Oral fluorography: Detection of corneal vascularization

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Oral fluorography: Detection of corneal vascularization

Abstract
Corneal vascularization is a pathological process occurring in a cornea suffering physical and/or physiological insult. Its presentation could impair clear vision and thus its detection is important. Unfortunately current methods to detect and grade corneal vascularization are largely subjective and therefore a uniform system to identify and grade its presence does not exist. This study has explored a method using oral fluorescein angiography to examine corneal vessel growth. The results show that corneal vascularization fluoresces and leaks fluorescein during an oral fluorescein study. This information indicates that a method potentially exists to detect, grade and follow corneal vascularization through an objective means.

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ORAL FLUOROGRAPHY:
DETECTION OF CORNEAL VASCULARIZATION

BY
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A thesis submitted to the faculty of the
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for the degree of
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May, 1991

ADVISOR:
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ORAL FLUOROGRAPHY:
DETECTION OF CORNEAL VASCULARIZATION

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ABSTRACT

Corneal vascularization is a pathological process occurring in a cornea suffering physical and/or physiological insult. Its presentation could impair clear vision and thus its detection is important. Unfortunately current methods to detect and grade corneal vascularization are largely subjective and therefore a uniform system to identify and grade its presence does not exist.

This study has explored a method using oral fluorescein angiography to examine corneal vessel growth. The results show that corneal vascularization fluoresces and leaks fluorescein during an oral fluorescein study. This information indicates that a method potentially exists to detect, grade and follow corneal vascularization through an objective means.

Key Words: corneal vascularization, oral fluorescein, fluorescein angiography, fluorescein leakage
ACKNOWLEDGEMENTS

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ORAL FLUOROGRAPHY: DETECTION OF CORNEAL VASCULARIZATION

INTRODUCTION

Since the advent of contact lenses, practitioners have been faced with many ocular health problems arising from their use. Although the possible side effects from wearing contact lenses are numerous, the most common are infections, hypersensitivity reactions, corneal oxygen deprivation, and corneal abrasions. Each of these has potential to produce irreparable corneal damage, including corneal vascularization.

Corneal vascularization is a frequent consequence from contact lens wear, but can also occur from corneal insult not related to contact lenses. Its appearance is concerning since it indicates a process is upsetting the cornea's environment and denying the cornea its most basic needs.

It is difficult for practitioners to identify vascularization in its earliest stages and to consistently grade its severity once it is detected. There is poor agreement among observers as to the degree of vascularization present in a given vascularized cornea. A subjective measurement such as "mild" may be graded as "moderate" or possibly "severe" by another practitioner. This confusion exists because there is no objective method to grade vascularization or even detect it in its earliest stages.

The goal of this paper is to develop and present a method for detecting and grading corneal vascularization through objective means. However, to appreciate the method, one must understand the processes involved with vascularization and its presentation. These provide a foundation for understanding why subjective gradings are difficult and underlines the need for establishing a standard to detect and grade vascularization.
ROLE OF THE CORNEA:

The cornea’s role in vision is vital. It is not only an instrument of refraction, but one of transmission as well. For optimum transmission, the tissue must be transparent and the surface must be smooth. The epithelium, Descemet’s membrane and endothelium are all transparent due to uniformity of their refractive indices. Although the refractive indices of the fibrils and the interfibrillar substance differs in the stroma, the regularity of the separation between the fibrils allows minimum light scattering and therefore transparency. (1)

The cornea must also remain in a relative state of dehydration to be transparent. This relative deturgescence of the cornea is attained through the barrier actions of the epithelium and endothelium, and the pumping function of the endothelium. If the integrity of either structure is compromised, the relative deturgescence is lost and corneal opacification occurs. Any unnatural structure or physiology present in the cornea will upset the cornea’s delicate makeup. Clear vision may be threatened as a result. (1)

