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Two Types of Visual Dysfunction in Autosomal Dominant Retinitis Pigmentosa

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Visual thresholds and rhodopsin levels were determined in nine subjects with autosomal dominant retinitis pigmentosa. The subjects fell into two groups, corresponding to two subtypes of the disease revealed by two-color, dark-adapted static perimetry. In the first of these subtypes, rod-mediated function was variably reduced and was accompanied by a corresponding reduction in cone function in the same retinal region. Dark-adapted threshold elevations varied in a way consistent with decreased quantal absorption by the rods as a result of reduced rhodopsin levels. In the second subtype, rod function was greatly reduced or absent throughout the retina, while cone function was much less severely affected. Although the levels of rhodopsin were only about half of normal, they were much too great to account for the visual threshold elevations on the basis of decreased probabilities of absorption by the visual pigment. Rhodopsin regeneration appeared to follow normal kinetics in patients from both groups. The results indicate that the examples of the two psychophysical subtypes of AD RP investigated here have very different disease manifestations. Invest Ophthalmol Vis Sci 29:1235–1241, 1988

Retinitis pigmentosa (RP) is a number of diseases that primarily affect the outer cells of the retina.[1] Differentiation between the various types of RP is of obvious importance if progress is to be made in the search for the origins of the disease processes and their ultimate cure. Over the last 20 years several investigators have set out to determine whether the loss of visual sensitivity which occurs as the disease progresses is due to an inability of the photoreceptors to capture incident light quanta because they lack their visual pigment, or to some deficiency in the subsequent transduction apparatus of the cell. In order to do this, fundus reflectometry, a noninvasive objective technique, has been used to measure the amount of visual pigment present in localized regions of the retina and the results compared with visual thresholds determined psychophysically for the same loci.[2–6]

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Studies of small numbers of patients with the autosomal dominant (AD), autosomal recessive and X-linked forms of the disease,[2,3] have reported that in general the loss of sensitivity in the affected areas of the retina could be wholly accounted for by a reduction in the level of rhodopsin, reducing the probability of light absorption. Perlman and Auerbach,[4] however, reported that patients fell into two groups and suggested that only those with AD inheritance showed the established pattern, while the others possessed much more pigment than would be expected on the basis of the elevation of their visual threshold. The question arises as to whether any of these fundus reflectometric data in AD RP patients were obtained from only one or from both of the psychophysically distinguishable subtypes now known to occur within this genetic type.[7,8]

In a previous study,[6] we used an imaging fundus reflectometer[9] to examine the visual pigments in AD RP patients having the psychophysical subtype characterized by regionalized retinal disease and parallel deterioration of both rod and cone function (Type 2[7] or pattern R[8]). In the present study we confirm our findings in this subtype and extend our observations to include AD RP patients with the subtype that has generalized loss of rod function across the retina occurring at a much earlier age than that of cones (Type 1[7] or pattern D[8]). Our results show that the two forms differ fundamentally: those subjects with the regionalized form of the disease showed a considerable loss of

visual pigment in affected areas of the retina,[6] while those with generalized loss of rod function had fairly substantial amounts of rhodopsin despite their profound night blindness. A preliminary report of this work has been published.[10]

Materials and Methods

Subjects

The nine patients in this study, representing five families, were selected from those in the Moorfields Eye Hospital RP register for whom complete genetic details and clinical histories were available. In two of the families there were two generations known to be affected while in the others there were at least three generations affected. Testing with two-color, dark-adapted static perimetry had been performed on each patient and many of their affected family members to establish the psychophysical subtype.[8,11] Criteria for inclusion in this study were that the patient had to possess relatively good visual acuity, minimal or no cataracts, substantial Goldmann kinetic visual fields and measurable visual function in at least part of the retinal area over which fundus reflectometric measurements were to be made. Although not selected on this basis, all patients showed greater visual field loss in the superior than inferior field and correspondingly more marked pigmentary disturbances in the inferior than superior retina (likely to be "Type 2" according to Fishman et al[12]). Informed consent was obtained after the nature of the procedures (described below) had been fully explained.

