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Does dark adaptation exacerbate diabetic retinopathy? Evidence and a linking hypothesis

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Abstract

The paper reviews evidence that before any change in diabetics' fundi, changes occur to blood flow, ERG and visual functions. In the case of colour vision and contrast sensitivity, the changes are partially reversed by breathing oxygen, and therefore are the result of retinal hypoxia. There are also other evidences that hypoxia is a major factor in the development of diabetic retinopathy (DR). Therefore in diabetics with early retinopathy, but normal photopic vision, functional disturbance might appear in dark adaptation, since in such circumstances, (as shown by Linsenmeier and his colleagues) the already low retinal PO_2 markedly decreases. This hypothesis has been tested and results consistent with the hypothesis (and with a number of older reports) have been obtained. The significance of this finding to early DR is discussed, and a mechanism suggested whereby prolonged periods of hypoxia during dark adaptation could generate changes in retinal capillaries. Such periods occur each night, and their elimination in diabetics could be therapeutic. © 1998 Elsevier Science Ltd. All rights reserved.

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1. Introduction

In the briefest summary, diabetic retinopathy (DR) is considered basically a vasculopathy. The pericytes, basement membrane and the endothelial cells of retinal capillaries are first to show histological degenerative changes [1–3]. The resulting reduction in vessel diameter, coupled with rheological changes, is thought to lead to the production of microaneurysms, capillary dropout, leakage from vessel walls and the pattern of local hypoxia that is seen clinically [4]. Once tissue damage has occurred, secondary processes exaggerate and continue the damage, which may progress (in man and in experimental animals) even if the diabetes is cured by pancreatic transplantation [5,6].

Long before the retinal clinical signs of DR appear, there is evidence of loss of function: there are ERG changes [7–10]; colour vision changes [11–14]; reduction of contrast sensitivity [15]; alteration in dark adaptation [16–21] and reductions in retinal blood flow [22,23].

As the clinical condition increases in severity, various other manifestations can be seen (cotton wool spots, hard exudates, flame haemorrhages) and the pathological changes to blood vessels are accompanied by changes in the RPE. These are associated with macular oedema and maculopathies, which, together with proliferative retinopathy, are the chief immediate causes of loss of vision [4].

The underlying mechanism of DR is thought to be the metabolic load placed on retinal cells by the lack of insulin and the increased level of blood glucose, which leads to abnormal glycation and glycoxidation of specific compounds [24,2,25]. The abnormal metabolism has been investigated in detail, particularly in respect to the polyol pathway, abnormalities of which may directly cause damage [26–28]. The role of substances released from damaged tissues has been investigated [29,30] and it has been shown that in some cases, the effects on the RPE are controlled by hypoxia [31]. Although close control of insulin levels may slow the appearance of DR, laser photocoagulation of the retina remains the only effective treatment for severe background retinopathy which otherwise develops into proliferative retinopathy and for some forms of

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maculopathy [32,4]. The benefits of antisorbitol oxidase therapy remain undecided [27].

It has been pointed out [33] that the vascular endothelial and pericyte changes of DR (which initiate the later progressive retinopathy) only occur in retinal capillaries. In brain capillaries of diabetics, which otherwise resemble retinal vessels, only thickening of the basal membrane occurs. Therefore local factor(s) must be responsible for DR. The purpose of this communication is to suggest the nature of one local factor, and thus to provide a linking hypothesis for the development of DR.

What is peculiar to retina is of course photoreceptors, and particularly the more numerous rods (120000000 compared to 6000000 cones). Rods have a uniquely high metabolic rate, demanded by the processes which enable them to signal the absorption of single quanta. The outer limbs are depolarised in darkness, and hyperpolarise in light. Thus a circulating ionic 'dark current' flows extracellularly between inner and outer limbs, and the current path is completed intracellularly [34]. The pumps which maintain this current turn over the entire cell cytosol ionic contents in a few seconds [35]. Additional unusual features of rods include the 10% of the outer limb shed and resynthesised each day, and the external neurotransmitters and internal transmitters that are constantly catabolised and anabolised.

