Studies on intravitreal blood vessels.

II. Effectiveness of xenon arc and argon laser photocoagulation in blood vessel closure

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We produced complete displacement of the vascular and myelinated portion of the rabbit retina into the vitreous by intravitreal injection of NH₄Cl. This was followed by degeneration of the myelinated portion of the retina, leaving the retinal vessels free within the vitreous. We used this model to compare the short-term effectiveness of xenon-arc coagulation and argon laser coagulation in treating intravitreal vessels. Fluorescein angiography and angioscopy both immediately after treatment and one week later were used to confirm vessel closure. Xenon-arc coagulation was entirely ineffective in closing intravitreal vessels. Argon laser achieved immediate closure of all types of intravitreal vessels except small arteries. At the end of one week, 20 per cent of the large veins, 75 per cent of the large arteries, and 100 per cent of the small veins remained closed. Minimal cataract increased the amount of energy needed to achieve closure. Background reaction increased with pigmentation and decreased as the distance from the fundus of the coagulated vessel increased. The rate of hemorrhage was 18 per cent for argon laser. Most hemorrhages were less than one-half disc diameter in area. Argon laser caused vitreal hemorrhage when using high energy on vessels accompanied by fibrous tissue.

Key words: xenon arc photocoagulation, argon laser, intravitreal vessels, closure, hemorrhage.

Diabetes and sickle cell retinopathies are frequently manifested by new vessel formation which causes retinal and vitreous hemorrhage. Attempts to treat this pathologic process have sought to prevent bleeding by ablating the new vessels. Surface proliferation is effectively treated with both xenon-arc and argon laser photocoagulation, but it has been much more difficult to treat intravitreal vessels. Argon laser offers many advantages over the non-specific broad coagulation of the xenon arc. The argon laser enables precise focusing of a high-energy density beam of small diameter on the vitreous vessels. In addition, the narrow wavelength of the argon laser, complimentary to that of hemoglobin, results in significant absorption of energy. Unfortunately, it was impossible to test and
compare therapeutic methods prior to their use on human patients because no animal model of intravitreal vessels existed. Our laboratory has now developed an experimental model to study vitreal vessels. The procedure causes complete displacement of the entire retina into the vitreous cavity. The retinal tissue then degenerates, leaving the retinal vessels free within the vitreous.5

We used this model to evaluate the roles of xenon-arc photocoagulation, and argon laser photocoagulation in treating intravitreal vessels. The investigation was designed to control such variables as media opacity, size and type of vessel coagulated, and energy of coagulation. In addition, the treated vessels were studied with fluorescein angiography and histologic examinations.

Method

I. Production of intravitreal vessels. One hundred albino and pigmented rabbits, each weighing 2 to 3 kilograms, received intravitreal injection of 15 mg of NH₄Cl as previously described.6 Each rabbit received an intramuscular premedication of atropine and then intravenous sodium sac, the inferior and superior rectus muscles were grasped with toothed forceps. A tuberculin syringe containing 15 mg of NH₄Cl in 0.1 ml of normal saline was directed through the temporal pars plana. It was possible to visualize the needle within the vitreous and direct it inferiorly. When the needle was thus positioned correctly, paracentesis was performed on the anterior chamber. The NH₄Cl was then injected slowly; the needle was withdrawn, and the eye released.

The animals were observed at the end of a three- to four-week period by direct ophthalmoscopy. Only animals with undamaged nasal or temporal vessels, which were free of retinal tissue in at least one eye, were selected for use. Approximately half of the animals initially injected were so selected. In addition, six animals within this group had developed a minimal posterior subcapsular cataract; these were identified and included for use in further experimentation. Four animals in which the retinal tissue remained fixed to the vitreal vessels were also selected for further use.

