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The regional distribution of retinal circulation

David W Hill FRCS DO

Research Department of Ophthalmology, Royal College of Surgeons of England

Summary

The retinal circulation of the cat has been studied in vivo by high-speed cineangiography and post mortem by indian ink injection. Flow patterns in the arterial tree, relative volume inflow per unit area, and capillary circulation times were recorded. The retinal circulation shows progressive slowing of linear flow rate in arterioles and capillaries on passing centrifugally from the optic disc to the periphery; this is largely responsible for the reduced perfusion rate.

Introduction

The purpose of this lecture is to discuss the distribution of retinal blood flow within the anatomical constraints imposed by the structure of the eye. Human retinal circulation has the following anatomical characteristics: it is centrifugal, two-dimensional, and devoid of anastomoses, the central retinal artery and its branches being end arteries. The centrifugal nature arises from the development of the optic vesicle with a single vascular pedicle in the optic nerve. The central retinal artery branches dichotomously, spreading peripherally from the optic disc to supply the whole area of the retina, as illustrated in Figure 1. The thought at once arises, how is the blood flow distributed to maintain a balance between proximal and distal tissues? Though the final regulator is physiological control of peripheral resistance, dictated by local metabolic demand, an anatomical component in the vascular architecture might be expected;

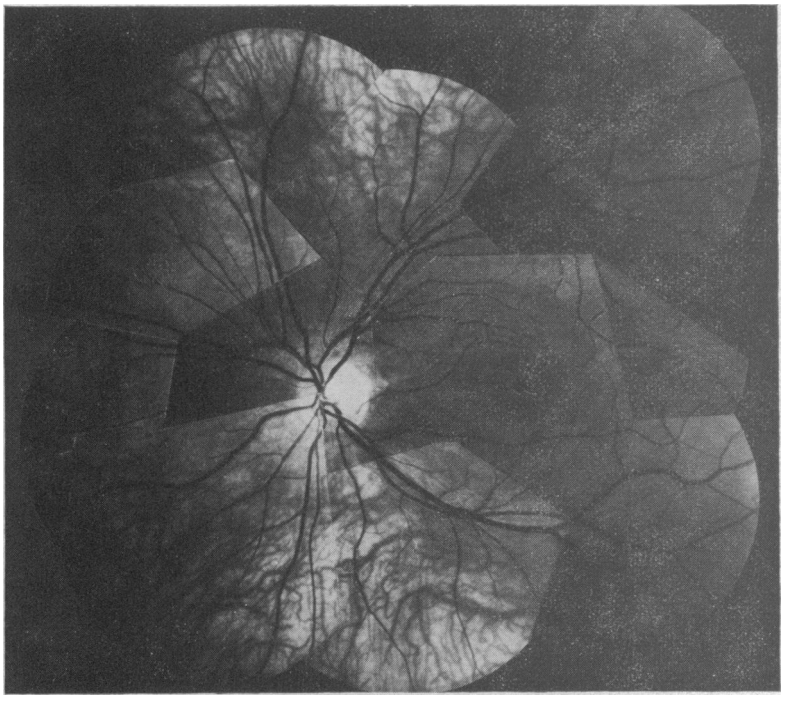

FIG. 1 *Montage of a human retinal vascular tree, left eye, from red-free fundus photographs. The central retinal artery divides at the optic nerve head to give four principal branches, which pass towards the periphery, branching as they go. The arteries may be distinguished from the veins by their relative pallor.*

Based on an Arris and Gale Lecture delivered on 10th January 1977.

so studies in my department have been directed to the correlation of blood flow and vascular anatomy in the retina.

Critical comparison of flow data in the living organism with anatomical detail after death could be made only on an animal model, and for this purpose the cat was selected on the grounds of feasibility and knowledge of the retina available from previous studies. The cat retina has a capillary network distributed in layers similar to the human[1], and arterial occlusion results in a similar retinal necrosis extending to the inner nuclear layer[2]. It differs in being supplied by a number of branches from the ciliary arteries which pass through the optic disc to spread centrifugally over the retina. These vary in size; some small arteries supply the retina within a short distance of the disc, large arteries supply big sections of the retina but seldom branch near the disc, while intermediate arteries may supply a narrow tongue of retina passing peripherally from the disc.

