Assessment of Microcirculation of an Axial Skin Flap Using Indocyanine Green Fluorescence Angiography

Serdar Eren, M.D., Albert Rübben, M.D., Rainer Krein, M.D., Grahame Larkin, M.D., and Rolf Hettich, M.D.

Cologne, Germany

In many cases the complexities of skin-flap microcirculation are difficult to assess despite all the subjective and objective examination techniques available today. Adequate microcirculation is essential for tissue viability, so any method employed for studying microcirculation should provide as accurate an assessment of the prevailing conditions as possible. Of all the clinical methods, the fluorescence technique using the dye sodium fluorescein has so far provided the most reliable results. However, the pharmacokinetic properties of this tracer have prevented the technique from becoming established in clinical practice. The fluorescent dye indocyanine green (Cardio Green), on the other hand, has far more favorable pharmacokinetics.

In an experimental animal model, the fluorescence technique using indocyanine green (indocyanine green angiography, ICGA) was used to study postoperative changes in the microcirculation of a skin flap. On the day of operation, indocyanine green angiography revealed a state of hemodynamic imbalance for which the organism was able to compensate in the postoperative phase with the aid of humoral, physical, and metabolic factors. With indocyanine green angiography it was possible to quantify objectively the new hemodynamic equilibrium. Basically, microcirculation may be quantified in temporal and spatial terms. The significant objectivity of indocyanine green angiography and short intervals between each examination favor its possible and meaningful use in clinical practice and give cause for continuing studies. (*Plast. Reconstr. Surg.* 96: 1636, 1995.)

The microcirculation of the skin is a very complex chain of events which, even under physiologic conditions, is characterized by its heterogeneous behavior. Of the total blood flow through the skin, functional circulation plays a greater role than nutritive circulation. The heterogeneity of resting perfusion is, on the one hand, dependent on anatomic factors and, on the other, adapts itself to local conditions and requirements. Adequate microcirculation is, however, essential for tissue viability and functionality. Autoregulatory mechanisms over time and space control the basic demands of the skin. These mechanisms include first and foremost the neural regulation of the vascular system. In addition, humoral, metabolic, and physical factors exert their influence on the microcirculation.

Similarly, adequate microcirculation is essential for the viability of a skin flap. With regard to the physiologic conditions of skin perfusion, even the dissection of the flap has a significant effect on its microcirculation, as well as on its autoregulatory mechanisms, leading to a hemodynamic imbalance in its circulatory system. The organism tries to compensate for this by humoral, metabolic, and physical mechanisms. The otherwise dominant neural regulation of the microcirculation is disrupted by flap dissection. If the organism is not able to restore an adequate microcirculation, then necrosis of the skin flap will occur. Skin perfusion is complex even under physiologic conditions; with a skin flap, one is confronted with even more difficulty when assessing microcirculation.

McCarthy[1] describes various subjective and objective methods of examination to assess microcirculation, the most common being the clinical examination. Inspection of skin color and capillary blanching on pressure gives clues to the current state of microcirculation. Monitoring skin temperature also can be very helpful. Stabbing with a cannula or a no. 11 scalpel

From the Clinic of Plastic and Hand Surgery at St. Agatha Hospital and the Clinic for Burns and Plastic and Reconstructive Surgery and the Dermatological Clinic at the RWTH Aachen. Received for publication October 26, 1993.

blade to induce capillary bleeding is another effective test for judging flap circulation. Interpretation of these examination results, however, requires a sufficient degree of clinical experience. On the whole, these tests are easy to apply yet must be considered unreliable.

These purely subjective tests are contrasted by objective procedures for assessing microcirculation that are employed in clinical practice and research with varying degrees of success and significance. These methods include measuring transcutaneous P_{O_2} (tcP_{O_2}), vital capillaroscopy, photoplethysmography, laser Doppler flowmetry, thermography, isotope clearance, dermofluorometry and dermofluorography, and measurements using radioactively labeled corpuscular blood components.

