*Exp. Eye Res.* (1999) **69**, 145–153
Article No. exer.1999.0683, available online at http://www.idealibrary.com on IDEAL®

# Central and Peripheral Arteriovenous Passage Times of the Retina in Glaucoma

H. FERDINAND A. DUIJM[a,b,*], THOMAS J. T. P. v.d. BERG[b,c] AND ERIK L. GREVE[d]

[a] *Department of Ophthalmology, Academic Medical Center, University of Amsterdam, The Netherlands;* [b] *Department of Medical Physics, Academic Medical Center, University of Amsterdam, The Netherlands;* [c] *The Netherlands Ophthalmic Research Institute, Amsterdam, The Netherlands and* [d] *Department of Ophthalmology, Saint Lucas and Andreas Hospital, Amsterdam, The Netherlands*

(*Received Lund 27 May 1998 and accepted in revised form 9 February 1999*)

The purpose of this paper was to estimate arteriovenous passage (AVP) times, taking into account the non-uniform distribution of arrival times over the vessel diameter, and assessment of respective differences between 15 normal controls (N), 30 primary open-angle glaucoma (POAG) and 30 normal-pressure glaucoma (NPG) patients.

Arrival times in retinal vessels were assessed from digitized scanning laser fluorescein angiograms. The arrival times were assessed as a function of position (juxtamural versus axial) in the vessel. This differentiation, based on the measurement position in the vessel, enabled the estimation of AVP times of the posterior pole and of the peripheral retina.

The overall, juxtamural and axial AVP times were prolonged in POAG as compared to both N and NPG ($P < 0{\cdot}03$). The difference in axial AVP times between POAG and normal subjects was considerably larger than the juxtamural values. The distribution of AVP times was considerably larger in POAG patients than in N subjects and NPG patients.

Retinal AVP times are prolonged in POAG patients as compared to N and NPG. The wider distribution of AVP times in POAG patients may point to a generalized microvascular alteration. Since the axial AVP times seem to provide the largest differences between NPG and POAG patients, this measurement may be preferred over more general AVP times. The axial AVP times may possibly reflect peripheral vascular changes, e.g. increased vascular resistance. The underlying mechanisms causing these differences are at present unknown.


*Key words:* fluorescein angiography; image analysis; retinal circulation; arteriovenous passage time; glaucoma.

## 1. Introduction

Angiography can be regarded as the classic method for the investigation of the circulation anywhere in the human body. With the advent of fluorescein angiography, this method has become probably the most important method in ophthalmology for the evaluation of retinal–circulatory disturbances. In daily clinical use, fluorescein angiography supplies qualitative information on the circulation of the posterior pole. However, since the early days of fluorescein angiography attempts have already been made to create a quantitative analysis of the retinal circulation. Hickam and Frayser (1965) were the first to attempt to quantify retinal hemodynamics using dye build-up curves. Others followed using various imaging techniques, e.g. photographic (Bulpitt and Dollery, 1971), video (Fonda and Bagolini, 1977; Heuven et al., 1977; Koyama et al., 1990; Suzuki, Sugihara and Kurimoto, 1992) or the scanning laser ophthalmoscope (Jung et al., 1983; Nasemann, Kantlehner and Kirsch, 1989; Wolf et al., 1993; Duijm, Van den Berg and Greve, 1997a).

In general, the retinal arteriovenous passage (AVP) times are determined using the time lapse between the average arterial and venous arrival times. This time difference can be assessed through various estimates. Some methods use a log-normal approximation to describe the arterial and venous dye-curves, from which a theoretically average AVP time is estimated (Riva, Feke and Ben-Sira, 1978; Koyama et al., 1990). Others have used the time of first appearance of the dye (Fonda et al., 1977; Koyama et al., 1990; Wolf et al., 1993; Duijm et al., 1997a), the time of maximal fluorescence, or the time of a specific percentage of fluorescence, e.g. 50 percent of the maximal fluorescence (Koyama et al., 1990; Duijm et al., 1997a). It is likely that all these different analyses have their own specific physiological significance. Methods using the very first moment of fluorescence are likely to reflect the shortest circulation path in the vascular bed. The values based on higher fluorescence intensities, e.g.75%, may reflect more peripheral parts of the circulation (Koyama et al., 1990).

Apart from technical, instrumental and analytical problems, in the first reports on quantitative as-

* Corresponding author: H. Ferdinand A. Duijm, Department of Ophthalmology (G2), Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 De Amsterdam, The Netherlands.

0014–4835/99/080145+09 $30.00/0

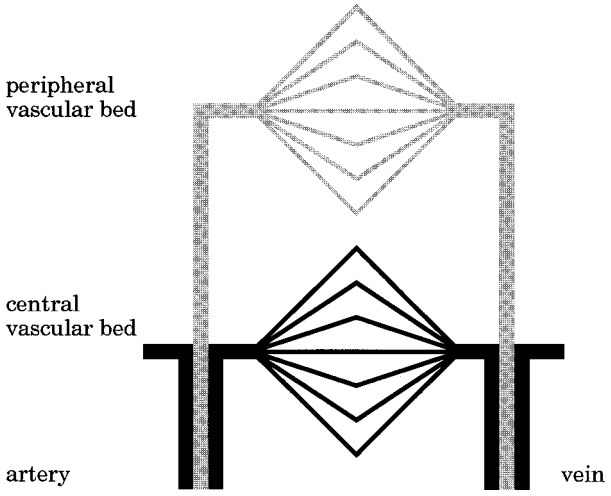


FIG. 1. A schematic view of the hypothetical separation in the peripheral and central vascular bed, based on the stratification pattern in the vessels.

sessment of the retinal circulation using angiography, the inhomogeneity of dye in the vessel is mentioned as a possible disturbing phenomenon (Hickam and Frayser, 1965). Others have given a more quantitative approach of the differences over the vessel cross section (Goldmann, 1973; Riva et al., 1978).