II. Xenon-arc photocoagulation. Four albino rabbits and one pigmented rabbit chosen from Part I each had a clear lens and vitreal vessels free of retinal tissue. After the animals were anesthetized as previously described, the pupils were dilated with one to two drops of Neo-synephrine and cyclopentolate hydrochloride. The animals were then positioned so that the vitreal vessels were visible through the direct ophthalmoscope of the Zeiss xenon-arc photocoagulator. The energy level at which a background reaction, and segmentation of the vitreal vessels first occurred was recorded. This was accomplished in the albino rabbits by increasing the instrument settings stepwise from Green I to Red III and by increasing the length of coagulation at each level from 0.5 second to 10 seconds. The iris and field diaphragm remained at 0 and 6, respectively. In the pigmented rabbit, the energy level was increased at Green I by changing the iris diaphragm from 3 to 0. In albino rabbits, a background reaction was defined as segmentation in the choroidal arteries and veins; in pigmented animals it consisted of a whitish reaction in the fundus. The settings at which this occurred were noted. The energy was then increased in attempts to produce segmentation of the vitreal arteries and veins. If segmentation occurred, we attempted to coagulate these vessels by repeated applications at this setting. Fundus photographs were taken before and after treatment. In addition, fluorescein angiography was performed with an indirect ophthalmoscope at the end of each treatment session.

III. Argon laser photocoagulation. In this part of the experiment, 41 albino rabbits and four pigmented rabbits were divided into six groups for use in six different experiments. The method of treatment in each group was basically the same; the animals were all prepared as previously described. A low-vacuum contact lens was then placed on the eye to be treated. The animal was positioned on a platform and the vitreal vessels identified through the slit lamp delivery system of the argon laser. In each animal, the background reaction was first measured by focusing on the fundus and recording the lowest amount of energy necessary to produce segmentation of the choroidal vessels in albino rabbits. The aiming beam was then focused upon the vitreal vessels and the wattage gradually increased until segmentation occurred after a single impulse; occlusion was then attempted by repetitive treatment at this same energy level. The area coagulated on each vessel was estimated to be 1½ disc diameters long. Coagulation was attempted by first placing a lesion on either end of the area to be coagulated and then coagulating the blood trapped in the center. The length of each impulse was kept constant at 0.1 second and the diameter of the beam remained at 50 microns. The energy was increased by increasing the milliwatts and the total amount of energy delivered was recorded by counting the number...
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Fig. 1. Fundus photograph showing segmentation of a large artery and vein (arrows) by xenon-arc.

Fig. 2. Fundus photograph showing the background reaction (outlined by arrows) secondary to coagulation by xenon-arc. Note vitreal vessels which have re-opened immediately following termination of xenon-arc coagulation.

of impulses delivered. Fundus photographs were taken before and after treatment; fluorescein angiography was performed immediately after treatment. The animals were then observed for one week by direct ophthalmoscopy; then fluorescein angiography and angiography were repeated and the patency of the vessel was recorded.

Experiment I. Ten albino rabbits in which the vitreal vessels were free of tissue and the lens was clear were chosen for a study of the differential effect of argon laser coagulation on a large and small vein. We defined a large vein as the main trunk or first division emerging from the optic disc; a small vein was the third division of that trunk. In six rabbits a large vein and in four rabbits a small vein were coagulated as previously described.

Experiment II. 10 albino rabbits were used in this experiment. Five large arteries and five small arteries were coagulated. The definition of a small artery was expanded to include both second and third divisions of the main trunk.

Experiment III. Four albino rabbits in which the vitreal vessels were free of tissue and the lens was clear were chosen for a study of the effect of combined treatment of a large artery and vein by laser coagulation.

Experiment IV. Eight albino rabbits, of which four had a posterior subcapsular cataract and four had a clear lens, were subjects for a study of the effect of lens opacity on coagulation of a large vein. In all rabbits, the vitreal vessels were free of retinal tissue.

Experiment V. Four pigmented rabbits and three albino rabbits, each with a clear lens and vitreal vessels free of retinal tissue, were chosen for a study of the effect of pigment on the amount of energy necessary to produce a background reaction while focusing on intravitreal vessels.

