the microcirculation system to the interstitium, and vice versa, by diffusion through the capillary membrane and bulk flow through the capillary channels (pores).

Blood flow measurements, theoretical background

Blood flow may be defined as the amount of blood that passes through a given point in the circulation in a given period of time. It may be expressed in millilitres per time unit or as any other unit of flow per unit of time.

Several techniques for measuring blood flow have been developed, among them the indicator dilution technique, in which an indicator substance is injected into the circulation as a bolus, the indicator mixes in the system, i.e. the blood, it is transported and it is then measured at a determined sampling point. The data are then collected and curves are created. The results are expressed in volume per time units. Certain criteria have to be fulfilled: the amount of indicator injected must be known, there must be total mixing of the indicator, there must be free-diffusion (extraction) of the indicator and no back flux of the indicator to the system, no recirculation of the indicator and the system should be in steady state.

In this study the indicator used was *sodium fluorescein* and the results are expressed in *density units per second*.

Sodium fluorescein

History

Sodium fluorescein was synthesised in 1852. Thirty years later Ehrlich (1882) used sodium fluorescein for the first time to study the secretion, flow and absorption of the aqueous humour in the rabbit's eye. In 1922 Kock determined the circulation time by injecting sodium fluorescein into a vein of one arm and collecting samples of blood from a vein of the other arm and examining them for the first appearance of fluorescence. Lange and Wollheim (1931) demonstrated that the dye could be seen in the blood vessels of the lip when observed in appropriate light. Lange and Boyd (1934) made an extensive study of capillary blood flow, and with the use of ultraviolet light they made the dye become visible. This led to a series of clinical studies of capillary blood flow, circulation time and tissue viability in various clinical situations in association with various surgical techniques and conditions, such as flaps, acute arterial embolism and thrombosis and vasospastic disorders. Lange and Krewer (1943) introduced the dermofluometer, which permitted objective measurement of fluorescence. In clinical practice sodium fluorescein has been used to evaluate the viability of different tissues, for example the small intestine in strangulated hernia (Herrlin et al, 1942), tubed flaps (Dinwall and Lord, 1943; Lord 1944), skin sloughs at the time of operation (Myers, 1962), colonic anastomosis (Meyers and Cherry, 1969) and flaps during breast reconstructions (Singer et al, 1978). Intraoperative determination of intestinal viability following ischaemic injury was achieved by the use of sodium fluorescein and controlled with clinical judgement (Bulkley et al 1981, Marfuggi and Greenspan, 1981). Intestinal viability after blunt trauma was studied by Johansson (1984), using the fluorescein angiography technique.

The use of sodium fluorescein has led to the development of several techniques for the study of the circulation: fluorescein angiography allows visualisation of the retinal vessels and study of the cerebral circulation, since the dye does not leak from the circulation under normal conditions (Wessin 1968; Rosén 1969; Feindel et al 1969; and Järpe 1968, 1969). Increased permeability in pathological conditions causes leakage of the dye and can be measured quantitatively (Lund, Anderssen and Lassen, 1981). Lund and Lund (1972) introduced dynamic fluorescein angiography. They measured changes in the fluorescence pattern in relation to time by rapid-sequence still photography followed by a video technique (Lund 1981). By measuring the appearance time and time for maximal fluorescence a semi-quantitative measurement of flow velocity could be achieved. Perbeck (1985) introduced fluorescein flowmetry as a method for measuring relative capillary blood flow in the intestine.

Physical and biochemical characteristics

Sodium fluorescein is a resorcinolphthalein with a molecular weight of 376.27 Daltons and a radius of 0.55 nm. In clinical medicine it is used as a sodium salt. Sodium fluorescein is an orange-red, odourless, almost tasteless, hygroscopic, water-soluble powder. The aqueous solution is red in colour but presents a green fluorescence that is maximal on exposure to ultraviolet or blue light. When diluted, the solution is yellow-green. The fluorescence disappears when the solution is acidified and reappears when it is alkalised. Sodium fluorescein is soluble in one to ten parts of alcohol, and insoluble in chloroform and ether. A 3.34% solution in water is iso-osmotic with serum. A 0.5% solution has a pH value of 8.2 to 8.7.

Metabolism

When injected intravenously, sodium fluorescein is free and dissociated and does not bind to proteins within the first two minutes. Thereafter it binds successively to albumin but not to

globulins or fibrinogen (Dollery et al, 1962). About 17% of the dye binds to the erythrocyte surface by adsorption (Crismon and Fuhrman, 1947). In the microcirculatory system it diffuses through the capillaries to the interstitium and back to the circulation. Diffusion is limited, however, by the size of the capillary pores, which differs in different tissues. In the mucosa of the small intestine and in the muscularis layer, diffusion is unrestricted because of the large size of the capillary pores, which have a ten times greater diameter than sodium fluorescein (Perry and Granger, 1983). Diffusion is partly restricted in the skeletal muscle (Renkin, 1959), and in brain vessels diffusion does not occur on account of the absence of pores in the brain capillaries. Elimination takes place through the liver and kidneys (Lange and Boyd, 1944). No metabolism occurs in these organs.

Dosage

Sodium fluorescein is given at a dose of 5-7 mg/kg body weight (range 5-15 mg/kg) as a 5% or 10% solution in studies of the circulation, both in skin and viscera (Schatz, 1978).

Toxicity

There are no reports of serious reactions after oral administration of sodium fluorescein, and as much as 6g has been given without any toxic effects (Lange and Boyd, 1943). After intravenous injection of fluorescein a very low incidence of serious adverse reactions and a high incidence of minor untoward effects have been reported. Fatal complications have been reported to occur at a rate of one case per 49,557 angiographies (Zografos, 1983) and one case per 222,000 angiographies (Yannuzzi, 1986). No deaths were observed in a prospective study of 2,789 angiographies (Kwiterovich et al, 1991).

Severe adverse reactions: The overall frequency of such reactions was reported by Zografos (1983) to be one case per 18,020 angiographies and by Yanuzzi et al (1986) to be one case per 1,900 angiographies. Tonic-clonic seizures occurred in 1:13,700, respiratory adverse reactions in 1:3,800 and cardiac problems in 1: 5,300 angiographies (Yanuzzi et al, 1986). Kwiterovich et al (1991) found two isolated cases of dyspnoea and two of syncope.

Minor adverse reactions: Nausea, vomiting, itching and flushing may occur at frequencies of 0.05 to 21 %. Yanuzzi et al (1986) reported an estimated frequency of 1-10 % and Kwiterovich et al (1991) a frequency of 4.8 %. Watson et al, (1990) found an adverse reaction frequency of 48 % among individuals who had previously had an adverse reaction. Turetta et al (1985) noted a significant decrease in the plasma ionised calcium level in 84 patients during fluorescein angiography. In our studies nausea, vomiting, urticaria, rhinorrhoea and chills were observed in a few cases. Extravascular administration of 10 ml of 5 % solution in the cubital area caused pain but no tissue damage. Neither severe nor fatal adverse reactions have occurred in our studies.

CURRENT METHODS OF STUDYING BLOOD FLOW IN THE MICRO-CIRCULATION SYSTEM

The following methods are currently in clinical use for blood flow measurements and for detailed studies of flow through the microcirculatory system.

Television microscopy

The nailfold capillaries can be visualised through a television microscope (Zimmer and Demis, 1964). The images of one to four capillaries can be recorded on videotape. By playing back the tape the velocity of the erythrocytes can be analysed and calculated by measuring the distance between similar signals generated by aggregation of erythrocytes during their passage through the capillary vessel (Bollinger et al, 1974). Intaglietta (1975) described a cross-correlation method for measuring capillary erythrocyte velocity. Fagrell et al (1977) have reported on the velocity of blood cells in the finger nailfolds capillaries of normal subjects (Östergren and Fagrell, 1986). New methods include: Sidestream Dark Field (SDF) imaging, a stroboscopic LED ring based imaging modality used for clinical observation of the microcirculation (P.T Goedhart, 2007)

Fluorescence videomicroscopy

A bolus of sodium fluorescein is injected into a vein, and using a video microscopy system, including an incidence light fluorescence microscope, a television camera, a monitor and a tape recorder, the dye is observed when it appears in the capillary bed. The light intensities produced are determined at different sites in the capillary and recorded as arbitrary units or percentage of the maximal individual intensity obtained. Fluorescence video microscopy allows assessment of the microcirculatory system and dynamics in vivo by quantitative evaluation of transcapillary and interstitial diffusion of sodium fluorescein in a single capillary or in a defined tissue area, permitting evaluation of the nutritive flow of fluids into the interstitium (Bollinger et al, 1974, 1979). Intravital microscopy of microcirculation in different organs allows visualisation of various parameters during both physiological and pathophysiological changes in the microcirculatory system. Zeintl, H et al (1989), Klyscz, T et al (1997), Hungerer S et al (2010). Indocyanine green (ICG) is a fluorescence agent that produces maximum fluorescence in plasma at 840 nm (wavelength). Techniques, which allow imaging of tissue and capillaries have been developed and are being used for diagnostic purposes. (Kusano et al, 2008), (Murawa et al, 2009).

Capillary filtration techniques

The capillary filtration capacity was recognised by Starling in 1896. The net flow of fluids between the blood vessels and the interstitium was determined from differences in hydrostatic and osmotic pressures across the capillary wall. Changes in the filtration rate can be measured by means of a volume plethysmograph, which assesses the changes in volume of the tissues, as fluid flows to the interstitium, of a human leg or arm (tissue swelling rate). It may also be calculated from changes in the diameter of a limb by using a mercury gauge (Krough et al, 1932), (Landis et al, 1930, and 1933).

Electromagnetic flowmetry

A probe containing electrodes is placed in direct contact with the vessel, where the column of blood acts as a moving electrical conductor when exposed to a magnetic field. The induced potential is picked up by the electrodes, the signal is processed and amplified and the output expresses blood flow quantitatively. The probe embraces the vessel and may cause kinking,

compression and vasospasm, which may affect the measurements. The method is designed for intraoperative use.

Transcutaneous oxygen tension

The oxygen tension transducer was used for the first time by Huch et al. in 1972 to estimate central arterial oxygen levels in the neonate. A typical electrochemical transducer consists of a heat coil, a temperature sensor, a gas-permeable membrane, an electrolyte, an anode and a cathode. Oxygen diffuses across the membrane to the electrolyte and the current generated by the reduction of oxygen at the cathode surface flows between the anode and the cathode and is dependent on the applied voltage and the supply of oxygen. Thus the method monitors changes in PO₂ mainly from the skin blood flow in the superficial dermis. (Jakobsen 1987), (Newson 1987)

Radioactive washout techniques

Kety (1948) described a method for measuring tissue blood flow by monitoring the clearance rate of a radioactive tracer from a uniformly perfused area.

Several tracers have been used, either freely diffusible tracers such as ¹³³Xe and ¹²⁵I-4-iodantipyrine or tracers of restricted diffusibility such as ²⁴Na, ⁴²K, ¹²⁵I, ¹³¹I and ⁹⁹Tc. A known volume of the tracer is administered into a tissue, a scintillation detector records the emitted radiation and the results are collected as a logarithm of counts per second versus time (Young 1983). Although inexpensive, straightforward and easy to use, this technique is not free from artefacts.

Laser Doppler fluxmetry

The principle of laser Doppler fluxmetry (LDF) is based on the fact that when light encounters a moving object, it undergoes a frequency shift that is related to the velocity of the moving object; in other words monochromatic laser light becomes spectrally broadened when scattered by moving objects, in our case red blood cells, whereas light beams scattered in static structures alone, such as connective tissue, remain unchanged in frequency. In reality the light undergoes multiple frequency shifts depending on the number of red blood cells encountered and the velocity at which they pass through the microcirculatory system, creating a spectrum of shifted frequencies (Stern et al, 1977). Laser light was used to measure blood flow in the microvascular system by Stern (1975), who demonstrated that light that is backscattered from the skin surfaces showed spectral Doppler broadening which was the result of blood flow in the microvascular bed. Holloway and Watkins (1977) introduced an instrument for LDF clinical use and the technique was further developed by Nilsson et al (1980), who produced a model called Periflux PF 1C Mark II (Perimed, Järfälla, Sweden). This consists of a low power helium-neon laser, two photodetectors and a signal processor. Light from the laser is conducted to the tissue area to be studied, where it is scattered by both stationary and moving objects, in this case erythrocytes. Light scattered by stationary objects remains unaltered, whereas light, which encounters moving objects, undergoes a frequency shift related to the velocity of the moving objects. This light, now having different wavelengths, is backscattered to the two photodetectors, where it is mixed; creating beat frequencies equal to those of the Doppler shift. Thus the output of the signal from the processor is related to the flow of the red blood cells, and is defined as the product of the number of blood cells and their mean velocity within the measurement volume and expressed in arbitrary units, volts or perfusion units. Different models produce different voltage signals for the same blood flow, though results obtained with different instruments are not comparable.