The spatial distribution of the arrival times within the cross section of a vessel is far from homogeneous. This is particularly recognizable through the early venous laminar flow pattern during fluorescein angiography: early filling can be found near the vessel walls and later in the center of the vessel. A reverse pattern, though less obvious, can be found in the arteries. In arteries, a more or less parabolic velocity profile exists and consequently blood in the center of arteries will arrive slightly earlier as compared to locations near the arterial walls.

Already in the first papers on fluorescein angiography, the laminar flow pattern and its origin have been discussed (Novotny and Alvis, 1961; Dollery, Hodge and Engel, 1962; Hickam and Frayser, 1965). The laminar venous flow pattern is induced by differences in passage times. The blood near the wall of vein stems from the more central parts of the retina. Blood of the axial part of the vein is thought to come from more peripheral parts of the retina. Consequently, the appearance times in veins depending on the measurement location in the vein reflect very different passage times, due to the very different path lengths in the retina. In the arteries it may be assumed that blood in the axial part of the vessel will be transported to more peripheral parts of the retina, whereas blood near the vessel walls will flow to more central parts of the retina.

This heterogeneity of arrival times can be used for further delineation of arteriovenous passage (AVP) times. As illustrated in Fig. 1, the differentiation between axial and juxtamural locations within the vessel, theoretically, allows a differentiation between the AVP time of the more central vascular bed (peripapillary and macular area) and the AVP time of the more peripheral retinal bed.

In the present study the spatial differences of arrival times over the vessels, especially their effects on the determination of retinal AVP times are analyzed. This enables the afore-mentioned separation in axial and juxtamural AVP times. The results of this analysis in normal-pressure glaucoma and primary open-angle glaucoma patients and normal subjects are presented. It has been reported before that the AVP time is prolonged in POAG patients (Wolf et al., 1993; Duijm et al., 1997a). The discrimination between axial and juxtamural passage times, theoretically reflecting the central and peripheral retinal vascular beds, may provide more insight in the location of vascular alterations.

## 2. Patients and Methods

### *Patients*

The study was approved by the Medical Ethical Committee of the Academic Medical Center. Informed consent was obtained from all patients and volunteers. In this study, 72 glaucoma patients from the outpatient clinic of the Glaucoma Center of the Academic Medical Center participated. Twenty accompanying persons and staff members were asked to participate as normal, healthy subjects.

Subjects were not included in case of lenticular or corneal disease (precluding fluorescein angiography), diabetes mellitus, severe cardiovascular disorders, history of allergy or known allergy to fluorescein or filtering surgery. Patients were classified as primary open-angle glaucoma (POAG) or normal-pressure glaucoma (NPG), in case of open angles examined by gonioscopy, glaucomatous cupping of the disk, glaucomatous visual field defects (using the 30-2 program of the Humphrey Field Analyzer) and if other reasons, e.g. secondary glaucoma, could be excluded. Glaucoma patients were diagnosed as POAG if the mean intraocular pressure (IOP) derived from a tonometric day curve was greater than 21 mm Hg. The diagnosis of NPG was based on an average IOP smaller than 21 mm Hg. Inclusion criteria for normal subjects were: no history of ocular diseases, normal findings of the slitlamp examination, ophthalmoscopy and normal intraocular pressure (IOP $\leqslant$ 18 mm Hg), ophthalmoscopy.

### *Angiography*

The angiographical technique and parts of the image analysis have been described in detail elsewhere (Duijm et al., 1997a, 1997b). In short, tropicamide 0·5% was used for mydriasis. One and a half milliliters of sodium fluorescein 25% was injected into a catheterized antecubital vein (21 G butterfly needle). The fluorescein angiograms were recorded on super-

