Studies of the Retinal Circulation in Man

Observations on Vessel Diameter, Arteriovenous Oxygen Difference, and Mean Circulation Time

By John B. Hickam, M.D., and Regina Frayser, Ph.D.

Systemic disorders often cause pathological changes in the optic fundus. These changes have diagnostic value because of their empirical association with specific illnesses. The value of funduscopic examination would be even further increased by a better understanding of how certain of these fundic lesions are produced. With the ultimate object of investigating this problem, several photographic techniques for study of the retinal circulation and metabolism in man have been adapted or developed and used by the authors and their associates. These techniques are: (1) measurement by fundus photography of changes in the diameter of retinal vessels in response to various stimuli, a procedure previously used by others; (2) estimation by fundus photography of the arteriovenous blood oxygen difference, (3) a method of photographing fluorescence in circulating blood in the retina, and (4) a method for measuring the mean retinal circulation time using fluorescein. It is the purpose of this presentation to summarize some results obtained by these special techniques and to interpret them in relation to the work of others in this rapidly expanding field.

Methods

The methods have been described elsewhere.

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and will be outlined only briefly here. In most of this work the Zeiss fundus camera has been used. For the measurement of change in vessel diameter, fundus photographs were made with the subject usually in a seated position, first in the control state and then after various test procedures. From these photographs, measurements were made of the arteries and veins near the disk using a dissecting microscope with a scale in the ocular. Changes in diameter were expressed as a percentage of the control value, and the mean changes for arteries and veins were calculated separately. For examination of patients with retinopathy, the procedure most often used was the inhalation of 100% oxygen, which constricts retinal vessels. The terms, "retinal arterial reactivity" and "retinal venous reactivity," denote the mean percentage decrease in diameter of these vessels after inhalation of oxygen for 5 minutes.

The method for measuring oxygen saturation of retinal venous, or arterial, blood was similar in principle to other photometric methods for measuring blood oxygen saturation. However, relative light intensities were estimated by the density of the images which they produced on photographic film rather than by a photocell. For fundus photographs a beamsplitting device was used that allows making two simultaneous film exposures, one through a red interference filter with peak transmission at 640 mµ and the other through a green filter with peak transmission at 510 mµ. The optical density of reduced hemoglobin is much greater than that of oxyhemoglobin at 640 mµ, but is the same at 510 mµ. The per cent oxygen saturation of hemoglobin and whole blood bears a linear relationship to the ratio of optical densities at these red and green wavelengths. In retinal photography the light which strikes the optic disk is largely reflected. The vessels crossing the disk are traversed twice by this light, once on the way to the underlying disk and once after reflection. The effect is somewhat like placing a thin cuvette filled with blood in front of a large light source. When the photographic negatives are projected onto a screen which has a small perforation with a photocell mounted behind it, the density of any portion of the
negative can be easily read. Within the proper exposure range, the difference in density between the image of a vessel and that of the adjacent disk is proportional to the optical density of the vessel which is primarily owing to the contained blood. The ratio of this density difference by red light to the density difference by green light equals the ratio of optical densities at these wavelengths and is, accordingly, linearly related to the blood oxygen saturation. The slope and position of this line were obtained by measurements on retinal arteries with the arterial blood oxygen saturation adjusted to different, known values by having the subject breathe low oxygen mixtures. The relationship is as follows:

\[
\text{Per cent blood oxygen saturation} = 118 - 188R,
\]

where \( R \) is the ratio of the red to the green density measurements. The standard deviation from regression was 4.2% saturation. For most purposes this equation was applied to the estimation of mean blood oxygen saturation in the retinal veins of both eyes of a subject, and the results were averaged.

The basic fluorescence method simply consisted of fundus photography after the intravenous injection of fluorescein, with use of appropriate filters to emphasize fluorescence by reducing the intensity of other wavelengths of light.

The method for measuring mean retinal circulation time was based on estimation of the relative concentration of fluorescein in the blood from density measurements of the image of the large retinal vessels in photographic negatives. When these measurements are made on a series of negatives (fig. 1) taken at 1- to 2-second intervals after the injection of fluorescein into a peripheral vein, it is possible to construct separate arterial and venous time-concentration curves. The mean time between injection of fluorescein and its arrival in the retinal arterial system was determined from the arterial curve by the conventional Stewart-Hamilton formula.

**Figure 1**

Serial photographs, taken every 1.5 seconds, showing the passage of fluorescein through the retinal circulation in a normal eye. The photograph marked "O" is the last in the series before the appearance of fluorescein. The next picture, 1.5 seconds later, shows the arteries filling with fluorescein. Subsequently, the veins fill. Venous stratification is prominent.
tion. The mean time between injection and arrival in the large retinal veins at the disk was similarly determined. The venous time less the arterial time is the mean transit time through the retinal vascular system. The time required for a detectable quantity of fluorescein to traverse different parts of the retinal vascular tree can be relatively easily determined by cinematography, but this information has more limited quantitative value than the determination of mean time.