Experiment VI. In six albino rabbits, a large vein and artery were each treated with high-level energy in an attempt to purposely damage the vessel wall and produce hemorrhage. In three rabbits, the vitreal vessels were accompanied by retinal tissue; in the other three rabbits, the vessels were free of this tissue. This experiment studied the relation between tissue surrounding the vessels and hemorrhage secondary to high-energy photoacoagulation.

IV. Histology. Animals in which intravitreal vessels were coagulated by argon laser and were found closed by fluorescein angiography were killed. The coagulated vessels were studied by electron microscopy. Tissue to be examined by electron microscopy was immediately immersed in one per cent formaldehyde, one per cent glutaraldehyde mixture in phosphate buffer (pH 7.35), postfixed with Dalton’s chromic osmium for 2 hours, again washed in phosphate buffer, dehydrated in graded ethanols and propylene oxide, and embedded in araldite.

Thin sections were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a JEM 100B electron microscope.

Results

I. Xenon-arc photoacoagulation. We could identify large and small arteries and veins with an indirect ophthalmoscope prior to
Table I. Results of treatment of large and small veins with argon laser*

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Type of vessel</th>
<th>Average milliwatt segmentation</th>
<th>Average No. lesions</th>
<th>Average milliwatt background</th>
<th>No. vessels closed immediately after treatment</th>
<th>No. vessels closed at one week</th>
<th>No. of animals in which hemorrhage occurred</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Large vein</td>
<td>125 (100-150)</td>
<td>210 (80-300)</td>
<td>110 (100-125)</td>
<td>5</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Small vein</td>
<td>125 (100-150)</td>
<td>195 (70-255)</td>
<td>110 (100-125)</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

*Range of values given in parentheses.

Table II. Results of treatment of large and small arteries with argon laser*

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Type of vessel</th>
<th>Average milliwatt segmentation</th>
<th>Average No. lesions</th>
<th>Average background reaction</th>
<th>No. vessels closed immediately after treatment</th>
<th>No. vessels closed at one week</th>
<th>No. of animals in which hemorrhage occurred</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Large artery</td>
<td>150 (125-175)</td>
<td>260 (205-350)</td>
<td>110 (100-125)</td>
<td>4</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Small artery</td>
<td>750 (300-1000)</td>
<td>345 (295-465)</td>
<td>110 (100-125)</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
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*Range of values given in parentheses.

treatment: the veins were larger, darker, and more tortuous than the arteries. These observations were also confirmed during fluorescein angioscopy of untreated vessels. Intravitreal vessels could not be closed with xenon-arc photocoagulation. In the albino rabbits, background reaction first appeared when the energy level increased from Green I to Green II; an impulse of 0.5 to 1.0 second would produce such a reaction. Segmentation occurred in the vitreal vessels when the energy level increased to Red III (Fig. 1). An impulse of 5 to 20 seconds was necessary to produce segmentation of the vein. This high-energy impulse caused an intense choroidal reaction. Immediately after treatment, the segmented vessels opened. A fundus photograph taken at this time (Fig. 2) showed the intense background reaction. Fluorescein angioscopy confirmed that the vessels were patent in all albino rabbits.

We produced a background reaction in the pigmented rabbits using an energy of Green I and 0.3 second, with an iris diaphragm of 3 and field diaphragm of 6. When the energy level was increased by changing the iris diaphragm from 3 to 0, a massive hemorrhage from the choroidal vasculature occurred. It was not possible to increase the energy further to coagulate vitreal vessels.

II. Argon laser photocoagulation.

Experiment I. Table I records a comparison of the treatment of large and small veins. The amount of energy necessary to produce a background reaction was slightly less than that needed to produce segmentation in the intravitreal veins. The same amount of energy produced a reaction in both the large and small veins; the number of lesions was, however, less for the small veins. A fundus photograph (Fig. 3) shows the appearance of a large vein after laser coagulation.