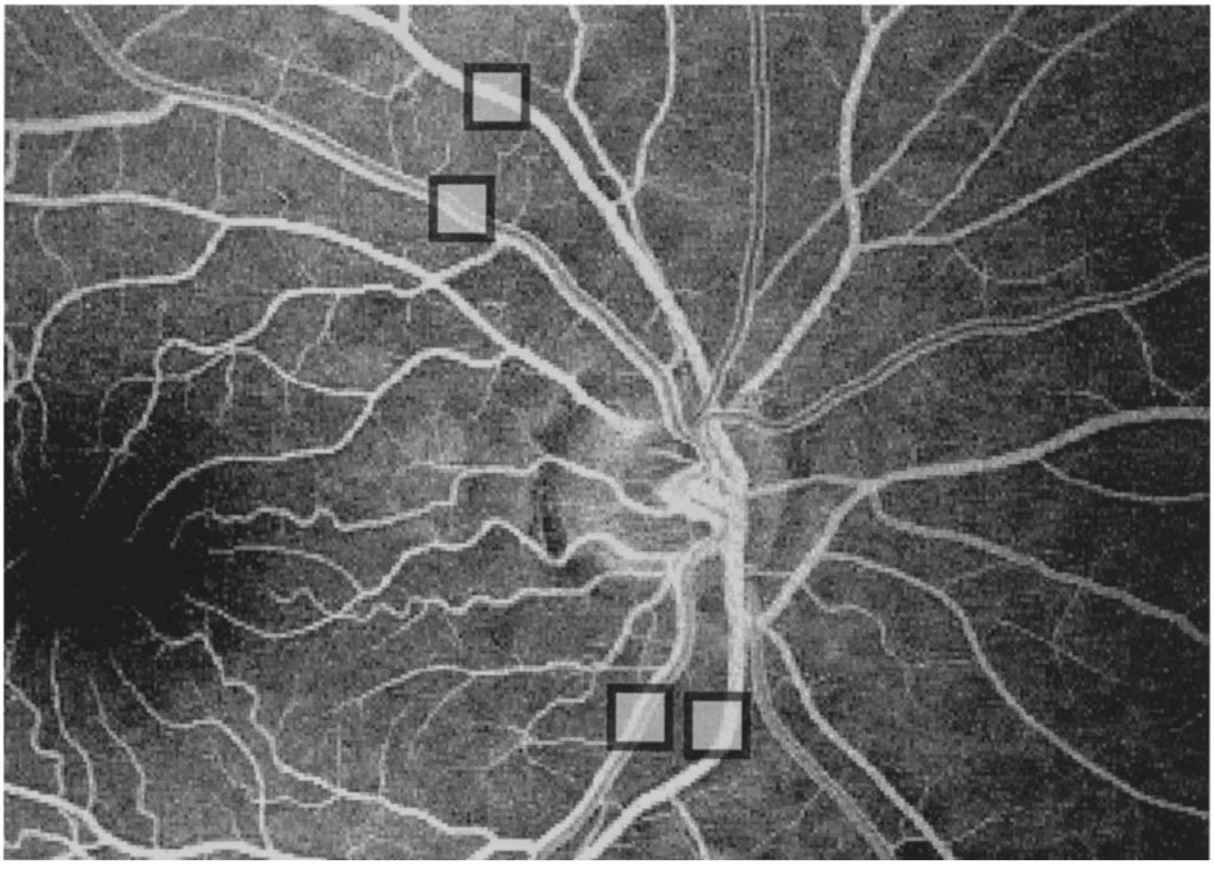

FIG. 2. An example of the locations of the two artery-vein pairs. Only pixels actually overlying the vessel are used to calculate the arteriovenous passage time.

VHS video tape (Panasonic AG-7350) using Rodenstock scanning laser ophthalmoscope. The pictures were centered around the disk. After the angiogram the IOP and blood pressure were measured; the ocular perfusion pressure was defined as:

$$\textit{perfusion pressure} = p_{diastolic} + \tfrac{1}{3}(p_{systolic} - p_{diastolic}) - IOP.$$

*Analysis*

The angiogram was digitized into 100 pictures (spatial resolution: $380 \times 228$ pixels; temporal resolution varying from 1 picture second$^{-1}$ to a maximum of 5 pictures second$^{-1}$). The pictures were automatically aligned using software as described previously (Appledorn, Oppenheim and Wellman, 1980). Interactively, four regions of interest of $16 \times 16$ pixels, (ROI) were chosen near the disk (within approximately 2 disk diameters from the center of the disk) overlying large retinal vessels; because of the relative crowding of vessels, the ROIs were not located near the disk rim (Fig. 2). They were located in such a way that in both the superior and inferior temporal quadrant, an artery-vein pair was obtained. The measurements may be influenced by the location of the ROIs. However, the standard deviation of the location of the ROIs is on average 0·017 disk diameter, corresponding to a time lapse of 0·046 seconds; this variation when compared to the arrival times, which are in the range of 17·5 seconds, is rather small (Wolf et al., 1993). As there exists a relatively high velocity in arteries and veins as compared to the capillaries, the influence on the arteriovenous passage time will be small.

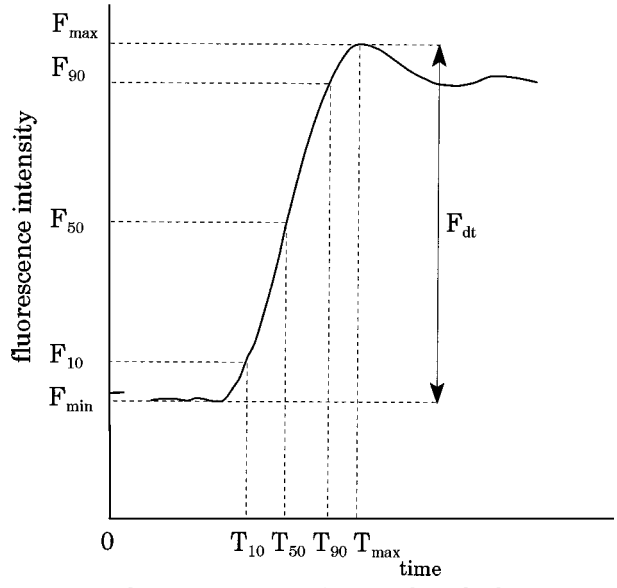


FIG. 3. Schematic diagram of a retinal dye build-up curve. $F_{dt}$ depicts the difference between $F_{max}$ and $F_{min}$ and is used to determine the relative fluorescence intensities: $F_{50}$ indicates the 50% level of $F_{dt}$ and $T_{50}$ the belonging time lapse.

