Foveal Densitometry in Retinitis Pigmentosa

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Cone density and psychophysical thresholds were investigated in patients with retinitis pigmentosa. Our aim was to assess possible disturbances of foveal cones, especially in patients with good visual acuity. Using the continuously recording densitometer described by van Norren and van der Kraats, we examined ten patients (19 eyes). With the same apparatus it was possible to determine cone final threshold. In all patients double density values were lower than in an age-matched and sex-matched control group of an equal number, and the half time of regeneration was increased in eight eyes. In 12 eyes of eight patients the foveal final threshold was raised above normal. Pseudoprotanomaly was found in seven eyes in four males. Possible explanations for these findings are discussed. Since reduced double density was found in all patients with retinitis pigmentosa, we conclude that disturbed foveal cones are probably a common and rather early feature in this disease. Invest Ophthalmol Vis Sci 24:1123–1130, 1983

Retinitis pigmentosa is a progressive form of tapetoretinal dystrophy. Typical observations in the disease are the characteristic fundus picture of bone-spicule pigmentation, a lowered or undetectable scotopic b-wave on electrophysiologic examination, an annular scotoma on perimetric examination, and a raised final threshold on examination with the Goldmann-Weekers adaptometer. The disease affects primarily the receptor layer, particularly the cells of the midperipheral retina; in a later stage the cones in the fovea may also become involved. It is well known that the central field of vision may be retained for a long time and that the visual acuity can remain relativily unimpaired, even in the far advanced stages of the disease.

When the technique of retinal densitometry came into use, it was to be expected that attention would be especially focused on measuring the properties of the rods, and several investigators demonstrated that a reduction in the density of rhodopsin correlated well with the loss of visual sensitivity.[1,2] Perlman and Auerbach[3] distinguished two groups of patients: one group with a recessive inheritance, in which the relation between the *log* threshold elevation and the fraction of rhodopsin was probably linear, and another group with principally dominant inheritance in which *absolute* threshold elevation was linearly related to the fraction of rhodopsin present.

It was our aim to investigate more thoroughly whether densitometric examination could demonstrate an impaired function in foveal cones in patients with retinitis pigmentosa. For this purpose we used the retinal densitometer described by van Norren and van der Kraats,[4] an instrument that enables reliable and relatively easy measurements in patients. To assess the functional state of the fovea, we measured psychophysical cone dark adaptation to all patients with the same apparatus. Our findings show that in all patients tested by densitometry, the double density values were significantly less than normal, and that in several of these subjects the half-time of pigment regeneration was slower than in the normal.

Materials and Methods

Subjects

For the patient group only those patients were selected who had a confirmed retinitis pigmentosa, established by the following criteria. Except for patients 1 and 2, all patients had a family history of retinitis pigmentosa. Patient 5 is a sister of patient 6, patient 4 and 8 are brothers, and patient 9 and 10 are brothers (Table 1). All patients had complaints of night blindness. Ophthalmoscopic examination showed bone corpuscle pigmentations, except for patient 8 who showed atrophy of the retinal pigment epithelium and choriocapillaris and pigmentations as little spots. Except for patient 1 and 2 all showed wax-like optic discs and narrow retinal vessels. The following investigations were done. Visual field with the Goldmann perimeter showed in six patients complete and in two patients incomplete annular scotomata. Patient 5 was not available for further investigation. Dark adaptation showed in all patients an elevated

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Table 1. Clinical data and genotypes of patients with retinitis pigmentosa

Patient	Sex	Age (yr)	Visual Acuity		Scot. B-wave (μV)		EOG (%)		Perimetry isopter	Central field		Genotype
			OD	OS	OD	OS	OD	OS		OD	OS	
1	M	47	1.25	1.25	150	150	170	150	I2	35	35	Isolated
2	F	40	1.0	1.0	40	40	175	129	I2	20	20	Isolated
3	F	31		1.0	Extinct	Extinct	100	140	I2	20	25	Dominant
4	M	35	1.0	1.0	50	50	100	100	I2	10	10	Recessive or x-linked
5	F	18	1.0	1.0	Extinct	Extinct						Recessive
6	M	24	1.0	0.5	Extinct	Extinct	125	125	I1	5	5	Recessive
7	M	34	0.8	0.8	Extinct	Extinct			I2	8	8	Recessive
8	M	40	0.5	1.0	Extinct	Extinct			I2	10	15	Recessive or x-linked
9	M	36	0.5	0.5	Extinct	Extinct	100	120	I4	30	30	Recessive or x-linked
10	M	40	0.5	0.5	Extinct	Extinct	100	115	I4	20	20	Recessive or x-linked