After repetitive coagulation we often observed a change in color of the treated area; this area would become darker than the untreated area. The large veins also showed engorgement of the distal portion and attenuation of the proximal portion near the disc. At the end of coagulation, fluorescein angioscopy revealed closure of five out of six large veins and three out of four small veins (Table I). In the area of coagulation, fluorescein had leaked from
the vitreal veins. At one week, one large vein and three small veins remained closed. Two small vitreal hemorrhages developed within 24 hours after treatment. These were estimated to be 3/8 to 3/4 disc diameters in area.

*Experiment II.* Table II records the comparison of the treatment of large and small arteries. Background reaction was the same as in Experiment I. The amount of energy necessary to produce segmentation of a small artery was significantly larger than the amount needed to produce segmentation of a large artery. Fundus photographs demonstrated the appearance of a large artery before and after treatment (Figs. 4 and 5). At the end of coagulation, fluorescein angiography showed closure of four large arteries and one small artery (Fig. 6). In the area of coagulation there was leak-
Fig. 7. Fundus photograph showing an artery and vein coagulated by argon laser. Approximately equal areas were coagulated on the large vein (top arrows) and the large artery (bottom arrows).

Fig. 8. Fluorescein angiogram showing an artery and vein closed by argon laser. The closed vein (V) slightly overlaps the closed artery (A).

Fig. 9. Fundus photograph showing background reaction in the choroid (arrow) secondary to coagulation by argon laser in an albino rabbit.

Fig. 10. Fundus photograph showing a vitreous hemorrhage secondary to rupture of the choroidal vasculature in a pigmented rabbit (arrow).

Experiment III. Table III summarizes the results of treating both an artery and vein. The background reaction occurred with slightly less energy than did segmentation of the artery and vein. Although we recorded the average milliwatts needed, we noted during treatment that about 25 milliwatts more energy was needed to segment an artery than a vein. A fundus photograph demonstrated the appearance of...
Table III. The results of combined treatment of a large artery and vein with argon laser*

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Type of vessel</th>
<th>Average milliwatt segmentation</th>
<th>Average No. lesions</th>
<th>Average background reaction</th>
<th>No. vessels closed immediately after treatment</th>
<th>No. vessels closed at one week</th>
<th>No. of animals in which hemorrhage occurred</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Large artery and vein (100-150)</td>
<td>(340-620)</td>
<td>110</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Range of values given in parentheses.

a large vein and artery immediately after treatment (Fig. 7). Fluorescein angioscopy done at this time showed a closed artery and vein in each animal and leakage of fluorescein proximal to the treated area. Repeat fluorescein angiography at one week demonstrated a closed artery and vein in only two animals (Fig. 8).

Experiment IV. Table IV reports the effect of a minimal posterior subcapsular cataract on the coagulation of a large vein. The lens opacity increased the amount of energy necessary to produce both a background reaction and segmentation of a large vein. Fluorescein angioscopy performed immediately after treatment showed two veins closed when a cataract was present and three veins closed when none were present. At the end of one week, however, all veins were open. A small area of hemorrhage occurred in three of the treated eyes. In all cases the area was small, estimated at $\frac{1}{6}$ to $\frac{1}{4}$ disc diameters.

Experiment V. In the pigmented rabbits, it was possible to produce a background reaction with between 25 and 50 milliwatts less energy than in the albino rabbits when the beam is focused on the choroid. If a point on a large vein estimated to be three disc diameters from the fundus was focused upon and segmented a background reaction occurred in the pigmented rabbits but not in the albino rabbits. In the pigmented rabbits this reaction occurred at 125 mw., the amount of energy necessary to segment a large vein. In the albino rabbits the energy level had to be increased to 300 mw. before a background reaction occurred (Fig. 9), although when the beam is focused on the choroid, a background reaction occurred at 110 mw. During the course of treatment in a pigmented rabbit, a choroidal hemorrhage developed (Fig. 10). A narrow column of blood flowed downward from the point of injury of the choroidal vasculature.