During the last few years new techniques have been developed for studies of the micro vascular system: The laser Doppler imager allows visualisation of micro vascular blood perfusion. It scans

a low power laser beam in a raster pattern over the skin or other tissue surfaces. Moving blood in the microvasculature causes a Doppler shift that is processed to build up a colour coded image of blood flow. The technique is non-contact and can be used to record chronic changes or for measuring repetitively in quick succession (Wårdell et al, 1990), Essex et al. (1991), Naizi et al. (1993). New probes have also been developed and the new systems equipped with green and red and near infrared light sources that allow studies at different vascular depths (periflux systems, Perimed AB, Järfälla, Sweden).

Recently, another approach has been developed for assessing skin blood flow based on laser speckle analysis. Laser speckle flowgraphy (LSFG) evaluates the normalized blur rate, which indicates the relative velocity of erythrocytes, to represent the blood flow volume. Nakagami G, et al. 2009.

Fluorescein angiography

Lund and Lund (1972) introduced dynamic fluorescein angiography. After injection of sodium fluorescein into a vein, changes in the fluorescence pattern in relation to time were determined by a rapid sequence of still photographs, later followed by the video technique (Lund 1981). By determining the fluorescence appearance time and the time of maximal fluorescence in a defined area, a relative value of flow velocity could be achieved. Thus it is not the capillaries per se that are visualised with this technique, but the tissue they perfuse, and the technique represents the nutritive perfusion of the area. Further, this method has been developed by Scheffler et al (1995) to study skin perfusion patterns topographically. Fluorescence appearance times in the skin are calculated and their topographical distribution is displayed as a functional image in pseudocolours on a video screen. The method has lately been developed by video technique and is called Fluorecein Video Imaging, Lund F et al. 1998.

New techniques are being used for diagnostic purposes: Vovadaq Spy Intraoperative System (Novadaq Technologies Inc., Concord, Ontario, Canada), Vogt PR Bauer EP, Graves K 2003. Katsuhisa et al (2009).

Fluorescein flowmetry

Perbeck et al (1985) introduced fluorescein flowmetry as a method for measuring a relative capillary blood flow in the intestine, expressed as a flow index in arbitrary units (density units per second). Technologically the method is based on dynamic fluorescein angiography in which fluorescence is recorded either photographically or by a video technique. The blood flow is measured as the uptake of sodium fluorescein and expressed as a fluorescence index, which is the ratio between the maximum fluorescence obtained during the first circulatory passage of sodium fluorescein and the rise time, defined as the time interval between 10 and 90 per cent of the maximum fluorescence. This index is an expression of the slope of the curve of the uptake function of sodium fluorescein. The method will be described in detail later in the material and methods section of this book.

AIMS OF THE PRESENT STUDY

The overall aim of this study was to evaluate the use of fluorescein flowmetry for measurements of the skin circulation.

The specific aims were as follows:

- to evaluate the use of fluorescein flowmetry for measuring the circulation in the human skin by correlating the uptake of sodium fluorescein to ¹³³xenon clearance and to laser Doppler flowmetry in measuring a change in skin circulation (Paper I);
- to study the effect of exposure to external heat and intake of alcohol on the skin circulation and skin temperature both in healthy subjects and in patients with ischaemic limbs (Paper II);
- to evaluate the feasibility of repeated measurements of skin circulation on the same individual on a day to day basis by calculating the coefficient of variation for both fluorescein flowmetry and laser Doppler fluxmetry (Paper III);
- to elucidate the change both in the superficial skin circulation, as measured by fluorescein flowmetry, and in the deeper skin circulation as measured by laser Doppler flowmetry, after successful vascular reconstruction (Paper III);
- to evaluate the influence of weight-bearing pressure on the plantar skin circulation in subjects with diabetes mellitus by developing a method to study the skin circulation in the sole of the foot during standing and walking (Paper IV);
- to study the skin circulation in the nipple-areola complex during different surgical steps of reduction mammoplasty with a bipedicle vertical dermal flap and after completion of the operation (Paper V);
- to evaluate the skin circulation in the nipple-areola complex after subcutaneous mastectomy and immediate reconstruction by implantation of a sub muscular prosthesis in patients with breast cancer (Paper VI);
- to evaluate the influence of the site of skin incision on the circulation in the nipple-areola complex following subcutaneous mastectomy in breast cancer (Paper VII).

MATERIALS AND METHODS

Patients and healthy subjects

A total of 133 patients and 55 healthy subjects were studied in different situations (papers I to VII; see Table 1).

Table 1. Characteristics of patients and healthy subjects in studies I to VII.

Study	I	II	III	IV	V	VI	VII	Total n
Patients								
Age		65	65	41	47	50	53	
		47-80	47-80	36-64	33-66	35-73	32-78	
Occlusive artery disease		14*	14*					14
Diabetes				10				10
Macromastia					16			16
Breast cancer						24	69	93
Healthy subjects								
	24	14	9	8				55
Age	33	33	35	34				
	24-59	21-43	28-47	24-44				

* The same subjects, five of them were diabetics.

In study I, we correlated the uptake of sodium fluorescein after an intravenous injection to ¹³³xenon clearance and laser Doppler fluxmetry in measuring a change in skin circulation after provocation of vasodilatation, in healthy subjects of both sexes.

The patients undergoing vascular reconstruction in studies II and III were suffering from occlusive arterial disease, and all of them had rest pain and reduced ankle systolic blood pressures. Five of the patients had insulin dependent diabetes mellitus. Arteriography was performed preoperatively in all patients; in the affected limb it showed three open crural arteries in eight patients, two in four patients and one in two patients. Fourteen patients underwent femoro-popliteal bypass surgery with a reversed vein, one of them bilaterally. Graft patency was checked intraoperatively either by angiography or by electromagnetic flowmetry. (Studies II and III were carried out on the same patients.) Individual characteristics of the patients are shown in table 2. Blood pressure values are missing for patients 10 and 14, as they underwent emergency surgery.

Table 2. Characteristics of the patients in study II

Patient No.	Age Years	Diagnosis Occlusive arterial disease	Diabetes mellitus	Angiographic outflow*	Ankle mmHg	G-Toe mmHg	Clinical outcome
1	63	yes	yes	3/3	-	20	Successful
2	70	yes	no	1/	0	0	Foot amputated one month later
3	80	yes	yes	3/3	-	25	Successful
4	67	yes	no	3/3	35	20	Successful
5	63	yes	yes	3/3	80	40	Successful
6	66	yes	yes	2/3	65	20	Successful
7	64	yes	no	3/3	30	20	Successful
8	73	yes	no	3/3	65	20	Successful
9	77	yes	no	2/3	0	0	Successful
10	67	yes	no	1/3	-	-	Successful
11	47	yes	no	3/3	70	30	Successful
12	69	yes	no	3/3	90	35	Successful
13	65	yes	yes	2/3	135	5	Toes amputated one month later
14	53	yes	no	2/3	-	-	Successful

* Outflow in the three branches of the popliteal artery was graded according to the degree of arteriosclerotic involvement (open or occluded): 0/3 = no open artery; 1/3= one open artery; 2/3 = two open arteries; 3/3 = three open arteries.

The diabetic patients in study IV exhibited severe neuropathy and autonomic failure. All of them had a previous history of foot ulcers and had an ankle systolic blood pressure ratio of >1.0; the foot pulses were palpable in all patients. All of them had retinopathy and eight had proteinuria. The blood glucose level was stable during the skin circulation measurements.

In studies V-VII the skin circulation of the breast was evaluated during and after different surgical procedures. Study V comprised patients with macromastia, who underwent a reduction mammoplasty with transposition of the nipple-areola complex according to the method described by McKissock, which involves the construction of a bipedicle vertical dermal flap. In one of the breasts adrenaline was injected in the incision lines, which nowadays is the usual procedure to diminish blood loss. The other breast did not receive any adrenaline, partly so as to make comparison between the breasts possible, and partly to allow comparison of the data obtained with this operative technique with previous data obtained by the method of Strömbeck (1960) (Perbeck et al, 1988).

Studies VI and VII comprised patients with invasive breast cancer not suitable for breast conservative treatment. The skin circulation was monitored in the nipple-areola complex following different surgical techniques of subcutaneous mastectomy and immediate surgical reconstruction.

In study VII the influence of the site of skin incision on the circulation in the nipple-areola complex after subcutaneous mastectomy and immediate reconstruction was evaluated.

All healthy subjects were recruited among volunteers from personnel staff at our hospital (Huddinge University Hospital, Stockholm, Sweden).

Methods

The methods used in the present studies, including the equipment and the experimental procedures, are described below:

Fluorescein flowmetry

Photographic equipment and techniques used in evaluation of the images

Seven milligrams of sodium fluorescein per kg body weight dissolved in 10ml isotonic saline was given as a bolus injection into a cubital vein. Photographs of the area of interest were taken with a Hasselblad electric motor-driven camera 500 EL/M (Hasselblad, Gothenburg, Sweden) with a yellow Barrier filter (Scott glass GGA 95; Eastman Kodak Company, Rochester, New York, USA), a Kodak wratten filter 15 (Eastman, Kodak Company), and a ringflash (Pro-2 Ringflash, Pro, Stockholm, Sweden) with a blue excitation filter: Kodak Gelatine filter Wratten 47 A (Eastman Kodak Company) was used. A fast built repetitive generator (Pro) fed the camera. An objective lens (Planar, F 120mm, and 1:2.8) supplemented with an intermediate ring No. 55 was used. Black and white film was employed for imaging and exposure to 800 ASA. Images of the skin at a scale of 1:5 in size were used for the numerical evaluation of the fluorescence intensity, which was achieved by measuring the optical density of the negative film with a densitometer (Macbeth TD 501, Kallmorgen Corporation, Newburg, New York, USA). Circular areas of the images, 2 mm in diameter, were analysed.

Photographs are taken every 5 seconds for the first 120 seconds and thereafter every 30 seconds for another 180 seconds. Fluorescence is measured on the film within a circular area 2mm in diameter, corresponding to an area 1cm in diameter in natural size. A curve is drawn with the recorded fluorescence data and the blood flow or rather the transcapillary exchange of sodium fluorescein expressed as a fluorescence index, in density units per second. The index is calculated as the ratio between the maximum fluorescence obtained during the first circulatory passage of sodium fluorescein and the rise time, defined as the time interval between 10 % and 90 % of the maximum fluorescence. As recirculation of sodium fluorescein is assumed to be negligible and as the tissue is considered to act as a sink in the same time interval, the tissue receives the same fraction of cardiac plasma output as the fraction of the bolus received, thus the maximum fluorescence reflects the fraction of cardiac output distributed to the tissues according to Sapirstein's indicator fractionation principle (Sapirstein 1956). The rise time as defined above indicates the duration of the dispersion of 80 % of the bolus, thereby eliminating the uncertainty as to when the first and the last part of the bolus become trapped in the tissue. The rise time, which is an expression of blood velocity, correlates both with the mean transversal time of the bolus proper (correlation coefficient 0.96) and with the mean transit time of the system (correlation coefficient 0.74). It is inversely proportional to cardiac output but is also influenced by peripheral resistance (Perbeck et al 1985).

Laser Doppler fluxmetry

The instrument used in our studies was a Periflux PF 1C, Perimed AB, Järfälla: Sweden, with a differential detector system containing a 2 mw Helium-Neon laser that produces continuous monochromatic light within the visible wavelength of 632.8 nm. Light is led through an optical fibre to the skin, where it penetrates to a depth of 0.7mm (Andersson and Parris, 1981), (Bachen et al, 1931), 0.14mm to 1.5mm (Tamaru et al, 1988) and to 6.0mm in the intestine (Johansson et al, 1987). The final output from the instrument, as stated previously, is related to the velocity and number of red blood cells moving in a hemisphere of tissue approximately 1-1.5mm in radius. Thus a large number of slowly moving cells could produce a flow signal similar to that of a small

number of rapidly moving cells. The backscattered light is transmitted to two photo detectors, where shifted and unshifted light is mixed, resulting in the creation of beat frequencies (Bonner et al 1981), which are recorded on a chart and expressed in volt units. These two photo-detectors, together with the optical fibre, which guides the beam, constitute the optical lead, the tip of which forms the probe.