Such a considerable degree of metabolic activity demands a high blood supply. Rods are avascular but are supplied by the extensive choroidal capillary network. The evidence that oxygen requirements are very high is that in cats and monkeys, while the PO_2 at Bruch's membrane approximates to the arterial PO_2, it falls precipitously to very low values in the outer plexiform layer, despite the richness of the choroidal vascular supply. The oxygen tension reduces still further in dark adaptation. The maximum decrease in PO_2 in darkness (and the trough of the oxygen profile) occurs about 110 μM from the choroid where (in dark adapted cats) the tension is approximately zero [36–40] though it is higher in monkeys [41]. In darkness, an unusually low PO_2 continues into the retina up to 150 μM from the choroid, because the rods act as 'sink' for oxygen supplied by the retinal circulation. In light adaptation, the dark current and the oxygen sink decrease very considerably. Quantitative analysis shows that $>50\%$ of all the oxygen is consumed by the photoreceptors, and that this is halved in light. Light does not influence the use of oxygen by the remaining layers of the retina, at least in monkeys.

We suggest that in diabetes the retinal circulation is initially embarrassed to the same degree as are other capillaries and small arterioles, but dark adaptation imposes additional periods of more profound hypoxia (at the retinal depths indicated above). It is a consequence of this hypothesis that in persons with background DR, even if photopic visual function is unaffected, scotopic function might be abnormal. The experiments described below were designed to test this prediction.

If in otherwise normal retina, hypoxia in dark adaptation can lead to loss of rod function, there is a further implication. The actual deficit in sensitivity of rods may well be unimportant, but demonstrates the hypoxia, which (by a mechanism detailed in Section 4), could allow dark adapted hypoxia to exacerbate the inner retinal vascular changes, thus contributing to the DR.

2. Methods

2.1. Subjects

Twenty five normal subjects served as controls for the dark adaptation procedures. Twenty two were untrained students aged 20–30: the remaining were experienced observers aged 40, 55 and 67. There were no significant differences between their results and those of the younger group. All had best corrected vision of 6/6 or better and no ocular disease. Four diabetics aged 25–33 were investigated. All had rapid onset insulin dependent diabetes mellitus (IDDM) in childhood. They had had no systemic or eye disease (other than the background DR) They were all under the supervision of the Diabetic screening service and the Department of Ophthalmology of St. Thomas' Hospital, and had had fluorescein angiograms and clinical examinations in the last six months. All had best corrected vision of $\geq 6/6$. Of these, three had mild background DR with few microaneurysms only. The fourth patient had more microaneurysms, and the fluorescein angiograms showed extensive areas of peripheral capillary closure. No haemorrhages or exudates were present in any patient, and there was no maculopathy. The patients had no other evidence of other eye or retinal disease, and no systemic disease. All subjects and patients gave informed consent for the procedures.

2.2. Procedures

Colour and achromatic contrast sensitivity was determined using the systems described previously [42]. A subset of Sloan optotypes were used as stimuli: these subtended 2° at the pupil. The stimuli were flashed for 50 ms with a repetition rate of 1 Hz and appeared against a uniform background. In the case

of achromatic contrast, the contrast threshold was defined and determined in the usual way. For coloured targets, colour contrast was varied along colour confusion axes for protan or tritan axes, and the threshold expressed as a percentage of the maximum modulation possible with the given phosphors in terms of the (XY) primaries introduced by the Committee Internationale d'Eclairage (CIE). Some of these tests were part of a standard clinical routine. They were however extended to mesopic levels, by interposing neutral filters between the subjects' eye and the monitor. The filters were mounted in spectacle frames. In all cases thresholds were determined by a modified binary search (MOBS) technique. The diabetics' results were obtained after a training session.