One such pathological insult threatening the cornea’s integrity is vascularization. Vascularization is defined as the process of blood vessel growth occurring in a previously unvascularized cornea. Since the cornea is naturally an avascular tissue, the presence of blood vessels creates a condition which is incompatible with the cornea’s physiology and can negatively affect transmission and refraction. Many practitioners consider mild vascularization to be inconsequential, but the mere presence of vessels may have significant side effects. It has been documented that decreased vision, lipid degeneration of the cornea, pannus, scarring, intrastromal hemorrhages, ghost vessels, and corneal graft rejections are either the result of corneal vascularization or an accompanying pathological process. (2,3,4)
ANATOMY OF VASCULARIZATION:

Corneal vascularization occurs at two different levels: superficial and deep. (3,5) Superficial vascularization is considered to originate from or be continuous with the conjunctival vessels. Its appearance is most frequently tortuous and irregular, with anastomoses forming a series of arches or arcades. On occasion, superficial vessels do not form arcades and appear as a more orderly or linear arrangement with the arterial and venous portions lying very close together. These can look as if they are one vessel. This linear arrangement represents early or new vascularization; however, as the vascularization matures it begins to appear as an arcade pattern. (3,6,7) Superficial vascularization is the level from which the fibrovascular condition referred to as pannus develops. (6,8)

Deep vascularization is believed to be associated with more serious ocular pathology. It originates from scleral vessels and presents in the corneal stroma. Its appearance is usually linear due to the highly structured stromal elements, although it may present in a deeper tortuous pattern when corneal insult leads to softening and loss of tissue regularity. (3,6)

Although the anatomy is easily described, vascularization is not easily detected. The reasons for this will be discussed later.

EVENTS OF VASCULARIZATION:

The sequence of events associated with corneal vascularization has been studied through animal experiments. The correlation between these experimental results in animal corneas and vascularization in human corneas can only be extrapolated. One study described the events of vascularization in the rabbit cornea arising from experimentally induced corneal lesions. Vascularization began with engorgement of the limbal capillaries,
venules and arterioles in the region nearest the corneal lesion. This engorgement was followed by the development of saccular aneurysms in the previously engorged venules and capillaries. Several days later these aneurysms led to corneal hemorrhages with subsequent loss of local limbal vessel engorgement. Two days following the hemorrhages, new capillary growth appeared in the region previously occupied by the hemorrhages. Within one to two days, this new capillary growth became more defined and some of these newly defined vessels enlarged to form an extension loop from the original capillaries. Other newly formed channels regressed and disappeared. This pattern of capillary growth was a frequent occurrence, and repetition of this cycle characterized the progressive movement of vessels into the cornea. (7)

METHODS TO DETECT VASCULARIZATION:

As said earlier there is poor agreement among practitioners when judging the extent of vessel ingrowth present in a vascularized cornea. The cause for this uncertainty is due to the undefined nature of the corneoscleral limbus. (3) Since the limbus is a zone or region, there is no precise and consistent landmark in individual corneas from which to measure the extent of vessel ingression. The definition of this region is further compromised by the presence of limbal pigmentation and by the reduction of transparency typically found in the peripheral cornea, especially in older patients. Since small superficial capillaries originating from the episcleral branches of the anterior ciliary arteries are often found adjacent to the limbus or within the limbal region, their presence may easily be misdiagnosed as corneal vascularization. (2,3,5,6,9) In addition, corneal vascularization does not elicit complaints of pain or discomfort from the patient. (3) Thus its identification is dependent upon keen observation from the practitioner.
Due to this zone of uncertainty in the limbal region, researchers have attempted to identify vessel growth at early stages using different techniques. Several researchers have attempted to correlate the amount of continuous contact lens wear time and the occurrence of vascularization. The problem with this is knowing how long vascularization has existed prior to its visible detection. It is also difficult to correlate vessel ingrowth from continuous lens wear to vessel ingrowth from daily lens wear, as well as to other factors or conditions which create vascularization. (3,4) Similarly, through the use of animal studies that have induced corneal lesions, researchers have studied the changes preceding noticeable vascularization and clocked the time which passes before vessel growth is noticed. (10,11,12) Again it is difficult to know how long the vascularization existed prior to its visible detection and whether these animal studies correlate to human conditions.