Two probes PF 108, one with a specially designed adaptor with a concave incision for the nipple, were used to direct the laser beam perpendicular to the tissue. The probe was held by hand. Our system has a filter at 4 kHz. We used a time constant of 1.5 seconds and gain $\times 10$.

Xenon clearance

With the subject lying in the supine position, an airtight, waterproof chamber (Atlantic, Mediate, Borlänge: Sweden) 1 cm in diameter was placed on the skin of the sole of the right foot. Thereafter 100mq ^{133}Xe in 0.6 ml saline was injected into the chamber and the xenon was allowed to remain in contact with the skin for 5 minutes. The xenon solution was then removed and the chamber was carefully detached from the skin. A gamma camera (Maxicamera 400 T, General Electric, Milwaukee, Wisconsin, USA) was then placed in front of the sole of the foot, as close as possible without making contact with the skin, and consecutive 1 minute scintigrams were continuously recorded in a Computer during a period of one hour. The gamma camera registration was started immediately after the chamber was removed. Regions of interest were drawn around the depot area and a background area. Curves were generated and the background was subtracted. A fast and a slow clearance curve were separated and analysed as described by Sejrsen (1972). Since only a change in skin circulation and not absolute blood flow was measured, there was no need to calculate the tissue-blood partition coefficient of the tracer.

Skin temperature measurements

The subjects were placed in the supine position, in a room with a constant temperature of 22°C. The skin temperature was measured in the middle of the plantar region of the forefoot with an electronic thermometer (Universal TE3, ELBA Instruments A/B, Copenhagen: Denmark) to an accuracy of 0.03 % at room temperature.

Provocation of vasodilatation with alcohol and an external heat box

After basal measurements of skin circulation and skin temperature as described above, the skin vessels were dilated by placing a heat box built of wood over the subject; this covered the upper part of the abdomen to the knee and provides a constant temperature of 34°C. After 30 minutes' exposure to heat, measurements were repeated. The subject then drank 30 ml of 50 % alcohol diluted in juice in study I, and 15 ml in studies II and III, and 30 minutes later, with the heat box still in position, the measurements were repeated and were followed by fluorescein flowmetry measurements.

Weight-bearing pressure and gait analysis

An EMED Gait Analysis System (Novell GmbH, Munich, Germany) was used in study IV. This consists of a footplate placed in the middle of a 4m long way covered by a carpet. The subjects walked at normal speed over the plate three times; the first two were preparatory and the third was recorded. Pressure recordings are retained in an analytical Computer system and expressed in Ncm^{-2} . During static recording the subject stands for 5 seconds with one foot on the footplate, keeping the other parallel close to the plate. No support for the hands is provided. The recorded pressures were divided into four regions: the toes, the tarsal and metatarsal regions and the heel.

In each zone the maximum pressure and surface contact area were identified and recorded. A mean of four pressure points within a 1 cm² area was calculated.

Podoscope

The podoscope had previously been designed at Huddinge Hospital to allow observation of the plantar aspect of the feet with the subject standing. It consists of a glass platform, with a mirror beneath, placed at an angle of 45° to enable the sole of the feet to be viewed in front of the subject. The podoscope has a frame for hand support. The subject stands or simulates walking on the tray for measurements.

STATISTICS

The results in study I are expressed as the ratio between the second measurement, after intake of alcohol and application of external heat, and the first measurement, of basal flow. Correlation coefficients were calculated between fluorescein flowmetry, laser Doppler fluxmetry, ¹³³xenon clearance and fluorescence appearance times.

In studies II experimental data are expressed as median and interquartile ranges. For comparisons of data before and after vascular reconstruction and in healthy subjects, for evaluating the change in skin circulation after intake of alcohol and after alcohol intake and heat exposure, Wilcoxon's matched pairs signed rank test was used. For comparisons between groups regarding the effects of intake of alcohol and heat exposure, the Mann Whitney U test was used.

In study III, for comparisons of patients before and after vascular reconstruction Wilcoxon's matched pair signed rank test was used, and in healthy subjects the coefficient of variation was calculated for fluorescein flowmetry and laser Doppler fluxmetry in order to assess the value of measurements in different occasions in the same individual.

In study IV the medians and the interquartile range or ranges were calculated. Significance between groups was tested by the Mann Whitney U test. A two-way analysis of variance was used to study the relationship between weight-bearing pressure and fluorescence index.

In study V data are expressed as mean \pm SEM. Statistical hypotheses were tested by Wilcoxon's matched pair signed rank test.

In study VI data are presented as means (SD). Statistical hypotheses were tested by Student's t test for paired and unpaired data.

In study VII data are expressed as means (SD). Statistical hypotheses were tested by Student's t test for paired and unpaired data or Wilcoxon's rank sum test.

RESULTS

Comparison between fluorescein flowmetry, ¹³³xenon clearance, laser Doppler fluxmetry and fluorescence appearance time (I)

The correlation coefficient (r) between FF and the fast slope of the ¹³³xenon elimination curve was (r) 0.46 (p<0.05), between FF and the slow slope of the ¹³³xenon elimination curve (r) 0.66 (p<0.001) and between FF and LDF laser Doppler flowmetry (r) 0.86 (p<0.001) regarding assessment of the change in skin circulation between the initial measurement under basal conditions and the second measurement after intake of alcohol and application of external heat. The correlation coefficients, including those for fluorescence appearance time (AT) are shown in Table 2. FF showed high correlations with the fast and slow ¹³³xenon clearance curves and LDF. Fluorescence AT also showed correlations with the slow ¹³³xenon clearance curve and LDF, though of a lower degree than FF. There was also a correlation between FF and fluorescence AT. There were no changes in ambient temperature during the measurements (22.3 ± 0.7 before and 22.6 ± 0.6 after the measurements).

Table 3. Correlation coefficients (r) between fluorescein flowmetry (FF), the fast slope of the ¹³³xenon elimination curve (Xe-fast), the slow slope of this curve (Xe-slow), laser Doppler fluxmetry (LDF) and the fluorescence appearance time (AT) regarding assessment of the change in skin circulation between the two measurement occasions, calculated as the ratio between the second and first occasion. n = number of subjects. n.s. = non-significant.

		n	r	p
FF	Xe-fast	24	0.46	< 0.05
	Xe-slow	24	0.66	< 0.001
LDF	FF	24	0.86	< 0.001
	Xe-fast	24	0.28	0.18 (n.s.)
	Xe-slow	24	0.72	< 0.001
Xe-fast	Xe-slow	24	0.15	0.48 (n.s.)
AT	Xe-fast	24	0.17	0.42 (n.s.)
	Xe-slow	24	0.47	< 0.05
	LDF	24	0.63	< 0.001
	FF	24	0.63	< 0.001

Repeated measurements of the skin circulation

Repeated measurements with fluorescein flowmetry could be performed with intervals of 100 min. The maximal fluorescence, including the background fluorescence, after the first circulatory passage of sodium fluorescein at rest, was 0.10 ± 0.08 and after provocation of vasodilatation 0.18 ± 0.10 density units.

Influence of heat and ethanol on skin circulation measured with laser Doppler fluxmetry (II)

No increase in skin circulation or in skin temperature was observed after exposing patients suffering from occlusive arterial disease to external heat alone or to ethanol and heat combined, either before or after vascular reconstruction. In contrast, in healthy individuals laser Doppler flowmetry showed a significant increase in skin circulation after 30 minutes of application of external heat, by 126 % from 9.5 (8-17) V ($p < 0.01$; $n = 14$). After alcohol intake and a further 30 minutes of heat exposure there was a further increase by 81 % from 21.5 (11-34) V ($p < 0.01$); compared with the pre-warming up period, the increase after 60 minutes of warming was 310 %, up to 39 (13-52) V ($p < 0.01$). The increases in skin temperature at the corresponding times were 2.7°C from 26.9 (25.2-29.0) °C ($p < 0.05$), 3.1°C from 29.6 (24.2-31.5) °C ($p < 0.05$), and 5.8°C ($p < 0.05$), respectively.

Changes in skin circulation after vascular reconstruction measured by fluorescein flowmetry and laser Doppler flowmetry (III)

Skin circulation was recorded both by FF and LDF before and after vascular reconstruction. The measurements were performed both in patients and healthy subjects after intake of alcohol and application of external heat. After vascular reconstruction, an increase in circulation by 140 % ($p < 0.01$) was noted with FF and by 48 % ($p < 0.01$) with LDF. The skin temperature rose by 3.2°C ($p < 0.01$). The correlation coefficient (r) between the increase in circulation after vascular reconstruction measured by LDF and that measured by FF was 0.80. No change in circulation was observed in the contralateral non-operated limb with either LDF or FF.

There were no differences in skin circulation in the healthy individuals between an initial measurement and a second measurement after an interval of one month with either FF or LDF, or when measured in terms of skin temperature. The coefficients of variation for these three methods were 0.46, 0.34, and 0.03 respectively.

Weight-bearing pressure and plantar circulation in diabetes mellitus (IV)

Plantar circulation in relation to weight-bearing pressures was assessed in 10 patients with severe diabetic polyneuropathy. The nerve conduction studies revealed neurophysiological signs of severe motor/sensory polyneuropathy in all patients and autonomic failure in all but one patient.

Significantly higher pressures during walking were noted in previously ulcerated feet in diabetic patients than in healthy feet in controls ($p < 0.05$), this difference was entirely attributable to a higher maximal weight-bearing pressure in the metatarsal region ($p < 0.05$), while no difference in pressure was founded in the standing position between diabetic patients and controls. After sodium fluorescein injection in the supine position there were no differences in plantar circulation in the great toe, metatarsal region and heel region compared to either in the diabetic patients or in the control subjects. The fluorescence was homogeneous within the different regions of the feet in all subjects. Those areas in which the weight-bearing pressure while standing was 3 N cm⁻² or more did not show any circulation at all except at the edges, where there was a marginal zone 2-8 mm wide with increased fluorescence. There was no difference in the fluorescence index in this marginal zone between the previously ulcerated and non-ulcerated feet in the diabetic subjects or between diabetic subjects and controls. However, more irregular and more intensive fluorescence was observed in the areas with no weight-bearing pressure and preserved circulation in the diabetic foot in the standing position compared to controls (I V, Fig. 3 b).

The skin circulation was independent of weight-bearing pressure below 3 N cm⁻², including a pressure of 0 N cm⁻², both in diabetic and control subjects (correlation coefficients $r = -0.01$ and -

0.19, respectively) when the complete range of 0-3 N cm⁻² was included in the analysis. However, when the plantar circulation was analysed in these two groups at separate pressure intervals of 1.0 to 2.0 and 2.1 to 3.0 N cm⁻², different results were obtained. In the former range of pressures no difference in skin circulation, measured by FF was found between the groups, with median values (and interquartile ranges) of 0.006 (0.004 - 0.009) and 0.010 (0.004 - 0.009) density units/s, respectively. But in the pressure range of 2.1-3.0 Ncm⁻² there was a successive decline in skin circulation in the diabetic patients compared with that in the control subjects, and the corresponding values were now 0.001 (0.001- 0.003) and 0.008 (0.002 -0.013) density units/s, respectively (p<0.05) (Fig. 2).

During walking, the circulation returned to the areas that had shown no circulation in the standing position. Homogeneous fluorescence was observed over the entire plantar region and the increased fluorescence in the marginal zone disappeared.

There were no differences in the fluorescence index irrespective of whether the measurements were performed in the foot with weight-bearing pressure, or in the other foot, during the period when the photographs were taken, either in the diabetic subjects or in the controls.