**Observations**

**Visible Reactions of the Normal Retinal Vessels**

The effect of a number of different procedures on the size of the larger retinal vessels in normal young adult males is summarized in table 1. Cusick and associates first reported that the retinal vessels dilate at low ambient oxygen tensions and constrict at high tensions, and this finding has been amply confirmed. Vasoconstriction is apparent within a minute after the start of oxygen breathing, but the full effect takes nearly 5 minutes to develop. The response to oxygen is unaffected by stellate block and is still present after thoracic sympathectomy. Hyperbaric oxygen causes even greater vasoconstriction than oxygen at atmospheric pressure. Dollery and associates have found that the smaller retinal arteries and veins constrict proportionally more than the larger vessels near the disk at both atmospheric and hyperbaric oxygen tensions.

The mechanism by which changes in blood oxygen bring about changes in retinal vascular caliber is not understood. Conceivably the vessel wall may be responding directly to changes in oxygen tension or to local metabolic changes in retinal tissue induced by variations in oxygen tension. Table 1 shows the marked vasodilation which developed 30 seconds after release of pressure applied to the eye for 10 seconds with enough force to obstruct diastolic flow at the disk margin. This pronounced reactive hyperemia is compatible with the suggestion that local metabolic changes may be important in regulating retinal vascular tone.

Carbon dioxide inhalation has been reported to dilate retinal vessels in both man and the dog, but in our experience with man inhalation of 10% CO2 has had little effect on the diameter of the larger vessels. When combined with 90% O2, however, 10% CO2 prevents a significant part of the vasoconstriction which otherwise results from breathing high concentrations of oxygen. In addition, evidence to be presented later indicates that increasing arterial CO2 tension increases the retinal blood flow, apparently by dilating vessels smaller than those being measured by the present technique. Changing from the seated to the recumbent position constricts retinal vessels, as shown in table 1. This change in position increases retinal arterial pressure. Presumably, the retinal venous pressure, which remains above the intraocular pressure, is less affected by the change in body position so that the net retinal perfusion pressure is increased.

The change in vessel caliber, if shared by

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**Table 1**

*Change in Diameter of the Larger Retinal Vessels after Different Stimuli in Normal Subjects*

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of subjects</th>
<th>Retinal vascular response, %*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Arterial</td>
</tr>
<tr>
<td>Change from air to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% O2</td>
<td>50</td>
<td>−11.6 ± 4.7</td>
</tr>
<tr>
<td>10% O2</td>
<td>6</td>
<td>+10.1 ± 3.6</td>
</tr>
<tr>
<td>10% CO2, 21% O2</td>
<td>10</td>
<td>−2 ± 5</td>
</tr>
<tr>
<td>10% CO2, 90% O2</td>
<td>10</td>
<td>−3 ± 4</td>
</tr>
<tr>
<td>After pressure on eye</td>
<td>8</td>
<td>+13.8 ± 5.9</td>
</tr>
<tr>
<td>Seated to recumbent</td>
<td>8</td>
<td>−10.1 ± 3.9</td>
</tr>
</tbody>
</table>

*Per cent change in vessel diameter from control state, mean ± standard deviation.
†Change from inhalation of air.*
the resistance vessels, is consequently in a direction to oppose changes in retinal blood flow resulting from gravitational effects on the arterial blood column. These postural changes in retinal vessel size are unaffected by stellate block.

In a few trials in normal subjects, the combination of recumbency and oxygen breathing produced more retinal vasoconstriction than either procedure alone.14 Changes in the caliber of retinal veins might be active in the sense of reflecting alteration in the tone of the vessel, or they might simply be a passive consequence of change in venous pressure secondary to the change of blood flow with arterial reactions. In an attempt to differentiate between these possibilities a sphygmomanometer cuff was inflated around the neck of two subjects to a pressure of 60 mm Hg. This caused moderate dilation of the retinal veins, but breathing oxygen with the cuff still inflated reduced venous caliber below the control level while breathing air.14 This result supports the concept of active venoconstriction during oxygen breathing.

The reactions of the retinal vessels to change in blood gases and body position apparently tend to stabilize the environment of the tissue by adjusting blood flow according to metabolic requirements and by reducing fluctuations in blood flow caused by changes in perfusion pressure. Because of the high metabolic rate of the retina, it is probable that these vascular reactions are important to the maintenance of function under stress of various kinds.

Retinal Vascular Reactivity in Disease

In generalized vascular disease the retinal vessels are often changed in appearance. To determine how vascular disease may affect the response of retinal vessels to a stimulus, observations were made on the effect of breathing oxygen for 5 minutes in patients with hypertension, diabetes, and atherosclerosis.3, 17, 18 The percentage decrease in vessel diameter caused by oxygen was designated "retinal vascular reactivity." The results of these studies are summarized in table 2.