OD = right eye; OS = left eye.
The right eye of patient 3 could not be measured because of amblyopia.
Minimal amplitude of the scotopic B-wave of the ERG with this intensity is 200 μV for normal subjects.

final rod threshold (Goldmann-Weekers adaptometer) and in patient 9 and 10 there was only cone adaptation. The Arden ratio in all patients was abnormal and the scopotic b-wave of the ERG was diminished in three patients and extinct in seven patients which is typical for retinitis pigmentosa. Visual acuity can be found in Table 2, and the results of EOG, ERG, and perimetric examination are listed in Table 1. These ten patients were all examined twice with a time interval of one year. Densitometric data on both these occasions were almost the same, but in all patients only the outcomes of the second mea-

Table 2. Results of densitometry, psychophysics, and anomaloscope settings

Visual acuity	Patient	Double density	Densitometric half-time value(s)	Psychophysical threshold elevation (log unit)	Psychophysical halftime(s)	Anomaloscope setting
1.0 or better	1	OD 0.14	75	OD 0.32	87	42.5
		OS 0.10	90	OS 0.44	96	41.5
	2	OD 0.16	69	OD 0.36	66	43
		OS 0.16	63	OS 0.52	66	43
	3	OS 0.17	84	OS 0.48	72	44
	4	OD 0.08	150	OD 0.72	102	43
		OS 0.10	210	OS 0.44	87	43
	8	OS 0.07	210	OS 0.56	144	44
	5	OD 0.23	93	OD 0.04	78	42
		OS 0.17	87	OS 0.24	66	41.5
	6	OD 0.19	93	OD 0.32	60	46
0.8	7	OD 0.04	—	OD 0.88	150	48
		OS 0.03	—	OS 1.04	210	49
0.5	6	OS 0.10	112	OS 0.60	63	49
	8	OD 0.02	—	OD 1.00	—	44
	9	OD < 0.01	—	OD 1.04	30	53
		OS < 0.01	—	OS 1.12	45	50
	10	OD 0.03	—	OD 1.00	45	52
		OS 0.03	—	OS 1.00	—	53

OD = right eye; OS = left eye.
The patients are arranged in 3 groups according to visual acuity. The two eyes of patients 6 and 8 with different visual acuity are therefore listed in different groups.

surement are discussed in this paper. We did foveal densitometry in patients with retinitis pigmentosa after the following selection criteria: First, a minimal visual acuity was required of 0.5 or better, since we were most interested in cone pigment kinetics in patients with good visual acuity. Second, central fixation was necessary, because eccentric fixation is often unstable and it is impossible to measure exactly in the fovea. Third, clear media are required in order to decrease the amount of straylight. A group of ten normal naive subjects, age-matched and sex-matched with the patient group, served as a control group for both densitometry and psychophysics.

Retinal Densitometer

The densitometer has been described in detail by van Norren and van der Kraats.[4] In brief, the densitometer consists of an optical and an electronic part. In the optical part a xenon high pressure lamp is used as the source of three different beams of light; viz, the bleaching beam, the reference beam, and the measuring beam (Fig. 1). The bleaching beam passes through a yellow filter that eliminates wavelengths shorter than 530 nm. For the measuring beam, a wavelength of 554 nm was chosen in examining all patients. The reference beam had a wavelength of 711 nm. The three channels are converged near a sector disc that produces a repetitive sequence of 7.5 msec pulses of darkness, reference light, bleaching light, and measuring light. The beams are focused onto an optical fiber that provides the input to a modified Zeiss fundus camera. The light is projected into the eye with a field width of 3.8°, and the reflected light is measured by a photomultiplier. The photomultiplier "sees" only the central 2.5° of the stimulus field. This photomultiplier is used as a photon counting device; the counts are separated for each of the four timed sequences. For measuring, reference, and bleaching counts, the dark light counts are subtracted and the difference plotted on a penwriter together with the logarithm of the measuring to reference ratio. The latter trace provides a relative measure for the density of visual pigments (provided straylight is negligible). The difference in this trace between the fully bleached and the fully dark-adapted condition defines the double density.