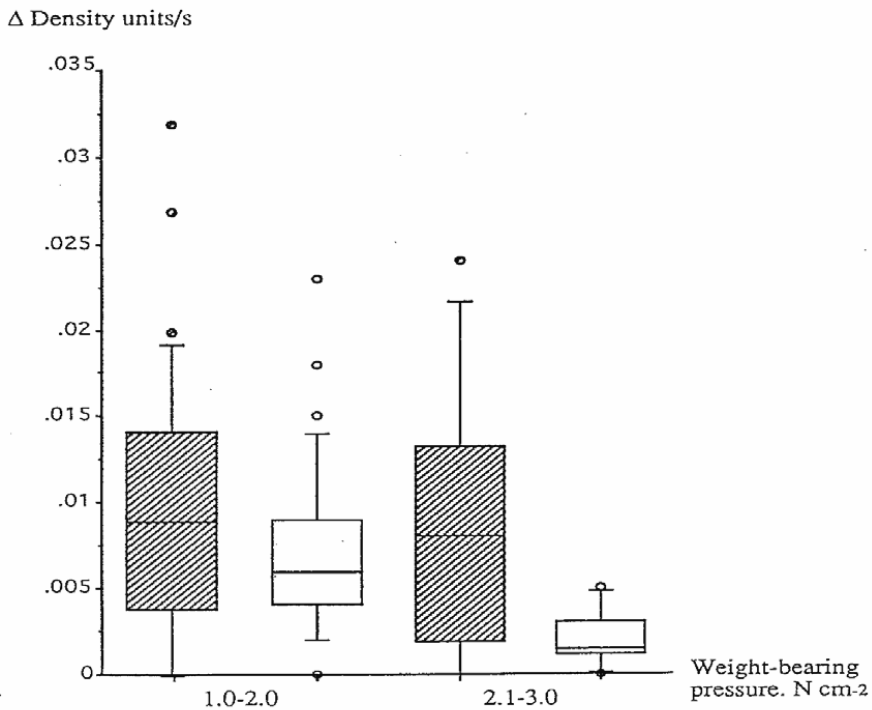


Fig. 2. Skin circulation measured by fluorescein flowmetry in relation to weight-bearing pressure in diabetic and control subjects. Medians and interquartile ranges and 10th and 90th percentiles. Points show subjects outside these percentiles.
 Controls ■ Patients □

Influence of reduction mammoplasty on the circulation of the nipple (V)

In 16 patients with macromastia undergoing reduction mammoplasty, the skin circulation in the nipple was measured on both breasts before, during and after surgery. The mean weight of the glandular tissue removed was 636 g (range 153-1,767 g) and in the breasts in which adrenaline was injected it was 702 g (range 186-2,197 g). As measured by LDF, the circulation in the nipple increased after the de-epithelialization to 245.7 ± 39.3 % of the pre-operative value (100 %) ($p < 0.01$, $n=16$). In the breasts that received adrenaline the corresponding increase in circulation was 153.4 ± 15.6 % ($p < 0.01$, $n=16$), which is significantly lower than when adrenaline was not given ($p < 0.05$, $n=16$). After the medial and lateral glandular resections, the circulation in the nipple of the vertical dermal bridge was reduced by 128.1 ± 26.9 % of the circulation noted after the de-epithelialization ($p < 0.01$, $n=16$) to 125.6 ± 21.2 % of the preoperative circulation. In the breasts in which adrenaline was given, the corresponding reduction was 74.1 ± 19.4 % of the circulation observed after the de-epithelialization ($p < 0.01$, $n=16$) to 79.3 ± 6.5 % of the preoperative circulation measured after the adrenaline injection into the incision lines. Postoperatively the circulation was 128.4 ± 25.9 % of the preoperative value and in the breasts in which adrenaline was injected it was 177.0 ± 96.9 % of the preoperative circulation after the administration of adrenaline. One to four days postoperatively the circulation was 123.1 ± 19.9 % of the preoperative circulation, and in the breasts that received adrenaline it was 130.1 ± 24.0 % of the preoperative circulation measured before adrenaline was given (100 %).

At fluorescein flowmetry, the postoperative fluorescence index was 0.007 ± 0.003 density units/s, with a range of 0.001-0.032 density units/s ($n=12$). At FF the postoperative fluorescence index in the breasts that received adrenaline was 0.007 ± 0.004 density units/s, with a range of 0.000-0.055 ($n=13$). Homogeneous fluorescence was observed in the nipple in all patients except one. In this patient a glandular resection of 2,197 g was performed and the patient also received adrenaline. There was no visible fluorescence, but the LDF signal was 6, corresponding to 75 % of the preoperative value after administration of adrenaline. On the fourth day it was obvious that the nipple was not viable, and it became necrotic, most likely as a result of strangulation of the approximately 20 cm long distal vertical pedicle.

The skin circulation in the medial lower corner as measured by LDF increased after the de-epithelialization to 196.7 ± 22.9 % of the preoperative value (100 %) ($p < 0.01$, $n=15$). In the breasts in which adrenaline was injected, the circulation increased after de-epithelialization to 155.9 ± 27.6 % of the pre-operative value obtained after adrenaline was administered (100 %) ($p < 0.05$, $n=16$). After the medial and lateral glandular resections the blood flow in the skin was 169.7 ± 26.0 % of the preoperative blood flow and in the breasts in which adrenaline was injected it was 155.6 ± 16.6 %. When the skin had been sutured postoperatively, the blood flow in the skin was 201.6 ± 33.3 % of the preoperative blood flow and in the breasts that received adrenaline it was 104.8 ± 17.9 %. One to four days postoperatively the corresponding values were 318.8 ± 109.6 % and 192.4 ± 45.1 % respectively. At FF the postoperative fluorescence index was 0.044 ± 0.036 density units/s, with a range of 0.001-0.048 density units/s, and in the breasts in which adrenaline was injected it was 0.006 ± 0.001 density units/s, with a range of 0.001-0.014 density units/s ($n=13$).

Influence of subcutaneous mastectomy in the circulation of the nipple-areola complex (VI)

After subcutaneous mastectomy through a lazy-S incision, neither FF nor LDF showed a decrease in circulation in the nipple-areola complex compared with that in the complex of the untreated, contralateral breast. In the reduction mammoplasty group, however, the circulation in the nipple-areola complex was decreased by 74 % ($p < 0.01$) on FF and 70 % ($p < 0.05$) on LDF, compared

with the circulation in the contralateral breast in which a conventional reduction mammoplasty or mastopexy had been done.

When the circulation in the nipple-areola complex of those breasts treated with subcutaneous reduction mammoplasty was compared with that in the untreated breasts of the patients in whom a lazy-S incision had been used, a reduction of 87 % was found as measured by fluorescein ($p<0.01$), but only of 52 % as measured by LDF. In five patients partial or complete epidermal necrosis developed in the nipple-areola complex, and in one patient total dermal necrosis occurred, but there was no deep necrosis that included the whole layer of skin and fat. There was no fluorescence at all within the areas in which necrosis later developed in any of these six cases. The laser Doppler signal in the corresponding areas was not reduced.

The influence of the site of the skin incision on the circulation in the nipple-areola complex after subcutaneous mastectomy in breast cancer (VII)

I. Influence of the site of skin incision on the skin circulation in the breast.

There was no difference in skin circulation in any of the three areas of measurement, namely: 2 cm above the nipple-areola complex (position 1), in the nipple-areola complex (position 2), or 2 cm below the complex (position 3), measured either by LDF or FF (Table 4), between the group of patients with a lazy-S horizontal incision and the group with a sub mammary incision. However, when the ratio of the circulation in the surgically treated group to that in the opposite intact breasts was calculated, it was found that in position 3, 2 cm below the complex, FF but not LDF indicated that the skin circulation was impaired in the group with a sub mammary incision compared with the lazy-S group ($p<0.01$) (Table 4). There was no skin necrosis in any of the breasts.

II. The skin circulation in the treated breast compared with the opposite, intact breast

As measured by LDF, the skin circulation in the lazy-S incision group was increased in all three areas compared with the intact breast, and as measured by FF it was increased in position 3 but not in position 1 or 2 (Table 4).

Corresponding measurements of skin circulation by LDF in the group with the sub mammary incision also showed an increase in all positions compared with the intact breast. The skin circulation was not increased in either position on FF

III. Skin circulation within different regions of the breast

In the treated breast, both with the lazy-S incision and the submammary incision, the circulation in the skin as measured by LDF was higher in position 2 than in both position 1 ($p<0.01$) and position 3 ($p<0.01$). Fluorescein flowmetry showed no difference in skin circulation between any of the three positions.

In the intact breast, both in the group with the submammary incision and in the lazy-S incision group, the skin circulation as measured by LDF was higher in the nipple-areola complex (position 2) than in positions 1 and 3 ($p<0.001$ for both comparisons), and it was also higher in position 3 than in position 1 ($p<0.05$). When measured by FF, the skin circulation in position 3 was again higher than in position 1 in the submammary incision group ($p<0.05$).

Table 4. Skin circulation in three areas of the breast, 2cm above the nipple-areola complex (position1), within the complex (position 2) and 2cm below the complex (position 3), measured by fluorescein flowmetry or by laser Doppler fluxmetry depending on the operation performed. Values are mean (SD) except where otherwise stated.

	n	Fluorescein flowmetry (Δ Density units x 10 ⁻² /s)			Laser Doppler fluxmetry (volts)		
		Position 1	2	3	Position 1	2	3
Lazy-S incision	26	0.39 (0.31)	0.35 (0.29)	0.45 (0.33)	10.8(3.4)	20.4(9.1)	11.9(4.6)
Intact breast	26	0.31 (0.21)	0.34(0.39)	0.33 (0.18)	8.7 (2.8)	15.0 (5.5)	9.9 (5.1)
p value		0.22	0.84	< 0.01	< 0.01	< 0.01	< 0.01
Submammary incision	43	0.29 (0.26)	0.27 (0.22)	0.29 (0.26)	11.5(4.5)	23.9 (12.2)	12.9 (6.8)
Intact breast	43	0.27 (0.20)	0.24 (0.23)	0.32 (0.25)	7.9 (2.6)	21.2 (13.0)	9.4 (4.9)
p value		0.49	0.48	0.30	< 0.001	< 0.05	< 0.01

GENERAL DISCUSSION

The ideal method for measuring blood flow or skin perfusion should preferably be non-invasive, inexpensive, and easy to use and reproduce. Further, it should allow continuous recording, have diagnostic reliability and validity and permit qualitative and quantitative measurements that are easily understood by all who use it (Spence, 1987). At present none of the current methods used for these purposes meet these criteria.

In clinical practice it is important to measure the nutritive circulation, which in reality represents the transport of nutritive elements through the capillaries to and into the cells. Since sodium fluorescein, with its molecular weight of 376 and size of 0.55 nm, mimics the nutritive transport of small solutes, even intracellularly, and is used as an indicator in fluorescein flowmetry, the method can be used for this purpose.

In order to calculate blood flow by an indicator dilution technique, certain prerequisites have to be fulfilled, such as: unrestricted diffusion, no back-flux and no recirculation of the indicator (Lassen and Perl, 1979). Fluorescein flowmetry is an indicator dilution technique. In studies in the intestine, Perbeck et al (1985) demonstrated that the method is valuable for measuring blood flow (expressed in arbitrary units), as the amount of indicator is known, the extraction of sodium fluorescein is 100 % in the intestinal capillaries, there is no back-flux, since the tissue acts as an effective sink for sodium fluorescein diffusing out of the capillaries, and there is no recirculation of the dye, since the fluorescein index is calculated from the tissue from the time interval between 10 % and 90 % of the maximum fluorescence, thus representing the first circulatory passage of the dye in the tissue.

In the skin, however, the extraction of sodium fluorescein is not known, and it is possible that it might change with changes in blood flow; for example there may be higher extraction during low flow, and vice versa (Renkin, 1959).

At present there is no method that can be regarded as a golden standard for measuring skin blood flow. All methods have their advantages and disadvantages. Today laser Doppler fluxmetry is the most frequently used method and it is especially suitable for studying changes in skin circulation even though it is very sensitive to spatial variation of the circulation in the skin in that it measures only a small volume, a sphere 1-2 mm down in the tissue (Nilsson et al, 1980). By measuring the circulation at five points and calculating a mean value within 1cm², this disadvantage could be partly overcome in our study. ¹³³Xenon clearance has the advantage of measuring a capillary blood flow, but the drawback of this method is that ¹³³xenon has a strong affinity for fat tissue, which reduces the clearance from the tissue and makes the interpretation of the elimination curve difficult.