In normal persons the retinal arterial reactivity tends to decline gradually with age.3

Table 2

Retinal Vascular Reactivity to Breathing 100% Oxygen in Normal Persons and Patients with Vascular Disorders

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of subjects</th>
<th>Arterial Retinal vascular reactivity*</th>
<th>Venous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control patients without</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>vascular disease (age 13-76 yrs.)</td>
<td>47</td>
<td>11.3 ± 4.3†</td>
<td>14.5 ± 5.8</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>without proteinuria</td>
<td>17</td>
<td>5.2 ± 4.9‡</td>
<td>12.9 ± 3.9</td>
</tr>
<tr>
<td>1+ Proteinuria</td>
<td>11</td>
<td>4.4 ± 5.5‡</td>
<td>8.5 ± 4.7‡</td>
</tr>
<tr>
<td>2+ Proteinuria</td>
<td>7</td>
<td>0.6 ± 0.6‡</td>
<td>4.5 ± 2.8‡</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>without retinopathy</td>
<td>20</td>
<td>8.2 ± 4.7‡</td>
<td>12.8 ± 5.3</td>
</tr>
<tr>
<td>Normotensive,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>with retinopathy</td>
<td>15</td>
<td>3.8 ± 3.8‡</td>
<td>10.8 ± 5.5‡</td>
</tr>
<tr>
<td>Hypertensive</td>
<td>15</td>
<td>4.1 ± 4.3‡</td>
<td>8.3 ± 6.8‡</td>
</tr>
<tr>
<td>Atherosclerosis (myocardial</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>infarction, angina pectoris, or</td>
<td>15</td>
<td>9.0 ± 4.4</td>
<td>12.3 ± 4.1</td>
</tr>
<tr>
<td>oblitative atherosclerosis)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Expressed as percentage decrease in vessel diameter after breathing 100% oxygen for 5 minutes.
†Mean ± standard deviation.
‡Significantly different from control group (P < 0.05).
From about age 20, arterial reactivity decreases at the rate of about 1% every 10 years. The correlation between age and venous reactivity, however, is not at a significant level.

Patients with arterial hypertension (blood pressure greater than 150/100 mm Hg) show a significant loss of retinal arterial reactivity. The group of hypertensives of table 2 included primarily patients with essential hypertension, but also a few with chronic glomerulonephritis and with pyelonephritis. Whether the patients were grouped, as in table 2, by the presence or absence of proteinuria, or by such other clinical indices of renal impairment as normal or abnormal serum nonprotein nitrogen concentration, or normal or abnormal phenolsulfonphthalein excretion, there was a marked loss of retinal arterial reactivity before clinical abnormalities of renal function appeared. The degree of proteinuria was chosen as a clinical index of the severity of renal vascular involvement. In the absence of proteinuria venous reactivity was normal, but with proteinuria venous reactivity was also significantly diminished. With heavy proteinuria, arterial reactivity was virtually absent.

In order to determine whether loss of retinal arterial reactivity in hypertension is directly dependent upon the elevation of pressure or results from secondary changes in the vessels, observations were made on four patients with chronic hypertension before and after they became normotensive on therapy.23 Retinal arterial reactivity was severely impaired before treatment and remained severely impaired after the pressure returned to normal.

Patients with diabetes, even in the absence of retinopathy or hypertension, show a significant loss of retinal arterial reactivity. With retinopathy, apart from hypertension, the loss of reactivity is more severe. This loss of reactivity may be related to the special changes found in the retinal vessels of diabetics19, 20 or to the hemodynamic and structural abnormalities found in small blood vessels of diabetics more generally through the body.21-24

Persons with uncomplicated atherosclerosis usually show only occasional discrete lesions in the retinal vessels by ophthalmoscopic examination. Correspondingly, nonhypertensive, non diabetic patients with atherosclerotic heart disease or occlusive peripheral atherosclerosis do not show significant decrease in retinal arterial reactivity.18

In persons with vascular disease the appearance of the retinal vessels is often considered an index to the state of vessels elsewhere in the body, especially those of the central nervous system. To assess the relation between disease of the retinal and the cerebral vessels in functional terms, observations on retinal arterial reactivity and cerebral blood flow were made in a group of 31 subjects which included normal persons, persons with overt cerebral vascular disease, and persons having illnesses commonly associated with generalized arteriosclerosis. A significant positive correlation was found between retinal arterial reactivity to oxygen and both the control cerebral blood flow rate and the increase in flow on inhaling 5 or 7% CO2. It was concluded that arteriosclerosis which is severe enough to produce functional changes of this kind is apt to affect retinal and cerebral vessels together.25

In summary, hypertension and diabetes cause diminution or loss of a retinal vasconstrictor response which is normally present. Presumably this functional change is caused by pathological alterations in the vessel wall. As pointed out, retinal vascular reactions apparently tend to stabilize conditions in the retina by appropriate adjustment of the blood flow to meet changing metabolic requirements. Impairment of vascular reactivity may, in itself, hasten the progression of retinopathy by interfering with this stabilizing function.