Psychophysics

By making some adjustments the densitometer can also be used to record foveal dark adaptation with the advantage of having nearly identical conditions in both types of measurements. Figure 1 shows diagram of the densitometer with the modifications that enable the psychophysical measurements. Mirror M1

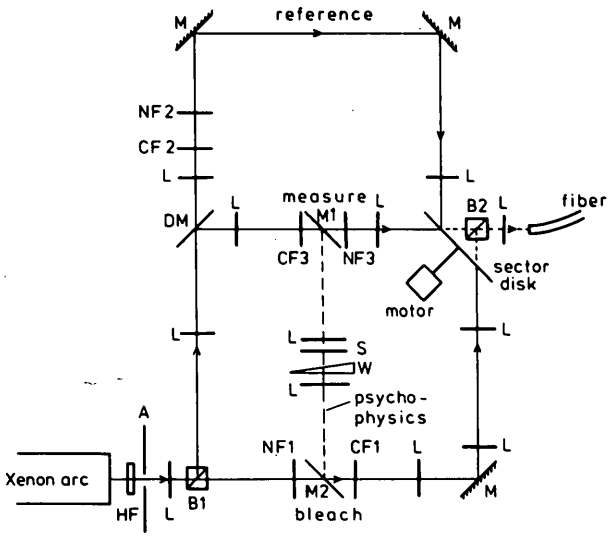

Fig. 1. Diagram of a part of the optics of the densitometer with the modification to enable the psychophysical measurements. HF, heat filter; A, aperture; B, 1, 2 beamsplitters; DM, dichroic mirror; CF 1, 2, 3, colour filters; NF 1, 2, 3, neutral filters; M, mirror; L, lenses. To enable psychophysical measurements the mirror M1 is inserted into the measuring light path. The light passes the shutter (S) and the wedge (W), and is combined with the bleaching light through half-mirror M2.

is placed in the path of the measuring beam. The motor of the sector disc is switched off and the disc is positioned in such a way that the stimulus beam (which passes through a neutral density wedge [W] via CF 3) is transmitted. The stimulus field is 1.5°, to ensure that the influence of rods is as small as possible. In the bleaching phase the full available 30° field is employed. The stimulus flash is 50 msec, presented every second. By pushing or releasing a button the subject manipulates the rotation of the wedge, so that the stimulus light becomes dimmer or brighter. The subject is asked to keep the stimulus at the just visible level. The position of the wedge is recorded on the penwriter.

Anomaloscope

A Nagel type I anomaloscope was used. The subject was asked to make a red-green setting for each eye with a yellow brightness setting of 12.5. The mean value of five matches for each eye was calculated. Thereafter the patients were asked to handle the yellow brightness setting knob in order to make a match with different red-green settings.

Fluorescein Angiography

Fluorescein angiography was performed in five of the ten patients. In patients 2, 3, and 6 this investigation revealed no distinct foveal abnormalities, but patient 9 and 10 had cystoid macular edema.

