Fluorescein Angiography in Microvascular Surgery: A Study Using the Rodent Artery

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Abstract:
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- Fluorescein angiography was carried out on 45 Ostpon Mendelson rats with a view toward detecting injury areas following microvascular anastomoses. Decreased fluorescence was seen regardless if the injury occurred prior to or after the injection of fluorescein, and also if postinjury angiography was filmed during direct arterial injection (mainly fluorescence from the blood stream) or one to five minutes later (mainly vessel wall fluorescence).

- The effects of injury paralleled the effects of positive direct current applied to the fluorescent vessel. Focal absence of fluorescence also occurred at sites of clotting or intraluminal adventitia deposition at anastomotic sites in spite of good vessel wall pulsations.

- The procedure may prove to be worthwhile and preferable to risky contrast x-ray angiography during intra-operative transcranial microvascular anastomoses for cerebrovascular insufficiency.

Additional Key Words
vascular injury direct current

Introduction
- At the end of the last decade microneurosurgical arterial reconstruction was initiated. At a recent symposium on microneurosurgical anastomoses for cerebral ischemia it was calculated that over 300 anastomoses between the superficial temporal artery and the middle cerebral artery had been performed for cerebrovascular insufficiency. It is thus apparent that we are entering a new era in the treatment of stroke where microvascular techniques will play a significant role.

Our study began as an evaluation of methods of relieving vasospasm where fluorescein angiography was used as an indicator of vessel diameter. We found that fluorescein also was an excellent indicator not only of microvascular blood flow but also of the extent of vessel wall injury caused by the dissection and repair.

Methods
Forty-five Ostpon Mendelson rats were anesthetized with 0.3 ml of 10% diethylamine allylisopropylbarbituric acid ("Numal," Roche) for these experiments. The arteries studied were the aorta, carotid, axillary and femoral vessels. Exposure was carried out with the aid of microdissection instruments and the Zeiss operating microscope at 4 x to 25 x magnification using a 300-mm objective and a 20 x eyepiece.

Fluorescein angiography was of two types: (1) direct injection with immediate filming, and (2) delayed filming about one to five minutes after injection. The former was done on the left carotid artery by the injection of 0.5 ml of 5% fluorescein into the aorta via the right carotid artery, on the right carotid by the injection of 0.5 ml of 5% fluorescein into the right axillary artery, and on the abdominal aorta by the injection of 0.5 ml of 0.25% fluorescein directly into the aorta via the celiac artery. The delayed filming was carried out either after the above injections or after 1 ml of 5% fluorescein intravenously. The agent used was sodium fluorescein (uranine) injected into a catheter (Dow Corning Silastic tubing 0.012 inch ID X 0.025 inch OD).

Fluorescein was excited in a darkened room using a 100-watt ultraviolet lamp (Black Light Eastern Model B-100) positioned about 30 cm from the vessel. Records were made on Kodak Tri-X pan film (ASA 400) exposed for one second using a Nikon camera (Nikkormat) and a Micro-Nikkor-P auto 55-mm lens 24 to 25 cm from the subject. When fluorescence was brilliant and dark nonfluorescent zones were of particular interest, then exposures as short as one-eighth second were used. The shutter was set at f/3.5 and the lens was covered by a green number 58 Kodak Wratten gelatin filter. By this method sufficient fluorescent light reached the film for exposure. Magnification was achieved by making positive prints (contrast number 5) enlarged from the 35-mm negative film. We obtained maximum contrast by slight underexposing of the negative film during angiography, increasing the exposure of the film when making the positive print, and carrying the print in the developer a bit longer until ideal contrast was seen.

The effects of direct (dc) electric current on the angiographical phenomenon were studied and are reported in detail elsewhere. Essentially dc + or − current (2 to 10
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volts, 0.1 to 1 mAmp, 30 seconds) was applied to the vessel either by platinum wire or by a stainless steel jeweler's forceps. Other rats had various types of carotid artery (0.8 to 1.1 mm diameter) end-to-end or end-to-side anastomoses performed by various neurosurgeons learning microvascular surgical techniques in the neurosurgical laboratory at the Kantonsspital Zürich. Fluorescein angiography was used to test the functioning of these anastomoses.