Another method attempted to define early vascular changes in the limbal area that associate with beginning vascularization. Terminal capillary engorgement followed by the sprouting of new vessels from existent arcades were described as the early markers of vascularization. From this observation it is hypothesized that sustained engorgement of the limbal vessels from chronic irritation may be a precursor to corneal vascularization. (3,4)

Perhaps the most fascinating method that has been used in identifying and following corneal vascularization is intravenous dyes, such as sodium fluorescein, trypan blue, or benzo blue 6BA. In experiments that utilized animal corneas, intravenous dyes were injected into the animal after corneal insult had been inflicted. The dye allowed researchers to investigate the changes in limbal vessel permeability that were associated with vascularization. In each experiment, increased permeability of the limbal vessels was demonstrated, but controversy existed about the latency between corneal insult and increased permeability. (7,13) Regardless of this time differential, it was obvious from these experiments that limbal vessel permeability increased prior to new vessel growth and that all newly formed blood vessels leaked the intravenous dye. (2,3,4,6,7,13,14,15) This increased
vessel permeability appears to be an early marker of beginning corneal vascularization and is the foundation upon which the premise of this paper originates.

**INTRAVENOUS FLUORESCIN:**

Another application of intravenous dyes is the use of fluorescein angiography to assist the examination of corneal vascularization in contact lens wearers. This method documents the changes in vessel patterns before, during and after contact lens wear. It is an effective procedure for revealing corneal vessels of lower contrast. It also allows exact and permanent records to be obtained for future comparison. One can see precisely where vessel changes are occurring and can relate these changes to local factors stimulating vessel growth. In addition, the effects of therapy for corneal vascularization have been assessed and followed more accurately through this method. (16)

Intravenous fluorescein has also been used to investigate normal anterior segment vascular anatomy. These studies revealed that the permeability characteristics of the conjunctival, episcleral, limbal and iris vessels are very different from the retinal vessels. (17,18,19,20) Normal retinal vessels do not leak fluorescein. Leakage of dye from the retinal capillaries implies pathology of those vessels, whereas leakage from vessels in the anterior globe is normal and varies in degree at different sites. Interpretation of such leakage is therefore difficult, but there are diseases of the anterior globe where leakage is sufficiently gross to be denoted as pathological. Examples include scleritis, pannus, rosacea keratitis, and herpes stromal keratitis. (14,18,21)

Fluorescein angiography has proven to be an effective and relatively safe means of studying the vascular anatomy of the anterior segment. This approach was used by the authors of this paper to investigate corneal vascularization. There is one primary difference to the method of fluorescein introduction used in previous research compared to our
Previous experimenters utilized intravenously injected fluorescein whereas we have elected to use oral fluorescein.

**ORAL FLUOROGRAPHY:**

Fluorescein angiography has been used as a diagnostic agent in ophthalmology. Its usual route of administration is intravenous injection; however, this may not be an available avenue to many optometrists. Recently oral fluorescein has received attention as a valuable diagnostic tool. Although injected fluorescein has the distinct advantage of revealing circulatory details in the early phase and measuring velocity of flow, oral fluorescein cannot show this. Oral fluorescein is introduced to the vascular system through gastrointestinal absorption. Subsequently, this slow introduction reveals itself as a gradual fluorescence within the vascular system in contrast to the acute presentation that a bolus of fluorescein reveals upon intravenous injection. It is this difference of fluorescence within the vasculature that accounts for the lack of information given by oral fluorescein in contrast to injected fluorescein. However, both injected and oral fluorescein will reveal barrier integrity; therefore, oral fluorescein has gained acceptance in assisting in the diagnosis of conditions which influence tissue permeability. (22,23,24,25)

Not only is oral fluorescein accessible to optometrists, but it is also believed to be safer than injected fluorescein. Reactions to injected fluorescein have included nausea, wheezing, pruritis, edema, shock, urticarial reactions, respiratory arrest, laryngeal edema, exanthema, and cardiac arrest. (26,27) These reactions are believed to be anaphylactic in nature, but the exact mechanism of their occurrence is uncertain. (26) Although oral fluorescein poses a similar risk, the occurrence of complications is less common. (22,23,24,25,28,29,30,31) Because of this, oral fluorescein might prove to be the better choice
when examining a young patient, one with no visible veins, one psychologically unsuited for injections or one undergoing multiple fluorescein studies. (22,23)

