
Fluorescence Microlymphography

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SUMMARY Microneedles, 0.2 mm o.d., were connected to a microsyringe and mounted on a micro-manipulator. Under microscopic control, 0.01 ml of a 25% solution of FITC-labeled dextran-40 or dextran-150 were injected into the subepidermis at the big toe near the nailfold or in the medial ankle region. Fluorescence intravital microscopy revealed a network of lymphatic microvessels. The comparison with recent anatomic studies reveals that the reticular network visualized by FITC-dextran corresponds to the network in the stratum papillare. In 20 healthy subjects lymphatic capillaries were detected in a restricted area on the lateral aspect of the big toe. In 10 patients with primary lymphedema, the dye expanded to almost the entire dorsal skin surface of the big toe. In two cases, enlarged and tortuous microvessels of pathologic shape were observed.

Fluorescence microlymphography is a simple and nearly atraumatic approach for depicting the intravital anatomy of human skin lymphatic capillaries.

THE ANATOMY of human skin capillaries has been studied by noninvasive intravital microscopy. The excellent light absorption properties of red blood cells and the presence of plasma gaps between them allow one to measure the flow speed if videomicroscopy systems are used. Unlike blood capillaries, lymphatic microvessels are not visible with conventional intravital microscopy. After local injections of dyes or contrast media, however, lymphatics may be visualized. Different techniques were applied in experimental animals, where it became possible to evaluate prelymphatic pathways.

In man, the interdigital injection of patent blue delineates the lymphatic vessels of the foot to be cannulated for lymphography with contrast media. Macroscopic images of larger trunks have been obtained in various clinical conditions. However, patent blue and other nonfluorescent dyes are not suited to depict lymphatic capillaries because they provide insufficient contrast (personal observation).

In the present report we introduce a method of studying lymphatic microvessels in human skin previously not accessible to visualization in vivo. The fluorescence video microscopy system used was developed to measure transcapillary diffusion and inter-
stitial clearance of i.v. Na-fluorescein. For microlymphography, FITC-labeled dextran is injected in minute amounts into the subepidermal layer.

**Materials and Methods**

The apparatus is similar to that described for measurements of diffusion, pericapillary distribution and clearance of Na-fluorescein. The skin section is placed on a specially equipped stage of an incident light fluorescence microscope (SM-Lux, Leitz). For transcutaneous intravital observation the skin is made transparent by a drop of paraffin oil. Plan fluorar objectives 10/0.3 and 3/0.1 were used. With the first, the final magnification on the television screen reaches 512 X, with the second 170 X. Light is provided by a mercury vapor lamp (HBO 100 W, Osram) connected to a DC power supply (E, XHSP/L, Irem). It passes the fluorescence excitation filter (450-490 nm) and is directed to the skin. The emitted fluorescent light goes through a K 515 barrier filter.

Images were recorded with a cadmium-selenide camera (Siemens) that provides a sensitivity comparable with that of a multidiode vidicon, and were stored on videotape (Grundig BK 204). Single frames were selected for photography on the monitor by replay of the tape.

Steel microneedles with an external diameter of 200 μm (fig. 1) were adapted to a Hamilton microsyringe (W. Niederhauser, H. Schneider and Co.). The microsyringe is fixed on a micromanipulator (Leitz) (fig. 2). The syringes are filled with a sterilized solution containing 25% FITC-labeled dextran (fluoresceinylthiocarbamoyl-dextran) with a mean molecular weight of 40,000 or 150,000 (Pharmacia). Minute standard amounts of the dye (0.01 ml) are injected into the subepidermal layer (fig. 3), which contains the superficial capillary loops.

The subjects were sitting comfortably on an elevated chair so that they could put their foot on the stage of the microscope. The knee joint was positioned at a right angle and the foot fixed by a plastic mass. The room temperature was 22-24°C.

The injection site was the area just proximal to the nailfold of the big toe. The needle was carefully advanced with a micromanipulator under microscopic control. The fluorescence filters were set by a switch mechanism and the injection was performed (fig. 3).

At the big toe, five main observation sites were defined (fig. 4). The presence of the fluorescent dye at these locations was followed for 1 hour. The second site of injection was the medial ankle region with the subjects resting in supine position. Again, the dye deposit and its surroundings were observed for 1 hour.

FITC-labeled dextrans were well tolerated when injected into the subepidermal layer; there were no adverse reactions. The 0.01-ml dosage is sufficient to visualize the deposit in the subepidermal interstitium (fig. 3) and the microvessels arising from it (figs. 5-7). The acceptable contrast was enhanced by the use of FITC-dextran-150 instead of FITC-dextran-40.

**Subjects and Patients**

Twenty healthy volunteers, 10 males and 10 females, were examined at the big toe using fluorescent...
dextran-40. The mean age of the volunteers was 29 years (range 21-57 years). Ten patients with primary lymphedema, nine females and one male (14 legs) were also studied. The mean age of these patients was 41 years (range 28-57 years). Eight patients received FITC-dextran-40 and two received dextran-150. In four other normal subjects, dextran-150 was injected into the medial ankle region.