Measuring tcP_{O_2}, a selective technique, has so far not produced the expected results with regard to the complexities of skin circulation, and because of its susceptibility to interference, it is now rarely used. Vital capillaroscopy is an excellent method for recording local mechanisms of microcirculation (flow distribution, erythrocyte flow rate, capillary diameter, etc.). Furthermore, qualities of transcapillary and interstitial diffusion can be determined by injecting a fluorescent dye.[2,3] Despite modifications of the method, photoplethysmography[4] has so far not produced unequivocal results in the assessment of skin-flap circulation, so this technique is now seldom used. Great expectations were placed on the laser Doppler flowmeter. As a noninvasive method, it is simple and can be repeated several times in succession. Serial monitoring allows registration of local autoregulatory fluctuations of skin perfusion. Plastic surgery in particular can look back on a number of interesting studies.[5–9] The disadvantages of this procedure are its susceptibility to artifacts due to movement and its inability to provide an overall picture of the microcirculation. Extensive data are provided by thermography, which, despite its good approach, however, is not particularly popular. The most exact study results have so far been provided by the clearance method using Xe, H, and Te and by the process using radioactively labeled corpuscular blood components.[10,11] But for reasons of time and cost, these tests are used principally in research.

The fluorescence technique currently provides the most accurate information on the microcirculatory state of skin. It was first used by Lange and Boyd[12] to study circulation time, capillary permeability, and tissue circulation in peripheral vascular diseases. They used the dye sodium fluorescein to gain an impression of the conditions of microcirculation from the intensity, rate, and homogeneity of the appearance of fluorescence in the tissue.

Sodium fluorescein is a water-soluble fluorescent dye with a molecular weight of 376. In plasma and whole blood it displays an absorption maximum at 495 nm and an emission maximum at 515 nm.[13] Approximately 50 percent of systemically applied sodium fluorescein is bound primarily to albumin, as well as to the surface of erythrocyte membranes.[14,15] Fluorescein is freely dissolved in plasma and, as a result of its low molecular weight, can diffuse into the interstitium by a transcapillary route.[2]

It was Myers[16] who in 1962 used sodium fluorescein in operations to predict skin necrosis by differentiating between fluorescent and nonfluorescent areas. By using the dermofluorometer, Silverman et al.[17] quantified the uptake of sodium fluorescein in the skin and correlated it with flap viability. Since then, various studies have shown good correlations of the fluorescence technique with blood flow and the viability of tissues.[18–26]

Although dermofluorometry is commonly used by various authors, it only allows a punctate registration of tissue fluorescence. This is why Lund employed a rapid-sequence photographic recording of dye distribution to gain a spatial analysis of skin perfusion.[27,28] This technique was modified further by Rieger and Scheffler[29] using a digital image processing system. However, the pharmacokinetic properties of sodium fluorescein have until now prevented this method from becoming further established in clinical practice. Its routine use for assessing blood circulation of skin or a skin flap was unsuccessful, not least because of the long intervals (7 to 8 hours) between examinations.

The vital dye indocyanine green (ICG) was first introduced into clinical medicine by Fox et al.[30,31] in 1957. Indocyanine green is used primarily in hepatology as a liver function test[32,33] and in cardiologic diagnostics.[34–37] In 1973, Flower and Hochheimer[38,39] introduced it into the fluorescence technique. They used the dye for fluorescence angiography of the choroidea. Indocyanine green has a molecular weight of 775 and is almost completely bound to plasma proteins following intravenous application, with alpha$_1$-lipoproteins and albumin being the principal binding partners.[40,41] Tight binding to plasma proteins guarantees that the fluorescent

dye will remain intravasal. During its elimination from the blood, indocyanine green undergoes biphasic plasma clearance. In the initial phase, $t_{1/2}$ amounts to 3 to 4 minutes at a dose of 0.5 mg/kg of body weight, with a $t_{1/2}$ of 66 minutes in the second phase.[42] More than 90 percent of the applied dye is eliminated in the first phase. These pharmacokinetic properties offer a considerable advantage over the behavior of the dye used hitherto. Further studies on the choroidea,[43] as well as those using fluorescence videomicroscopy,[3,44] also have produced good results with indocyanine green, so it would seem reasonable to use this dye in a broader model using skin or a skin flap. The rapid elimination of the dye also makes the possibility of a time-oriented analysis of skin perfusion more likely. The present animal study provides data on the microcirculatory state of an axial-pattern flap by means of a computer-assisted analysis of the influx and efflux dynamics of the dye and its fluorescent intensity.