HYPOTHESIS:

Our goal is to evaluate the effectiveness of oral fluorescein angiography in detecting corneal vascularization. It is our hypothesis that corneal vascularization induced through soft contact lens wear will leak fluorescein during an oral fluorescein study. This pilot study is also intended to determine correct methods and dosage needed for repeatable oral fluorescein studies.
METHODS

Four subjects were chosen from the patient population of Pacific University College of Optometry. All subjects met the screening criteria set for the project. (See Table I.) Patients were included only if they were between the ages of 21 and 45, were not pregnant, and could demonstrate a negative history for allergies, hypertension, and systemic health problems.

The subjects ages ranged from 23 to 27 years. There were three males and one female. These subjects were divided into two groups. Group One (GI) consisted of two subjects who had never worn contact lenses, had a negative history of ocular trauma and ocular disease, and no apparent vascularization of the cornea. Group Two (GII) consisted of two subjects who possessed at least Grade 2 vascularization as described by Efron. (32,33) Selection of GII was not made on the basis of having superficial or stromal vascularization. Each of the subjects in both groups were randomly assigned a letter for identification: A or B.

Before participation in the study the subjects were advised of all foreseeable risks and side effects inherent to the study. (See Table II.) If the risks were acceptable to them, they signed an Informed Consent form.

In designing the experiment, it was known that once fluorescein was ingested, its digestion is affected by other food products in the stomach. Previous research indicates that the most influential factor in achieving quality photographs in posterior segment oral fluorography was the amount of time since the last meal prior to ingesting the fluorescein. (29) The presence of food products in the stomach reduces the quality of fluorography. (34) Since the stomach usually empties its contents within two to six hours, a fasting period of at least six hours prior to fluorescein ingestion was established. Therefore before each subject was given fluorescein, they were questioned about the time since their last meal and how they were feeling at the present. (See Table III.)
Each of GII's cornea's were inspected for signs of vascularization and the region of most pronounced vessel growth was chosen as the area of study. An area of normal limbal vasculature was selected from each subject in GI. For both groups, the area was photographed with the Nikon FS-2 photo slit-lamp with an attached Nikon MF-12 camera. A Nikon electronic power supply was used to increase the flash intensity. Kodak Ektachrome 200 ASA colored slide film was used.

Following these initial photographs the subject was instructed to drink a prepared, chilled mixture of 4 grams of sodium fluorescein mixed with enough Tang™ orange drink to yield 5 ounces of solution. Eight 5 ml. vials of 10% sodium fluorescein solution were used to yield the 4 grams.

The decision to use four grams of fluorescein was based on preclinical studies performed on the authors. Various concentrations were attempted on a six hour fasting period. Initially one gram was tried, but fluorescence was not detected. By gradually increasing the concentration, it was found that four grams was the minimum amount that provided reasonable photographic results. Previous research suggests it is not always true that more fluorescein ingested provides brighter photographs. (23,29) Although earlier research has tried to correlate the weight of the subject with the amount of fluorescein delivered, this was not attempted in this study.

After preparing the mixture for ingestion, the subject drank the solution from a cup with the aid of a straw to decrease fluorescein staining of the teeth and lips. Each subject was instructed to place the straw on the back of the tongue and to drink the entire mixture as quickly as possible, preferably in one breath. This technique was devised to decrease staining of the teeth and decrease the amount of after taste from the fluorescein. To further eliminate staining to the lips, each subject was given a tissue with which to blot his/her lips and tongue immediately upon drinking the mixture. A hard candied mint was available to remove the after taste. Although each subject reported that the taste was unpleasant, all
subjects were able to drink the entire sodium fluorescein-orange drink mixture within twenty seconds.

After ingesting the fluorescein solution the subject was seated in a completely darkened room before a Nikon AS-1 endothelial cell photo slit-lamp. The slit lamp was equipped with a 7X powered telephoto lens and a 1.4x teleconverter attaching the Nikon MF-12 camera to the slit-lamp mounting. An f-stop of 2.6 was used on the telephoto lens. The Nikon electronic flash power supply was set on a flash setting of "5" and a lamp setting of "high". Spectro-Tech™ filter #501 was used as a barrier filter and Spectro-Tech™ filter #32 was used as the excitation filter. Black and white photography utilizing Tri-X Pan 400 ASA 36 exposure print film was used to capture the fluorescence.

