STUDIES OF RETINAL CIRCULATION AND A-V OXYGEN DIFFERENCE IN MAN

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Systemic metabolic and circulatory disorders often cause changes in the appearance of the optic fundus which are helpful in evaluating the nature and severity of the causative disorder. Although good correlations have been established empirically between the appearance of retinal lesions and the nature of associated systemic disorders, there is little information about how such lesions are produced. With the ultimate object of obtaining such information, techniques have been developed for investigating certain aspects of retinal circulation and metabolism in man. These techniques are: 1) Measurement by fundus photography of diameter changes in retinal vesssels in response to various stimuli,[1] and 2) Estimation by fundus photography of the per cent oxygen saturation of retinal venous blood. It is the purpose of this presentation to describe the information which has been obtained with these techniques in normal subjects and in patients with hypertension, diabetes, and atherosclerosis.

Methods

Measurement of Diameter Change in Retinal Vessels

Fundus photographs were made, with the subject seated unless otherwise stated, first in the control condition and then after various procedures intended to alter the caliber of the retinal vessels. From these photographs, measurements were made of the visible diameter of the larger arteries and veins near the disc, using a low-power dissecting microscope with a scale in the ocular. Changes in diameter were expressed as per cent change from the control diameter, and the mean arterial and venous changes were calculated separately. The inhalation of oxygen, which constricts retinal vessels, was the most commonly used procedure, and the terms "retinal vascular reactivity" or more specifically arterial or venous

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reactivity, denote the per cent shrinkage in vessel diameter which has developed five minutes after changing from breathing air to breathing 100% (tank) oxygen.

Estimation of Retinal Venous Blood Oxygen Saturation

The method is similar to other photometric procedures for the measurement of blood oxygen saturation except that relative light intensity is measured by the density of the image produced on photographic film rather than by a photoelectric cell. Fundus photographs are made, using infra-red sensitive film and appropriate filters to allow exposures to be made separately by red and by infra-red light. The red filter, which consists of a Wratten 29F filter and a 3.4 mm. layer of 0.25 molar aqueous $CuSO_4$ solution allows a maximum light transmission of 50 per cent at 640 millimicrons. Here, reduced hemoglobin absorbs much more light than oxyhemoglobin. The infra-red filter is a Wratten 88A, and the combination of this filter and Kodak High-Speed Infra-Red film yields a broad light band in the vicinity of 800 millimicrons which is absorbed to about the same extent by reduced hemoglobin and oxyhemoglobin. The negatives of the fundus pictures made with these filters are projected onto a screen in which a photocell is mounted, so that the density of any portion of the photographic image can be measured. Density measurements are made of the veins as they lie over the disc, and of the portions of the disc next to them. These vessels are so thin that most of the red and infra-red light which strikes them is transmitted to the underlying disc and reflected back through the vessel. For present purposes the vessel is like a cuvette filled with blood and placed across the surface of a large light source. In such a system, which resembles that of conventional oximeters, the per cent oxygen saturation of the blood sample bears an approximately linear relationship to the ratio of its optical density by red light to its optical density by infra-red light. In the present case an estimate of this ratio is obtained from the density measurements made on the negatives. If the disc be considered a light source, then the optical density of a vessel crossing it is proportional to the difference in optical density between the photographic images of disc and vessel. On taking the ratio between these density differences by red and infra-red light for a particular vessel, the proportionality constant, which depends upon film and developing conditions, disappears. That is:

$$R = \frac{\text{"Red" optical density}}{\text{"Infra-red" optical density}}$$

$$= \frac{\text{Disc density less vessel density by red photograph}}{\text{Disc density less vessel density by infra-red photograph}}$$

To confirm that R, as estimated in this way, would bear a usefully linear relationship to the per cent oxygen saturation of blood, estimates of R were made from photographs of a model eye containing glass capillaries filled with blood samples having oxygen saturations between 24 and 100%. For 20 samples over this range, and different capillary sizes, the following regression lines were obtained:

For 500 micron diameter capillaries, % Sat. = 201 − 101R, with a standard deviation from regression of ±6%; and for 250 micron capillaries, % Sat. = 163 − 67R, with a standard deviation of 9%. In each case, the linearity is good. The difference in slope with change in cuvette thickness is characteristic of whole blood samples.[2] To obtain the coefficients of the regression line for the blood vessels of actual eyes, similar photographic measurements were made on the retinal *arteries* of 10 normal subjects whose arterial blood oxygen saturation was varied by having them breathe air and low oxygen mixtures. The actual value of the arterial blood oxygen saturation at the time of photography was determined by analysis of brachial arterial blood samples. Per cent oxygen saturations ranged between 99.2 and 63.0. From 20 such measurements, the regression line obtained for the actual eye was:

$$\% \text{ Sat} = 128 - 34R$$

The standard deviation from regression was 6.5%. The assumption has been made that the line calculated from data obtained on arteries can be used with veins. For collecting this data the largest available retinal arteries were used, and it seems likely that relatively little error will be introduced because of size differences between these vessels and the usual veins on which measurements are made.

Results

Reactions of the Retinal Vessels in Normal Subjects

A summary of the effects of various stimuli on the diameter of retinal arteries and veins in normal subjects is presented in Table I. As first reported by Cusick, Benson, and Boothby,[3] a pronounced constriction of the retinal vessels occurs on changing from breathing air to breathing 100% oxygen, and a dilation on changing from air to 10% oxygen. A distinct vasoconstriction is evident within a minute of changing from air to oxygen, and the full constriction occurs within 5 minutes. The presumptive effect of these changes in vessel size would be to adjust retinal blood flow so as to minimize changes in retinal oxygen tension caused by changes in arterial blood oxygen content. These vessel reactions are not mediated by the sympathetic nervous system; they are present in persons

TABLE I

Change in Diameter of Retinal Vessels After Different Stimuli in Normal Subjects

Procedure	Number Subjects	Retinal Vascular Response[1] (%)	
		Arterial	Venous
Air to 100% Oxygen	50	−11.6 ± 4.7	−14.9 ± 6.5
Air to 10% Oxygen	6	+10.1 ± 3.6	+10.0 ± 3.3
After Pressure on Eye	8	+13.8 ± 5.9	+18.0 ± 6.8
Seated to Recumbent	8	−10.1 ± 3.9	−7.6 ± 4.1

[1] Expressed as per cent change in vessel diameter from control state, means and standard deviations.

who have had a thoracic sympathectomy, and their magnitude is not altered in normal subjects by stellate block. The possibility that these changes in vessel size may depend upon local metabolic changes induced by variations in retinal oxygen tension is supported by the marked degree of reactive hyperemia which follows brief, partial obstruction of the retinal blood flow. Application to the eye for 10 seconds of pressure sufficient to obstruct diastolic flow is followed by dilation of both arteries and veins, which becomes greatest about 30 second after pressure is released. The magnitude of this response is shown in Table I.

Changing from the upright to the supine position also causes constriction of both arteries and veins in normal subjects, as indicated in Table I. These changes in arterial size would tend to oppose retinal blood flow changes caused by the gravitational effect on net perfusion pressure with variation in body position. In the eye, this gravitational influence on perfusion pressure is considerable because the effect on retinal arterial pressure of a change in body position is not counterbalanced by an opposite effect on venous pressure; despite change in body position, the retinal venous pressure remains above the relatively high and constant level of the intra-ocular tension, about 25 mm.Hg. These postural changes in the caliber of retinal vessels are not affected by stellate block. They have also been found to occur in a patient with severe postural hypotension.

In a few normal subjects the combination of breathing oxygen and changing from the upright to the supine position has been found to cause more constriction of retinal arteries and veins than either procedure alone.

In order to examine the question of whether retinal venoconstriction is an active process or simply a passive consequence of reduced venous pressure secondary to arterial constriction and a drop in blood flow, an attempt was made to resist venoconstriction by raising the venous pressure. To this end, a sphygmomanometer cuff was inflated around the neck of

2 subjects to a pressure of 60 mm.Hg. and, in one case, also to a pressure of 100 mm.Hg. This caused a moderate distention of the retinal veins, but oxygen breathing with the cuff inflated still reduced venous size below the control level while breathing air. It was concluded that constriction of the retinal veins under the influence of oxygen is an active process.