Fluorescein flowmetry cannot be considered to measure skin blood flow per se, but in the skin it rather measures, the transcapillary exchange of sodium fluorescein, which is dependent on the blood flow, the permeability of the capillary to this indicator and the available capillary surface area. The uptake of sodium fluorescein as measured by fluorescein flowmetry correlated to the fast slope of the ¹³³Xenon elimination curve, with a correlation coefficient (r) of 0.46, and also to the slow slope of this elimination curve (r = 0.66). Sejrsen (1972) suggested this separation of the elimination curve into a fast and a slow component. The exact anatomical background of this dual compartment functional system is not known. The fast slope of the elimination curve represents the superficial blood flow, and the slow slope the blood flow in the deeper layers of the skin, including the subcutaneous adipose tissue. By measuring the change in skin circulation, we did not need to calculate the blood-tissue partition coefficient of ¹³³Xenon, which might be a factor of uncertainty. Fluorescein flowmetry also correlated to laser Doppler fluxmetry in assessing a

change in skin circulation (from the basal level to a level at most 16 times higher as measured by LDF), with a correlation coefficient of 0.86. The possible correlation between the three methods in low flow and high flow states were not evaluated. However, in study II patients with ischaemic limbs exhibited a low flow state, which was improved after successful vascular reconstruction, but not to the same level as in healthy subjects. The correlation coefficient concerning this change in skin circulation measured by fluorescein flowmetry and laser Doppler fluxmetry was $r = 0.80$. To increase the blood flow more than 16-fold in healthy subjects without giving too potent drugs that will allow measurement of a constant blood flow during a period of 1 to 1.5 hours is not easy. If this is achieved by reducing the initial basal flow, for example by cold, the blood flow might decrease differently in different layers of the skin, and since the three methods used measure the skin circulation at different tissue depths, comparison between the methods in measuring a change in skin circulation might be hazardous. Laser Doppler fluxmetry also correlated to the slow slope of the elimination curve of $^{133}\text{Xenon}$, but not to the fast slope of this curve, the reason for which might be that the deeper level has greater influence on the laser Doppler signal than the superficial level. The velocity of the blood is higher in the arterioles than in the capillaries, which means that the arterioles have much more influence on the laser Doppler signal than the capillaries, despite a larger capillary network. The measuring depth of the laser Doppler signal is a matter of some uncertainty. This signal has been reported to reach 0.7mm (Andersson and Parris, 1981), and 0.14 -2 mm in the skin (Stern et al, 1977) (Nilsson et al, 1980) (Tamaru et al, 1988), but up to 6 mm in the intestines (Johansson et al, 1987). Fluorescein flowmetry measures fluorescence down to 0.6 mm in the tissue (Perbeck et al, 1985), which might imply that it measures both the superficial and the deeper skin circulation.

The weak correlation of fluorescein flowmetry to $^{133}\text{Xenon}$ and its somewhat stronger correlation to laser Doppler fluxmetry suggest that fluorescein flowmetry is not suitable for studying minor changes in skin circulation in the individual subject but can be used for comparisons of larger groups of individuals. So far fluorescein flowmetry has only been evaluated regarding changes in skin circulation in healthy subjects. In patients the permeability of the skin vessels might be altered, leading to an increased leakage of sodium fluorescein in the tissue. This again emphasises the importance of determining what type of flow needs to be studied, i, e. the true blood flow with flux of erythrocyte, the plasma flow or the transcapillary exchange of small solutes, also intracellularly.

Several methods have been used for evaluating skin circulation in ischaemic disease. Those employed in our studies include laser Doppler fluxmetry, fluorescein flowmetry and skin temperature, with and without exposure to external heat and intake of alcohol.

External heat is frequently used to reduce spatial and temporal variations in skin blood flow under basal conditions prior to measurements. When Lund and Lund (1972) introduced fluorescein angiography, which is the technical and experimental set-up for fluorescein flowmetry, they used routine external heat and ingestion of ethanol to dilate the skin vessels. One aim of our studies was therefore to evaluate the necessity for this procedure. Since fluorescein flowmetry can only be performed twice, because of the too high background fluorescence, measurements were made with laser Doppler fluxmetry. Our studies suggest that in patients with severe limb ischaemia it is unnecessary to apply external heat and administer alcohol to dilate the skin vessels before circulation measurements (II). We are aware that the amount of alcohol given in our study, 15 ml of 50 % alcohol (roughly 7 g) dissolved in a glass of juice, does not cause maximal dilatation of the skin vessels, but it was chosen and administered for practical reasons: the dose was well tolerated by our elderly patients, and our younger patients and controls were able to drive their own car back home. In study I, where we wanted to achieve a large increase in skin circulation, the healthy subjects were given 30 ml of 50 % alcohol.

When LDF was used for measuring skin perfusion, patients with occlusive vascular disease did not show any changes in skin perfusion or skin temperature after exposure to external heat alone or external heat combined with alcohol intake, whereas healthy individuals showed a significant increase in both parameters. The reason for this difference in the responses might be that the number of patients with occlusive vascular disease was too small to allow statistical detection of a small increase in skin circulation. However, another reason might be that in patients with ischaemic limbs or in patients who had undergone successful vascular reconstruction within the last 10 days, the skin vessels were fully dilated before heat exposure or alcohol intake because of post-reconstruction hyperaemia (II). After successful vascular reconstruction in our patients the skin circulation increased by 140 % as measured by fluorescein flowmetry, and by 48 % as measured by laser Doppler fluxmetry (III), which might imply that during ischaemia the superficial nutritive circulation measured by FF is reduced more than the deeper circulation measured by LDF and that after a successful vascular reconstruction the relative increase in skin circulation will be higher with fluorescein flowmetry. It has been established that only 15 % of the skin blood flow is nutritive and that the other 85 % takes part in the thermoregulatory system (Took, 1987). This fact supports the idea that the skin circulation is maximal both during ischaemia and during hyperaemia.

In study III it was found that fluorescein flowmetry could be used for repeated measurements in the skin. There was no difference in the median skin circulation value, obtained either by fluorescein flowmetry or by laser Doppler fluxmetry, or in skin temperature, between two measurements made with an interval of one month in our group of healthy subjects. It is known that the basal skin blood flow shows large spatial and temporal variations in spite of local application of heat, indicating that repeated measurements are hazardous (Tenland et al, 1983) (Nilsson et al, 1980). We found a coefficient of variation of 0.46 for fluorescein flowmetry and of 0.34 for laser Doppler fluxmetry. Our results are thus in concordance with those of Lukkari-Rautiarinen et al (1989), who noted a coefficient of variation of 0.30 for laser Doppler flowmetry and 0.37 for transcutaneous oxygen tension in day-to-day measurements. They concluded that single values can hardly be expected to predict the outcome for the foot in a prospective series, nor can they be used in therapeutic trials of drug action. Major changes in the peripheral circulation, for example the effects of revascularisation procedures, can be evaluated by use of either of the two methods studied.

In study IV the circulation of the plantar skin was assessed. According to Brand (1983) the insensitive diabetic foot may be injured by external forces in three principal ways: by repetitive moderate stress, by constant pressure maintained for a long time, or by short periods of high pressure. Our results indicate that the nutritive circulation in the plantar skin is arrested when the weight-bearing pressure exceeds 3N cm^{-2} . Below 3N cm^{-2} the circulation was independent of weight-bearing pressures, both in diabetic and control subjects (correlation coefficients r -0.01 and -0.19, respectively), when the complete range of $0\text{-}3\text{ N cm}^{-2}$ was included in the analysis. However, when the weight-bearing pressure interval of $2.1\text{-}3.0\text{ N cm}^{-2}$ was analysed, it was found that there was a significant decline in circulation in the diabetic subjects, indicating a lower capillary closing pressure. No differences were found in the nutritive circulation of either diabetic subjects or controls in the 2-8 mm wide marginal zone around the areas that displayed circulatory arrest in the standing position. During walking the circulation rapidly returned to the areas that had shown circulatory arrest during weight-bearing pressure. The fluorescence pattern in the diabetic subjects was irregular and intensive, indicating an increased capillary leakage due to capillary damage.

The use of sodium fluorescein to assess the viability of the skin in relation to different surgical procedures is informative. In our studies the findings with this technique influenced the choice of surgical strategy and predicted tissue viability.

In study V, we examined the skin circulation in the nipple-areola complex after reduction mammoplasty with a bipedicle vertical dermal flap according to the method of McKissock (1972). After the skin had been sutured the skin circulation was 128% of the preoperative value and one to four days postoperatively it was 123% of the preoperative value. The skin circulation in the vertical pedicle was of the same magnitude as or even higher than in the medial pedicles when the method of Strömbeck (1960) was used, which showed a value 70% of the preoperative value when the skin had been sutured (Perbeck et al 1988). In study VI, in which the skin circulation was measured after subcutaneous mastectomy, it was found that the circulation in the nipple-areola complex was extensively reduced after subcutaneous reduction mammoplasty, for the reason that the circulation to the complex was based on a thin inferior subcutaneous dermal pedicle. When possible this operative technique was therefore avoided in favour of a submammary or lateral S-shaped skin incision and a subcutaneous location of the implant, in which the circulation in the nipple is twice that of the surrounding skin (VII), and when it was necessary to perform a subcutaneous reduction mammoplasty the blood flow to the nipple-areola complex was based on a superior dermal flap which reduces the distance between the nipple and the non de-epithelialised skin border. The circulation can easily be checked preoperatively by illuminating the tissue with UV light or blue light and inspecting the tissue fluorescence. If there is no fluorescence within 4-5 minutes (3 minutes in the foot) there is a high probability that the circulation is not sufficient to maintain tissue viability, and the surgical strategy must be accordingly reassessed.

In study VII the influence of skin incision on the circulation in the nipple-areola complex after subcutaneous mastectomy in breast cancer was studied. There was no difference in the skin circulation in this complex or in the skin 2cm above it between the group of patients with the lazy-S-shaped horizontal incision and the group with the submammary fold incisions, the two most commonly used skin incisions in subcutaneous mastectomy. However, 2 cm below the complex fluorescein flowmetry, but not LDF, showed 36% lower skin circulation in the group with the submammary fold incision than in the other group. The reason for this discrepancy might be that the superficial circulation is reduced more than the deeper one. The circulation was sufficient; however, to avoid necrosis irrespective of which of the two incisions was made. The two surgical incisions were used during different time periods. During the first part of the period (1990-1991), a lazy-S-shaped horizontal incision beginning at the upper part of the nipple-areola complex and extending laterally was made. In the second part (1991-1993) the incision chosen was a transverse submammary incision 1.5 cm above the submammary fold, for the reason that by 1991 it had been found that the cosmetic result was better with this type of incision. During this two time periods all other factors that might have influenced the result, such as adjuvant radiotherapy, chemotherapy and antioestrogenic therapy, anaesthesia and available drugs, were constant. As a submammary incision gives a better cosmetic result, it is still recommended despite a somewhat lower skin circulation, as the circulation was sufficiently high to avoid skin necrosis.

When the tissue circulation in the nipple-areola complex (V, VI and VII) was studied with fluorescein flowmetry and laser Doppler fluxmetry, different predictions of tissue viability were obtained. The difference can be explained by the fact that the two methods measure the circulation at different depths, as discussed above. There is probably a true difference in circulation in different layers of the skin due to differences in skin architecture, but the above discrepancy can also be explained by different pigmentation of the skin. Epidermal necrosis of the nipple-areola complex showed no tissue fluorescence but nevertheless an LDF signal (VI) (Perbeck et al, 1992).

Fluorescein flowmetry is to be considered a non-invasive method, since the fluorescence is recorded from the surface of the organs, although sodium fluorescein has to be injected as a

bolus. This method is inexpensive but technically quite complicated to perform, since the equipment has to be strictly standardised (film, film development, illumination and filters). The analysis of the film negatives, the construction of curves and the calculation of the fluorescence index are time-consuming and not suitable for routine examinations. In the future, the video and/or image analysis technique might simplify the measurements and provide immediate results. The method does not allow continuous recordings, but it can be used for repeated measurements, at least once as demonstrated in study I, provided that the background fluorescence and the increase in tissue fluorescence after a new injection are within the linear dose-response relationship between tissue fluorescence and tissue concentration of sodium fluorescein (up to 0.60 density units, Perbeck et al, 1985). The biological variation of the skin circulation makes day-to-day measurements uncertain and our results of measurements with a one-month interval showed a high coefficient of variance. This is also true for laser Doppler fluxmetry and transcutaneous oxygen tension. One way of reducing the coefficient of variation is, whenever possible, to express the results obtained by FF in relation to a reference area, e.g. as in paper VII, where they are given as the ratio of the values in the operated to the non-operated breast in corresponding areas. Fluorescein flowmetry is reliable in predicting necrosis of large areas of tissue and permits relative measurements of the superficial circulation in the skin (0.6 mm, Perbeck et al, 1985). The method measures a relative transport of small solutes in the skin. Absolute blood flow, corresponding to that obtained by the microsphere or ^{86}Rb technique (Karlberg et al, 1982), can theoretically be measured by FF provided that an arterial reference blood sample is taken. However, at present the extraction of sodium fluorescein in the skin cannot be measured.