Dark adaptation was measured with a stimulator, powered by light-emitting diodes (LEDs). [43,44]. An internally reflecting hollow tube was terminated in a translucent 5 cm internal diameter hemisphere, concave forward. Different types of LED were mounted facing forward along the hollow tube, which contained light diffusers. The interior of the bowl appeared uniformly bright. When used as an adapting light, the bowl was placed on the subject's face to provide a source which covered 180° of visual angle. (a 'miniGanzfeld') The adapting light appeared orange and had a peak wavelength 610 nm) giving a retinal illumination of 16000 photopic Td. (1800 sc. Td.) for 120 s. For dark adaptation, the same bowl was placed 57 cm from the eye, and subtended 5° at 10° in the horizontal nasal field. Blue LEDs (peak wavelength 440 nm.) were used as stimuli. For times ≤ 200 s, a computer recorded the times at which stimuli of preset intensities were seen. Test flashes were repeated at 1 s intervals. Later in the experiment, thresholds were obtained by the method of adjustment by the operator. For normals, each point represents the average of several subjects' determinations. The time of the recording thresholds differed by <10 s. For diabetics, single results are plotted. The subjects were provided with an appropriate fixation point. Tests were carried out with the pupil dilated to >6 mm with 0.5% Tropicamide. Each dark adaptation curve for every observer was repeated twice. No systematic variation between trials was observed. Only one set of results was used for each observer. The effectiveness of the stimuli was altered by changing both the luminance of the flashes and their duration (variable between 1 and 3000 μs.).

The orange light used for pre-adaptation was sufficient for the 'knee' on the dark adaptation curve to occur after 1–3 min. This equipment and regime was used to obtain dark adaptation which was completed more rapidly than in the standard clinical test, because we wished to minimise any change in the diabetic patients clinical state.

3. Results

3.1. Photopic and mesopic visual function

Fig. 1 shows the mean achromatic and colour contrast sensitivities of two older normal observers and the four diabetics as a function of screen illumination. The right hand points for all observers and test situations are at full screen luminance, and represent the normal clinical test we employ. The results for the normals and diabetics are within the clinic normal limits. The reduction of screen luminance to -1.78 log cd m^{-2} represents an extension to low mesopic levels. For achromatic targets, contrast threshold increased to nearly 100% with this reduced luminance. There is no difference between the normals and the diabetics. For coloured targets, protan discrimination was within clinic normal results at maximum luminance, and again no difference was seen between normals and diabetics, although it was not possible to reduce screen luminance much below 1 cd m^{-2} and obtain colour discriminations. The diabetic subjects had slightly worse tritan colour discrimination than the normals, but the mean threshold at standard luminance is not significantly different to the normals. However, at reduced luminance, (1 cd m^{-2}) it was not possible to obtain any threshold from the diabetics. This result is in agreement with a number of papers which have shown that in early DR, there may be a reduction in tritan discrimination only.

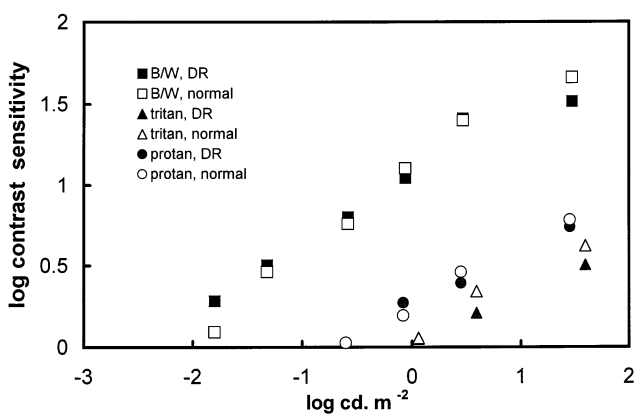

Fig. 1. Achromatic contrast sensitivity (□, ■), protan (△, ▲) and tritan (○, ●) colour contrast sensitivity in two normals (open symbols) and four diabetic subjects (solid symbols) as a function of mean luminance of the test screen In all cases, the test objects were a subset of the Sloan optotypes, subtending 2° at the eye at 1.15 m. Thresholds were determined with pupils dilated by 1% tropicamide. The letters appeared for 200 ms at a 1 Hz repetition rate. The short duration was chosen to reduce any photoreceptor adaptation during the stimulus. Only in the tritan thresholds is there any suggestion of a loss of function.