Contrary to the finding of others,[4] inhalation of 5% CO_2, 21% O_2, and 74% N_2 caused very little dilation of the retinal vessels. However, the inhalation of 10% CO_2, 90% O_2 produced only a slight constriction of the retinal vessels, so that CO_2 appears capable of blocking the response to oxygen.

The reactions of the retinal vessels to change in arterial blood oxygen and in body position are in directions which should have the effect of reducing fluctuations in retinal oxygen tension. Since the retina has the highest rate of oxygen consumption of any body tissue, such vascular reactions may well be important to maintaining the function, and even the integrity, of the retina under adverse circumstances.

Retinal Vascular Reactivity in Vascular Disorders

In persons with vascular disorders, retinal vascular reactivity was measured as the per cent decrease in vessel diameter which resulted from breathing tank oxygen for 5 minutes in the seated position.

Patients with arterial hypertension (blood pressure usually exceeding 150/100 mm.Hg.) show a marked reduction in arterial reactivity.[5] Table II summarizes the results obtained in 35 hypertensives, classified accord-

TABLE II

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

Condition	Number Subjects	Retinal Vascular Reactivity[1]	
		Arterial	Venous
Normal Control	47	11.3 ± 4.3	14.5 ± 5.8
Hypertension			
Without Proteinuria	17	5.2 ± 4.9	12.9 ± 3.9
1 + Proteinuria	11	4.4 ± 5.5	8.5 ± 4.7
2 − 4 + Proteinuria	7	0.6 ± 0.6	4.5 ± 2.8
Diabetes			
Normotensive, without Retinopathy	20	8.2 ± 4.7	12.8 ± 5.3
Normotensive, with Retinopathy	15	3.8 ± 3.8	10.8 ± 5.5
Atherosclerosis	15	9.0 ± 4.4	12.3 ± 4.1

[1] Expressed as per cent decrease in vessel diameter after breathing 100% oxygen for five minutes.

ing to the severity of their proteinuria. Before proteinuria appears, there is already a significant ($P < .01$) impairment of arterial reactivity, and with the development of marked proteinuria there is a further significant ($P < .01$) reduction in reactivity. Venous reactivity is significantly reduced in the groups with proteinuria. The reduction in reactivity does not depend upon the hypertension per se; reducing the blood pressure to normal levels produces no immediate significant change in reactivity of arteries or veins.

Although the vascular changes of diabetic retinopathy characteristically affect capillaries and veins rather than arteries, the data of Table II indicate that normotensive diabetics, even without retinopathy, show a significant ($P < .01$) reduction in arterial reactivity below normal. With the development of retinopathy, arterial reactivity is further reduced significantly ($P < .01$) below that of diabetics without retinopathy, and the venous reactivity also becomes reduced ($P < .05$) below normal.

In uncomplicated atherosclerosis, manifested primarily by coronary disease in the absence of hypertension or diabetes, no significant change was found in retinal arterial or venous reactivity.

Retinal Venous Blood Oxygen Saturation

In 34 normal young adult subjects breathing air in the seated position the mean retinal venous oxygen saturation was 57% (S.D. ±11%). As shown in Table III there was no significant change on assuming the recumbent position, indicating that the vasoconstriction of recumbency is not due to an increased oxygen tension in the retina. Breathing 10% oxygen (Table IV) caused a decrease in retinal venous blood oxygen which was

TABLE III

Effect on Retinal Venous Blood O_2 Saturation of Changing from the Seated to the Recumbent Position

Number Subjects	Position	Retinal Venous % O_2 Saturation
11	Seated	59 ± 6
	Recumbent	50 ± 17

TABLE IV

Effect on Retinal Venous Blood O_2 Saturation of Breathing 10% O_2

Number Subjects	Gas Breathed	Arterial % O_2 Saturation	Retinal Venous % O_2 Saturation
10	Air	97.9	54 ± 15
	10% O_2	70.1	38 ± 20

much less than the simultaneous decrease in arterial oxygen. Presumably, this decrease in arterio-venous oxygen difference reflects, at least in part, the accompanying retinal vasodilation.