Retinal Venous Blood Oxygen Saturation

The retinal arteriovenous oxygen difference is proportional to the ratio of oxygen consumption to rate of blood flow in that portion of the retina, the inner portion, that derives its oxygen supply from the retinal vessels. In 65 normal seated subjects, by the photographic method described, the retinal venous
blood oxygen saturation was found to be 59 ± 11% (SD).\textsuperscript{6} This is slightly lower than the mixed cerebral venous blood oxygen saturation.\textsuperscript{26-28}

Table 3 shows that changing from the seated to the recumbent position does not produce a statistically significant change in retinal venous oxygen saturation.\textsuperscript{4} From this it appears that the vasoconstriction associated with recumbency is not mediated by metabolic changes in the retina resulting from an increased perfusion rate. If anything, the rate of blood flow, as judged by the arteriovenous oxygen difference, may be slightly reduced in spite of the probable increase in net perfusion pressure due to recumbency. The present evidence suggests that changing from the upright to the recumbent position increases the retinal vascular resistance as it does the cerebral vascular resistance.\textsuperscript{28}

Breathing 10% oxygen reduces the oxygen saturation of retinal venous blood.\textsuperscript{6} The data of table 4 demonstrate that the arteriovenous oxygen difference is decreased to about three quarters of the control value. Presumably, this reflects an increase in blood flow associated with the vasodilation already described.

The effect of breathing 100% oxygen was unexpected. In a normal subject this increases the arterial oxygen content by about 2.5 vol% in part through binding more oxygen to hemoglobin but primarily through increasing oxygen in physical solution. If retinal blood flow and oxygen consumption were unchanged, this might be expected to increase the venous oxygen saturation by about 12%. Actually, the vasoconstriction caused by breathing oxygen suggests a reduction in blood flow, so that the increase in venous oxygen should be less than 12% if oxygen consumption did not change. In fact, however, as seen in table 5, the mean venous oxygen saturation in a large series of subjects increased by 24%.\textsuperscript{6,14} These observations were made after 5 minutes of oxygen breathing. In trials of prolonged oxygen breathing in a few subjects, the venous oxygen saturation was maintained at a similarly high level for 15 minutes but at 30 and 60 minutes was increased only about 10% above the control level.\textsuperscript{14}

To investigate this phenomenon further, a graded series of oxygen-enriched gas mixtures was administered in random order to a group of normal subjects, with adequate recovery periods of air breathing allowed between mixtures. The results are presented in table 5. The 40% and 60% O\textsubscript{2} mixtures caused only small increases in venous oxygen, while 100% O\textsubscript{2} produced a very large further increase.\textsuperscript{4} The most likely explanation for this marked increase in venous blood oxygen is that high oxygen tensions can bring about a decrease in the oxygen consumption of that portion of the retina which is supplied by the retinal vascular system, perhaps by interfering with enzyme systems which normally facilitate retinal oxygen consumption.\textsuperscript{29} This would not necessarily imply an over-all decrease in energy supply to the retina since glycolysis would presumably still be available. The fall in retinal venous oxygen saturat-

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Gas breathed</th>
<th>Arterial O\textsubscript{2} saturation, %</th>
<th>Retinal venous O\textsubscript{2} saturation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>Air</td>
<td>98 ± 1</td>
<td>57 ± 15</td>
</tr>
<tr>
<td></td>
<td>10% O\textsubscript{2}</td>
<td>70 ± 7</td>
<td>39 ± 17</td>
</tr>
</tbody>
</table>

\textsuperscript{307}
tion after a few minutes of breathing oxygen suggests that oxygen consumption has been at least partially restored toward the normal rate. The retinal vessels, however, remain constricted during prolonged oxygen breathing, indicating that both blood flow and oxygen consumption may continue to be depressed to some extent.

Glucose oxidation and glycolysis are major sources of energy for the retina. The effects on retinal venous oxygen of elevating the blood glucose level in normal subjects are presented in table 6. During air breathing, increasing blood glucose by an intravenous infusion caused a significant rise in retinal venous blood oxygen from 56% to 63% (P < 0.01). has shown that the developing retina and, to a lesser extent, the adult retina exhibit a fall in oxygen consumption when glucose is added to the medium. This phenomenon, the so-called Crabtree effect, is thought to be due to a competition between respiration and a high-capacity glycolytic system for phosphorylative co-factors such as ADP. The rise of retinal venous blood oxygen caused by high glucose concentrations in the subjects of table 6 may be an in vivo demonstration of the Crabtree effect in the human retina. Table 6 also shows that the venous blood oxygen remains lower (P < 0.02) with oxygen breathing when the glucose level is high than when it is normal. Expressed in another way, the venous blood oxygen rises by 21% during oxygen breathing at a normal glucose level, but when the blood glucose is high it increases only 9%. In effect, a high substrate concentration appears to protect against the partial inhibition of glucose oxidation caused by high oxygen tensions. These interpretations of the data of table 6 carry the implicit assumption that changes in retinal venous oxygen brought about by glucose infusion depend primarily upon changes in retinal oxygen consumption rather than changes in retinal blood flow rate. Similar infusions of large quantities of lactate in water were without significant effect on retinal venous blood oxygen during air and oxygen breathing. The glucose infusions produced no apparent change in retinal vessel diameter during the breathing of either air or oxygen.