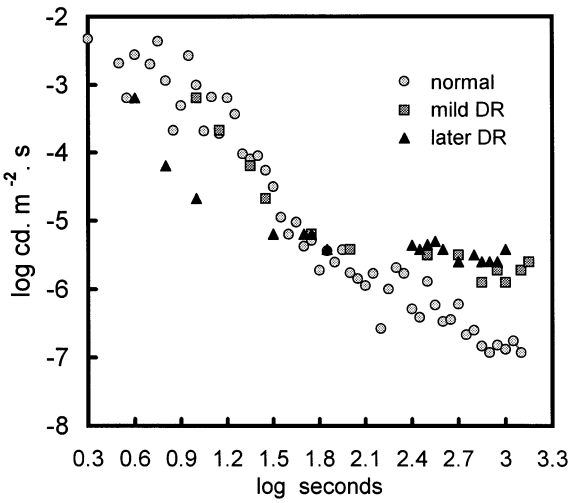

Fig. 2. Dark adaptation curves obtained in a group of 25 normals (●) and two diabetics. Patient JT (■) only had a few microaneurysms. Patient NA (▲) had many more microaneurysms and regions of peripheral capillary drop-out. The preceding light adaptation was by a 5 cm. diameter bowl apposed to the subjects face (a 'miniGanzfeld') The light was provided by light emitting diodes (peak wavelength 610 nm) giving a retinal illumination of 16000 photopic Td. (1800 sc. Td.) for 120 s. For dark adaptation, the same bowl was placed 57 cm from the eye, and subtended 5°. Blue LEDs (peak wavelength 440 nm.) were used as stimuli. For times ≤ 200 s, a computer recorded the times at which stimuli of preset intensities were seen. Later in the experiment thresholds were obtained by the method of adjustment by the operator. Thresholds were obtained at 10° in the nasal field on the horizontal meridian Light intensity was altered by changing both the intensity of the flashes (1 Hz) and their duration (variable between 1 and 3000 μs).

3.2. Dark adaptation

Fig. 2 shows the dark adaptation curves obtained by our non standard technique in the normal control group and in two diabetics. Note the logarithmic time scale. There is considerable variation in the initial part of the normal curve. This is partly due to the fact that the equipment could not change light intensity at sufficient speed to track the rapid increase of sensitivity which occurs in the first seconds. After 300 s the rate of dark adaptation slowed so that the method of adjustment could be used. The decline in normal values continues to >1000 s. The final values reached are similar to those reported in the literature, for large fields in the near periphery.

In the diabetics, the initial thresholds were also normal. Subject NA who had the most advanced condition had an initial fall of threshold which was one of the fastest in the entire group. His threshold fell to log -4.8 cd m^{-2} s^{-1} in 3 s. The normal group's timing was between 3 and 21 s. There is no significant difference between the diabetic group and the normals. Thus, for normals the mean duration for threshold to decrease below log -4.8 cd m^{-2} s^{-1} was 11.1 s with a standard deviation of ± 1.4 s, while for the diabetics (including NA) it was 7.8 ± 1.6 s. Previous workers have reported that dark adaptation slows in DR: it is possible that they investigated patients with more severe disease. However, after 5 min, when diabetic and normal thresholds were equal, the diabetics' dark adaptation stopped abruptly. Table 1 shows the results. There is almost no fall of threshold in the diabetics, while the normal threshold falls 0.8 log. unit. This difference is highly significant.