A series of photographs was taken with the first photograph taken at three minutes after ingestion. Photography was then repeated every twenty seconds for a period of twelve more minutes. The film was developed using a standard procedure for black and white print film at a local photography firm.

Each patient was questioned as to how they felt upon completion of the photography and then again in 24 hours.
RESULTS

Photographs taken at equal time periods for both group's blood vessels were compared to determine how soon the vessels fluoresced and leaked. The amount of leakage, and the distance the leakage advanced in a certain period of time was also noted. The time needed for fluorescein to enter the ocular vessels and to fluoresce depends upon several factors including digestion and circulation time, time since the last meal, length of the circulation path to the eye, and other individual characteristics. (18,29) The twenty second time interval between photographs was sufficient to capture even small differences in the amount of fluorescein leakage between exposures. In all subjects the first visible fluorescence captured on film was that of the conjunctival vessels. This fluorescence occurred at an average time of five minutes twenty-five seconds with times ranging from five minutes to five minutes forty seconds. The first visible limbal vessel fluorescence occurred on an average of seven minutes twenty seconds with times ranging from seven minutes to eight minutes. (See Table III.)

The time frames used for leakage comparisons were those of nine minutes, eleven minutes forty seconds, and fourteen minutes. These times were chosen as being evenly spaced intervals that allowed consistent comparisons between the groups. After nine minutes all of the limbal vessels appeared to be filled with fluorescein and some leakage was evident in all subjects. This leakage is manifested as haze that forms around individual vessels. (See Photos GI-Ab, GI-Bb, GII-Ab, and GII-Bb.) As discussed previously, it is customary for normal limbal vessels to leak some fluorescein. Therefore when looking at the pictures and comparing only the leakage and not the vessel pattern, it is hard to distinguish GI from GII at the nine minute time frame.

At the eleven minute forty second and fourteen minute time intervals there is a subtle, yet apparent difference in the amount of leakage between GI and GII. Subjects GI-A and GII-A have the most distinct examples of this difference. Subject GI-A has a very
distinctive vessel formation at the limbus and when looking at this vessel, it's clearly fluorescing in both the eleven minute forty second and fourteen minute photographs. However, the zone between the vessels and the central cornea is almost entirely without fluorescence and therefore appears dark. The zone between the vessel and the rest of the limbus shows only slight fluorescence. (See Figure 1.) Subject GII-A on the other hand, shows a larger, more apparent zone of fluorescence between the limbal vessels and the dark central cornea and an even more distinctive difference in the leakage between the limbal vessels themselves. (See Figure 3.) There was an attempt to quantify the distance of the leakage, but the fluorescence fades gradually making a definite endpoint impossible to determine. In Subject GII-A the interlimbal spaces are completely filled with fluorescence indicating a larger amount of leakage than that from GI-A. In subjects GI-B and GII-B there is also this difference in leakage, but is not as apparent in these photographs. Whether this was due to the photography technique or individual characteristics will be discussed later.

Upon completion of the photography, there was no report of nausea and most subjects felt relatively well. All subjects experienced a slight yellowing of the skin and conjunctiva, which reportedly increased until about the second hour and then gradually declined over a period of six hours. All subjects reported a strong discoloration of the urine for a period of 36 to 48 hours. Subject GII-B did report a slight rash with pruritis on the forearms. This was the only allergic side-effect experienced.
DISCUSSION

The purpose of this pilot study was to design and test a method of studying corneal vascularization with the intention of using the findings to guide future research. The results have delivered enough evidence to support continued study in this area.

In designing this experiment, the authors established a fasting period of at least six hours prior to fluorescein ingestion. Although one subject, GI-B fasted only four hours, inspection of the photographs shows that fluorescence does not appear to have been affected.

It is important to mention that the fluorescence observed by the photographer was not as bright as that indicated by the film. It appears the film was more sensitive to fluorescence than the human eye. This was discovered during preclinic trials and made photography especially difficult.