Fig. 11. Fundus photograph showing a vitreal hemorrhage (arrow) secondary to high-energy coagulation of a large artery.

Experiment VI. High-energy coagulation of 1,000 to 1,800 milliwatts resulted in rupture of the vessel walls when these vitreal vessels were accompanied by retinal tissue. High-energy coagulation of these vessels produced numerous areas of hemorrhage immediately after coagulation (Fig. 11). When the vitreal vessels were free of tissue, hemorrhage did not occur readily. Only two hemorrhages developed in this group; one animal developed a small choroidal hemorrhage and another developed a small hemorrhage from a treated vein. The hemorrhage was about $\frac{1}{6}$ to $\frac{1}{4}$ disc diameters.
diameters in size and appeared one day later.

III. Histology. The structure of an uncoagulated vessel (Figs. 12 and 13) demonstrates the relative portion of the lumen occupied by the erythrocytes and plasma. Following treatment with argon laser vessels that remained closed at the time of fixation all contained closely packed erythrocytes within the lumen (Figs. 14 and 15). In addition to characteristic clumping of erythrocytes, the lumen of coagulated vessels also contained small plasma bubbles (Figs. 15 and 16). Platelet-like structures were noted in some areas of the coagulated vessels (Figs. 16 and 17).

The changes seen in the vessel wall depended upon the distance from the point of coagulation at which sections were taken. In the area of direct coagulation with the argon laser a portion of the vessel wall was completely disrupted (Fig. 14). Distal to the area of direct coagulation degeneration of the endothelial cells was
Fig. 13. Higher magnification electron micrograph of thin-walled intravitreal venule showing erythrocytes (E), thin basement membrane (arrow), and collagenous adventitia (COL). Note three terminal bar areas of junctional complexes (opposing arrows). Intravascular granularity is due to blood plasma (P). (×10,434.)

<table>
<thead>
<tr>
<th>No. vessels closed immediately after treatment</th>
<th>No. vessels closed at one week</th>
<th>No. of animals in which hemorrhage occurred</th>
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<tbody>
<tr>
<td>2</td>
<td>0</td>
<td>2</td>
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<tr>
<td>3</td>
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<td>1</td>
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evident (Fig. 18). In many sections of laser-treated vessels, macrophages were in close proximity to the treated vessels (Fig. 18).

Discussion

The use of NH₄Cl to simulate neovascular growth within the vitreous provides a simple model in which intravitreal arteries and veins are easily produced and can readily be identified by direct or indirect ophthalmoscopy. The limitation of this method is, of course, the fact that the intravitreal vessels do not represent vascular proliferation, but are in fact retinal vessels displaced into the vitreous. Unlike the clinical situation, a progressive pathologic process does not exist; only a simulation of a stage within that process is produced.

Xenon-arc photocoagulation could not produce permanent closure of intravitreal vessels. In the absence of a pigmented fundus it was, however, possible to produce transient segmentation of the intravitreal vessels. This occurred at an energy level much higher than the level necessary to create a background reaction. We believe the intravitreal vessels failed to close permanently because not enough energy was absorbed to cause sufficient coagulation. The lack of focusing ability of xenon-arc photocoagulation on intravitreal vessels, the
large spot size of the beam, and the diffuse wavelength of the light source all contributed to the lack of absorption of energy. In a pigmented rabbit, this disparity between amount of energy necessary for background reaction and for segmentation of the vitreal vessels was greatly increased; it was not possible to produce even segmentation without causing a massive vitreal hemorrhage secondary to choroidal bleeding. We concluded that xenon-arc photoagulation is entirely ineffective in treating intravitreal vessels and can be damaging when treating vessels in a pigmented fundus.

Unlike xenon-arc photoagulation, argon laser photocoagulation was generally effective in closing intravitreal vessels. As demonstrated by fluorescein angiography, only small arteries were resistant to argon laser therapy. Large and small veins could be closed using approximately the same amount of energy; large arteries needed slightly more energy than veins, but less than the small arteries. The velocity of the flow within the vessel might explain the difference between the amount of energy needed for segmentation of a large vein and a large artery. The amount of hemoglobin and its state of oxygenation within the lumen of each vessel may be another important factor for absorption of the energy needed for coagulation.