4. Discussion

4.1. Abnormalities of dark adaptation in background DR

Even though we have only investigated a few diabetics, it is clear that in early background DR, with normal photopic functions, there is a change in rod dark adapted threshold. The form of the diabetics' dark adaptation curve implies that sensitivity is limited by an 'equivalent background illumination' [45], 10–30 times the intensity of the absolute threshold of vision. The 'equivalent background' represents either the inability of the rods to increase their dark current to the maximum, or a reduction in rod 'gain' or a postsynaptic failure to detect minimal rod signals. Earlier investigations of dark adaptation in DR are consistent with our findings [46–48,16–21]. Some of these authors report slowing of dark adaptation as well as an elevated final threshold. It is not clear whether this is related to their different technique or choice of patients. None have shown that abnormal rod function develops even when refined test of photopic vision are normal.

We (work in progress) are determining whether this loss of dark adaptation is reversed by breathing oxygen. Until then, we cannot directly relate the dark adapted

Table 1
Statistical analysis of rod thresholds after 5 and 10 min dark adaptation, in normals and diabetics

	Thresholds[a] After 5 min	After 10 min	Threshold[a] decrease 5–10 min
Normals			
Number	25	25	25
Mean	−5.93	−6.74	0.81*
S.D.	0.51	0.74	0.54
S.E.	0.1	0.15	0.11
Diabetics			
Number	4	4	4
Mean	−5.99	−6.05	0.06*
S.D.	0.44	0.39	0.20
S.E.	0.22	0.20	0.11

[a] Thresholds in log cd m^{-2} s^{-1} of 440 nm light
* The probability that normals and diabetics come from same population (t-test) = 0.0012

abnormality to a relative anoxia, related to the increased oxygen demand of rods, though this seems highly likely. Below we consider other evidence for retinal hypoxia in dark adaptation, and how hypoxia in dark adaptation, occurring in the outer retina, could cause vascular damage in the inner retina.

We anticipated that rod thresholds might reach a trough, and then rise again in diabetics as hypoxia developed. This was not seen, probably because (as recently demonstrated), the change in PO_2 caused by light is nearly as rapid as the electrical activity of the retina, and too fast to be detected by our technique [49]. This result is also consistent with ERG findings which show in background DR that the abnormalities in the b-wave are much more evident when the provoking flashes are weak [9].

4.2. Evidence that diabetic retina is hypoxic

The success of laser therapy supports this idea. Although it was first thought that laser coagulation worked by stopping the supply of harmful substances from pathologically damaged retina [50], in retinal photocoagulation it is not necessary to direct the laser to any particular region: it is the reduction in the number of photoreceptors which seems to be important (though superficial to the 'burn' inner retinal neurones survive, at least in part). The treatment increases retinal PO_2 [51–53].

Additionally, in early DR, ERG abnormalities are not limited to the photopic oscillatory potentials, generated in the inner retina [7,8,10]. There are also abnormalities in dark adaptation, that suggest loss of sensitivity and response amplitude of photoreceptors to dim flashes [9].

Further evidence comes from the discovery that contrast sensitivity loss [15] and the loss of Tritan (blue-yellow) colour vision in DR [54] (which begins before ophthalmoscopic changes can be detected) is rapidly and partially reversed by inhalation of oxygen (but not air) from a face mask. Tritan vision in normals is unchanged by breathing oxygen. Colour vision losses (similar to those of diabetes) occur in normal subjects after decompression to the equivalent of 12000 ft., when the arterial oxygen saturation decreases to about 90%, and are then relieved by oxygen inhalation which increases the saturation to 95% ([55], Arden and Hogg, unpublished). The steep S-shaped characteristic of the Hb/HbO_2 saturation curve means that both in normals under decompression, and in diabetics, the additional quantity of oxygen carried to the retina by breathing oxygen is quite small. Therefore, small decrements in the rate of oxygen supply must cause functional changes in normals, and be present in early DR. In the latter, the lack of oxygen occurs under ordinary lighting conditions, and must thus get worse in dark adaptation. Finally, direct measurement of PO_2 in the 'clinically' normal inner retina of long-term diabetic cats revealed an astonishingly low PO_2: in one case 1.7 mmHg, against the normal 18.5 mmHg [56].