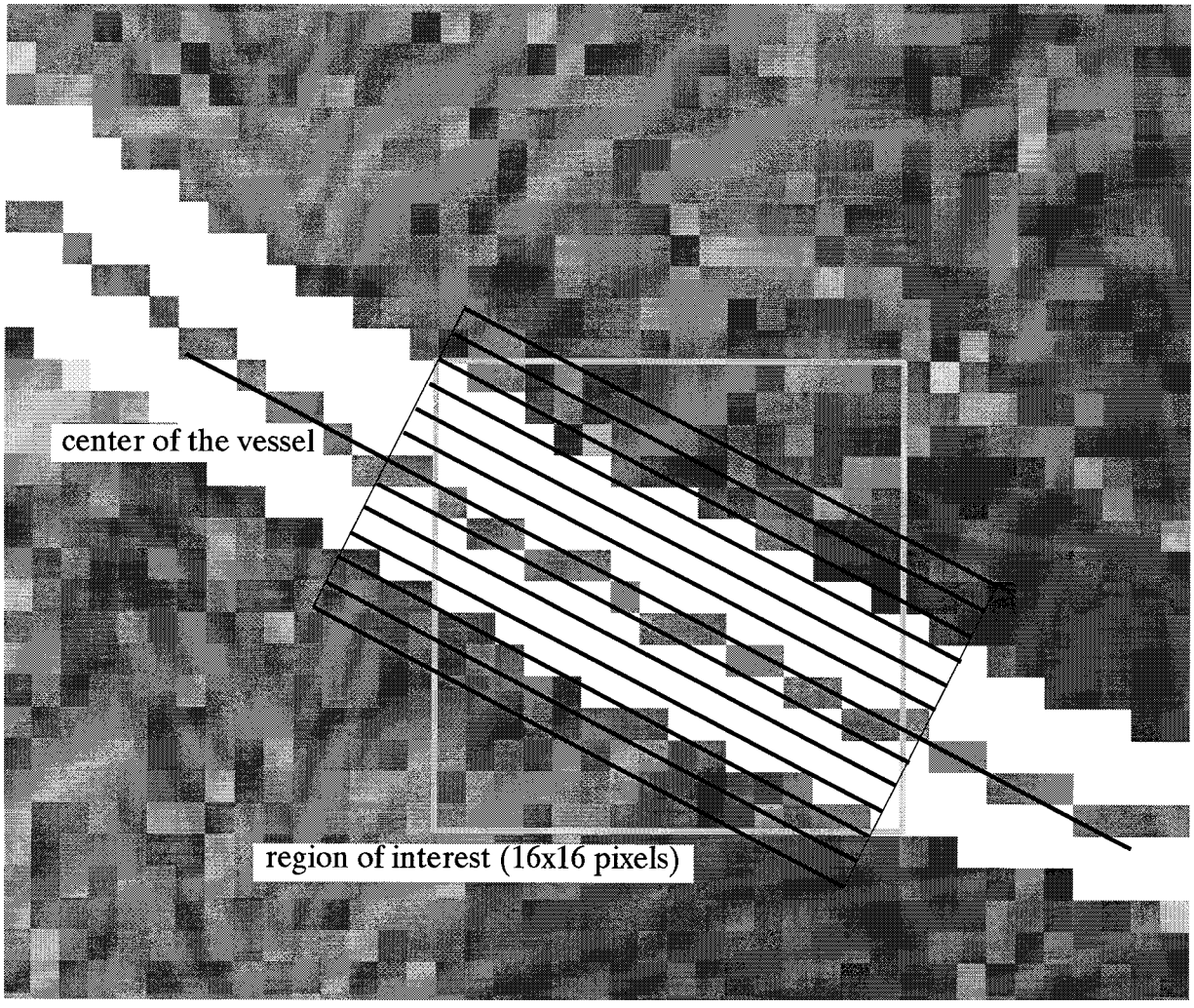


FIG. 4. Schematic overview of the construction of the cross sections of arrival times. The white pixels are the pixels actually overlying the vessel. The grey pixels in the middle of the vessel represent the pixels after 'skeletonizing', and are used for locating the straight line through the center of the vessel. The smaller rectangular regions parallel to the straight center line are used to calculate an arrival time cross section of the vessel.

By means of software, using the level of maximal fluorescence, pixels actually overlying the analyzed vessel in the region of interest were determined (usually approximately 80 pixels). For each of the $4 \times 80$ pixels, a dye build-up curve (fluorescence intensity versus time) was constructed. For each of the dye curves several parameters were calculated: the minimal, maximal and difference fluorescence intensities ($F_{min}$, $F_{max}$, $F_{dt}$), the time of maximal fluorescence ($T_{max}$) and the times at which certain percentages of $F_{dt}$ were reached: $T_p$ with $p = 10$, 50, 90% (Fig. 3). For each region of interest these parameters were averaged, only taking values from the 80 pixels actually located on the vessel.

To study the spatial distributions of the arrival times in the arteries $T_{p,a}$ and veins $T_{p,v}$ a profile of arrival times across the vessel was constructed. The center of the vessel was determined by skeletonizing pixels overlying the vessel in the region of interest. In Fig. 4, white pixels indicate pixels overlying the vessel, and grey pixels in the center of the vessel indicate the skeleton of the vessel. Through these skeleton pixels in the region of interest a straight line was fitted. Average $T_p$ values were calculated as a function of distance from the straight center line. The stripe-shaped averaging areas are schematically indicated in Fig. 4. Thus, for all afore-mentioned parameters a distribution is calculated perpendicular to the axis of the vessel. In Fig. 5, examples are given for the cross section distribution of $T_p$ values of an artery and a vein. Each vessel was divided into three parts of the vessel diameter: a central one-third–axial, and two peripheral one-thirds–juxtamural.

The spatial resolution limits the analysis of cross sections. Vessel diameters smaller than 6 pixels were excluded from the analysis. In such vessels the cross sectional analysis is less reliable because central and peripheral parts cannot be clearly separated. Therefore, the results of 5 controls, 5 POAG and 7 NPG patients were excluded from the original population. In the following, the results of 15 controls, 30 POAG patients and 30 NPG patients are presented.