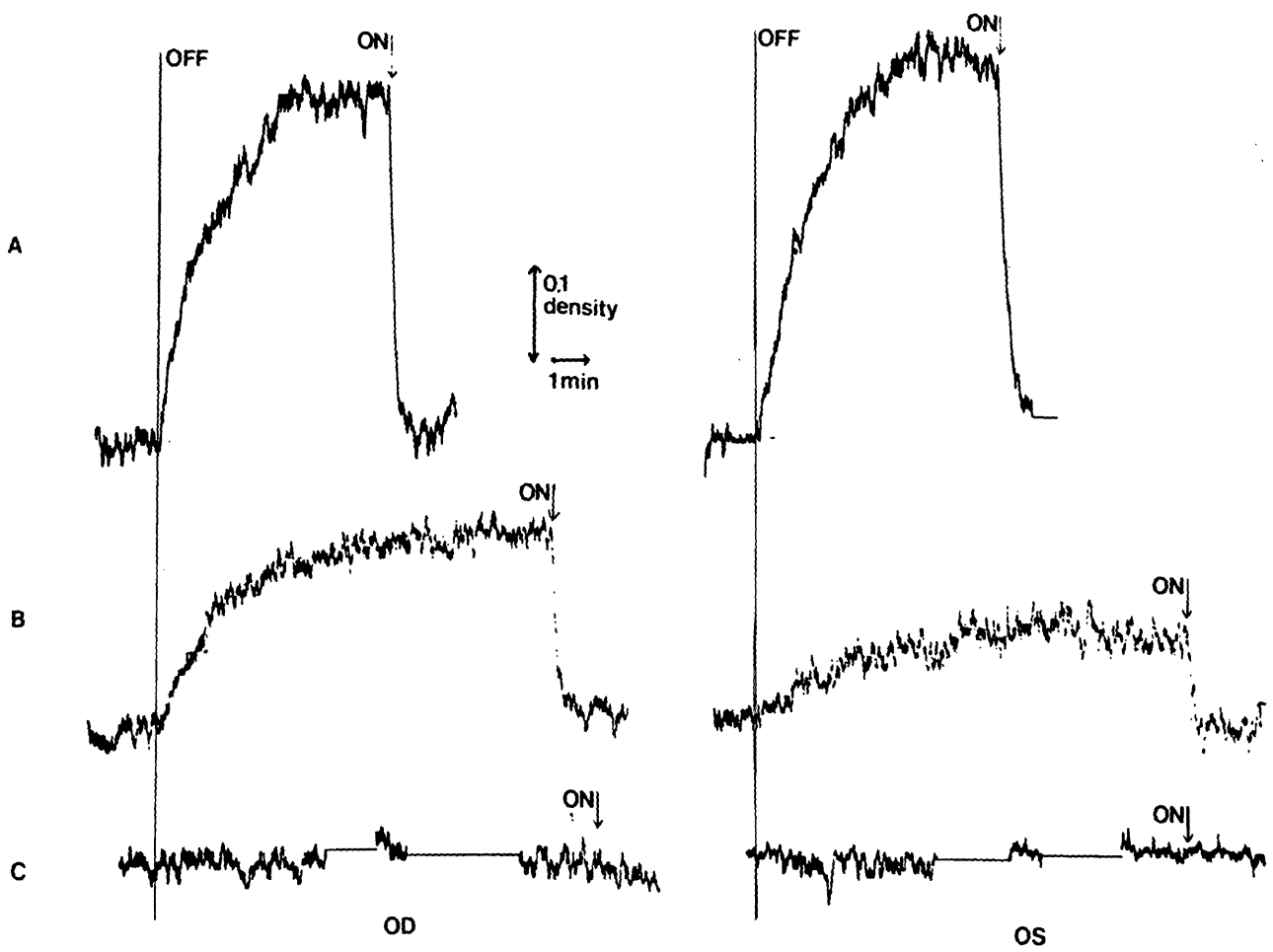

Fig. 2. Foveal densitometry in a normal subject and two patients with retinitis pigmentosa. A, Measuring sequence in a normal subject. After a bleach of 6 log trolands during two minutes a dark period starts at "OFF," during which the pigment regenerates. At "ON" the bleaching light is switched on again, resulting in rapid photolysis of the pigment. Double density in this example is 0.34, half time is 54 sec. B, Densitometry in patient 6. Same sequence as in A but with larger dark period. OD: double density 0.19 and half time 93 seconds, OS: double density 0.10 and half time 112 seconds. C, Densitometry in patient 9. After about 12 min of dark adaptation no change in density is observed when the bleaching light is switched on again. Straight lines in the curves indicate periods in which patient closed his eyes to avoid fatigue.

Procedure

The nature of the experiments was explained carefully. The pupils were dilated with tropicamine (Mydriaticum Roche) and phenylephrine 10%. Temple pads and a bite bar, made of a dental impression compound, were used to immobilize the subject's head during measurements. By removing a field stop, the examiner can obtain a 30° view of the fundus, which can be focused by shifting the position of the camera. Focusing is performed with a field intensity of 5 log trolands. Subjects are asked to fixate a pair of crosshairs centered with the stimulus field. Adjustment of the camera was aimed at the highest possible fundus reflection. In practice we looked for a position in which the total perimacular reflex was present. This position, just avoiding corneal reflexes, proved to yield the highest reflection. The adjustment procedure generally takes 1 min. After adjustment the measurement starts with a 2-min bleaching exposure at 6 log trolands. The bleaching light is then switched off and the recovery of density is recorded as a function of time to indicate the regeneration of the visual pigments. In healthy subjects regeneration is generally completed within 7 min. Next, the bleaching light is switched on again for 2 min. The density trace returns again to the fully bleached condition, enabling a check on the reliability of the measurement.

Results

Control Group

In Figure 2A an example is given of a foveal densitometric recording from both eyes of a healthy sub-

ject. Double density is, in this case, 0.34 for the right eye and 0.34 for the left eye. For all normal subjects we found a mean double density of 0.30 with a standard deviation of 0.03. Half time, which is the period necessary to reach half the double density, was calculated by taking the position of the penwriter recording at intervals of half a minute; these values were then plotted on a logarithmic scale as a function of time, and a best-fitting straight line was positioned by eye through the data points. The average half time was 69 sec with a standard deviation of 8 sec.