Breathing 100% oxygen had an effect which was unexpected. If the arterio-venous oxygen difference remained unchanged, the retinal venous oxygen saturation might be expected to have a maximum increase of about 15% in normal subjects as a result of breathing 100% oxygen. Actually, less increase than this was anticipated because of the attendant vasoconstriction, which should reduce blood flow and widen the arterio-venous oxygen difference if retinal oxygen consumption remained unchanged. Actually, however, as shown in Table V, there was a mean increase of 23% in the retinal venous oxygen saturation, after 5 minutes of oxygen breathing. In observations on 3 subjects during prolonged oxygen breathing this increase was found to be maintained for 15 minutes or more, but at 30 minutes and 60 minutes of oxygen breathing, the mean venous oxygen saturation was elevated only about 10% above the control level. The effect of breathing a graded series of oxygen-enriched gas mixtures is shown in Table VI. The mixtures were administered in random order with adequate rest periods between. It is apparent that 60% oxygen produces only a modest increase in venous oxygen saturation, while 100% oxygen causes a very substantial further increase. The readiest explanation for this marked increase in venous blood oxygen is that high oxygen tensions cause a paradoxical, temporary decrease in the oxygen consumption of that portion of the retina which is supplied by the retinal vessels. The effect of supplying a high concentration of glucose during this procedure

TABLE V

Effect on Retinal Venous Blood O_2 Saturation of Breathing 100% O_2

Number Subjects	Gas Breathed	Retinal Venous % O_2 Saturation
28	Air	55 ± 9
	100% O_2	78 ± 9

TABLE VI

Effect on Retinal Venous Blood O_2 Saturation of Breathing Various Oxygen Mixtures for 5 Minutes

Number Subjects	Gas Breathed	Retinal Venous % O_2 Saturation
9	Air	55 ± 8
	40% O_2	56 ± 5
	60% O_2	61 ± 7
	100% O_2	82 ± 10

TABLE VII

The Effect on Retinal Venous Blood Oxygen Saturation of Infusing 10% Glucose Intravenously During the Breathing of Air and 100% Oxygen (9 normal subjects)

Control				Glucose Infusion			
Air		Oxygen		Air		Oxygen	
G[1]	% Sat	G[1]	% Sat	G[1]	% Sat	G[1]	% Sat
85 ± 6	56 ± 11	88 ± 7	77 ± 12	315 ± 39	63 ± 11	421 ± 54	72 ± 12

[1] G = Blood glucose in mg. %.

by the intravenous administration of 10% glucose is presented in Table VII. When oxygen was given during the administration of glucose, the increase in retinal venous blood oxygen saturation was only 9%, as against 21% in the control period, a highly significant difference ($P < .01$). Similar administration of sodium lactate was without effect.

Observations have also been made on 12 diabetics, most of whom had retinopathy and nearly all of whom were abnormally hyperglycemic at the time of study. The control retinal venous oxygen saturation was 54 ± 15%, which is normal, but administration of oxygen caused an increase to only 68 ± 10%, which is significantly ($P < .05$) less than the normal rise, but close to that of the normal subjects who were made hyperglycemic by glucose infusion.

Discussion

Decrease in retinal arterial reactivity appears to be a sensitive index of pathological vascular change in patients with hypertension and diabetes. It appears early in the course of these disorders, often before any abnormality is evident by ophthalmoscopic examination, and the development of retinopathy and other evidences of vascular damage are attended by progressive loss of retinal vascular reactivity. It seems possible that the development of retinopathy in patients with diabetes and hypertension may depend in part upon loss of the ability to adjust the retinal blood supply to meet changing metabolic requirements.

The relatively low value of the retinal venous oxygen saturation in normal subjects presumably reflects the active metabolism of this tissue. Fundus photography, including the preliminary positioning of the eye before making the actual picture, is carried out in bright light, and it is possible that the metabolic rate of the retina may be particularly high under these circumstances because of the need for continuously re-synthesizing visual purple and related substances.

The responses of retinal venous blood oxygen saturation to changing body position and arterial oxygen content are about as expected from the effect of these procedures on the retinal vessels, except for the very high venous oxygen temporarily resulting from the inhalation of 100% oxygen. This marked narrowing of the arterio-venous oxygen difference, together with vasoconstriction, strongly suggests a decrease in retinal oxygen consumption. This does not necessarily imply a reduction in energy consumption, since glycolysis would presumably still be available as an energy source. It is postulated that high oxygen tensions may partially interfere with the activity of enzyme systems which normally facilitate oxygen consumption by the retina. Barron[6] has advanced experimental evidence which supports this view. The infusion of glucose prevented the apparent drop in retinal oxygen consumption during oxygen breathing, possibly by providing a high enough concentration of substrate to overcome the partial inhibition of oxidative pathways.