The greatest advantages of fluorescein flowmetry are the possibilities of studying a heterogeneous skin circulation and predicting tissue viability in large areas of tissue.

CONCLUSIONS

- The uptake of sodium fluorescein as assessed by fluorescein flowmetry correlates to two established methods of measuring skin circulation, namely $^{133}\text{xenon}$ clearance and laser Doppler fluxmetry. In measuring changes in the skin circulation in healthy subjects, the correlation coefficients (r) between FF and the fast and slow slopes of the $^{33}\text{xenon}$ elimination curves were 0.46 and 0.66, respectively, and between FF and laser Doppler fluxmetry, $r=0.86$. In view of the low correlation coefficients, FF is not suitable for studying small changes in skin circulation in individual subjects, but can be used for comparisons of larger groups. Fluorescein flowmetry cannot be considered to measure a relative blood flow per se in the skin, since the extraction of sodium fluorescein in relation to flow cannot be measured at present, but it does measure the transcapillary exchange of sodium fluorescein, which mimics the transport of small solutes to the interstitial and intracellular compartments.
- Application of external heat and intake of alcohol increase the skin circulation in healthy subjects. In patients with severe ischaemia of the limbs there was no increase either in skin circulation or in skin temperature after such treatment, suggesting that the vessels were already fully dilated. This suggests that in this category of patients it is unnecessary to apply external heat and administer alcohol to dilate the skin vessels before skin circulation measurements.
- Fluorescein flowmetry can be used for repeated measurements, provided that the background fluorescence and the increase in tissue fluorescence after the new injection of sodium fluorescein are within the linear dose-response relationship between tissue fluorescence and tissue concentration of sodium fluorescein, but the variation in skin circulation between two measurements is quite high; In the present studies the coefficient of variation for the two measurements of skin circulation with an interval of one month was 0.46. The corresponding coefficient of variation with laser Doppler fluxmetry was also high, 0.34, and in both cases this was most likely due to biological variation in skin circulation.
- Fluorescein flowmetry is suitable for studying changes in the superficial skin circulation after successful vascular reconstruction. The skin circulation increased by 140 % as measured by fluorescein flowmetry and by 48 % as measured by laser Doppler fluxmetry in patients who had undergone such surgery. The correlation coefficient (r) between the increase in skin circulation as measured by the two methods was 0.80.
- A weight-bearing pressure of 3 Ncm⁻² or higher was required to arrest the circulation in the sole of the foot both in diabetic and control subjects. However, a successive decrease in fluorescence index was observed at a weight-bearing pressure of 2.1-3.0 Ncm⁻² in diabetic subjects, suggesting a lower capillary closing pressure.
- The circulation in the nipple-areola complex is not significantly reduced after a reduction mammoplasty with transposition of the nipple with a bipedicle vertical dermal flap according to the method of McKissock.
- Better circulation in the nipple-areola complex is observed when subcutaneous mastectomy is performed through a horizontal lazy-S incision than with subcutaneous reduction mammoplasty. By using a textured prosthesis filled with saline, which allows it to be placed subcutaneously instead of at the otherwise mandatory submuscular location, the need of a subcutaneous reduction mammoplasty to elevate the nipple-areola complex is reduced.

- The site of skin incision does not influence the circulation of the nipple-areola complex. Although the skin circulation as measured by fluorescein flowmetry was somewhat reduced when a submammary incision was used, the skin viability was never jeopardised. The submammary incision is still preferred because of its better cosmetic results. Other factors such as loss of sensibility of the nipple might influence this decision in the future.
- The greatest advantages specific for fluorescein flowmetry are the possibilities of studying heterogeneous circulation and predicting tissue viability in large areas of the skin.

ACKNOWLEDGEMENTS

The following persons have in one way or another participated in bringing about this work. I should like to express my sincere gratitude to:

Associate Professor Leif Perbeck, my tutor and friend, for sharing his knowledge on fluorescein flowmetry and breast surgery, for his excellent sense of humour, personal support and never-failing enthusiasm;

the late Professor Dag Hallberg, for opening the doors of Sweden to me and for introducing me and teaching me the art of surgery. Unfortunately I did not finish this work in time for him to see it;

Hannu Määttänen, Lars Westerberg, Ibrahim Turan, Göran Solders and Leif Svensson, my co-authors and colleagues, for sharing their knowledge and time and for all their efforts on my behalf;

the late Professor Fredrik Lund for developing the technical equipment which made this study possible, for being an inspiration source and for providing the most inspirational discussions in front of the lake Mälaren;

Professor Jörgen Larsson, Former Head of the Department of Surgery, Huddinge University Hospital, for making this thesis possible;

Associate Professor Johan Westerdahl and *Professor Jan Frisell*, for support and encouragement during the final stages of this work.

Professor Jesper Lagergren, for support, encouragement and appreciated suggestions.

Associate Professor Göran Hellers, former head of the Department of Surgery, Karolinska University Hospital Huddinge, for support and for making this work possible;

all my colleagues at the Department of Surgery, Karolinska University Hospital Huddinge, for those hours in the OR and on the ward;

Associate Professor Jan Eklund, former head of the Department of Anaesthesia and Intensive Care, Karolinska University Hospital, Huddinge, for helping me out when things were most difficult and for kind support;

all my colleagues at the Department of Anaesthesia and Intensive care at Karolinska University Hospital Huddinge, for friendship and support and for those long hours in the OR and the Intensive Care Unit;

my colleagues and the nursing staff at the Department of Cardiothoracic Surgery and Anaesthesia at Karolinska University Hospital Huddinge, for their enthusiasm and co-operation;

the department of Anaesthesia at Mälar Hospital, Eskilstuna for their trust and co-operation;

the Clinics of Cardiothoracic Surgery and Anaesthesia at Blekinge Hospital, Karlskrona, for support and tolerance during the concluding part of this work;

the patients and healthy volunteers whose invaluable participation made these studies possible;

Dr Patricio Jarramillo, Former Medical Director at Hospital Metropolitano, Quito, Ecuador, for showing me the way and for sharing his knowledge and friendship;

Chatrin Lindahl, Hélène Jansson, Astrid Ericsson and Solveig Nettelblatt, for first class secretarial assistance not only with the manuscripts but also with all the many practical details;

Teresa Urria, for excellent library assistance;

Maud Marsden, For reviewing the manuscripts and making the English language understandable even for English speaking people;

Jan Holm and Viveka Åberg, for first class and invaluable technical assistance, for their excellent sense of humour and for making the routine always fun;

Björn Löfgren, Medical illustrator at Karolinska Hospital for the art illustration of the microcirculation system in the skin;

The department of Media at Huddinge University Hospital and at Blekinge Hospital for excellent technical assistance;

Gila, por todas aquellas razones que Vos conoces, aunque a veces no hayan sido tan aparentes;

A Marina Romero, mi madre, por haberlo hecho todo posible, a mi hermano **German Proaño Romero, Camila Proaño, Emilia Proaño y a toda mi familia** por hacerlo todo valedero.

Thank you all.

This work was financed and supported by The Instituto Ecuatoriano IECE, the Swedish Institute, the Seraphimer Foundation, the Swedish Medical Foundation, the Swedish Diabetic Foundation, the Swedish Hoechst Foundation and the Nordic Insulin Foundation.

REFERENCES

- Andersson R and Parris J A. The optics of human skin. *J. Invest. Dermatol.* 1981; 77:13-19.
- Bachem A, Reed CI. The penetration of light through human skin. *Am J Physiol.* 1931; 97:86.
- Baer-Suryadinata CH and Bollinger A. Transcapillary diffusion of Na-fluorescein in skin areas measured by a "large window technique". *Int J Microcirc Clin Exp.* 1985; 4:217-228.
- Benson RC, Kues HA. Fluorescence properties of indocyanine green as related as related to angiography. *Phys Med Biol* 1978;23:159-163.
- Bloch E H. The illumination, sensing and recording of the living microvascular system. *Bibl. Anat.* 1973; 11:185.
- Bollinger A and Fagrell B. *Clinical capillaroscopy. A guide to its use in clinical research and practice.* Göttingen: Hogrefe & Huber Publishers, 1990
- Bollinger A, Butti P, Barras J P, Trachler H, Siegenthaler W. Red blood cells velocity in nailfold capillaries of man measured by a television microscopy technique. *Microvasc. Res.* 1974; 7:61-72.
- Bollinger A, Jäger K, Geser A, Sgier F and Seglias J. Transcapillary and interstitial diffusion of Na-fluorescein in chronic venous insufficiency with white atrophy. *Int J Microcirc. Clin Exp.* 1982; 1:5-17.
- Bollinger A, Jäger K, Roten A, Timeus CH and Mahler F. Diffusion, pericapillary distribution and clearance of Na-fluorescein in human nailfold. *Pflugers Arch Physiol.* 1979; 382:137-143.
- Bollinger A, Partsch H and Wolfe JHN (Eds). *The initial lymphatics. New methods and findings.* Stuttgart and New York, Thieme. 1985:94-97.
- Bonner R F, Clem T R, Bowen P D and Bowman R L. Laser Doppler Continuous real-time monitor of pulsatile and mean blood flow in tissue microcirculation. In: scattering techniques applied to supramolecular and non-equilibrium systems. pp. 685-701. Eds. Chen S H, Chew B, Nossal R pp, Plenum Press, New York, 1981.
- Brand PW. The diabetic foot. In Elleberg M, Rifkin H, eds. *Diabetes Mellitus: Theory and Practice.* New York: Medical Exam Publishing Company. 1983; 829-849.
- Brousseau D A, Kein S G and Weinstock B A: Fluorometric assessment of perfusion in multicoloured skin. *Surg. Form.* 1983; 34, 641.
- Bulkley G, Zuidema G, Hamilton S, O'Mara C, Klacsmann P and Horn S. Intraoperative determination of small intestinal viability following ischemic injury. *Ann Surg.* 1981; 193:628-637.
- Burton A C. Special features of the circulation of the skin. In *Blood vessels and circulation Vol. II. Advances in biology of the skin.* Eds. Montagna W and Ellis R. 117-122. Pergamon Press, New York. 1960-1961.

Carrier E B and Rehberg P B. Capillary and venous blood pressure in man. *Scand. Arch. Physiol.* 1923; 44:20-31.

Cochrane T and Sherriff SB. Stimulus response measurement of skin blood flow by laser Doppler flowmetry. Does it provide useful information? In: *Practical aspects of skin blood flow measurement*. Eds: Spence VA and Sheldon. Ed Biological Engineering Society, PP. 1985; 36-41.

Crismon JM and Fuhrman FA. Studies on gangrene following cold injury. The use of fluorescein as an indicator of local blood flow, distribution of fluorescein in body fluids after intravenous injection. *J Clin Invest.* 1947; 26:259-267.

Danzer CS and Hooker DR. Determination of capillary blood pressure in man with the microcapillary tonometer. *Am J Physiol.* 1920; 52:136-137.

Dimoni M, Nishimoto S, Matsumoto S, Hatanaka H, Ishikawa K and Baba S. Evaluation of perflax blood flow measurement in diabetic patients with autonomic neuropathy. *Diabetes Res and Clin Pract.* 1985; 1:81-85.

Dingwall J A, Lord I W. The fluorescein test in the management of the tubed pedicle flaps. *Bull John Hopkins Hosp.* 1943; 73:129-131.

Dingwall I A, Lord I W: The fluorescein test in the management of tubed pedicle flaps. I-Iynes W: The blood vessels in skin tubes and flaps. *Br Arch Intern Med* 74:17.5, 1944. 12.

Dollery CT, Hodge JU and Engel M. Studies of the retinal circulation with fluorescein. *Br Med J.* 1962; 10:1201-1215.

Dowd GSE, Linge K and Bentley G. Measurement of transcutaneous oxygen pressure in normal and ischemic skin. *The Journal of Bone and Joint Surgery* 1983; 65-B No. 1:79-83.

Ehrlich P. Ueber Provocierte florescenzer-scheinungen am Auge. *Deutsche Med Wchnsehr.* 1882; 8:35-37.