Materials and Methods

The Test

A total of seven Sprague-Dawley rats (weighing approximately 300 gm) (Central Laboratory for Experimental Animal Studies, Aachen, Germany) were anesthetized with a subcutaneous injection of 0.4 ml Hypnorm (Jansen GmbH, Neuss, Germany). After complete removal of abdominal hair, axial-pattern skin flaps, modified according to a model by Vidas et al.,[45] were raised. Measuring 2×8 cm^2, they were based on the left inferior epigastric neurovascular bundle (Fig. 1). Before returning the flaps to their wound beds, collagen sheets were sutured in to delay revascularization from the wound bed and its edges. The right femoral vein was punctured for injection of indocyanine green. An in vitro study demonstrated maximum fluorescence at plasma dye concentrations that indicated a dose of 0.5 mg/kg of body weight. The animals were divided into two groups. Group I was studied on the day of operation and on the first and third postoperative days. The operation day and days 2 and 4 were selected for studying group II. On the seventh postoperative day, the viability of the skin flap was assessed clinically in all the study animals. Room temperature was maintained at 25°C during the entire study period, and standardized conditions (focusing of the camera, distance of the object from the camera or the light source, etc.) were strictly adhered to.

Indocyanine Green Angiography

For indocyanine green angiography, a 2000-W halogen lamp was used to excite the dye (Strand Lighting GmbH, Wolfenbüttel, Germany). Because of the considerable development of heat, a water filter was included to protect the excitation filter 750-FS 40 (LOT, Darmstadt, Germany). A long-pass filter RG 850 (LOT, Germany) was used as a barrier filter. The emission of the excited fluorescent dye was then recorded with a Sanyo CCD camera, a videotimer (VTG-33, FOR A), a Sony U-Matic videorecorder, and a high-resolution monitor. The signal was entered into a digital image processing system, where it was further evaluated. The test construction is depicted in Figure 2.

Digital Image Processing and Evaluation of Indocyanine Green Angiography

The pictures received from indocyanine green angiography were digitalized by a 768×512 pixel, 8-bit image processor (VP 1100-768-E-AT; OFG). A series of images was stored at a rate of two per second during the first 25 seconds. Subsequent rates were slower as a result of computer capacity. Processing of the digitalized images was then undertaken with a Software Bisc Optimas (Stemmer, Puchheim, Germany). Ten different regions of interest were defined on the skin flap, and a mean fluorescent intensity was measured over time (see Fig. 1). The regions of interest were numbered 1 to 10 from proximal to distal to facilitate evaluation. The evaluation of indocyanine green angiography was then performed with a model that took into account influx and efflux as well as lag time, which allowed for the dye to spread from the injection site to the region of interest. The formula for the time course of the mean intensity $f(t)$ is as follows:

$$f(t) = f_{max}(1 - e^{-t'/C_{inf}})\, e^{-t'/C_{eff}}$$

where

f_{max} = maximum intensity
t' = $t - t_{lag}$
t_{lag} = influx lag time
C_{inf} = influx time constant
C_{eff} = efflux time constant

To determine the time constants, f_{max} was set at $f_{max} - \max[f(t)]$ under the assumption that