Results
Direct injection of fluorescein into an artery resulted in fluorescence from two sources. Initially the fluorescein in the blood itself sent photons through the vessel wall for visualization provided the ultraviolet light was strong enough, the fluorescein concentration was not too weak or too strong, the vessel wall was not too thick, the vessel wall did not selectively absorb the light, and the background light was adequately filtered. But within a few seconds or less the vessel wall itself continued to fluoresce and this fluorescence gradually increased as the blood recirculated with fluorescein, which gradually leaked out to stain most organs of the body. After about five minutes the fluorescence of the vessel wall gradually began to recede.

The effects of the application of dc + and — current are detailed elsewhere, but of interest is the similarity we found between the effects of dc + current and vessel wall injury. Simple dissection of the aorta would leave areas of nonfluorescence if insufficient care was taken (fig. 1). This occurred with pinching, squeezing, or pulling on the vessel wall resulting in microhemorrhage into the wall or other changes preventing adequate absorption of dye. Application of dc + current caused the identical phenomenon (fig. 2). Whether this was due to adsorption of normally negatively charged blood cells to the vessel wall or to other phenomena is unknown at present. The application of dc + current often led to clotting along the

FIGURE 1
Aorta after dissection from vena cava with microscopic technique. Filmed three minutes after direct arterial injection. Darker, poorly fluorescent areas due to vessel wall injury during dissection. x6.
vessel wall if the current was sufficiently strong and was applied for several minutes.

There were additional similarities between application of dc + current and injury. Firstly, if the current was applied or the injury occurred before or after the injection of fluorescein, the same phenomenon occurred. In other words, squeezing the vessel deliberately before injection caused local decreased fluorescence. But if the vessel wall was stained first with fluorescein and then the wall injured (fig. 2), the same zone became less fluorescent. This latter also occurred with dc + current, but this can be explained by the precipitation of a nonfluorescent metallic-fluorescein compound at the electrode, a phenomenon we confirmed in vitro. Secondly, the reduced fluorescence at the + electrode or at injury sites was seen during immediate direct angiography (fig. 3) as well as during delayed filming (figs. 1 and 2). In other words, for some reason photons in these areas were not well detected even though fluorescein was in the blood stream. Yet we suspect fluorescein in the blood stream will fluoresce because we could see changing eddy currents of early nonfluorescence in zones of focal vasodilation. These currents were visible until the nonfluorescent pockets of blood became fluorescent and the vessel wall itself began to absorb dye.

After microvascular anastomoses of the carotid artery, we could detect a relatively perfect anastomosis by complete absence of nonfluorescent areas during immediate and delayed filming. After doing both in several rats, it appeared that delayed filming about two minutes after the intravenous injection of 1 ml of 5% fluorescein gave sufficient information. If we waited more than two minutes, some nonfluorescent areas of the vessel wall were not seen due to gradual diffusion of the dye from normally fluorescent zones into the injured zone. Also, exposure times of one-eighth second allowed better portrayal of the nonfluorescent areas with these fluorescein dosages.

FIGURE 2
Aorta. Filming three minutes after direct arterial fluorescein injection following injury. Short arrows: zone of squeezing by forceps. Long vertical arrow: zone of dc + current application (5 volts, 0.6 mAmp, 30 seconds). Oblique arrow: zone of focal hemorrhage in vessel wall. x6.
Nonfluorescence often occurred at the anastomotic site due to local clot formation (usually platelet clot) or due to adventitia pulled into the lumen, allowing fluorescent blood to come to this point from above and below but preventing passage of blood (fig. 4). In this situation the vessel still may appear to pulsate normally. On the other hand, nonfluorescence at the anastomotic site did not predict occlusion, for trauma to the wall without early clot formation would still cause nonfluorescence (fig. 5). Temporary clamps too tight on the vessel also could cause a nonfluorescent band (fig. 6). Failure to keep the vessel continually wet also caused some reduced fluorescence.