To determine the retinal arteriovenous passage (AVP) times, the arterial times $T_{p,a}$ are subtracted from

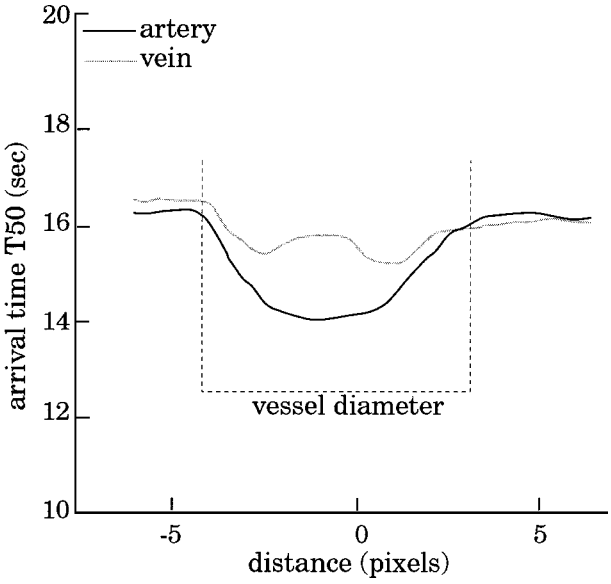


FIG. 5. An example of the arrival time cross section of an artery and a vein. Evident is the laminar flow pattern in the vein.

the corresponding venous $T_{p,v}$ values, resulting in $\Delta T_p$. Arteriovenous passage times are calculated for three intensity levels, 10, 50 and 90 percent of $F_{dt}$. The AVP times were calculated for the total of all vessels pixels, $\Delta T_{p,all}$, as well as for the axial and juxtamural parts of the vessels, $\Delta T_{p,axi}$, and $\Delta T_{p,jxm}$ respectively.

*Statistical Analysis*

For evaluation of the differences between study groups non-parametric statistical tests were used. To examine the differences in model parameters between patient groups, the Kruskal–Wallis analysis of variance (ANOVA) by ranks and the Mann–Whitney U test (MWU) were used.

## 3. Results

Table I shows some characteristics of the study groups. As follows from the definition of NPG and POAG, the IOP is considerably lower in controls and NPG patients as compared to the POAG patients. Furthermore all POAG patients received topical medication, whereas only half of the NPG patients received topical antiglaucomatous medication. The controls tended to be younger; however, correlation analysis of age on the arteriovenous passage times and other parameters showed that age did not have a significant influence. Moreover, analysis of age-adjusted parameters showed approximately the same differences between the groups. Therefore, age influences were not taken into account.

It is noted that the axial $T_{p,a/v,axi}$ and juxtamural $T_{p,a/v,jxm}$ are estimated from approximately 25 and 50 dye build-up curves respectively. In the case of, e.g., $T_{10,a,axi/jxm}$ the standard deviations are approximately 0·6–0·7, corresponding to standard errors of 0·1 and 0·09 for axial respectively juxtamural arrival times, whereas the latter are in the order of 18–19 seconds. Thus, by the relatively large averaging areas, parallel to the axis of the vessel, as indicated in Fig. 4, low standard errors are obtained.

In each patient two artery–vein pairs are analyzed: one pair is located on the superior and the other is located on the inferior temporal vascular arcade. For each of the pairs $3 \times 3$ AVP time are calculated: $\Delta T_{10,all}$, $\Delta T_{50,all}$ $\Delta T_{90,all}$ (for the overall cross section of the vessels), $\Delta T_{10,axi}$, $\Delta T_{50,axi}$, $\Delta T_{90,axi}$ (for the axial thirds) and $\Delta T_{10,jxm}$, $\Delta T_{50,jxm}$, $\Delta T_{90,jxm}$ (for the juxtamural thirds) of the vessels. Since there proved to be no statistically significant difference between superior and inferior locations, only average values are presented. In Table II the average values and standard deviations for the AVP times are given for the three study groups: the overall AVP times, $\Delta T_{10\ldots90,all}$ the juxtamural AVP times, $\Delta T_{10\ldots90,jxm}$ and the axial AVP times, $\Delta T_{10\ldots90,axi}$. Significant differences exist between the study groups for all AVP times (KW ANOVA $P <$ 0·01). The results of the MWU showed that, compared to controls, all APV times (excluding $\Delta T_{10,axi}$) were significantly longer in the POAG group (MWU $P <$ 0·03). Compared to the NPG group, all AVP times were significantly longer in the POAG group as well (MWU $P < 0{\cdot}03$). There were no significant differences

TABLE I

*Number, gender, age, corrected pattern standard deviation (CPSD), intraocular pressure, topical medication of the patients (average ± standard deviation)*

| | Normals | POAG | NPG |
|---|---|---|---|
| No. | 15 | 30 | 30 |
| Sex (m/f) | 7/8 | 17/13 | 16/14 |
| Age (years) | 56·8 ± 11·3 | 61·0 ± 12·5 | 62·7 ± 9·1 |
| CPSD (dB) | 3·4 ± 2·1 | 8·8 ± 3·4 | 9·4 ± 4·1 |
| IOP (mm Hg) | 15·1 ± 1·4 | 23·3 ± 12·6 | 15·0 ± 6·4 |
| No topical medication | 15 | 0 | 16 |
| Topical beta blockers | 0 | 27 | 14 |
| Other topical medication | 0 | 3 | 0 |

TABLE II

*Mean values ± S.D. of the average* ($\Delta T_{10,all}$, $\Delta T_{50,all}$, $\Delta T_{90,all}$), the border ($\Delta T_{10,jxm}$, $\Delta T_{50,jxm}$, $\Delta T_{90,jxm}$) *and the axial AVP* ($\Delta T_{10,axi}$, $\Delta T_{50,axi}$, $\Delta T_{90,axi}$), *as well as the differences between axial and border AVP times* ($\Delta T_{10\ldots90,jxm} - \Delta T_{10\ldots90,axi}$). *The* P *values indicate the outcome of the Kruskal–Wallis ANOVA (KWA)*