Elbers PW, Ozdemir A, van Iterson M, van Dongen EP, Ince C. Microcirculatory imaging in cardiac anesthesia: ketanserin reduces blood pressure but not perfused capillary density. *J Cardiothorac Vasc Anesth.* 2009 Feb; 23(1):95-101. Epub 2008 Dec 5.

Essex T J H, Byrne P O.A lasser Doppler scanner for imaging blood flow in the skin. *Journal of Biomedical Eng.* 1991; vol 13,May, 189-194.

Fagrell B. The dynamics of skin microcirculation in man. *J cardiovasc Pharm.* 1985;7:S53-S58.

Fagrell B and Östergren J Capillary flow measurements in human skin. *Clinical investigation of the microcirculation* Ed J E Tooke and Smaje (Boston: Martinus Nijhoff). 1987; pp 23-24.

Fagrell B, Fronck A, Intaglietta M. A microscope television system for studying flow velocity in human skin capillaries. *Ann. J. Physiol.* 1977; 233(2): H318-H321.

Fagrell B, Fronck A, Intaglietta M. Capillary blood flow velocity during rest and post-occlusive reactive hyperaemia in skin areas of the toes and lower leg. *Biblthea Anat.* 1977; 16:159-161 (Karger, Basel).

- Fagrell B. Microcirculation of the skin. In the physiology and pharmacology of the microcirculation (ed. Mortillaro N), Vol. 2, 133-180, Academic Press, New York.1983-1984.
- Fagrell B. The dynamics of skin microcirculation in man. *J. Cardiovasc. Pharm.* 1985; 7:S53-S58.
- Feindel W, Yamamoto Y L. and Hodge Ch P. Intracarotid fluorescein angiography. A new method for examination of the epicerebral circulation in man. *Canad. Med. Ass. J.* 1967; 96(1): 1-7.
- Fisher B and Wolmark N.: Limited surgical management for primary breast cancer: A commentary on the NSABP reports. *World.J: Surg.* 1985; 9:682.
- Fisher B, et al Five-years result of a randomised clinical trial comparing total mastectomy and segmental mastectomy with or without radiation in the treatment of breast cancer. *N. Engl. J. Med.* 1985; 312:665-670.
- Franzeck UK, Isenring G, Frey J and Bollinger A. Videodensitometric pattern recognition of Na-fluorescein diffusion in nailfold capillary areas of patients with acrocyanosis, primary vasospastic and secondary Raynaud«s phenomenon. *Int Angiol.* 1983; 2:143-152.
- Goedhart PT., Khalilzada M, Bezemer R, Merza J, and Ince C. Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. *Opt Express.* 2007 Nov 12;15(23):15101-14.
- Gratti J E, La Rossa D D, Silverman D G and Hartford C E. Evaluation of the burn wound with perfusion fluorometry. *J Trauma.* 1983; 23 (3) 202.
- Gush RJ, King TA, Jayson MIV. Aspects of laser light scattering from skin tissue with application to laser Doppler blood flow measurement. *Phys Med Biol.* 1984; 29:1463.
- Guyton A C, Coleman G and Granger J. Circulation: overall regulation. *Ann. Rev. Physiol.* 1972; 34:13.
- Guyton A C. Integrative hemodynamics. In Sodeman W A Jr, and Sodeman W A (Eds.): *Pathologic physiology: mechanisms of disease*, 5th edition, Philadelphia, W B Saunders Company, 1974, p. 149.
- Guyton A C. *Textbook of Medical Physiology.* 7th ed., Saunders, Philadelphia. 1986.
- Haeger K. *Kirurgins Historia.* A B Nordbok, Gothenburg. Sweden. 1988.
- Hassan A, Rayman G, Tooke JE. The effect of indirect heating on the postural control of skin blood flow in the human foot. *Clin Sci* 1986 Jun; 70(6): 577-582.
- Herrlin J, Glasser S and Lange K. New methods for determining the viability of the bowel. *Arch Surg.*1942; 45:785-791.
- Holloway Jr GA, Watkins DW. Laser Doppler measurement of cutaneous blood flow. *J Invest Dermatol.* 1977; 69:306.

Huch R, Lubbers DW and Huch A. Quantitative continuous measurements of partial oxygen pressure on the skin of adults and newborn babies. *Pflugers Archiv.* 1972; 337:185-198.

Huch R, Lubbers DW and Huch A. The transcutaneous measurement of oxygen and carbon dioxide tensions for the determination of arterial blood gas values with control of local perfusion and peripheral perfusion pressure. Payne JP and Hill JW (Eds) *Oxygen measurements in biology and medicine*, Butterworth, 1975.

Hungerer Sven, Nolte Dirk, Elstner Beate & Pröhl Monika, Messmer Konrad. Fiber Optical Spatial Filter Anemometry – Intravital Measurement of Red Blood Flow Velocity (RBCV) in the Microcirculation. (2010). *Artificial Cells, Blood Substitutes, and Biotechnology*, 38: 119-128.

Ince C. Sidestream dark field imaging: an improved technique to observe sublingual microcirculation. *Crit. Care* 9, Suppl. 1, 72 (2005).

Intaglietta M, Silverman N R and Tompkins W. Capillary flow velocity measurements in vivo and in situ by television methods, *Microvasc. Res.* 1975; 10:165-179.

Jakobsen J, Christensen KS. Transcutaneous oxygen tension measurements over the sacrum on various anti-decubitus mattresses. *Dan Med Bull.* 1987; 34, 330-331.

Johansson KL, Ahn H, Lindhagen J, Lundgren O. Tissue penetration and measuring depth of laser Doppler flowmetry in the gastrointestinal in the gastrointestinal application. *Scand J Gastroenterol.* 1987; 2:1081-1088.

Johansson L, Norrby K, Lennquist S. Fluorescein angiography in the evaluation of intestinal intramural haemorrhage after trauma. *Acta Chir Scand.* 1984; 150:57-61.

Johnson P C. The microcirculation and local and humoral control of the circulation. In Guyton A C (ed.): *MTP International Rev. of Science: Physiology*. Baltimore, University Park Press. 1974, Vol. 1, p. 163.

Järpe S. Cortical fluorescein angiography in microsurgery. *Acta Neurol Scand.* 1968; 44:177-182.

Järpe S. Preoperative fluorescein angiography of the cerebral cortex. *Fluorescence photography of the eye.* 1969; 299-305.

Karlberg L, Källskog Ö, Öjteg G and Wolgast M. Renal medullary blood flow studied with the 86 Rb extraction method. *Acta Physiol Scand.* 1982; 115:11-18.

Katsuhisa Waseda, Junya Ako MD, Takao Hasegawa, Yoshihisa Shimada, Fumiaki Ikeno, Toshihiro Ishikawa, Yoshitaka Demura, Kazuyoshi Hatada, Paul G. Yock, Yasuhiro Honda, Peter Fitzgerald J and Masao Takahashi. Intraoperative fluorescence imaging system for on-site assessment of off-pump coronary artery bypass graft. *JACC: Cardiovascular Imaging* Vol 2, issue 5, May 2009, Pages 604-612.

Kety SS. Measurement of regional circulation by the local clearance of radioactive sodium. *Am Heart J.* 1948; 38:321-328.

Kety SS. Quantitative measurement of regional circulation by the clearance of radioactive sodium. *Am J Med Sci.* 1948; 215:352-353.

Klyszcz, T., Jünger, M., Jung, F., Zeintl, H. Assisted video image analysis system for dynamic capillary microscopy. (1997). *Cap Image - a new kind of computer-* Biomed. Tech. Berl. 42(6):168–175.

Kock E. Velocity of blood stream. *Deutsch Arch F Klin Med.*1922; 140:39-66.

Korner P I. Circulatory adaptations in hypoxia. *Physiol. Rev.* 1959; 39:687.

Krogh A, Landis EM and Turner AH. The movement of fluid through the human capillary wall in relation to venous pressure end to the colloid osmotic pressure of the blood. *J Clin Invest.* 1932; 11:63-95.

Kusano Mitsuo, Tajima Yusuke, Yamazaki Kimiyasu, Kato Masanori, Watanabe Makoto, Miwa Mitsuharu. Sentinel Node Mapping Guided by Indocyanine Green Fluorescence Imaging: A New Method for sentinel Node Navigation Surgery in Gastrointestinal Cancer. *Dig Surg* 2008;25:103-108.

Kwiterovich Kris A, Maguire Maureen G, Murphy Robert P, Schachat Andrew P, Bressler Neil M, Bressler Susan B, Fine Stuart L. Frequency of adverse systemic reactions after fluorescein angiography. Results of a prospective study. *Ophthalmology.* 1991; 98: 1139-1142.

Landis E M. Microinjection studies of capillary blood pressure in human skin. *Heart.*1930; 15:209-228.

Landis EM and Gibbon Jr L. The effects of temperature and of tissue pressure on the movement of fluid through the human capillary wall. *J Clin Invest* 1933; 12:105-138.

Lange K, Boyd L. The technique of the fluorescein test to determine the adequacy of circulation in peripheral vascular diseases, the circulation time and capillary permeability. *Bull New York M Coll Flower and Fifth Ave Hosps.*1934: 78-81.

Lange K, Boyd L. The use of fluorescein to determine the adequacy of the circulation. *M Clin North America.* 1942; 26:934-952.

Lange K, Krewer SE. The Dermofluometer, *J. Lab & Clin Med.* 1943; 28:1746-1750.

Lange K, Wollheim E. Die Kreislaufzeit und ihre Beziehung zu anderen Kreislaufsrößen. *Verhandl. D. Deutsch. Gesel.f. Inn Med Kongr.*1931; 43:134-140.

Lassen NA and Holstein P. Use of radioisotopes in assessment of distal blood flow and distal blood pressure in arterial insufficiency. *Surg Clin N Amer.* 1974; 54:39-55.

Lassen, N. and Perl, W. *Tracer kinetic methods in medical physiology.* 76-101, 1979. Raven Press Books Ltd, new York.

Lisca Development AB. Linköping, Sweeden.1994.

Lukkari-Rautiarinen E, Lepäntalo M. Pietilä J. Reproducibility of skin blood flow, perfusion pressure and oxygen tension measurements in advanced lower limb ischaemia. *Eur J Vasc Surg.* 1989; 3:340-350.

Lund F and Fahlgren H. Supraorbital fluorescein angiography. In: *Non-invasive cardiovascular diagnosis II* Ed. E.B. Diethsich. 1980; 119-134. PSG Publishing comp, Littleton, Mass, USA.

- Lund F and Lund S. Dynamic fluorescein angiography. A new method for assessment of skin circulation of the limbs in peripheral arterial disease. In: Arthrogenesis II. Proc. II Int. Symp. On Atherogenesis, thrombogenesis and pyridinolcarbonate treatment. Tokyo, 1972. Eds. T Shimamoto et al, 333-346, 1973. Excerpta Medica. Int. Congr. Amsterdam. Ser No 269.
- Lund F, Lund S and Johansson R. Dynamic fluorescein angiography of the "macro-surface". In circulatory studies of abdominal viscera in the rat. *Biochem Exper Biol.* 1976; 12:193-200.
- Lund F. An atlas of clinical fluorescein angiography in functional evaluation of nutritional blood perfusion and microvascular permeability of the skin, mucous membranes and visceral organs. In: non-invasive methods on cardiovascular haemodynamics, Ed. A. Jageneau. 1981; 357-392 a. Elsevier/Nord, Holland Biomedical Press. The Netherlands.
- Lund F. Clinical fluorescence angiography: Macro, micro and combined techniques. 11th Europ Conf Microcirculation, Garmisch-Partenkirchen 1980. *Bibl Anat.* 1981; 20:656-666. b. Karger, Basel.
- Lund F. Fluorescein angiography of the upper extremities. *Acta Chir Scand, Suppl.* 1976; 456:60-70.
- Lund F. Fluorescein angiography, an overview of technical improvements and recent experiences. In previous and new clinical areas of application. *Biblhca anat, Vol 18:* Basel, Karger. 1979:322-327.
- Lund-Anderssen H and Lassen N. Microvascular permeability in diabetic patients. 11th Europ Conf Microcirculation Garmisch-Partenkirchen 1980. *Bibl. Anat.* 1981; No 20:675-678. Karger, Basel.
- Marfuggi R A and Greenspan M. Reliable intraoperative prediction of intestinal viability using a fluorescent indicator. *Surg Gynecol Obstet.* 1981; 152:33-35.
- Mc Grow J B, Byers Band Shanklin K D: the value of fluorescein in predicting the viability of arterialized flaps. *Plast. Reconstr. Surg.* 1977; 60, 710-19, 1977.
- McKissock PK: Reduction mammoplasty with a vertical dermal flap. *Plast Reconstr Surg.* 1972; 49:245-252.
- Mellander S. Systemic circulation. *Ann. Rev. Physiol.* 1970; 32:313.
- Meyers M B, Cherry G. Use of vital dyes in the evaluation of the blood supply of the colon. *Surg Gynecol Obstet.* 1960; 128:97-102.
- Milstein DM, te Boome LC, Cheung YW, Lindeboom JA, van den Akker HP, Biemond BJ, Ince C. Use of sidestream dark_field (SDF) imaging for assessing the effects of high-dose melphalan and autologous stem cell trans plantation on oral mucosal microcirculation in myeloma patients. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2010 Jan;109(1):91-7.
- Murawa D, Hirche C, Dresel S and Hunerbein M. Sentinel lymph node biopsy in breast cancer guided by indocyanine fluorescence. *British Journal of Surgery* 2009; 96: 1289-1294.