Summary

1. The retinal vessels normally show well-marked caliber changes in response to a number of stimuli. In general these vascular responses appear to have the effect of reducing fluctuations in retinal oxygen tension.

2. The constrictor response of the retinal arteries which normally occurs on breathing 100% oxygen is significantly reduced in patients with hypertension and diabetes, but not in patients with uncomplicated atherosclerosis. It is suggested that this change may be a sensitive index of damage to small arteries.

3. The retinal venous blood oxygen saturation is normally low (57 ± 11%) under the conditions of fundus photography, and, for the most part, it varies as expected in response to stimuli which produce retinal vascular reactions.

4. Evidence is presented that inhalation of 100% oxygen may cause a partial, temporary inhibition of retinal oxygen consumption.

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DISCUSSION

DR. LEVY (New York): I have two questions.

First, what is the constancy of these findings in the same individual, whether he is normal or diseased, from hour to hour and from day to day?

Second, have you studied the effects of other factors such, for example, as digestion, exercise and emotional status?

DR. HICKAM: We have investigated the constancy of the findings in normal individuals by making repeated measurements over a period of about two hours. The standard deviation from the mean of individual readings in a given person is about seven or eight per cent oxygen saturation. The fluctuation is less when a series of observations is made in rapid succession. The method itself is not capable of a high degree of precision, so that part of the fluctuation must be ascribed to the method. Some part of the variability probably reflects actual change in the per cent oxygen saturation of the blood.

We have not yet made any observations on the effect of other factors such as digestion, exercise, and emotional status.

DR. WOOD (Philadelphia): Have you investigated the effect of age?

DR. HICKAM: We have made observations on the change in reactivity of normal retinal vessels to the inhalation of oxygen with advancing years. In normal persons retinal vascular reactivity shows only a very gradual decrease with age amounting to about 1 per cent every 10 years beginning at age twenty.

DR. HOWARD F. ROOT (Brookline, Massachusetts): This effect of oxygen is particularly interesting in relation to the matter of retrolental fibroplasia which causes blindness in premature infants. You will remember that the administration of high concentrations of oxygen to these premature infants has been implicated as being very significant in causing this disorder. It is a curious finding that the diabetic patient with proliferative retinopathy has some pathological changes in the retina which resemble very much some of the changes in retrolental fibroplasia.

I wonder, Dr. Hickam, if you have made any observations with these techniques in relation to the matter of retrolental fibroplasia or whether you know of any similar observations made by others relating to this disorder?

DR. HICKAM: I know of none, Dr. Root, except the observations in the literature that the blood vessels in young animals particularly tend to constrict when they are in a high oxygen atmosphere. This is true of kittens and it is much more true of the kitten than of the adult cat.

I have made no observations on this.

DR. ELLIOT V. NEWMAN (Nashville): I would just like to raise a question of interpretation, John, to see what you think about it. This relates to the question of the initial size of the artery and your reactivity index. The resistance which governs flow in vessels is related to the fourth power of the radius. Therefore, I think if the initial size of the hypertensive artery is half what the normal is it would take only 1/16 of the change in diameter to accomplish the same change in flow. I am not sure that this arithmetic is right but the initial size might have something to do with the small change in size in the hypertensive artery, which might have the same effect on flow as a larger size change in the vessels of a normal subject.

DR. HICKAM: I think that is a very shrewd point, Dr. Newman, and as a matter of fact, in the few hypertensives to whom I have given oxygen, the change so far in the retinal venous blood has been about the same in magnitude as in normal people, so this may well be right.

DR. HOWARD P. LEWIS (Portland, Oregon): Is there a change due to the effect of carbon dioxide?

DR. HICKAM: Yes, Dr. Lewis, I should have mentioned that. Five per cent carbon dioxide produces relatively little change in retinal vessels as against the considerable change which it has in cerebral vessels, but carbon dioxide does have an effect which can be best demonstrated in our hands by giving 10 per cent CO_2 and oxygen together. The addition of CO_2 largely blocks the constriction which would otherwise occur on oxygen.