Techniques and limitations

Flow in the intact animal was studied by high-speed cineangiography at framing rates up to 141 pictures per second. The calibre of retinal arterioles was measured from the photographic image of the fluorescein-filled vessel, either by densitometry[3] or by direct measurement using a low-power microscope with a screw micrometer eyepiece and observing the projected photographic image. The morphology of the vascular tree was observed in the retina injected with indian ink post mortem[4]. Cineangiography was accomplished with a modified Zeiss fundus camera in which the 35-mm format was reduced to 16-mm format and recorded by a Photosonics IP scientific cine camera[5]; 40 μl of 5% fluorescein was injected into the ipsilateral carotid artery through the lingual branch. The success of this technique depends on keeping the eye quite steady, and for this purpose a metal ring attached to the head holder was sutured to the periphery of the cornea in the anaesthetized animal; optical clarity was maintained by an afocal contact lens. Experiments in which the eye rings were applied to and removed from each eye at differing times showed no detectable alteration in flow rate due to the presence of the eye rings provided they were carefully applied without obvious pressure on the globe.

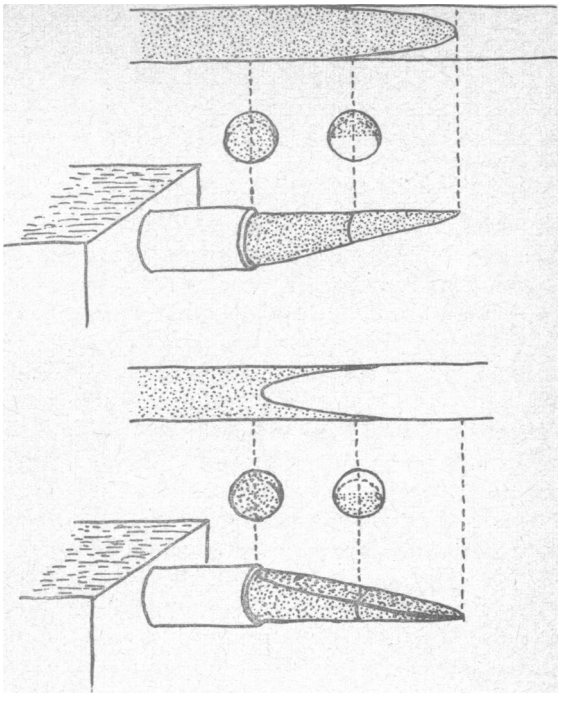

FIG. 2 *Model illustrating how the angiographic appearance of a fluorescein bolus is modified by the position of the dye front because fluorescence of the blood column is visible only from a superficial crescent of the arteriole. Above, a peripheral dye front is situated superficially on the aspect nearest the observer, and the angiogram is a projection of its outline. Below, the dye front lies deep and is hidden by the overlying blood; angiographically it becomes visible only in the later stages, when the superficial part of the artery is filled. The result is two peripheral streams of fluorescence which spread centrally and coalesce.*

Following the regional arterial injection of fluorescein a visible dye front enters the retinal arterioles, the exact instant of appearance differing a little for each vessel. In successive frames of the angiogram centrifugal advance of the dye bolus can be followed and its velocity calculated. Occasionally the bolus is well formed and its tip clearly defined; more often it is poorly defined owing to irregular distribution across the arteriole (Fig. 2). In addition, the apparent position of the tip may vary with slight changes in exposure due to photographic factors. Because of the rapid framing rate it is possible to use a 'digital' technique, counting the number of frames

which elapse while the dye passes between two selected points on an arteriole, rather than an 'analogue' technique, measuring the dye front advance directly; consequently the proportion of arterioles available for measurement is much greater.