In Figure 3A an example is given of a normal psychophysical dark adaptation curve with central fixation and a 1.5° stimulus field. We found an average final threshold value of 0.11 log trolands with a standard deviation of 0.17 log trolands. The mean half time (obtained as described above) was 74 sec with a standard deviation of 13 sec. The red-green settings on the anomaloscope with a fixed yellow setting of 12.5, were found to lie between 37 and 46 in normal subjects. None of the subjects preferred a yellow setting different from 12.5.

Patients

In Figure 2B the densitometry of patient 6 is shown. Visual acuity in the right eye was 1.0, visual acuity in the left eye was 0.5. The double density is 0.19 for the right eye and 0.10 for the left eye. Half time in the right eye is 93 sec. Half time in the left eye is 112 sec, which is precisely the same value reported by Ripps et al[2] for a patient with dominant retinitis pigmentosa, and is more than the normal mean value of 69 sec plus two times the standard deviation. The psychophysical dark adaptation curve of this patient is shown in Figure 3B. The final cone thresholds lie, respectively, 0.32 and 0.60 log units above the normal mean value for the right and the left eye.

The results of the densitometric measurements in all patients are summarized in Table 2. The eyes have been arranged in three groups in order of descending visual acuity. The results show that in all patients the double density is much lower than in the normal subjects. In one patient (9, Table 2) no change in density was observed between the light- and dark-adapted condition. His data are presented in Figure 2C. His psychophysical curves are given in Figure 3C. In those cases where the difference in density was large enough to render a calculation of the half time possible, it is listed in Table 2. The densitometric half time appeared to be increased in eight eyes. It could not be assessed in seven eyes and in four eyes it was normal. The final thresholds of cone dark adaptation after eight minutes of adaptation are listed in Table 2.

In 12 eyes of 8 patients final thresholds were found to be significantly raised above normal (above 0.45 log trolands). Four males (seven eyes) appeared to be pseudoprotanomalous, whereas all the others made a red-green setting within the normal range (Table 2). All yellow settings were within normal limits.

Fluorescein angiography in patients 2, 3 and 6 showed no distinct foveal abnormalities, but in patient 9 and 10 it revealed a cystoid macular edema. On ophthalmoscopic examination patient 1, 2, 3, 5, and 6 showed no abnormalities of the macular area, in the other five patients there was a beginning of cystoid macular edema.

Discussion

The results of the examination of patients with retinitis pigmentosa as described in the foregoing can be summarized as follows. All patients show a significantly decreased density of the cone visual pigments. In those cases in which it was possible to assess the half time of cone pigment regeneration, 8 out of 12 eyes showed a prolonged time course of regeneration (Table 2). Thus, major changes in the cone visual pigments of the fovea seem to be a common feature in patients with retinitis pigmentosa, even in those patients who have virtually no complaints about their visual acuity. Retinal densitometry generally is a rather difficult procedure, but the use of a modified Zeiss fundus camera in our densitometer is a great advantage and makes the task to obtain a clear and luminous fundus picture relatively easy.

The occurrence of pseudoprotanomaly only in those eyes with a visual acuity of 0.8 and 0.5 (seven out of eight eyes) is striking. All but one of these eyes have a foveal double density of 0.04 or less. Pseudoprotanomaly is an independent indication for reduced absorption in the cone visual pigments,[5] and can be described as an acquired defect of the color sense whereby the patient used an abnormally large quantity of red light in proportion to green light, when making a match to the yellow half field of the anomaloscope.

Smith and Pokorny[5] suppose that pseudoprotanomaly in central serous retinopathy is caused by distortion of the photoreceptor layer because the sensory retina is lifted. The effective optical density decreases since in such cases the light strikes the outer segments of the cones at an angle. Young and Fishman[6] also find that color matches in patients with retinitis pigmentosa were more protanomalous in trichromats and more protanopic in anomalous trichromats or dichromats. They also suppose that these changes, when viewed in the light of the known his-

