Prediction of leg viabililty and amputation level by fluorescein uptake

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Abstract
There is a continuous search for more reliable, locally non-invasive tests for prediction of leg viability and for the selection of amputation levels in dysvascular lower limbs. Refinement of the dermofluorometer by addition of a shielded probe and fibreoptic bundles has reduced the bulk of the instrument and permitted rapid testing. Excitation light is carried to the tissues and emitted fluoresence is returned to a photo multiplier. The Index of Fluorescein Uptake is 20–30 units in control areas. Healing is constant above 15 units, inconstant from 5 to 15 units, and absent below 5 units. Reliability has been virtually 10% in preliminary tests.

Introduction
Tissue fluorescence following intravenous injection of sodium fluorescein has been used for many years as a visual indicator of capillary blood flow. It has been used in limbs compromised by peripheral vascular disease and in other tissues whose viability was compromised by acute and chronic ischaemia (Lange and Boyd, 1942, and 1944; Myers, 1962). The dye is distributed throughout the patent vascular system. It diffuses through the capillary walls to equilibrate with the extracellular fluid. Exposure of skin or tissue surfaces to blue or ultraviolet light produces a yellow-green fluorescence. Hypoperfusion is presumed in absence of fluorescence.

Estimation of the degree of fluorescence by the naked eye is not accurate and means of

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measurement were sought in the early 1940's. Lange and Krewer reported the dermofluorometer in 1943. Unfortunately the equipment was cumbersome and was not well accepted as a clinical instrument. Modifications and adaptations of the basic concepts have aided in the production of an instrument that separates the bulk of the equipment from the patient. A relatively small shielded probe is used in a normally lighted room. Sterilization of the probe shield permits use of the instrument during surgery.

The fibreoptic dermofluorometer has been developed by Silverman et al, (1980) and the Johnson Research Foundation. The present perfusion fluorometer is manufactured by Diversatronics, Inc., Broomall, Pennsylvania, U.S.A.

Action of the perfusion dermofluorometer
Incandescent light passes through an excitation filter which selectively transmits wavelengths between 450 and 500 nanometers, within the range of maximal excitation of fluorescein in vivo (Delori et al, 1978). This light passes along fibreoptic pathways to the probe which is held on the tissues being tested. The probe also contains fibreoptic bundles which carry the emitted fluorescence plus reflected excitation light back to the instrument. Both fluorescent emissions and reflected excitation light pass through a selective filter before reaching the photo multiplier tube. This filter passes wavelengths between 530 and 660 nanometers. With such selective transmission there is minimal overlap and a resultant high signal to noise ratio. There are two photo multiplier tubes which allow for simultaneous

measurements of experimental and control areas. The emitted fluorescence is quantified by the photo multiplier tube and is read as DF (dye fluorescence) on an arbitary scale.

Tissue uptake of fluorescein appears to be a multiexponential function which can be empirically converted to a "straight line" by graphing "DF readings vs. log time." The slope of this line provides an index of fluorescein uptake (IFU).

Control areas, e.g., forehead, abdomen, and upper limbs, have indices between 20 and 30 units after intravenous injection of fluorescein at 4 mg/kg. All regions with an index greater than 15 remained viable and were able to heal an amputation when performed at that level. In areas where the IFU was below 5 units healing did not occur. Healing was inconsistent in transitional zones with the IFU between 5 and 15 units.

Preliminary studies at Veterans Administration Medical Center, Philadelphia, Pennsylvania, were conducted on laboratory animals (Wagner 1979). There appeared to be a high correlation with tissue viability and tissue fluorescence. Further studies were performed on 9 human subjects with difficult lower limb problems secondary to decreased circulation. Fluorometric studies were contrasted with clinical findings, skin temperature, pulses, pulse volume recordings, ankle/brachial Doppler pressure measurements, and angiography. Fluorometry was 100% accurate; the other tests combined were accurate in 44% of the cases (Table 1). Treatment was based on the "standard tests".

Approximately 30 cases have been studied in a prospective randomized study designed to run two years. Fluorometry has shown a reliability over 95%. At Rancho Los Amigos Hospital a similar study is in progress. Prediction of healing of leg and foot lesions and selection of amputation level are at a 100% level on the first 11 cases.