**Photography after Injection of Fluorescein**

In 1959, Flocks and co-workers reported observations by cinephotography on the retinal circulation of the cat after intravenous fluorescein injection. In 1961, Novotny and Alvis, in this laboratory, described the development of a technique for serial retinal photography in man using intravenous fluorescein and reported observations on normal subjects and on patients with hypertensive and diabetic retinopathy. Subsequently, the method and various modifications of it have been used extensively in the study of the human retinal circulation, notably in the careful, systematic work of Dollery and his associates. The introduction of cine and rapid-sequence photographic techniques has greatly improved the precision with which the circulatory dynamics of the retina can be observed.

**Table 5**

*Effect on Retinal Venous Blood Oxygen Saturation of Breathing 100% Oxygen and Various Oxygen Mixtures for 5 Minutes*

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Gas breathed</th>
<th>Retinal venous O2 saturation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>Air</td>
<td>55 ± 9</td>
</tr>
<tr>
<td></td>
<td>100% O2</td>
<td>79 ± 9</td>
</tr>
<tr>
<td>9</td>
<td>Air</td>
<td>55 ± 8</td>
</tr>
<tr>
<td></td>
<td>40% O2</td>
<td>56 ± 5</td>
</tr>
<tr>
<td></td>
<td>60% O2</td>
<td>61 ± 7</td>
</tr>
<tr>
<td></td>
<td>100% O2</td>
<td>82 ± 10</td>
</tr>
</tbody>
</table>

**Table 6**

*Effect on Retinal Venous Blood Oxygen Saturation of Infusing 10% Glucose Intravenously during the Breathing of Air and 100% Oxygen (Nine Normal Subjects)*

<table>
<thead>
<tr>
<th>Glucose, mg/100 ml</th>
<th>% Saturation</th>
<th>Glucose, mg/100 ml</th>
<th>% Saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td></td>
<td>Oxygen</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>85 ± 6</td>
<td>56 ± 11</td>
<td>88 ± 7</td>
</tr>
<tr>
<td>Glucose infusion</td>
<td>315 ± 39</td>
<td>63 ± 11</td>
<td>421 ± 54</td>
</tr>
</tbody>
</table>

*Circulation, Volume XXXIII, February 1966*
With fluorescein photography it is immediately apparent that there are considerable differences in the arteriovenous transit time of different portions of the retina, the time being much shorter in the region of the fovea than elsewhere, and longest in the peripheral parts of the retina. The central portion of the larger arteries fills more rapidly than the sides, and this observation indicates considerable difference in flow velocity within these small vessels. Occasional small arterial branches in normal subjects fill and empty much more slowly than the arterial tree in general. With arterial filling, and early in the venous filling phase, the retina as a whole begins to fluoresce, presumably due to retinal capillary and choroidal vascular filling. During venous filling, stratification of flow within the larger veins is often strikingly apparent. Fluorescent streams are first contributed to the large venous trunks by smaller branches which usually enter relatively near the disk. These fluorescent streams tend to maintain a lateral position in the large veins, on the side of entrance, all the way to the disk. Dollery and associates have pointed out that as many as five individual streams can be identified in occasional large veins. Lamination of flow in the retinal venules of the cat has also been observed by Friedman and his associates in their study of the retinal microcirculation under high magnification.

In patients with hypertensive and diabetic retinopathy, fluorescein injection brings out dramatically the new vessel proliferation which tends to replace the orderly, economical vascular pattern of the normal retina. Capillary aneurysms show clearly. In regions of abundant neovascularization the small vessels tend to remain fluorescent longer than vessels of the same size in other parts of the retina, thus indicating that the blood flow is relatively slow. Diffuse fluorescence appears in some areas of neovascularization, showing that vascular permeability is sufficiently great to allow plasma protein, to which fluorescein is attached, to pass through the vessel walls. Cotton-wool exudates often, but not always, show pronounced, diffuse fluorescence, evidently for the same reason. Dollery and associates have found that diffuse fluorescence may appear in the place where a cotton-wool exudate will later develop, and that the vessels of the region will still continue to leak fluorescein for a time after the exudate has cleared. Hard exudates and hemorrhages do not fluoresce.

Mean Retinal Circulation Time

Observations of the arteriovenous transit time across various parts of the retina have been reported by a number of investigators. Values have ranged from slightly over 1 second for the region of the macula to from 3 to 5 seconds for other portions of the retina. For quantitative purposes measurement of the mean circulation time is generally more useful. The procedure for making this measurement has been outlined in the methods section. Figure 2 shows typical fluorescence intensity-time curves for the retinal arteries and veins. The mean circulation time across the retina is calculated as the difference in mean time between the arterial and venous curves.