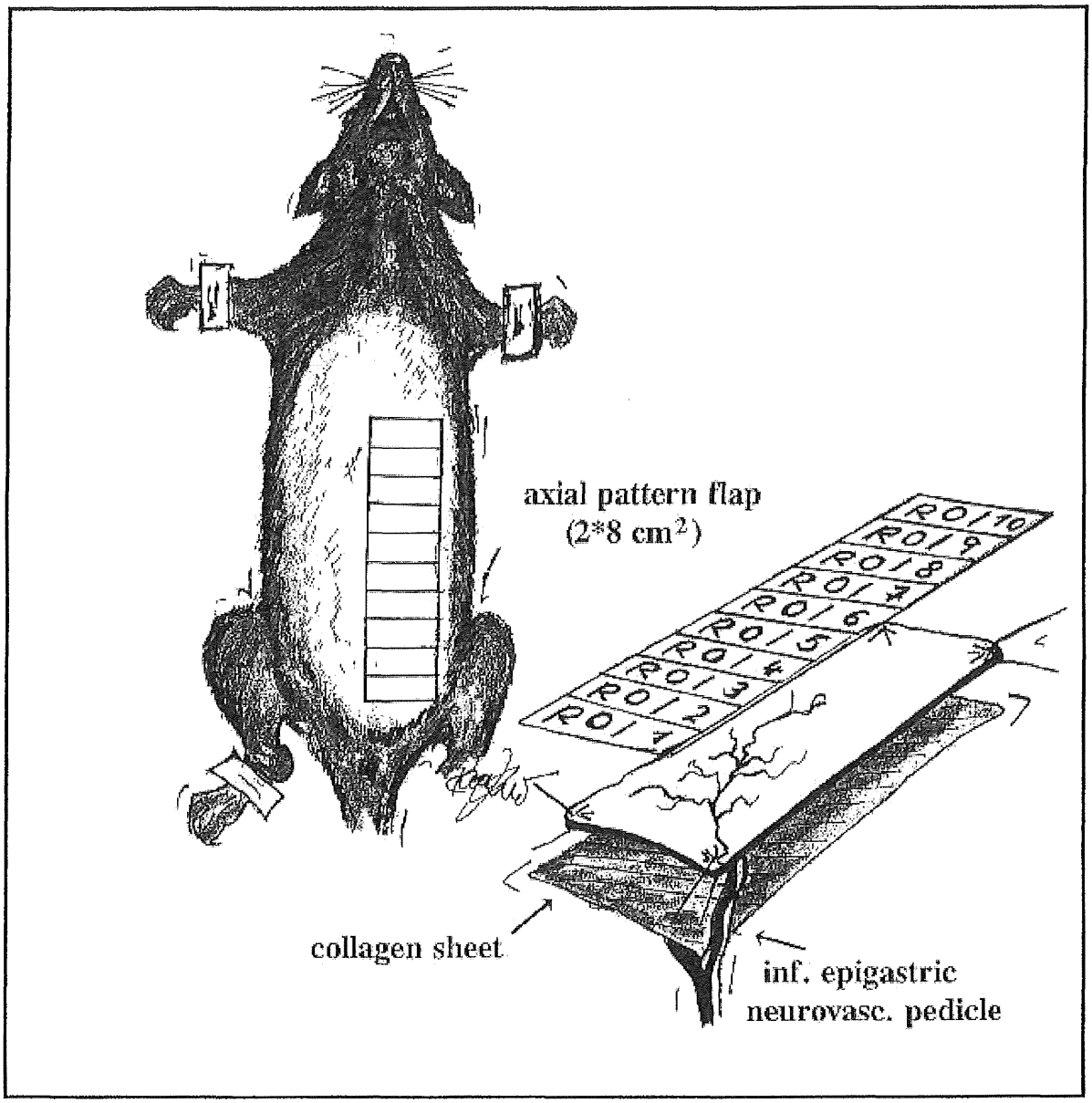

FIG. 1. A 300-gm Sprague-Dawley rat with a skin flap (2 × 8 cm²) based on the left inferior epigastric artery, vein, and nerve. Beneath the flap is a synthetic collagenous sheet.

C_{eff} is considerably larger than C_{inf}, that is, $C_{eff} \gg C_{inf}$. Calculation of the influx constant C_{inf} results from

$$g(t) = \log[f(t) - f_{max}] \qquad t < t_{max}$$

After finding the logarithms of the data up to the peak of the curve at $t = t_{max}$, a straight line is drawn by linear regression. The slope of the straight line provides the influx time constant. The efflux time constant is calculated in a similar fashion. In this case, however, f_{max} is not subtracted because these data follow the peak. The time constants thus calculated (C_{inf}, C_{eff}) and the maximum intensity (f_{max}) then allow conclusions to be drawn on the state of blood circulation. The quality of the calculated data was confirmed by determining the correlation coefficients.