A sample protocol outlines the steps for a patient with peripheral vascular disease. The tests are performed in a temperature regulated area.

A. Safety precautions
1. Well running intravenous line.
2. Blood pressure and cardiac monitoring available.
3. Resuscitative personnel and equipment available (highly unlikely to be needed).
4. A physician administers the fluorescein.

B. Grid pattern
1. The anterior aspect of each leg is marked at 5 cm intervals starting at the inguinal ligament. The leg is marked at 2 cm intervals in the areas where amputations are most likely to be performed, e.g. at above-knee, through-knee, below-knee, and Syme levels. In addition, the foot is marked at 2 cm levels on dorsal and plantar surfaces and readings are taken on dorsal and plantar aspects of the toes.
2. Adjustments in the grid pattern can be made before and during the study. Landmarks are recorded on a patient drawing and on the computer program if a computer is used.
3. Control areas are identified on abdomen, forehead, and upper limbs.

C. Pre-injection measurements
1. Temperature reading every third interval.
2. Background readings on both sides of each dot are generally quite low and are subtracted from post-injection readings.

D. Injection of fluorescein
1. Rapid injection through well-running intravenous line.

Table 1. "Standard test" treatment and Fluorometry predictions compared in 9 patients.

	Diagnosis	Treatment based on "standard tests"	Fluorometry prediction	Clinical outcome
1.	Gangrene of toe	Transmetatarsal amp.	Not heal	Not healed
2.	Failed transmet. #1	BK amp.	Heal	Healed
3.	Gangrene of toe	Transmetatarsal amp.	Not heal	Not healed
4.	Failed transmet. #3	BK amp.	Heal	Healed
5.	Infected foot	Medical prescription	Fail	Failed
6.	Extension of foot infection #5	BK amp.	Heal	Healed
7.	Infected AK amp.	Possible disarticulation	Surgery not necessary	Healed
8.	Foot ulcer	Medical prescription	Heal	Healed
9.	Ulcer of BK amp.	Medical prescription	Heal	Healed

2. At 45 seconds measurement is started-probe is moved to all points (1 second/point).
3. Points are measured sequentially for 20 minutes.
4. A computer aids markedly in assembling data. The reading and time for each data point are recorded, the time course of fluorometer readings are graphed and analysed for each point, and at 20 minutes ratios between the test sites and control areas can be provided. Areas showing critical changes can be further subdivided, further readings and ratios obtained.

E. Post examination
1. Patient observed for one hour post-injection.
2. The patient and those caring for him will be instructed in possible changes in skin and urine colour.

F. Statistical analysis
1. Patients will be randomly assigned to control or treatment groups. In the control group the surgeon will not be shown the perfusion results. Amputation will be carried out at the level determined by the hospitals "standard" means of assessment (Wagner, 1979).

   In the treatment group amputation will be performed at the level determined by the test showing the greatest amount of limb salvage. Successes and failures will be analysed by standard statistical methods.

G. Risks
Most articles report virtually no complications. However, a recent review of the literature reports two cardiac arrests and three fatal reactions (Buchanan and Levine, 1982). The same authors report a study on 38 flap procedures in 29 patients over a ten year period. An immediate blood pressure drop of more than 20 mmHg was noted on nine occasions. Pressure returned to base line with supportive measures in six patients, but ephedrine was felt to be necessary on three occasions.

The only side effect seen so far in our patients has been mild nausea.

Summary
Tissue fluorescence has been used to aid in determining nutritive blood flow. Quantification of fluorescence by the naked eye has not been accurate. Previous instruments to measure fluorescein dynamics were not acceptable for clinical use due to cumbersome size.

A new fibreoptic perfusion fluorometer has been developed that contains the basic elements of earlier instruments but removes the bulky components from the patient. The probe is placed on the area to be examined after fluorescein injection and a reading is obtained in one second.

Preliminary studies in animals and in humans show that ultimate viability of tissue can be predicted and amputation level selected with great reliability.

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