By this method, the mean retinal circulation time in 29 normal men between 20 and 35 years of age was found to be 4.7 ± 1.1 seconds. Table 7 shows the effect of various procedures on the mean retinal circulation time. It was significantly prolonged by the inhalation of oxygen, by hyperventilation, and by sublingual nitroglycerin. Inhalation of 7% CO₂ shortened the time, but the change in five subjects was not statistically significant. Inhalation of 10% O₂, which dilates retinal vessels, did not change the mean circulation time.

Calculated Changes in Retinal Blood Flow and Oxygen Consumption

From measurements of change in the diameter of retinal vessels, change in mean circulation time, and change in retinal arteriovenous oxygen difference in response to various stimuli, it is possible to estimate the relative change in rate of retinal blood flow and of oxygen consumption of that portion of
the retina which is supplied by the retinal vasculature. The mean circulation time, $t$, is proportional to the ratio of retinal vascular volume to retinal blood flow rate:

$$t = \frac{\text{Volume}}{\text{Flow}},$$

or

$$\text{Flow} = \frac{\text{Volume}}{t}. \quad (1)$$

Change in the vascular volume from one state to another can be estimated from change in the square of vessel diameter. Since the smaller vessels change proportionally more than the larger vessels, which can be measured more exactly, basing calculations on the larger vessels will tend to underestimate the actual volume change. Because the venous system of an organ, in general, contains most of the blood, the use of mean change in the diameter of the larger retinal veins is a conservative choice as a basis for roughly estimating change in retinal vascular volume from one state to another. The ratio of retinal blood flow ($F$) after an experimental stimulus to that in the control state may then be estimated as:

$$\frac{F_e}{F_c} = \frac{t_e}{t_c} \times \left( \frac{\text{diameter}_e}{\text{diameter}_c} \right)^2, \quad (2)$$

where the subscripts $e$ and $c$ indicate experimental and control states, respectively.

The rate of oxygen delivery from the retinal circulation is equal to the product of flow and arteriovenous (A-V) $O_2$ difference across the retina. If $QO_2$ is used to designate the oxygen

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**Table 7**

Effect of Various Procedures on Mean Retinal Circulation Time

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of subjects</th>
<th>Control time, sec</th>
<th>Experimental time, sec</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% $O_2$ Inhalation</td>
<td>5</td>
<td>$4.8 \pm 1.0$</td>
<td>$6.1 \pm 1.2$</td>
<td>$&lt; 0.01$</td>
</tr>
<tr>
<td>10% $O_2$ Inhalation</td>
<td>4</td>
<td>$4.7 \pm 1.5$</td>
<td>$4.9 \pm 0.7$</td>
<td>n.s.</td>
</tr>
<tr>
<td>7% $CO_2$, 21% $O_2$</td>
<td>5</td>
<td>$5.0 \pm 0.8$</td>
<td>$4.2 \pm 1.0$</td>
<td>$0.05 &lt; P &lt; 0.1$</td>
</tr>
<tr>
<td>Hyperventilation</td>
<td>5</td>
<td>$5.5 \pm 0.7$</td>
<td>$8.6 \pm 1.9$</td>
<td>$&lt; 0.025$</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>5</td>
<td>$4.8 \pm 1.3$</td>
<td>$6.9 \pm 1.0$</td>
<td>$&lt; 0.01$</td>
</tr>
</tbody>
</table>

Circulation, Volume XXXIII, February 1966
delivery rate, then the ratio of experimental to control $\bar{Q}_{O_2}$ may be estimated as:

$$\frac{\text{Experimental } \bar{Q}_{O_2}}{\text{Control } \bar{Q}_{O_2}} = \frac{F_e}{F_e} \times \frac{\text{A-V } O_2 \text{ difference}_{e}}{\text{A-V } O_2 \text{ difference}_{c}} \quad (3)$$

In order to make these calculations for the procedures of Table 7, additional data are necessary. Table 8 presents in a different form the findings previously given for the effect of breathing different mixtures of oxygen and carbon dioxide on the diameter of the retinal veins of normal subjects and adds data for the effects of hyperventilation and sublingual nitroglycerin.43

Table 9 presents the effect of these same procedures on the retinal arteriovenous oxygen difference, expressed as percentage blood oxygen saturation. The effect of 10% CO$_2$ in 21% O$_2$ is of particular interest. Breathing this mixture caused a considerable reduction in A-V $O_2$ difference, even though the diameter of the larger vessels showed very little change. Presumably, this results from an increase in blood flow. Evidently CO$_2$ has a considerable effect on the resistance vessels, which are not measured by the present technique. Correspondingly, hyperventilation caused a widening of the A-V difference, evidently because of a decrease in blood flow, but in this case a significant, though moderate, decrease in the diameter of the larger vessels occurred.