The time elapse until first fluorescence of the conjunctiva vessels and the time elapse until first fluorescence of the limbal vessels are nearly equivalent in all four subjects. (See Table III.) This is surprising considering all the factors which influence the distribution of fluorescein in the human body. The similar age of the subjects and/or the fasting time may have contributed enough influence to yield these results.

When studying the fluorescein pictures of GI and GII, several facts become apparent. The fluorescence of the iris vasculature is visible in some of the pictures. It is most easily identified in GI-B. (See GI-Bd and Figure 2.) Its presence in the pictures may make interpretation of the results difficult. In both groups, it is plainly seen that the conjunctival and episcleral vessels easily leak fluorescein. If one were to follow the progression of the photographs from nine minutes to eleven minutes forty seconds to fourteen minutes, conjunctival and episcleral fluorescence is seen to increase. If one directs attention only to GI, it is obvious that the limbal region leaks fluorescein readily. However the fluorescein rapidly dissipates and has negligible fluorescence within the cornea. When
comparison of the limbal region between the groups is made, it is apparent that the fluorescence found in the limbus of GII is greater than that found in GI. Obviously, something other than normal limbal vessel leakage is contributing to this fluorescence. The most likely candidate is the vascularization found in this region.

The vascularization in GII's photographs is highly visible from its fluorescence. The leakage from these vessels is easily seen between the vessels as well as forward from the apex of each vessel as a "soft cotton" appearance.

Subject GI-A possesses a unique normal vessel variant in the limbus zone. One limbal vessel fluoresces quite prominently. (See GI-A photographs and Figure 1.) However this vessel does not demonstrate the same degree of perivascular leakage or "soft cotton" appearance as vessels from subjects in GII. Some leakage from GI-A's vessel can be seen, but the fluorescence is very dim compared to the vessels from GII subjects. In addition, the small leakage present in the vicinity of the GI-A's vessel represents leakage from this vessel and from the surrounding limbal/conjunctival vessels. This would artificially create more apparent fluorescence than the vessel itself can be credited for.

Initially, subject GII-B was chosen for the large corneal vessel found superiorly in the right eye. Color photographs of GII-B document the existence of this extensive vascularization. However, none of this subject's oral fluorography pictures appeared to reveal this vessel as fluorescent. One explanation for this might be that the vessel is not patent, and therefore is not fluorescent. However, in the color photograph, a blood column is present within the vessel. It does not seem likely that this vessel would be patent to blood products but not fluorescein. Closer inspection of GII-B’s oral fluorography pictures reveal a faint fluorescence with a shape similar to the large corneal vessel pattern seen in the color photographs. (See Figure 4 schematic.) In the pictures this fluorescence was initially dismissed as an iris vessel. However the authors now believe this fluorescent pattern represents leakage from the large corneal vessel initially selected for study.
This explanation is very plausible since fluorescence seen by the photographer was considerably less than that recorded on film. As a result, the photographer was unable to detect subtle fluorescence and consequently did not make adjustments in the camera focus to accurately photograph the area of leakage. It appears the camera is focused on the limbal region and therefore the major vessel of interest was out of focus during oral fluorography and its detailed action during the event was lost.

Another interesting feature is the difference of fluorescein leakage found between GII-A’s and GII-B’s corneal vascularization. GII-B’s fluorescein leakage extends further beyond the apex of the vessels than does GII-A’s leakage. (Compare fluorescein photographs GII-B to GII-A.) An explanation for this might be that the vessel diameters of the abnormal vascularization differs between GII-A and GII-B. One might hypothesize that a larger diameter vessel has more available "leaky junctions" to allow the passage of fluorescein. This could account for the difference in leakage. However when comparing the photographs from each subject, it is not possible to accurately assess which vessel set is larger.