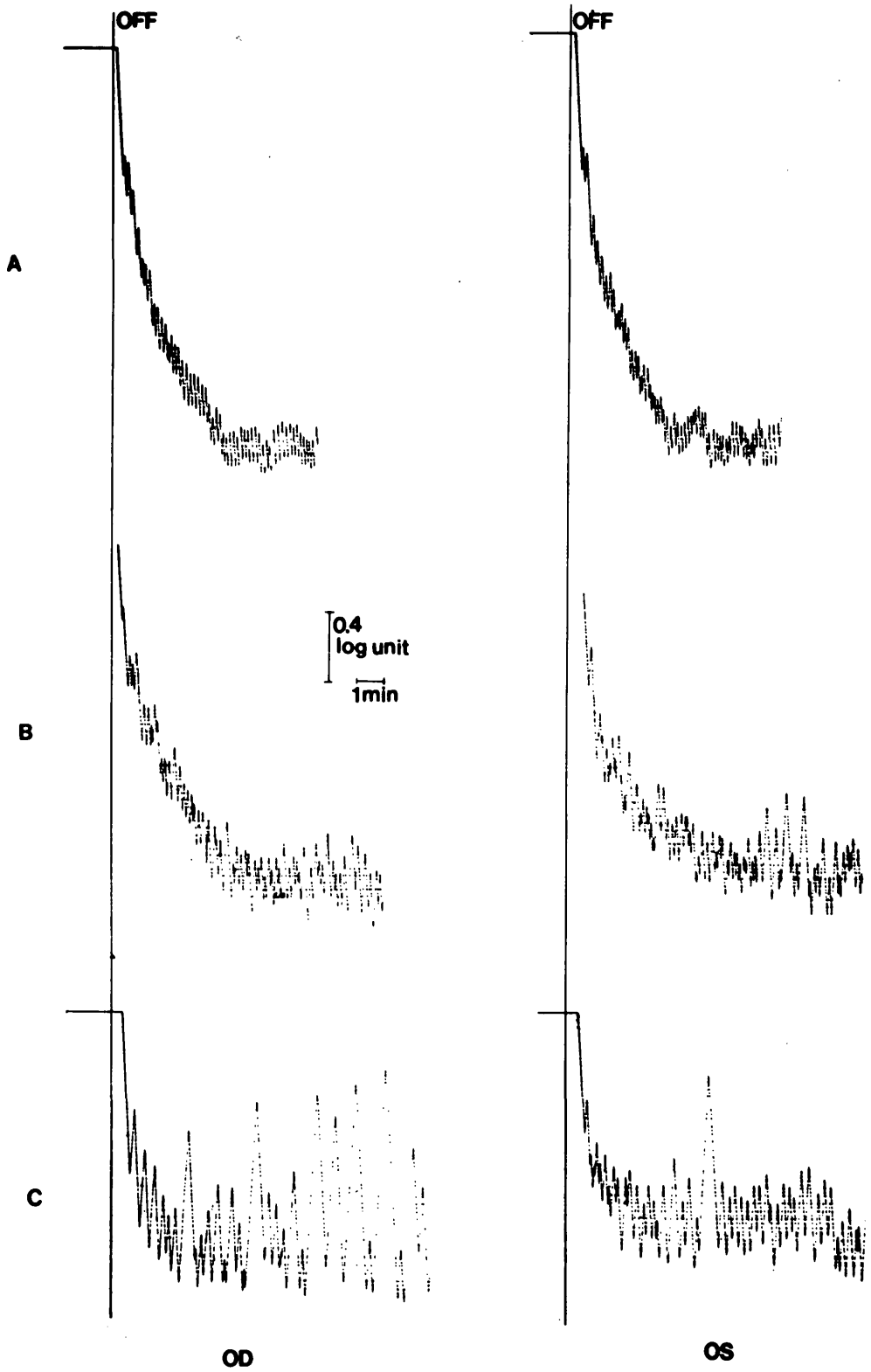

Fig. 3. Cone psychophysics in the same subjects as in Figure 2. A, Psychophysics in a normal subject. After a yellow bleach of two minutes with 6 log trolands and a field of 30°, the bleaching light is switched off ("OFF"). At that moment the field is reduced to 1.5°. Final threshold after about 8 min is 0.03 log trolands. B, Psychophysics in patient 6. OD: final threshold elevation compared to normal mean is 0.32 log unit and half time 60 seconds. OS: final threshold elevation 0.60 log unit and half time 63 sec. C, Psychophysics in patient 9. OD: final threshold elevation 1.04 log unit and half time 30 sec. OS: final threshold elevation 1.12 log unit and half time 45 sec.

tologic and densitometric alterations, can be best explained by a reduction of the cone optical density.