In the patients with lymphedema, the first symptoms appeared before age 45 years, in six before age 35 years. The family history was negative. Edema was localized in the lower leg and foot. Seven patients had unilateral involvement (right leg in two and left leg five) and three had bilateral involvement. Patients with elephantiasic deformities were not studied. Care was taken to rule out deep venous thrombosis and its sequelae (clinical findings and exploration with Doppler ultrasound). In five patients, conventional lymphography was performed on both legs. The examination revealed in each case aplasia or hypoplasia of the lymphatic vessels in the calf and thigh region. In these patients and in the other five, a patent blue test was performed. Extensive spreading of the dye over the dorsum of the foot or even on the lower leg indicated lymphedema.

**Figure 4.** Points of observation for fluorescence video microscopy at the big toe.

**Figure 5.** Reticular network of lymphatic microvessels arising from the original dye deposit (big toe of a healthy 24-year-old woman). The black points within the meshes of the network represent culminating capillary loops.

**Figure 6.** (A) Network of lymphatic microvessels arising from a dye pool in the medial ankle region in a 29-year-old woman. (B) Lymphatic microvessels of the ankle region at higher magnification in a 28-year-old man. The blurring of the vessels suggests a movement of dye from the intravascular to the extravascular compartment.
Results

Normal Subjects

After subepidermal injection just proximal to the nailfold of the big toe, the dye deposit extends to about 1-2 mm². In this field, bright fluorescence is detected (fig. 3). Fluorescent streamers arise from the original pool, forming a reticul network (fig. 5). The meshes of the network enclose two to six blood capillaries, which emerge in the dermal papillae from below. Not all the meshes contain the same amount of dye. There seem to be preferential pathways (fig. 7).

The diameter of the microvessels containing the fluorescent tracer is difficult to measure because of blurring of the walls. However, the diameter of these microvessels is greater than the diameter of the blood capillary loops.

Especially near the original deposit, the dye accumulates not only in the reticular network, but also in the pericapillary halo-like structures. Even the interstitial space between the meshes contains fluorescent material.

The extension of the dye expanding from the deposit is limited to the lateral aspect of the big toe (fig. 8). Only point 1 (fig. 4), just proximal to the original pool, is always reached by the fluorescent compound. At point 2, on the lateral side of the interphalangeal joint, microvessels were filled in 17 out of 20 cases. Two to 5 minutes were needed to reach the maximal superficial dye expansion. At point 3 (fig. 4), FITC-dextran appeared in only one instance. Points 4 and 5, at the base of the big toe, remained free of dye, even 30 and 60 minutes after the injection.

In the four cases in whom FITC-dextran-150 was injected into the malleolar region, the reticular network filled from the deposit is composed of larger meshes than those at the big toe (fig. 6). The interstitial space in the neighborhood of the microvessels emits fluorescent light of lower intensity than the streamers themselves. Some amount of the dye seems to move out of the microvessels.

Patients with Lymphedema

Starting from the deposit at the big toe nailfold, the dye spreads rapidly in direction of the proximal part of the big toe. Identical networks of microvessels as visualized in the healthy controls are filled by FITC-dextran-40 and dextran-150 (fig. 7). The contrast of the images was even better than that in normal subjects.

In eight of 10 patients, the extension of the dye was not limited to the lateral aspect of the big toe. The medial and central parts of the skin overlying the interphalangeal joint (point 3; fig. 4) were reached. Furthermore, the dye was observed over the base of the big toe in eight of 10 patients (points 4 and 5). The increased extension of the dye characteristic for lymphedema is illustrated in figure 8.

Two patients showed enlarged and tortuous microvessels. Once they were localized on the lateral aspect of the big toe and once at the base (fig. 9). In none of the normal control subjects were similar vessels found.

Discussion

Microvessels forming a network are visualized by fluorescence intravital microscopy after subepidermal injection of 0.01 ml of FITC-labeled dextran with a molecular weight of 40,000 or 150,000 (figs. 5-7).
animal preparations.\textsuperscript{18, 20} In man, these fluorescent compounds cannot be applied by the intravascular route because human toxicity studies are not available. In very small amounts (0.01 ml), however, FITC-dextran-40 and dextran-150 are well tolerated when injected into the subepidermal layer.

Our results are preliminary. However, two features emerged. First, the spreading of the dye from the original pool exceeds the restricted propagation characteristic for normal subjects (fig. 8). In patients with lymphedema, this increased extension of a standard amount of dye is known after intradigital injection of patent blue, which is used to mark the larger superficial lymphatic trunks for conventional lymphography.\textsuperscript{12, 13} Second, in two cases, enlarged and tortuous lymphatic microvessels that appeared to be definitely pathologic were filled by FITC-labeled dextran (fig. 9).

Fluorescence microlymphography is a means of studying the intravital anatomy of cutaneous lymphatic microvessels in clinical medicine and supplements conventional lymphography with contrast media, which depicts the large lymphatic trunks. In primary lymphedema, hypoplasia or aplasia of large lymphatics is associated with a well-developed microvascular network.

The diagnostic value of the method must be established, especially by comparison with patent blue test and conventional lymphography. Independent of diagnostic applications, the method might contribute to a better understanding of edema formation in different clinical conditions. The lymphatic clearance of interstitial fluid and proteins plays an important role for the fluid balance.\textsuperscript{81}

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