An alternative explanation arises from an experiment which utilized intravenously injected fluorescein to investigate corneal vascularization secondary to herpes simplex. The experiment performed serial fluorescein angiography on the newly formed vessels. On the initial fluorescein study, fluorescence and leakage, similar to that found in this study, was evident from these corneal vessels. However, once treatment was inacted, the vascularization stabilized and the amount of fluorescence and leakage became less. In fact some vessels failed to leak and others failed to fluoresce at all. (14) This suggests that active vascularization readily leaks fluorescein, and stabilized vascularization leaks comparatively less. If this assumption is true and is applied to this situation, it implies that GII-B’s vascularization is active and GII-A’s vascularization is more stabilized or less active. At the moment, there is no additional evidence to support or refute this supposition. Both subjects are currently wearing contact lenses.
Oral fluorography presents the potential for accurately following the degree of corneal vessel ingress. If a patient with vascularization presents to a practitioner for contact lenses, the issue of aggravating vessel growth exists. By performing an oral fluorography study before contact lens fitting and at regular intervals thereafter, one is able to make accurate comparisons and subsequently conclude if the condition is stable or progressing. Similarly this alternative exists for other situations which potentially stimulate additional vessel growth. This procedure provides a method of defining the level of stability or activity present in corneal vascularization.
CONCLUSIONS

It has been shown that the vessels within a vascularized cornea, which still possess its irritant or stimulus to vascularization, will fluoresce during an oral fluorography study. In addition, these fluorescent vessels emit fluorescein leakage that cannot be accounted for by normal limbal leakage. It has also been demonstrated that normal vessels within the limbal region do not possess the same degree of leakage as vascularization does. Therefore abnormal vessel growth of the cornea does not demonstrate the same characteristics that normal vessels demonstrate.

Oral fluorography is a promising technique for charting the progression of corneal vascularization. This method may serve as an early detector of corneal vascularization and answer the elusive questions about corneal vessel growth. Corneal angiography could possibly determine if vascularization is stable. More definitive answers will be found in additional research which utilizes specific population groups and which performs serial studies. Perhaps this method of investigation can reveal many secrets or confirm many theories that presently are unanswered. The potential is certainly there; time and effort are the missing ingredients to provide the answers.
### Table I: Screening Criteriae

* Between the ages of 21 and 45.
* No history of allergies.
* No prior history of fluorescein angiography.
* No systemic health conditions.
* Not pregnant.
* Healthy and feeling well the day of the study.

### Table II: Risks and Possible Side Effects

1. Staining of the inside of the mouth usually lasting no longer than one day.
2. Yellowing of the skin lasting six to twelve hours.
3. Yellow discoloration of the urine and fecal matter lasting 24 to 36 hours.
4. Staining of the lips and outside areas of the mouth. (Eliminated if a drinking straw is used.)
5. Nausea.
6. Vomiting.
7. Possible staining of clothes of spillage occurs.
8. Sweating.
9. Dizziness and/or fainting.
10. Drop in blood pressure.
11. Red and itchy rash.
**Table III: Subject Information**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Weight</th>
<th>Time of Last Meal</th>
<th>First Fluorescence</th>
<th>First Visible Vessel</th>
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</thead>
<tbody>
<tr>
<td>GI-A</td>
<td>23</td>
<td>160</td>
<td>14 hours</td>
<td>5 min 40 sec</td>
<td>7 min</td>
</tr>
<tr>
<td>GI-B</td>
<td>27</td>
<td>170</td>
<td>4 hours</td>
<td>5 min 20 sec</td>
<td>7 min</td>
</tr>
<tr>
<td>GII-A</td>
<td>24</td>
<td>165</td>
<td>12 hours</td>
<td>5 min</td>
<td>7 min 20 sec</td>
</tr>
<tr>
<td>GII-B</td>
<td>25</td>
<td>120</td>
<td>6 hours</td>
<td>5 min 40 sec</td>
<td>8 min</td>
</tr>
</tbody>
</table>
References

32. Clark ER, Clark EL. Observations on living preformed blood vessels as seen in a transparent chamber inserted into the rabbit's ear. Am J Anat 1932; 49.

Figure 1: Subject GI-A showing normal limbal vasculature.
Figure 2: Subject GI-B who shows normal conjunctival fluorescence and some deeper iris vessels that are fluorescing. (White arrows.)