Figure 4 shows the relation between the foveal double density, expressed as a percentage of the mean for the ten normal subjects (0.30), and the increase, related to the normal value, of the foveal threshold after 8 min of dark adaptation. All data fit well with a curve that describes the relationship expected when threshold is determined by the probability of quantal absorption. Highman and Weale[1] and Ripps et al[2] in

similar plots, also found a high correlation between the reciprocal of the absolute threshold and the rhodopsin density. However, in all subjects, including patients with retinitis pigmentosa, they found a normal rate of rhodopsin regeneration.

Perlman and Auerbach[3] suppose on the basis of rhodopsin density estimate, that retinitis pigmentosa patients can be divided in two categories according to hereditary pattern. In one group with a recessive inheritance the log threshold elevation showed a linear relationship with the fraction of rhodopsin present. In the second group with autosomal dominant inheritance absolute threshold was linearly related to the fraction of rhodopsin present.

In our data there is no indication for such a distinction between hereditary patterns.

Arguments for pathologic changes in cones can in principle also be derived from fluorescein angiography and from pathologic examination of eyes from patients with retinitis pigmentosa.

We did fluorescein angiography in ten eyes of five patients. Four eyes (patients 2 and 3, and patient 6, right eye) fell within the group of visual acuity of 1.0 or better. In none of these cases did fluorescein angiography of the fovea show significant abnormalities. In patient 6, left eye, fluorescein angiography showed the beginning of macular holes. Patients 9 and 10, with a visual acuity of 0.5, had cystoid macular edema.

Krill et al,[7] who mentioned mottled fluorescent abnormalities due to abnormalities in the retinal pigment epithelium in retinitis pigmentosa, state that they never saw these findings in patients with a visual acuity of more than 0.5. This is in agreement with our findings.

From the work of Fishman et al,[8,9] it appears that foveal changes examined by fluorescein angiography are rather frequent. In a study including 31 retinitis pigmentosa patients[8] with foveal lesions they found three categories of macular changes: group 1—hypopigmented macular lesions, group 2—foveal cysts and partial thickness holes without leakage from perifoveal capillaries, and group 3—foveal cysts with cystoid macular edema.

Seven eyes of these patients showed a visual acuity of 0.5 or better. Two of these eyes showed a cystic lesion or partial thickness hole; the other five eyes showed small retinal cysts associated with cystoid macular edema, which was confirmed by fluorescein angiography. In a subsequent publication,[9] they report on a group of 110 retinitis pigmentosa patients, in which 69 patients showed lesions within the fovea; the authors mention that all of these patients showed some reduction in central acuity.

Considering that all our patients show abnormal

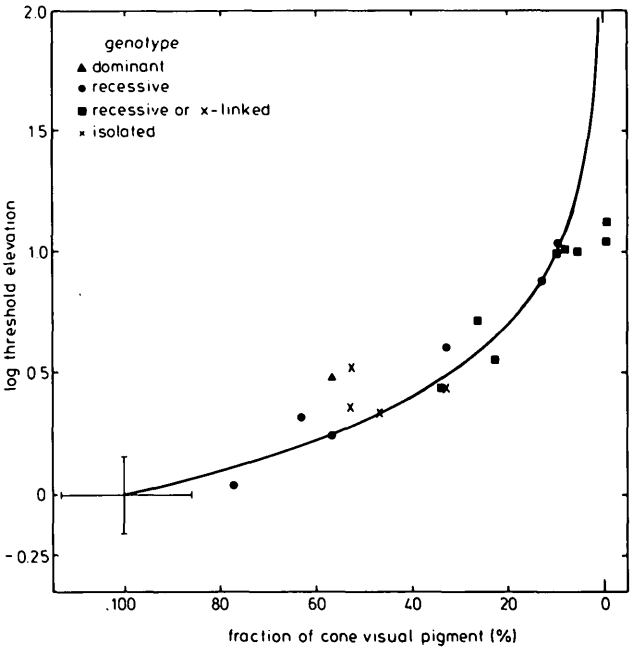

Fig. 4. Relation between the fraction of cone visual pigment and foveal threshold elevation in 10 patients (19 eyes) with retinitis pigmentosa. 100% cone pigment corresponds to a double density of 0.30. The continuous curve depicts the theoretical relation expected when threshold is determined by the probability of quantal absorption.

cone pigment densities, we conclude that specifically in patients with high visual acuity, densitometry provides an extremely sensitive method for the detection of foveal abnormalities.