Two other factors introduce uncertainty into the estimation of flow rate, cross-sectional velocity profile, and pulse wave. If blocks of consecutive angiograms are taken using first fluorescein and then indocyanine green (ICG) for infrared (IR) absorption angiography it is found that the respective mean flow rates have the ratio 1 : 1.19[6]. This is due to the translucency of the blood vessels to IR radiation[7], which allows peak, axial, flow to be recorded. whereas fluorescein permits only a superficial layer of the blood stream to be photographed. Unfortunately the present technique is not sufficiently precise to decide the form of the cross-sectional profile[8]. While both dyes show a pulse fluctuation of roughly ±10% about the mean[6], there is a phase difference between the two, the fast phase of the peripheral blood (fluorescein) lagging behind the axial stream (ICG).

Practically, the existence of a number of sources of error means that the significance of all flow measurements must be established statistically; experiments must be appropriately planned either to allow a comparison of variances due to experimental effect with the residual or to seek a correlation between flow and some other factor.

Distribution of arterioles

The reduction in thickness of the ganglion-cell and bipolar layers of the retina on passing from the centre to the periphery[9], coupled with reduction in the capillary bed density[1, 10], suggested a peripheral reduction in blood flow and prompted an investigation of the areas of retina supplied by arteriolar branches. For this purpose the retina was divided into three zones bounded by circles concentric with the optic disc, as shown in Figure 3.

The calibre of 243 arteriolar branches of varying size was measured and plotted according to the zone supplied, or predominantly supplied if more than one zone was involved. A significant positive regression of area supplied on calibre was demonstrated in all three

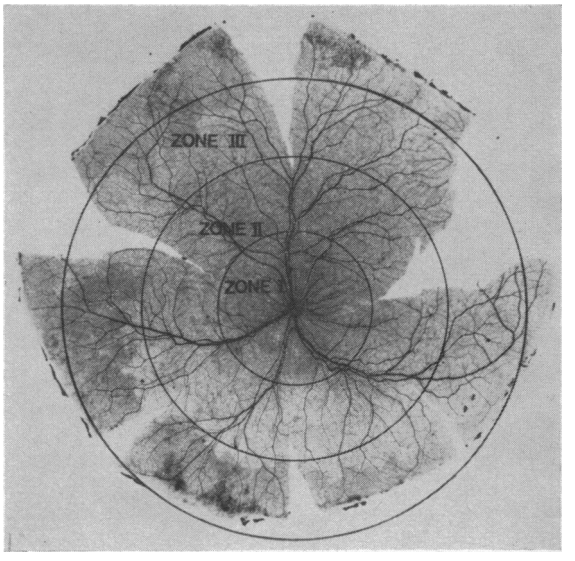

FIG. 3 *Ink-injected postmortem retina dissected and flattened, with circles superimposed to divide the surface into three concentric zones; the radii are in the proportions 1 : 2 : 3. The outer circle is placed to exclude as much retina as will balance the defects within the circle.*

zones[4]. Comparison of the areas supplied by arterioles of grand mean calibre showed that they increased on passing peripherally from Zone I to Zone III in the ratios 1 : 3.2 : 5.4.

In a separate study the total cross-sectional area of the arteriolar vascular tree was studied for 7 arterioles in 4 eyes, the calibre of the parent vessel being measured near the optic disc and those of all its major branches as they crossed the boundaries of Zones I–II and II–III. Since blood is leaving the arteriolar tree all along its length from the optic disc to the periphery, the cross-sectional areas were corrected by proportional increases in the peripheral values to bring them to the level which would have been expected if they had conducted the total flow without loss. The mean ratios of the corrected cross-sectional areas in Zones I–III were 1 : 1.90 : 6.74 and the mean calibres of the arterioles in Zones I–III were 95, 59, and 55 μm respectively. In such a hypothetical vascular tree the mean linear flow rate would be to a first approximation (disregarding the cross-sectional velocity profile) inversely proportional to the cross-sectional area; when the actual flow rates (presented in the next section) for vessels of

mean calibre in each zone were expressed as reciprocal ratios the figures were 1 : 1.92 : 3.33 respectively. The ratio for Zones I and II agrees with the cross-sectional ratio, but the Zone III disparity may relate to difficulty with Zone III flow rates.