Myers MB. Prediction of skin sloughs at the time of operation with the use of fluorescein dye. *Surg.* 1962; 51:158-162.

Myers B and Donovan W. An evaluation of 8 methods of using fluorescein to predict the viability of skin flaps in the pig. *Plast. Reconstr. Surg.* 1985; 75 245-50.

Niazi Z B M, Essex T J H, Papini R, Scott D, McLean N R and Black M J M, New laser Doppler scanner, a valuable adjunct in burn depth assessment. *Burns.* 1993; Vol. 19 (6), 485-489.

Nakagami, Gojiro; Sari, Yunita; Nagase, Takashi; Iizaka, Shinji MHS; Ohta, Yasunori; Sanada, Hiromi. Evaluation of the Usefulness of Skin Blood Flow Measurements by Laser Speckle Flowgraphy in Pressure-Induced Ischemic Wounds in Rats. *An Plast Surg.* 2010 Mar; 64 (3):351-4.

Newson T P and Rolfe P The use of transcutaneous oxygen tension measurements for the assessment of the cutaneous microcirculation. *Clinical investigation of the microcirculation* ed. J E Tooke and L H Smaje (Boston: Martinus Nijhoff) 1987.

Nilsson GE, Tenland T, and ÖBERG PÅ: A new instrument for continuous measurements of tissue circulation by light bearing spectroscopy. *IEEE Trans Biomed Eng.* 1980; 27:12-19.

Perbeck L, Olsson L, Thulin L. Comparison between fluorescein angiography and fluorescein microscopy. *Micr Endoth Lymph.* 1984; 1:611-628.

Perbeck L. Fluorescein Flowmetry: A blood flow measurement method. Dissertation thesis. Karolinska Institute. Stockholm. Sweden. 1985.

Perbeck L, Lund F, Svensson L and Thulin L. Fluorescein flowmetry: a method for measuring relative capillary blood flow in the intestine. *Clin Physiol.* 1985; 5:281-292.

Perbeck L, Lewis D, Thulin L, Thydèn G. Correlation between fluorescein flowmetry, ¹³³Xenon clearance and electromagnetic flow measurement. A study in the intestine of the pig. *Clin Physiol.* 1985; 5:293-299.

Perbek L, Alveryd A, Määttänen H, Walberg H. Skin circulation in the nipple after reduction mammoplasty by upper and lower glandular resections. *Scand J Plast Reconstr Surg* 1988; 22: 237-240

Perbeck L, Sevastik B and Sonnenfeld T. The transcapillar exchange of sodium fluorescein measured by fluorescein flowmetry in ischaemic limbs. *Clin Physiol.* 1989; 7:95-103.

Perbeck L, Proaño E and Määttänen H. Skin circulation in the nipple after reduction mammoplasty with a bipedicle vertical dermal flap. *Scand J Plastic Reconstr Hand Surg.* 1991; 25:41-45.

Perbeck L, Proaño E, Westerberg L. The circulation in the nipple-areola complex following subcutaneous mastectomy in breast cancer. *Scand J Plastic Reconstr Hand Surg.* 1992; 26:217-221.

Perry M and Granger N. Permeability of intestinal capillaries to small solutes and to plasma proteins. In: *Microcirculation of the alimentary tract.* Eds. A. Koo, SK. Law. & L.H. Smaje. 1983; 179-196. World Scientific Publ Co., Singapore.

- Raabe A, Beck J, Gerlach R, Zimmermann M, Seifert V. Near-infrared indocyanine green video angiography: a new method for intraoperative assessment of vascular flow. *Neurosurgery* 2003;52:132-139
- Rayman G, Hassan A and Tooke JE: Blood flow in the skin of the foot related to posture in diabetes mellitus. *Br Med J*.1986; Jan 11; 292 (6513): 87-90.
- Renkin EM. Transport of potassium 42 from blood to tissue in isolated mammalian skeletal muscles. *Am J Physiol*. 1959; 197:1205-1210.
- Renkin EM. Transcapillary exchange in relation to capillary circulation. *General Physiol. Suppl*. 1968; 52:96-107.
- Richardson D. Relationship between digital artery and nailfold capillary flow velocities in human skin. *Microcirculation* 1982; 2:283-296.
- Ring E F J. Skin temperature measurement. *Bioengin. Skin* 1986; 2 15-30.
- Rolfe P, Burton PJ, Crowe JA et al. Fetal scalp mass spectrometer blood gas transducer. *Medical and Biological Engineering and computing* 1982; 20:375-382.
- Rosen E.S. In: *Fluorescence photography of the eye*. 1969. Butterworth, London.
- Sapirstein LA. Fractionation of the cardiac output of rats with isotopic potassium. *Circ Res*. 1956; 4:689-692.
- Saner H, Boss CH and Mahler F. Demonstration of the pericapillary space after intravenous administration of sodium fluorescein. In: Bollinger A, Partsch H and Wolfe JHN (Eds). *The Initial lymphatics*, Stuttgart and New York, Thieme. 1985:94-97.
- Schatz, Burton, Yannuzzi, and Rabb: *Interpretation of Fundus Fluorescein Angiography*, Mosby Co., Saint Louis. 1978.
- Scheffler A, Rieger H. Topographical evaluation of the skin perfusion patterns in peripheral arterial occlusive disease by means of Computer-assisted fluorescein perfusography. *Eur J Vasc Endovasc Surg*. 1995; 10, 60-68.
- Sejrsen P. Measurement of cutaneous blood flow by freely diffusible radioactive isotopes. *Can Med Bull*. 1972; Suppl 3:1-38.
- Shepherd A P, Granger H J, Smith E and Guyton A C. Local control of tissue oxygen delivery and its contributions to the regulation of cardiac output. *Amer. J. Physiol*. 1973;225:747.
- Singer E R, Lewis C M, Franklin JD and Linch J R. Fluorescein test for prediction of flap viability in breast reconstructions. *Plast. Reconstr. Surg*. 1978; 61. 371.
- Spence VA, McCollun PT and Walker WF. Quantitative measurement of cutaneous blood flow using radioactive tracers. In: *Clinical investigation of the microcirculation*. Eds. Tooke J and Smaje LH. Boston: Martin Nijhoff Publishing. 1987:85-101.

Spence VA, McCollum PT, Mc Gregor IW, Sherwin SJ and Walker WF. The effect of the transcutaneous electrode on the variability of dermal oxygen tension changes. *Clinical Physics and Physiological Measurements*. 1985;6 No 2:139-145.

Starling EH: On the absorption of fluids from the connective tissue space. *J Physiol (Lond)*.1896;19:312-326.

Stern MD. In vivo evaluation of the microcirculation by coherent light scattering. *Nature*. 1975;254:56-58.

Stern M D, Lappe D L, Bowen P D, Chimosky J E, Holloway G A, Keiser H R and Bowman R C. Continuous measurement of tissue blood flow by laser Doppler spectroscopy. *Am. J. Physiol*. 1977;232(4):441-448.

Strömbeck JO. Mammoplasty. Report of a new technique based on the two-pedicle procedure. *Br J Plast Surg*. 1960;13:79-95.

Svensson H and Svedman P. Laser Doppler flow values during completely obstructed flow by occlusion. *Int. J. Microcirc: Clin. Exp. proceeding Int. Conf. Eur. Soc. Microcirc. Maastricht*. 1980, s. 90.

Svensson H, Svedman P, Holmberg J et al. Detecting changes of arterial and venous blood flow in flaps. *Ann. Plast. Surg*. 1985;15:35-40.

Svensson H, Svedman P, Holmberg J et al. Laser Doppler flowmetry and transcutaneously measured carbon dioxide tension for observing changes of skin blood flow in fingers. *Scand. J. Plast. Reconstr. Surg*. 1983;17:183-186.

Swain I D and Grant L J. Methods of measuring skin blood flow. *Phys. Med. Biol*. 1989; Vol. 34, N0 2, 151-175.

Tamaru T, Öberg Å. Studies of scattering volume geometry in laser Doppler flowmetry. *Innov Tech Biol Med*.1988;9:370.

Tenland T, Salerud EG, Nilsson GE and Öberg PA. Spatial and temporal variations in human skin blood flow. *Int J Microcirc: Clin Exp*. 1983;2:81-90.

Tooke J E and Rayman G. The investigation of foot skin microcirculation in diabetes mellitus. In: *Practical aspects of skin blood flow measurements*. eds: Spenceva, Sheldon CD, Biological Engineering Society 1985.

Tooke J E, Smaje L H. *Clinical investigation of the microcirculation*. Boston. Martinus Nijhoff Publishing.1987.

Tooke JE, Östergren J and Fagrell B. Synchronous assessment of human skin microcirculation. *Int J Microcirc: Clin Exp*. 1983;2:277-284.

Turetta F, Stefani R de, Milanese A, Cannizaro A, Padoan A M, Giorgio P de. Variation du calcium ionise plasmathique av cour de l'angiographie en fluorescence. *J. Fr. Ophtalmol*. 1985;8,12,785-788.

Tymk K, Ellis C G. Evaluation of the flying spot technique as a television method for measuring red cell velocity in microvessels. *Int. J. Microcirc.: Clin. Exp*. 1982;1:145-155.

Veronesi V, Zucali R and Delvecchio M. Conservative treatment of breast cancer with the Q.U.A.R.T. technique. *World J. Surg.* 1985; 9:676.

Vogt PR, Bauer EP, Graves K. Novadaq Spy Intraoperative Imaging System.current status. *Thorac Cardiovasc Surg* 2003;51:49-51.

Wallin L, Lund F, Westling H. Fluorescein angiography and distal arterial pressure in patients with arterial disease of the legs. *Clin Physiol.* 1989; 9,467-480.

Watson A P, Rosen E S. Oral fluorescein angiography: reassessment of its relative safety and evaluation of optimum conditions with the use of capsules. *British Journal of Ophthalmology.* 1990; 74, 458-461.

Wessing A. In: *Fluoreszenz-angiographic der Retina. Lehrbuch und Atlas.* 1968. George Thieme Verlag, Stuttgart.

Wårdell K, Jakobsson A and Nilsson G E. Laser Doppler Imaging of tissue perfusion. *Int. J. Microcirc. Clin. Exp.* 1990;9:72.

Yanuzzi Lawrence A, Rohrer Kathleen T, Tindel Lori J, Sobel Russell S, Constanza Marcelle A, Shields William, Zang Edith. Fluorescein Angiography complications survey. *Ophthalmology.* 1986; 93: 611-617.

Young CMA and Hopewell JW. The isotope clearance technique for measuring skin blood flow. *Br J Plast Surg.*1983;36:222-230.

Zeintl, H., Sack, F. U., Intaglietta, M., Messmer, K. (1989). Leukocyte adhesion measurement in intravital microscopy. *Computer assisted Int. J. Microcirc. Clin. Exp.* 8: 293–302.

Zimmer J G and Demis D J. The study of the Physiology and Pharmacology of the human cutaneous microcirculation by capillary microscopy and television cinematography. *Angiology* 1964;15:232-235.

Zografos L. Enquete internationale sur l'incidence des accidents graves ou fatals pouvant survenir lors d'une angiographie fluoresceinique. *J. Fr. Ophtalmol.* 1983; 6, 5, 495-506.

Östergren J and Fagrell B. Skin capillary blood cell velocity in man. *Int. J. Microcirc.: Clin. Exp.* 1986;5(1):37-51.