| | Normals | POAG | NPG | *P* KWA |
|---|---|---|---|---|
| $\Delta T_{10,all}$ | 2·12 ± 0·46 | 2·96 ± 0·99 | 2·28 ± 0·74 | 0·0031 |
| $\Delta T_{50,all}$ | 2·30 ± 0·66 | 3·21 ± 0·95 | 2·59 ± 0·79 | 0·0038 |
| $\Delta T_{90,all}$ | 2·50 ± 0·85 | 3·81 ± 1·41 | 2·96 ± 1·15 | 0·0035 |
| $\Delta T_{10,jxm}$ | 1·88 ± 0·48 | 2·57 ± 0·92 | 1·95 ± 0·66 | 0·0095 |
| $\Delta T_{10,axi}$ | 2·60 ± 0·57 | 3·70 ± 1·19 | 2·91 ± 0·96 | 0·0021 |
| $\Delta T_{10,jxm} - \Delta T_{10,axi}$ | 0·72 ± 0·45 | 1·13 ± 0·49 | 0·96 ± 0·49 | 0·06 |
| $\Delta T_{50,jxm}$ | 1·98 ± 0·65 | 2·74 ± 0·94 | 2·17 ± 0·73 | 0·014 |
| $\Delta T_{50,axi}$ | 2·91 ± 0·78 | 4·14 ± 1·05 | 3·41 ± 0·98 | 0·0013 |
| $\Delta T_{50,jxm} - \Delta T_{50,axi}$ | 0·94 ± 0·46 | 1·39 ± 0·47 | 1·24 ± 0·51 | 0·018 |
| $\Delta T_{90,jxm}$ | 2·06 ± 0·90 | 3·19 ± 1·37 | 2·59 ± 1·06 | 0·026 |
| $\Delta T_{90,axi}$ | 3·36 ± 1·00 | 5·05 ± 1·62 | 3·88 ± 1·29 | 0·0018 |
| $\Delta T_{90,jxm} - \Delta T_{90,axi}$ | 1·29 ± 0·78 | 1·86 ± 0·75 | 1·29 ± 0·77 | 0·011 |

TABLE III

*Mean values ± S.D. of the differences between* $\Delta T_{90,\ldots} - \Delta T_{10,\ldots}$ *indicating the width or spread of the distribution of passage times. The ratio* $\Delta T_{50,axi}/\Delta T_{50,jxm}$ *indicates the relation between the central and peripheral AVP times. The* P *values indicate the outcome of the Kruskal–Wallis ANOVA (KWA)*

| | Normals | POAG | NPG | *P* KWA |
|---|---|---|---|---|
| $\Delta T_{90,all} - \Delta T_{10,all}$ | 0·40 ± 0·66 | 0·97 ± 1·09 | 0·64 ± 0·93 | 0·13 |
| $\Delta T_{90,jxm} - \Delta T_{10,jxm}$ | 0·24 ± 0·74 | 0·71 ± 1·06 | 0·63 ± 0·84 | 0·24 |
| $\Delta T_{90,axi} - \Delta T_{10,axi}$ | 0·74 ± 0·63 | 1·50 ± 1·28 | 0·88 ± 1·08 | 0·05 |
| $\Delta T_{50,axi}/\Delta T_{50,jxm}$ | 1·52 ± 0·30 | 1·56 ± 0·25 | 1·61 ± 0·30 | 0·64 |

between controls and NPG patients. The differences between normal subjects and primary open-angle glaucoma patients are most prominent when the axial AVP times are compared.

From Table II it appears, as expected, that the juxtamural AVP times are significantly shorter than the axial AVP times. Moreover, AVP times increase when the fraction of fluorescence intensity, used for $T_p$ is increased.

In Table III the time difference $\Delta T_{90} - \Delta T_{10}$ for the overall, juxtamural and axial AVP times is given. Though this difference is twice as large in the POAG group as compared to normals and NPG patients, this difference reached only borderline significance for the axial AVP times.

In Table III the ratio $\Delta T_{p,axi}/\Delta T_{p,jxm}$ for $p = 10$, 50 and 90% is also given. No significant differences exist in these ratios between the groups; on the contrary, the ratios are rather constant.

There exist rather weak correlations between the various arteriovenous passage times and the IOP and to a lesser extent with the perfusion pressure (correlation coefficients ranging from 0·29–0·43). Naturally, as follows from the definition of the study groups, there exists a difference in IOP and perfusion pressure. The influence of the IOP and perfusion pressure on the arteriovenous passage times were investigated by analysis of variance after adjustment using the regression between e.g. IOP and studied arteriovenous passage time. ANOVA showed that the differences between the study groups could not be attributed to differences in IOP or perfusion pressure.

## 4. Discussion

Fluorescein—video—angiography and subsequent analysis of dye curves of arterial and venous locations enable estimation of retinal arterial-venous passage times. Comparing the results of controls and patients with POAG and NPG, it appears that, as reported before, AVP times are particularly slow in POAG patients (Wolf et al., 1993; Duijm et al., 1997a). The results show that the spatial distribution of the arrival times over the vessel cross section may profoundly influence the estimated arteriovenous passage times. The comparison of juxtamural and axial AVP times

show that juxtamural AVP times are considerably shorter.

In most studies, no explicit differentiation is made with regard to the nonuniformity of the flow patterns across the vessel cross section. Riva and co-workers theoretically showed that circular regions of interest, slightly larger than the vessel diameter (thus accounting for the nonuniformity), would give reliable results of an average AVP time (Riva et al., 1978).

The heterogeneity of arrival times may be used for the calculation of regional AVP times (of the peripheral and central retina). A basic assumption is that blood from a given area of the posterior pole has a preference for a certain position within the vessel cross section. The laminar flow pattern in the veins supports this concept as during venous filling stratification of the flow within the larger veins is usually apparent. Already after a very small time lapse after the appearance of the dye in the arteries, a small fluorescent stream, originating from smaller venules entering near the disk can be seen in larger veins. These streams tend to maintain a juxtamural location in the veins towards the disk. It is likely that these streams (small bands of dye) represent blood traveling the shortest pathways from the arteries to the veins. This part of the blood, traveling the shortest pathways available, is likely to originate from arterial locations near arterial vessel walls. On the other hand, blood located in the axis of larger arteries is likely to be transported to more peripheral parts of the retina. Consequently, this blood will appear in the more axial parts of veins. Therefore, it seems plausible to compare the arrival times of juxtamural parts of arteries with juxtamural arrival times in veins, and vice versa, axial arterial arrival times with axial venous arrival times.