4.3. Is the change in oxygen important?

Since more than half the retinal oxygen supply is absorbed by rods, and the uptake is rapidly and drastically reduced by halting the dark current, light adaptation reduces the oxygen requirements by as much as pan-retinal photocoagulation.

Quite mild degrees of cerebral hypoxia may cause cerebral symptoms. The maximum safe duration for the acute exposure of healthy young recruits to the German Air Force to decompression that affects tritan vision is deemed to be only 2 h. Even though photoreceptors are surprisingly resistant to acute anoxia, we have now confirmed that in cases of DR with normal photopic and mesopic psychophysics, dark adaptation reveals loss of scotopic function. Even if this is a protective mechanism, reducing oxygen consumption by the diabetic rod, it must mean that in our patients there is a unphysiologically low oxygen tension in dark adaptation. The retinal depth at which the greatest ΔPO_2 occurs, is associated with the generation of the b-wave, which recovers more slowly and incompletely after anoxia than is the case for the photoreceptors [57].

4.4. How can outer retinal hypoxia cause inner retinal capillary damage?

The photoreceptor layer is avascular, but a cellular mechanism for the vascular changes of diabetes is provided by vascular endothelial growth factor (VEGF)[1]. This is produced in the glial (Mueller) cells. The life time and quantity of its mRNA increases in hypoxia. VEGF moves from the cell of origin and binds to receptors on the capillary endothelium, and directly causes endothelial cell proliferation: it is present in higher amounts in diabetic retina, and thought to be a potent and crucial factor stimulating the angiogenic response in diabetic eyes [58,50]. Any small change caused by diabetes in capillary walls—for example, the thickening of basement membranes—could embarrass retinal oxygen supply to the point where more VEGF was produced in dark adaptation, and a vicious circle would then begin to operate. The retinal circulation would become slightly less adequate, so at the next period of dark adaptation, the relative hypoxia would be more profound, and even more VEGF would accumulate: and so on.

[1] Since submission of this paper, much work has further implicated VEGF in DR: e.g. Aiello LP. Vascular Endothelial Growth Factor—20th century mechanisms, 21st century therapies (minireview). Invest Ophthalmol Vis Sci 1997;38:1647–52.

4.5. Under what circumstances in diabetes can dark adaptation hypoxia alter retinal function?

In the natural history of diabetes, the period between the onset of functional abnormalities and the worsening of background retinopathy may be of peculiar significance. The large increase in oxygen consumption and the large change in PO_2 in dark adaptation might have a considerable effect on the progress of the condition. This would not be the same at all stages of the disease. At the onset of IDDM diabetes, the only abnormal stress on the retina is due to uncontrolled glucose metabolism. If this is corrected, dark adaptation need not be damaging. In the end stage (e.g. proliferative retinopathy) there is obvious anoxia. If normal animals are made hypoxic by respiring them with atmospheres containing reduced oxygen, the ΔPO_2 between dark and light is considerably diminished. Therefore, light and dark adaptation could not cause any change at the end stages of DR where anoxia is continuous.

4.6. Can possible hypoxic retinal damage be avoided?

In normals, prolonged dark adaptation occurs each night and therefore prolonged hypoxia must result. Even though this has no ill effect, it is difficult to avoid the conclusion that in DR, where the retina is already compromised, the oxygen requirements are higher, and the oxygen tension reduced, dark adaptation for prolonged periods is likely to contribute to the development of histological changes in the inner retina. It is possible that continuous exposure to a light intensity of the 'equivalent background' found in these experiments would be enough to avoid ill effects, and such light levels are easily achieved through closed lids.

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