The Dye: Indocyanine Green (Cardio Green)

Indocyanine green is supplied in 25- and 50-mg single packs (Paesel Company, Frank-

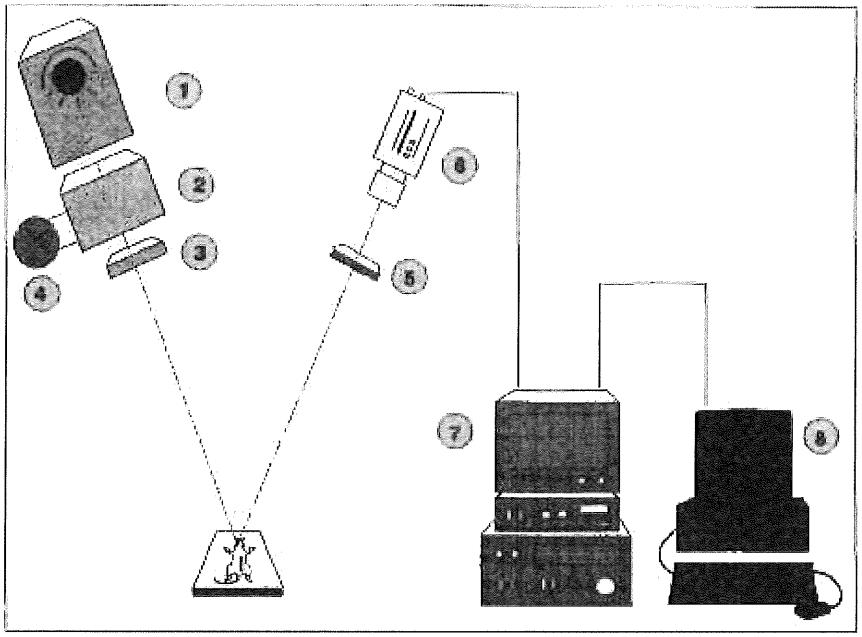

FIG. 2. Indocyanine green angiography. The dye was excited after its intravenous application and distribution in the bloodstream. The emitted light is selected out by the barrier filter and recorded by the CCD camera. The resulting images are stored and processed (*1*, 2000-W halogen lamp; *2*, water filter; *3*, barrier filter; *4*, water pump; *5*, excitation filter; *6*, CCD camera; *7*, videorecorder, videotimer, monitor; *8*, digital image processing system).

furt, Germany). As a finished solution, indocyanine green contains 5.0 to 9.5 percent sodium iodine. For this reason, the manufacturer recommends caution when applying it in patients with iodine allergy or thyroid disease. Similarly, administration during pregnancy or lactation is not guaranteed free of risk. The incidence of untoward reactions to indocyanine green is low. The literature cites a rate of 1 in 42,000.[46] An article by Carski et al.[47] mentions a case number of 4 reactions in over 240,000 applications. These reactions usually take a mild and harmless course with symptoms such as nausea, hot flushes, headache, and urticaria. On the other hand, exceptional cases also have been described with more severe side effects such as dyspnea, edema, fall in blood pressure, and tachycardia.[46–48] Michie et al.[49] report an increased incidence of reactions to indocyanine green in patients with chronic uremia. On the whole, side effects from indocyanine green are not considered genuinely allergic but rather a pseudoallergic reaction.[50,51] The lack of eosinophilia and no significant IgE increase support this view.[50] When using the fluorescent dye indocyanine green in clinical practice, however, the possibility of a reaction should be kept low or even excluded, so preventative measures or a reliable test to initiate prophylaxis deserves consideration. As a rule, atopic individuals and patients belonging to these groups (those with hay fever, allergic eczema, etc.) are considered at risk. A positive intracutaneous test provides reliable results, while a negative result would not exclude a later reaction. The intravenous injection of a small test dose would be the safest method of excluding sensitization, although it could interfere with the subsequent examination.

Before the examination, an antihistamine or a cortisone preparation could be applied as a direct form of prophylaxis. An important prerequisite for using this dye for fluorescence is an exact knowledge of its spectral properties. In serum, indocyanine green displays an absorption maximum at 805 nm and an emission maximum at 835 nm.

RESULTS

After intravenous injection of the dye, a rapid inflow was observed by means of the afferent vessel of the axial-pattern skin flap. The first fluorescence was registered after 1.20 s (SD = 0.54 s). Comparing the individual test days, vascularity was sparse on the operation day, while the following postoperative days showed clear

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