End-to-side (figs. 7 and 8) and side-to-side (fig. 9) carotid artery to jugular vein anastomoses also were studied, and zones of injury, clotting or stenosis were seen. Findings were confirmed by postangiography dissections.

Discussion
The very marked similarity of the effect of both injury and dc + current on the rat artery in terms of fluorescence suggests that dc + current may be injuring the vessel wall. Since it is known that the physiochemical reaction at the applied anode includes the formation of hydrogen ions, it is possible that a reaction between these ions and the vessel wall renders the wall less permeable to fluorescein. Why injury renders the vessel less fluorescent during immediate filming as well as late filming remains a mystery, but a possible explanation is the adsorption of blood cells onto the intima or the accumulation of microhemorrhages inside the vessel wall. The blood then absorbs the ultraviolet light so that fluorescein containing blood in the lumen cannot be excited to fluoresce at this point. But further changes occur also preventing absorption of the dye by the vessel wall at this site. This is somewhat similar to the findings of
Dohrmann and Wick: thioflavin S which stains vessel walls and fluoresces would not stain hemorrhagic areas in zones of cat spinal cord contusion.

These findings at the points of rat arterial injury or dc + current application can be compared with the recently discovered that dc — current causes marked, prolonged vasodilation in previously spastic rat arteries. Interestingly, there is increased fluorescence of the vessel wall at these sites apparently due to increased permeability of the wall following smooth muscle relaxation.

The use of fluorescein in the vascular system has received much attention by ophthalmologists investigating blood flow to the retina. Several investigators have used fluorescein extensively during craniotomy to study cortical flow patterns in various pathological states of the brain. In contradistinction to our technique most of these authors use a high-intensity strobe light or flash system with filters excluding all but the blue and ultraviolet light for excitation of fluorescence. We have not discovered any reference to the effect of injury on fluorescence of arterial walls during or after fluorescein angiography. Indeed, the fact that much of what is described as fluorescein angiography is actually vessel wall fluorescence and not necessarily intraluminal fluorescence seems not to have been emphasized. The rat arterial wall rapidly picks up and holds the fluorescein.

Reichmann noted in his study of lingual-basilar arterial micro-anastomoses that contrast radiographical angiography done immediately often showed spasm of both vessels and felt that early angiography may be detrimental to these vessels. We feel that fluorescein angiography may not only serve as a guide to the progress of the student as he becomes more proficient in carrying out micro-anastomoses but also may help indicate to the investigator and clinician the functional status of the anastomosis during the operative procedure.

**FIGURE 4**

Right carotid artery following end-to-end anastomosis and then three minutes after intravenous fluorescein injection. Arrow: zone of occlusion by platelet clot. x6.
FIGURE 3
Right carotid artery following end-to-end anastomosis and then three minutes after intravenous fluorescein injection. Arrow: zone of vessel wall injury at anastomotic site No clot x6.
Right carotid artery following end-to-end anastomosis and then three minutes after intravenous fluorescein injection. Note poor fluorescence due to drying of vessel and scattered trauma. Short arrow: anastomotic site. Long arrow: zone of proximal temporary occlusion by clip.
FIGURE 7A
Left carotid artery to jugular vein end-to-side anastomosis. Clip on cephalic end of jugular vein. x6.

FIGURE 7B
Filming during direct injection of fluorescein into aorta. Note fluorescence of carotid artery wall only. x6.
FIGURE 7C

Filming five minutes later. Note fluorescence of artery and vein as blood eventually passed constriction at anastomosis. Arrow: jugular vein. x 6.
Right carotid artery to jugular vein end-to-side anastomosis. Artery (above) and vein (below) fluoresce during filming three minutes after intravenous injection of dye. Platelet clot occludes cephalic end of vein (arrows) with extension into artery and proximal vein. x 6.
Left carotid artery (above) to jugular vein (below) side-to-side anastomosis. There is zone of injury (vessel patent) to proximal vein (left-below, short arrow) and artery (short arrow). The cephalic end of vein (normal flow in direction of long arrow) is totally occluded. x6.

References
3. Symposium on Microneurosurgical Anastomoses for Cerebral Ischemia, Loma Linda, California, June 14-15, 1973
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