The data of Tables 7, 8, and 9 on changes in mean circulation time, venous diameter, and A-V $O_2$ difference can now be combined to yield estimates of change in rate of blood flow and rate of oxygen delivery from the retinal circulation. The results of these calculations are presented in Table 10. There is a great decrease in blood flow and in oxygen delivery rate after 5 minutes of oxygen breathing. With more prolonged oxygen breathing, the arteriovenous oxygen difference returns to approximately normal values, but the vessels remain constricted. Measurements of mean retinal circulation time during extended oxygen breathing are needed to determine whether this remains prolonged. If it does, as suggested by the appearance of the vessels, then the oxygen delivery rate from the retinal vascular system during extended oxygen breathing might be expected to stabilize at about 60% of the rate during air breathing. CO$_2$ increases the retinal flow rate by about 25% without changing oxygen delivery. The relative decrease in blood flow on breathing oxygen is much greater than that reported for the cerebral blood flow as a whole, and the increase on CO$_2$ is very much less.27 On the other hand, the relative increase in flow caused by the inhalation of 10% O$_2$ is less than that reported for the cerebral circulation. The effect of hypoventi-

### Table 8

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of subjects</th>
<th>D*</th>
<th>Df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% O$_2$</td>
<td>50</td>
<td>0.85</td>
<td>0.72</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Inhalation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% O$_2$</td>
<td>6</td>
<td>1.10</td>
<td>1.21</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Inhalation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% CO$_2$, 21% O$_2$</td>
<td>10</td>
<td>1.01</td>
<td>1.02</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hyperventilation</td>
<td>7</td>
<td>0.96</td>
<td>0.92</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>7</td>
<td>1.05</td>
<td>1.10</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

$D* = \frac{\text{Experimental vein diameter}}{\text{Control vein diameter}}$. 

Circulation, Volume XXXIII, February 1966"
Table 9
Effect of Various Procedures on Retinal Arteriovenous Oxygen Difference

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of subjects</th>
<th>A-V difference, % saturation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation 100% O₂</td>
<td>28</td>
<td>44</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Inhalation 10% O₂</td>
<td>10</td>
<td>44</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Inhalation 10% CO₂ 21% O₂</td>
<td>9</td>
<td>43</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Hyperventilation</td>
<td>7</td>
<td>37</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>7</td>
<td>38</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Takes into account the effect of physically dissolved oxygen.

The effect on flow is about the same for the two circulations. The brain does not show the decrease in oxygen consumption at high oxygen tensions which is reported here for the retina.27

The effect of sublingual nitroglycerin on retinal flow is particularly interesting. The larger vessels are dilated by nitroglycerin.45 Blood pressure of the subjects did not change materially. Nevertheless, the mean circulation time was prolonged and the arteriovenous oxygen difference widened. These changes were compensatory in the sense that there was no substantial alteration in the rate of oxygen delivery. There was, however, a considerable decrease in the rate of blood flow. It is possible that the resistance of most intracranial vascular pathways may have dropped much more than the retinal vascular resistance, with a consequent decrease in the retinal arterial pressure to a level more than usually below that in the brachial artery, where the pressure was measured. Whatever the explanation, the simultaneous occurrence of vasodilation and slowing of the blood flow emphasize the need for caution in interpreting the total effects of drugs used to dilate the retinal vessels with the object of increasing blood flow.

This combination of photographic techniques which allows making estimates of relative change in retinal blood flow and oxygen consumption offers a new and interesting approach to study of the retinal circulation and metabolism in intact man.

A Quantitative Estimate of Retinal Oxygen Consumption, Blood Flow, and Blood Volume

By ingenious experiments on the persistence of vision during pressure-induced retinal ischemia in a hyperbaric oxygen chamber Carlisle and associates46 have been able to estimate the rate of retinal oxygen consumption as 5.0 ml/ml of retinal tissue per hour, or about 8.0 ml/100 g of tissue per minute. These observations have been confirmed by Anderson and Saltzman.47 These estimates are based on data obtained between 2 and 4 atmospheres, absolute, of oxygen, and in this interval the oxygen consumption appears to remain constant. Assuming that this is the rate of oxygen consumption which becomes established during extended oxygen breathing at 1 atmosphere when the retinal A-V O₂ difference has stabilized at about 8 vol %, and that this metabolic rate holds for the inner retina, then the retinal blood flow can be calculated by the Fick formula:

\[
\text{Flow} = \frac{(8 \text{ ml})}{(\text{min})(100 \text{ g})} \times \frac{8 \text{ ml}}{100 \text{ ml}} = 100 \text{ ml/100 g of tissue per minute.}
\]

This is the value estimated for extended oxygen breathing at 1 atmosphere. Allowing this flow rate to be about 60% of the rate during air breathing, for reasons discussed earlier, would yield an estimate of about 170 ml/100 g/min for retinal blood flow under normal conditions. This estimate for flow and the measurement of mean retinal circulation time can be substituted in equation 1 to yield an estimate of retinal blood volume as being 14 ml/100 g of tissue. These estimates are summarized in table 11.