However, there are some disturbing factors. First of all, the stratification pattern may be more complex: in some cases a number of individual streams can be observed, sometimes also in the center of a vein. This will interfere with the concept that the blood from peripheral retinal locations will be located in the center of the vessel.

Another complicating factor is that the flow pattern in the retinal vessels will be three dimensional. Angiography gives of course only two dimensional information. Yet, since the venules enter the veins in a more or less parallel plane, the stratification pattern will consist of bands approximately perpendicular to the retinal plane. Consequently, the two dimensional view of angiography will provide an adequate view of the stratification (Riva et al., 1978).

The results show that it is possible to estimate AVP times based on discrete positions within the vessel cross section. Theoretically, as illustrated in Fig. 1, the $\Delta T_{10\ldots90,axi}$ (calculated from the axial parts of the vessels) will reflect the passage time of blood with longer pathways, presumably of the peripheral retina; similarly, the $\Delta T_{10\ldots90,jxm}$ (calculated from the juxtamural parts of the vessels) will reflect the passage time through a shorter vascular bed, presumably of the central retina. The AVP times derived from juxtamural and axial parts of the vessel diameters are clearly different as expected: the $\Delta T_{10\ldots90,jxm}$ AVP times (representing the central retina) are considerably smaller than the $\Delta T_{10\ldots90,axi}$ AVP times (representing the peripheral retina).

To investigate the relation between the central vascular bed and the peripheral vascular bed, the ratios $\Delta T_{50,axi}/\Delta T_{50,jxm}$ have been calculated (Table III). These values are, on average, remarkably constant for the three study groups: for normals, POAG and NPG patients 1·52, 1·56 and 1·61 respectively. In other words, there exists a relatively fixed ratio between the central and peripheral vascular beds in terms of passage times. This fixed center-periphery relation is not influenced by the type of glaucoma, such as POAG or NPG.

Apart from the division between center and periphery, the difference between $\Delta T_{90},\ldots$ and $\Delta T_{10},\ldots$ also reflects a part of the time distribution in the vascular bed. It is plausible that the AVP time, based on an early moment of dye appearance, e.g. $\Delta T_{10,\ldots}$, reflects the shortest passage time in the bed. AVP times based on higher fluorescence intensities, e.g. $\Delta T_{90,\ldots}$, are apt to reflect more peripheral parts of the circulation (Koyama et al., 1990). Therefore, the time difference $\Delta T_{90}-\Delta T_{10}$ may be used as a measurement of the spread or width of the distribution of passage times. From Table III, it appears that the POAG patients have a larger spread in passage times, particularly the axial AVP time difference $\Delta T_{90,axi}-\Delta T_{10,axi}$, is approximately two times larger as compared to normals and NPG patients: 1·50 vs. 0·74 seconds in normal subjects and 0·88 seconds in NPG patients. For the overall and juxtamural AVP times approximately the same holds.

The retinal AVP time, $\Delta T$, reflects the time needed for a certain volume $\Delta V$, to travel a certain pathway $\Delta d$. What inferences can be drawn from a longer AVP time? First of all, if there is a lower blood flow, e.g. because of an increased vascular resistance, the arteriovenous passage time will be longer. Another speculation would be that if the vascular bed is larger, the AVP time will also be longer.

From the analysis of AVP times, we can conclude that the central and peripheral retinal vascular bed, expressed in $\Delta T_{50,axi}/\Delta T_{50,jxm}$, do not show different behavior in POAG in comparison with N and NPG. From the difference $\Delta T_{90}-\Delta T_{10}$, a measure for the distribution width of AVP times, it appears that this width is much larger in POAG patients. This might be explained by a decreased blood flow. If blood flow in e.g. the central retinal artery, would be reduced, both $\Delta T_{10}$ and $\Delta T_{90}$ would be larger; only if $\Delta T_{10}$ and $\Delta T_{90}$ increase equally, the difference $\Delta T_{90}-\Delta T_{10}$ would remain constant. However, if both parameters show a relative increase, the difference $\Delta T_{90}-\Delta T_{10}$ will be larger.

Apart from a decreased blood flow, the wider

distribution of AVP times might be due to a local change in the vascular bed. If the volume of the vascular bed is increased, e.g. under the influence of autoregulatory mechanisms, prolonged AVP times may be found as well. On the other hand, one could speculate that local vasoconstriction of e.g. arterioles causes these longer AVP times. Changes of the vascular bed have been reported in glaucoma. Generalized reduction of the retinal vessel diameters with chronic glaucomatous disease has been found (Jonas, Gusek and Naumann, 1988; Anderson, 1992), as well as more frequent localized narrowing of peripapillary arteries in glaucoma patients (Rader, Feuer and Anderson, 1994). Degeneration of capillaries has also been found (Henkind, 1967). Capillary collapse under the influence of high IOP has been mentioned as a pathogenetic mechanism (Hamasaki and Fujino, 1967). These and possibly other unknown mechanisms might account for an increased vascular resistance in POAG patients.

Apart from the changes, intravascular factors such as increased blood viscosity may play a role. Increased blood viscosity has been found in POAG patients and, particularly in combination with altered vascular structure, this may also cause increased resistance. Furthermore, retinal autoregulation may be impaired in glaucoma (Anderson, 1992; Hayreh, 1992). The increased vascular resistance in the POAG patients might be generalized, since the ratio $\Delta T_{50,axi}/\Delta T_{50,jxm}$ is rather constant between the study groups. From Table III it appears that the largest differences between normal subjects and POAG patients can be found if axial AVP times are compared: for $\Delta T_{50,axi}$ there exists a difference of 1·23 seconds. Therefore the measurement of axial AVP times may prove to be the preferred method.