A very minimal lens opacity increased the amount of energy necessary to achieve immediate closure of an intravitreal vessel, as has been demonstrated previously. We believe lens opacity absorbed and scattered the light of the argon laser and, thus, decreased the amount of energy transmitted to the vessel wall.

Many of the vessels that were closed initially opened within one week. This depended on the size and type of vessel...
Large veins demonstrated the highest percentage of re-opening—80 per cent. However, none of the small veins re-opened and only 20 per cent of the large arteries re-opened (Tables I and II). Too few small arteries could be closed initially to make any evaluation. We believe that a large artery remains closed because once the proximal portion is occluded, the distal portion undergoes degeneration secondary to stasis and hypoxia. In a large vein there were, generally, some collateral vessels allowing oxygenated blood supply from the uncoagulated artery; reparative changes caused the original coagulum to wash out. Although these factors also existed for a small vein, we believe that shunting through other collateral vessels excluded these vessels from the circulation entirely, causing them to remain closed. It appears, then, that closure of the arterial portion is necessary to prolong immediate coagulation; a slow flow also increases this possibility. Combined closure of an artery and vein in our experiment did not, however, increase the probability of the artery remaining closed and did not offer any advantage over treating the artery alone.

We found that the background reaction decreases as the distance from the point of treatment to the fundus increases. When a vessel in the midvitreous is focused upon there is a scattering of the beam; thus, more energy is needed to produce a background reaction. However, in treating with laser, a background reaction occurs in a pigmented rabbit at the lowest energy level necessary to coagulate a large vein three disc diameters from the fundus. Goldberg and Herbst also previously demonstrated the possible damaging effect of the background reaction on the optic nerve and paramacular nerve fiber bundle in proliferative diabetic retinopathy in the human.
Fig. 16. Electron micrograph at higher magnification of central portion of the lesion of a laser-treated vessel (arteriole). Note platelet-like components (p) and “plasma bubble” (B). The endothelial lining of the vessel is almost indistinguishable in one area (e) and apparently absent in another (e'). Collagenous adventitia is present and erythrocytes are in a characteristic intravascular “clump” (E). (b) Basement membrane. (x8,600.)

Fig. 17. Electron micrograph at higher magnification showing wall of a laser-treated vessel. Note extensive degeneration of vessel wall, possible merging of platelet-like components (p) into vestige of the endothelial lining (e), and random arrangement of outer collagen fibrils (COL). (x11,250.)
Vitreal hemorrhage was not a serious complication of argon laser coagulation, even when a high-energy level was used, unless glial tissue was present near the vessels. In all cases of hemorrhage occurring from the treated vitreal vessels, the size of hemorrhage was small—less than one-half disc diameter. Choroidal hemorrhage occurred twice, once in a pigmented rabbit receiving normal energy and once in an albino rabbit receiving high energy. Although larger than the hemorrhages from the vitreal vessels, choroidal bleeding stopped spontaneously in a short time. The rate of vitreous hemorrhage in all animals treated with argon laser was 18 per cent, except for those in Experiment VI, in which a very high-energy level was used.

We believe retinal tissue within the vitreous has an insulating effect. Although immediate rupture of a naked vessel was very difficult to achieve with high-energy levels, it was easy to do so at the same energy levels when glial tissue immediately surrounded this vessel. Two factors may have caused this. First, this tissue possibly prevented rapid energy loss by convection and resulted in more energy being transferred to the vessel wall. Second, it possibly inhibits flexible segmentation at the coagulation site, causing it to rupture.

In conclusion, we have demonstrated that argon laser photocoagulation is effective in closing intravitreal blood vessels, but this ability is dependent on the size and type of the vessel, opacity of the media, and the amount of fibrous tissue accompanying these vessels.

REFERENCES