This value for the rate of blood flow in the human retina is about three times the rate for the brain as a whole.26, 28 The flow rate varies widely for different portions of the
Inhalation of Nitroglycerin and Oxygenation: A Study in Animals

Some Observations on Ocular Blood Flow and Volume

A technique for following blood volume changes in the retina and choroid in experimental animals by the use of $^{32}$P was introduced in 1956 by Bettman and Fellows and has been used to study the effect of a large number of drugs. The retinal blood volume was found to be considerably increased by inhalation of CO$_2$ but, by this technique, 100% O$_2$ was without effect.

In 1961, Broadfoot and associates reported the development of a photoelectric method of investigating the amount and oxygenation of blood in the ocular fundus and reported observations on experimental animals and on man, showing that directional changes in the quantity and oxygenation of blood could be detected and recorded by this means.

Recently, Trokel using a reflective densitometric technique derived from the work of Rushton and associates developed ingenious methods for measurement of the ocular blood flow and blood volume in albino rabbits. These methods are particularly valuable because of the quantitative nature of the data that they provide. It was shown that exposure to 100% oxygen caused a 14% decrease in blood volume and a 32% decrease in flow; that 10% CO$_2$ in 21% O$_2$ caused a 35% increase in blood volume and a 61% increase in blood flow; and that 10% CO$_2$ in 90% O$_2$ caused a 15% increase in blood volume and an 18% increase in flow. Trokel has used similar techniques to demonstrate a difference in the ocular flow rate of the two eyes of human subjects caused by carotid occlusive disease.

Measurements of the uveal blood flow in dogs by the nitrous oxide technique and arteriovenous blood gas and glucose differences across the uveal tissue have been reported by Pilkerton and his associates. The results indicate a high ratio of flow rate to oxygen consumption and a relatively high rate of glucose utilization.

By graphic analysis of the radioactivity decay curve over the sclera after intracarotid injection of Krypton-85, Friedman and associates have obtained a value of 166 ml/g/min for the retinal blood flow of the cat and 1,200 ml/g/min for the choroidal flow. The volume of tissue supplied by the retinal cir-

Table 10

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of subjects</th>
<th>f Flow</th>
<th>f QO₂†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhalation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100% O₂</td>
<td>5</td>
<td>.57</td>
<td>.44</td>
</tr>
<tr>
<td>Inhalation</td>
<td>4</td>
<td>1.16</td>
<td>.85</td>
</tr>
<tr>
<td>Nitroglycerin</td>
<td>5</td>
<td>.77</td>
<td>.93</td>
</tr>
</tbody>
</table>

*†"f" indicates fractional value with respect to control value as unity; f flow = Experimental flow / Control flow.

†fQO₂ = Experimental QO₂ / Control QO₂.

Table 11

| Retinal O₂ Consumption (O₂ breathing) | 8 ml/100 g/min |
| Retinal Blood Flow (O₂ breathing)     | 100 ml/100 g/min |
| Retinal Blood Flow (normal pO₂)       | 170 ml/100 g/min |
| Retinal Blood Volume (Flow × t)       | 14 ml/100 g     |

Circulation, Volume XXXIII, February 1966
culation was estimated to be about twice that supplied by the choriocapillaris.

**Comment**

From all these observations the choroidal circulation appears as an unusually reactive, high flow system which is almost an arteriovenous shunt. Since the choroidal circulation supplies the outer portion of the retina by long-range diffusion, the utility of near-arterial blood in maintaining high diffusion, long-range supplies is obvious. The inner portion of the retina is supplied by a more conventional circulation, which nevertheless provides unusually rapid blood flow in conformity with the high metabolic rate of the tissue which it supplies. This vascular system reacts to changes in blood gases and perfusion pressure much like the over-all cerebral circulation, but it appears to be somewhat more sensitive to changes in oxygen tension and less sensitive to changes in the tension of carbon dioxide than in the cerebral circulation.

**Summary**

By photographic techniques measurements have been made in the human retina of vessel size, arteriovenous oxygen difference across the retina, and mean retinal circulation time. By combination of these methods estimates can be made of relative changes in retinal blood flow rate and in the rate of oxygen delivery from the retinal vascular system. Observations are presented on the response of the normal and diseased retinal circulation to a variety of stimuli, particularly changes in blood oxygen and carbon dioxide tensions.

**References**


that art without science is not slow to degenerate into routine. That hackneyed scepticism, which people so willingly oppose to all progress of the human mind, is a comfortable pillow for lazy heads; but the period in which we live allows no time for falling asleep.—J. M. Charcot. Clinical Lectures on Senile and Chronic Diseases. London, The New Sydenham Society, 1881, p. 20.
Studies of the Retinal Circulation in Man: Observations on Vessel Diameter, Arteriovenous Oxygen Difference, and Mean Circulation Time
JOHN B. HICKAM and REGINA FRAYSER

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