Conjunctival Fluorescence
Figure 3: Subject GII-A showing the diffuse leakage of fluorescein by the corneal vascularization.
Figure 4: Subject GII-B showing diffuse leakage of fluorescein as well as a large corneal vessel that was out of focus during photography. (White arrow.)
APPENDIX A

INFORMED CONSENT FORM
Informed Consent Form

Institution:

A. Title of project: Oral Fluorography: Detection of Corneal Vascularization
B. Principal Investigators: Craig Dockter 357-6949
Bryan Cook 357-9308
James Bell 357-2922
C. Advisor: Nada Lingel, O.D. 359-5906
D. Location: College of Optometry, Pacific University
Forest Grove, Oregon
Ocular Disease and Special Testing Clinic
E. Date: Spring, 1990

1. Description of project

This research project is designed to develop a new method to look for new blood vessels growing in the cornea (the clear part of your eye). Normally the cornea does not have blood vessels in it, so it may indicate a problem if they are found. Early detection of these growing blood vessels can be difficult, but with the use of fluorescein dye it may be possible to detect these blood vessels earlier. Each subject will drink a fluorescein-fruit drink mixture and their blood vessels will be observed for leakage and photographed.

2. Description of risks

Participants drinking oral fluorescein will experience one or more of the following symptoms/signs:
1) Staining of the inside of the mouth usually lasting no longer than one day
2) Yellowing of the skin lasting six to twelve hours
3) Yellow discoloration of the urine and fecal matter lasting 24 to 36 hours

Participants may also experience one or more of the following symptoms/signs:

1) Staining of the lips and outside areas of the mouth. (Eliminated if a drinking straw is used.)
2) Nausea
3) Vomiting
4) Possible staining of clothes if spillage occurs
5) Sweating
6) Dizziness and/or fainting
7) Drop in blood pressure
8) Red and itchy rash
9) Itching
10) Generalized anaphylaxis reactions (allergic reactions)

Although NONE of the following side effects have been reported with oral fluorescein they have been reported with the use of injected fluorescein:

1) Shock
2) Laryngeal edema (swelling of the throat cavity)
3) Pulmonary edema (swelling of the lungs)
4) Myocardial infarctions (heart attack, ratio of 1:20,000)

*Injected fluorescein is a process where fluorescein is injected with a syringe directly into the blood vein.

3. Description of benefits

It is hoped that this study will allow early detection of abnormal blood vessel growth on the cornea, which may be the result of disease or improperly fit contact lenses or other noxious stimuli. This will allow a doctor to treat, correct or eliminate the stimulus creating the vascularization before severe damage has occurred. If the vascularization was allowed to continue, vision could be severely threatened.

4. Alternatives advantageous to subjects

a) Current methods of finding this blood vessel growth include biomicroscopic observation of the cornea. Difficulties in this method include:

   1. Differences of opinion between examiners on the extent of the existing vascularization
   2. Poor detection of blood vessel growth in early stages

b) Injected fluorescein is another alternative that is more advantageous for the examiner, but poses a greater and more serious threat of side effects to the patient.
5. Records of the project will be maintained in a confidential manner and no name identifiable information will be released. Photographs of the eye will be taken, but will not be of a nature capable of identifying any individual.

6. Compensation and medical care

If you are injured in this experiment it is possible that you will not receive compensation or medical care from Pacific University, the experimenters, or any organization associated with the experiment. All responsible care will be used to prevent injury however.

7. Offer to answer any inquiries

The experimenters will be happy to answer any questions that you may have at any time during the course of the study. If you are not satisfied with the answers you receive, please call Dr. James Peterson at 357-0442. During your participation in the project you are not a Pacific University clinic patient. All questions should be directed to the researchers and/or the faculty advisor who will be solely responsible for any treatment (except for an emergency). You will not be receiving complete eye, vision or health care as a result of participation in the project; therefore you will need to maintain your regular program of eye, vision, and health care.

8. Freedom to withdraw

You are free to withdraw your consent and to discontinue participation in this project or activity at any time without prejudice to you.

I have read and understand the above. I am 18 years of age or older.

Printed name ____________________________ Date __________________________

Signed ____________________________ Phone __________________________

Address ____________________________ Phone __________________________

City ____________________________ State/Zip __________________________

Name and address of a person not living with you who will always know your address.

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