Flow studies

In 12 eyes of 6 cats the linear flow rates and calibres at 129 arteriolar sites distributed throughout the different zones of the retina were studied[11]. In 8 instances the studies covered the superior arterioles and in 4 the temporal. Post mortem the injected retinae were studied to assess the area supplied by each arteriole whose flow had been measured. A significant regression of flow rate on calibre was found in Zones I and II but not in Zone III; the ratios of flow rates for an arteriole of grand mean calibre were 1 : 0.7 : 0.6, the figure for Zone III representing the mean flow rate in this zone. The reason for the failure of arterioles supplying Zone III to conform to the pattern of the other zones is uncertain, but it may have been due to taking measurements at differing distances from the optic disc.

From the linear flow studies and calibre measurements relative volume flow rates were calculated. Significant positive regressions of volume flow on area supplied were found in all zones of the retina, despite the absence of a correlation between linear flow rate and calibre in Zone III already recorded. The relative volume flows in each zone, corresponding to the grand mean areas supplied, were expressed as ratios signifying relative volume flow per unit area; the results were 1 : 0.5 : 0.3 in Zones I–III respectively.

Additional studies were made on the effects of branching on linear flow rate in relation to the angle and calibre of the constituent vessels. Two situations were studied: dichotomous branching to form two vessels of approximately equal size in 14 cases and unequal branching in 42 cases[11]. In dichotomous branching there was a mean decrease of flow of 27% in the branches (range 0–60%) and vessel calibre was reduced by 15% (range 0–27%). These changes accord with a theoretical model of dichotomous branching with unchanged flow resistance. In unequal side-branching the mean decrease in linear flow rate in the main vessel was 25% (range 0–54%), whereas in the side-branch it was 48% (range 3–82%). The mean diameter of the side-branches was 58% of the parent vessel (range 21–91%) and of the main vessels 98% of the parent vessel before the branch (range 60–100%). Analysis of partial correlations with the linear flow rate showed a significant effect due to the calibre ratio of the branch and parent vessel but none due to the angle of branching.

Capillary circulation times

While the flow studies just described were being made it was discovered that the time taken for the dye to cross the capillary bed was longer in the peripheral areas than centrally. One explanation might have been that, in common with blood in the arterioles, flow was slower in the periphery. Alternatively, the path through the capillary bed might be longer. Further investigation of the capillary crossing time linked to a study of the capillary bed anatomy was undertaken[12].

The technique of cineangiography described in this lecture allows clear visualization of the filling of the terminal precapillary arterioles and, after an interval, of the postcapillary venules, but details of the capillary flow are not seen. The capillary circulation time (CCT) was determined from the interval between these two events.

In 4 eyes 40 sites were studied, distributed between the three zones; a mean CCT of 0.43, 0.63, and 0.93 s was found in Zones I, II, and III respectively, the corresponding standard deviations being 0.07, 1.7, and 2.3; the differences in the means were significant ($P<0.001$) by Student's t test. In the ink-injected retina the same sites were identified and studied by making camera lucida drawings of the flattened dissected retina. Two levels were discerned and drawn: the superficial contained precapillary arterioles and postcapillary venules but few true capillaries; the deeper layers consisted of capillary loops passing to various depths, the reduplication of deeper layers classically described[10, 13] being a random distribution at different depths rather than a system of stratified networks. From these drawings two measurements were

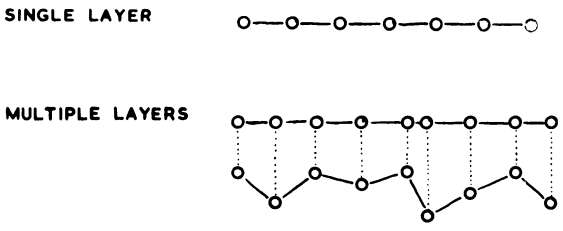

FIG. 4 *Model to illustrate how capillaries at different depths may be superimposed to give an appearance of crowding though their separation remains constant.*

made, the number of arteriolar terminals per unit area of capillary bed and the mean distance between adjacent capillaries in the deeper layer. Precapillary arteriolar terminals were defined as the points where straight precapillary arterioles gave way to sinuous capillaries, usually passing to the deeper layer of the retina. They were found to have a remarkably uniform population density over all three zones of the retina, the immediate peripapillary area and the extreme periphery being excluded for technical reasons. The mean intercapillary distance also showed little change in Zones I and II, where the deeper layers were stratified, but became slightly greater in the periphery, the figures for Zones I, II, and III being 56, 57, and 67 μm respectively; only the latter pair of means were statistically significantly different. Since the method of drawing results in superimposition of the capillaries at different depths, their apparent crowding in Zones I and II may be an artefact (Fig. 4).