Several reports have appeared in the literature on pathologic examination of eyes of patients with retinitis pigmentosa, and they demonstrate that morphologic changes in cones are common.[10-13] Mizuno and Nishida[14] examined an eye from a patient with retinitis pigmentosa with electron microscopy. The eye was removed because of an acute glaucoma. They only found remnants of cones with irregularly arranged lamellae in the outer segments. Kolb and Gouras[15] found in the eyes from a patient with retinitis pigmentosa who had had a visual acuity of respectively 0.7 and 0.8, that foveal cones showed short and broad outer segments while the discs were disoriented.

Our finding of reduced double density of the cones can be explained either by a reduced number of cones or by abnormalities in the outer segments (disorientation in the discs and/or shortening of the outer segments). The anatomic literature and recent Stiles-Crawford measurements on retinitis pigmentosa patients[16] strongly indicate some disturbance in the outer segments. A reduced number of cones would effectivily increase stray light which would cause the

measured half time to decrease[4]; we found either normal or increased half times.

The correlation between the psychophysical half times and the densitometric ones is not very high (correlation coefficient 0.71). This might be caused by noise in the patient data. First, half times have to be determined over rather limited dynamic ranges, and second the wedge settings in patients with low double density often show much larger deviation than in normal subjects (cf. Figs. 3A, C). We conclude that densitometry in patients with retinitis pigmentosa may give a much earlier indication of pathology of foveal cones than is assessed on the basis of visual acuity, fundus appearance, fluorescein angiography, anomaloscope settings, or elevation of cone thresholds.

Key words: densitometry, retinitis pigmentosa, foveal sensitivity, cone pigment density, dark adaptation, anomaloscope, pseudoprotanomaly

References

1. Highman VN and Weale RA: Rhodopsin density and visual threshold in retinitis pigmentosa. Am J Ophthalmol 75:822, 1973.
2. Ripps H, Brin KP, and Weale RA: Rhodopsin and visual threshold in retinitis pigmentosa. Invest Ophthalmol Vi Sci 17:735, 1978.
3. Perlman I and Auerbach E: The relationship between visual sensitivity and rhodopsin density in retinitis pigmentosa. Invest Ophthalmol Vis Sci 20:758, 1981.
4. van Norren D and van der Kraats J: A continuously recording retinal densitometer. Vision Res 21:897, 1981.
5. Smith VC, Pokorny J, and Diddie KR: Color matching and Stiles-Crawford effect in central serous choroidopathy. Mod Probl Ophthalmol 19:284, 1978.
6. Young RSL and Fishman GA: Color matches of patients with retinitis pigmentosa. Invest Ophthalmol Vis Sci 19:967, 1980.
7. Krill AE, Archer D, and Newell FW: Fluorescein angiography in retinitis pigmentosa. Am J Ophthalmol 69:826, 1970.
8. Fishman GA, Fishman M, and Maggiano J: Macular lesions associated with retinitis pigmentosa. Arch Ophthalmol 95:798, 1977.
9. Fishman GA, Maggiano JM, and Fishman M: Foveal lesions seen in retinitis pigmentosa. Arch Ophthalmol 95:1993, 1977.
10. Verhoeff FH: Microscopic observations in a case of retinitis pigmentosa. Arch Ophthalmol 5:392, 1931.
11. Hervouët F, Baron A, and Lenoir A: Anatomie pathologique de la rétinose hespéranopique. Arch Ophthalmol (Paris) 15:263, 1955.
12. Lucas DR: Retinitis pigmentosa; pathological findings in two cases. Br J Ophthalmol 40:14, 1956.
13. Szamier RB, Berson EL, Klein R, and Meyers S: Sex-linked retinitis pigmentosa: ultrastructure of photoreceptors and pigment epithelium. Invest Ophthalmol Vis Sci 18:145, 1979.
14. Mizuno K and Nishida S: Electron microscopic studies of human retinitis pigmentosa. Part I. Two cases of advanced retinitis pigmentosa. Am J Ophthalmol 63:791, 1967.
15. Kolb H and Gouras P: Electron microscopic observations of human retinitis pigmentosa, dominantly inherited. Invest Ophthalmol 13:487, 1974.
16. Birch DG, Sandberg, MA, and Berson EL: The Stiles-Crawford effect in retinitis pigmentosa. Invest Ophthalmol Vis Sci 22:157, 1982.