It is noted that the AVP time reflects passage times; as blood flow has the unit volume over time, and no information on the volume aspect of blood flow is provided, extrapolations from AVP times to blood flow are speculative. Both decreased blood flow and increased volume may cause prolonged AVP times. However, at present, no method is available to measure ocular blood flow in humans; a combination of methods may be used to unravel the described changes.

In summary, the analysis of the spatial distribution of arrival times over the vessel cross section can be used for the determination of central and peripheral retinal AVP times; analysis of AVP times based on the first moments of fluorescence and on the moment of approximately maximal fluorescence, may provide insight into the distribution of passage times. In POAG patients the distribution of AVP times is widened and on average the AVP time is prolonged. It is postulated that this might be the result of a low average blood flow caused by local vascular changes, or a larger vascular volume. The nature of these changes and the underlying mechanisms is at present unknown.

## References


Anderson, D. R. (1992). Autoregulation in glaucoma. In *International symposium on glaucoma, ocular blood flow, and drug treatment* (Drance, S. M. Ed.), pp. 82–9, Williams and Wilkins: Baltimore, U.S.A.

Appledorn, C. R., Oppenheim, B. E. and Wellman, H. N. (1980). An automated method for the alignment of image pairs. *J. Nucl. Med.* **21**, 165–7.

Bulpitt, C. J. and Dollery, C. T. (1971). Estimation of retinal blood flow by measurement of the mean circulation time. *Cardiovasc. Res.* **5**, 406–12.

Dollery, C. T., Hodge, J. V. and Engel, M. (1962). Studies of the retinal circulation with fluorescein. *Br. Med. J.* **2**, 1210–15.

Duijm, H. F. A., Van den Berg, T. J. T. P. and Greve, E. L. (1997a). Comparison of retinal and choroidal hemodynamics in POAG and NPG patients. *Am. J. Ophthalmol.* **123**, 644–56.

Duijm, H. F. A., Van den Berg, T. J. T. P. and Greve, E. L. (1997b). Choroidal haemodynamics in glaucoma. *Br. J. Ophthalmol.* **81**, 735–42.

Fonda, S. and Bagolini, B. (1977). Relative photometric measurements of retinal circulation (dromofluorograms). A television technique. *Arch. Ophthalmol.* **95**, 302–7.

Goldmann, H. (1973). Transfer of fluorescein from blood vessels to disc tissue: The theory. *Invest. Ophthalmol.* **12**, 475–84.

Hamasaki, D. I. and Fujino, T. (1967). Effect of intraocular pressure on ocular vessels. Filling with India ink. *Arch. Ophthalmol.* **78**, 369–79.

Hayreh, S. S. (1992). Vascular factors in the pathogenesis of glaucomatous optic neuropathy. In *International symposium on glaucoma, ocular blood flow, and drug treatment* (Drance, S. M. Ed.), pp. 33–41, Williams and Wilkins: Baltimore, U.S.A.

Henkind, P. (1967). New observations on the radial peripapillary capillaries. *Invest. Ophthalmol.* **6**, 103–8.

Heuven, W. A. J. van, Malik, A. B., Schaffer, C. A., Cohen, D. and Mehu, M. (1977). Retinal blood flow derived from dye dilution curves. Televised fluorescein angiography. *Arch. Ophthalmol.* **95**, 297–301.

Hickam, J. B. and Frayser, R. (1965). A photographic method for measuring the mean retinal circulation time using fluorescein. *Invest. Ophthalmol.* **4**, 876–84.

Jonas, J. B., Gusek, G. C. and Naumann, G. O. H. (1988). Parapapillärer retinaler Gefäßdurchmesser II. Kaliberverminderung in Glaukomaugen. *Klin. Monatsbl. Augenheilkd.* **192**, 693–8.

Jung, F., Kiesewetter, H., Körber, N., Wolf, S., Reim, M. and Müller, G. (1983). Quantification of characteristic blood-flow parameters in the vessels of the retina with a picture analysis system for video-fluorescence angiograms: initial findings. *Graefes Arch. Clin. Exp. Ophthalmol.* **221**, 133–6.

Koyama, T., Matsuo, N., Shimizu, K., Mihara, M., Tsuchida, Y. and Wolf, S. and Reim, M. (1990). Retinal circulation times in quantitative fluorescein angiography. *Graefes. Arch. Clin. Exp. Ophthalmol.* **228**, 442–6.

Nasemann, J. E., Kantlehner, R. and Kirsch, C. M. (1989). Die Fluoresenz-Perfusions-Szintigraphie. Eine neue Methode zur Quantifizierung okulärer Durchblutungsstörungen. *Fortschr. Ophthalmol.* **86**, 732–7.

Novotny, H. R. and Alvis, D. L. (1961). A method of photographing fluorescence in circulating blood in the human retina. *Circulation* **24**, 82–6.

Rader, J., Feuer, W. J. and Anderson, D. R. (1994). Peripapillary vasoconstriction in the glaucomas and the

anterior ischemic optic neuropathies. *Am. J. Ophthalmol.* **117**, 72–80.

Riva, C. E., Feke, G. T. and Ben-Sira, I. (1978). Fluorescein dye-dilution technique and retinal circulation. *Am. J. Physiol.* **234**, H315–22.

Suzuki, R., Sugihara, I. and Kurimoto, S. (1992). Retinal circulation in primary open-angle glaucoma tested by videodensitometric image analysis. *Ann. Ophthalmol.* **24**, 273–7.

Wolf, S., Arend, O., Sponsel, W. E., Schulte, K., Cantor, L. B. and Reim, M. (1993). Retinal hemodynamics using scanning laser ophthalmoscopy and hemorheology in chronic open-angle glaucoma. *Ophthalmology* **100**, 1561–6.