The stability of the population density of the precapillary arteriolar terminals implies that the mean capillary path length is similar in all three zones of the retina[12]. In this event the mean flow rate will be inversely proportional to the capillary circulation time, the ratios in Zones I–III being 1 : 0.68 : 0.46. Supporting evidence has been adduced from the volume inflow per unit area calculated from larger arterioles of measurable calibre. The capillary linear flow rate is directly proportional to the volume flow rate per unit area and inversely proportional to the cross-sectional area of the perfused capillary bed, which was estimated from the intercapillary distance[12]. The ratios for linear flow rate derived by this approach were 1 : 0.65 : 0.47, very little different from the CCT-derived ratios. These results support the hypothesis that linear flow in the capillaries is slower in the periphery of the retina than centrally.

Discussion

From the material presented here a dynamic concept of the retinal circulation can be formed. Arterial inflow near the disc is pulsatile, with a 20% fluctuation and some phase shift of the cycle in the central core relative to the peripheral laminae. Flow rate in the large arterioles is always greater than in the small but declines gradually in all vessels on passing from the disc towards the periphery, the degree being consistent with the expansion of the corrected cross-sectional area of the vascular tree. Studies of branch configuration and flow shows that the relative vessel calibres determine the division of blood flow; the effect of dynamic factors due to angle of branching did not reach a significant level.

Retinal perfusion, as measured by volume inflow per unit area, falls progressively towards the periphery, as might be expected from the reduction in number of neurones[9]. This is achieved principally by a big increase in CCT, by a factor of 116%, and to a much smaller extent by a reduction of the capillary bed. The figures for Zones I and II suggest that the increase in CCT preserves the same proportional reduction in linear flow rate as is occurring upstream in the arterioles suitable for direct angiographic measurement.

The result of reduced linear flow in the capillaries must be a reduction in the exchange of the smaller 'flow-limited' molecules; but other capillary exchanges, particularly that of oxygen, may be less reduced owing to the longer period available for equilibration[14].

As a corollary to this reduction of linear flow there must be either a considerable pressure drop over the distributing arterioles from centre to periphery or a greatly increased peripheral resistance in the precapillary arterioles, which maintain the same population density in the periphery.

The model of retinal circulation just described serves two obvious purposes. Firstly, it is a necessary prerequisite for further investigation of the retinal circulation in the experimental animal. Secondly, it establishes a fundamental difference in the nature of the circulation in different zones of the retina; it confirms the qualitative impression of a slower

peripheral circulation gained from clinical venous angiograms. This is a factor of importance in vascular retinopathies with peripheral ischaemia.

While discussion has centred so far on the distribution of blood flow under normal conditions, some preliminary experiments suggest that the artificial reduction of flow in a large retinal arteriole, by photocoagulation of a part of the capillary bed, may have a variable effect on the inflow into its smaller branches serving uncoagulated retina. The effect depends on the flow rate in the parent arteriole; when the reduction of flow following photocoagulation is slight the branch arterioles have a normal or occasionally increased flow; when it is marked the flow in the branches is reduced. The calibre of the vessels is not significantly changed in either instance. The effect may due to increased viscosity at low shear rates, a factor which could aggravate the reduction of peripheral circulation when retinal blood flow is pathologically reduced.

Conclusion

The microcosm of this Arris and Gale Lecture is far removed from the 'public anatomy' which was the founders' intention; yet I hope it is acceptable for it touches on an anatomical subject of clinical interest uniquely displayed in the eye and studied by methods of traditional dissection as well as modern photographic technology.

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