Psychophysical Testing

Each subject was first retested with Goldmann kinetic perimetry in order to confirm the extent of their visual fields in mesopic conditions. Then the pupil of the eye selected for further testing was fully dilated with cyclopentolate (1%) and phenylephrine (10%). The subject was allowed to dark-adapt for not less than 40 min. Static perimetric measurements were then made sequentially at 11 defined locations within a 30° area of retina in the temporal mid-periphery, coinciding with the region to be tested with fundus reflectometry.[6,9] The test stimulus used was a circular flashing (2 Hz) green (535 nm) light which subtended an angle of diameter 0.9° at the retina.[11] Some of the measurements carried out in the early stages of the test were repeated at the end in order to check that no further dark adaptation had occurred during the testing period. Normal data and a complete description of the instrument used for this testing have been published.[8,11]

For dark adaptometry the subject's pupil was fully dilated and then the eye was exposed to a 30 msec white Xenon flash of intensity sufficient to bleach essentially all the pigment in the mid-peripheral region of retina within which it was planned to carry out fundus relectometric testing. During dark adaptation the change in visual threshold was monitored at a point 25° from the fovea along the horizontal meridian in the temporal retina. The stimulus was the same as for the static threshold measurements described above. The test was continued until at least 40 min after the light-adapting flash.

Imaging Fundus Reflectometry (IFR)

In order to obtain estimates of the levels of visual pigment present in the retina, images of the retina are obtained (on a high sensitivity TV system) when it is illuminated with dim monochromatic measuring lights at several different wavelengths. The luminance levels of retinal images of the fully light-adapted eye are compared to those obtained when the eye has been allowed to dark-adapt. It can readily be shown that the density change (the differences of the logarithms of the reflectance values obtained for the two conditions) gives, at least approximately, a measure of the level of visual pigment regeneration which has accompanied the dark adaptation.[3,9,13,14]

Details of the IFR have been given elsewhere.[9,13] In brief, the optical system that provides the measuring and bleaching beams and collects the light reflected from the fundus back through the pupil is a Zeiss fundus camera with a 30° field of view. The retinal image yielded by the fundus camera is directed onto the face plate of an intensified silicon intensified target (ISIT) 16 mm TV camera (Thermal Imaging Ltd., Bodmin, UK). The output of the TV camera provides an image of the retina on a monitor screen which is used to observe the subject's eye position and quality of fixation during the test and is also digitized by an image acquisition system (Gems, Gems Ltd., Cambridge, UK). The image analyzer is equipped with a high-speed arithmetic unit which enables it to carry out real-time averaging of successive TV fields at TV scanning rates. For the present experiments 32 successive fields were averaged for each measurement, giving a measuring period lasting 640 msec, a duration which allows a considerable improvement in the image quality while being brief enough to minimize problems of eye movements. Before storage on magnetic disk, the arrays are reduced in size by spatially averaging them so that they consist of only 64 × 64 pixels (picture elements). This reduction in spatial resolution gives a further substantial improvement in

the signal/noise ratio of the data. Each element of this array corresponds to a rectangular retinal area of angular subtense of approximately 0.6°. For analysis, further spatial averaging was carried out, resulting in data values each corresponding to an area of angular subtense 1.2°.

Fundus reflectometry always followed the dark-adapted psychophysical measurements and was carried out on the same visit. In the procedure, the patient, with fully dilated pupil, was aligned with the fundus camera. Head position was stabilized by means of a bite-bar and forehead rests. As was the case for static perimetry and dark adaptometry, fixation was ipsilateral, with a red LED target arranged so that the center of the area of retina under test was located at 30° temporal to the fovea. The subject was then light-adapted on the instrument with a yellow-white light (from the tungsten source) delivered by Maxwellian optics and covering an area of retina concentric with, but larger than, the test area. This light was of intensity 6.0 log scotopic trolands and the period of illumination was 60 seconds. Such an exposure is expected to remove more than 95% of the visual pigment.[3,15] Images of the test area of the retina were collected immediately after the exposure and at intervals for the following 25 min. The subject was then light-adapted as before and the entire procedure repeated. The test concluded with a third 1 min light-adapting exposure and the recording of a final series of images from the light-adapted retina. Measured densities for normal subjects obtained with this instrument[9,14] are in agreement with previously published values.[3] The spectral criteria used to assess the quality of the data were as previously published.[9]

Results

Table 1 gives some clinical characteristics and electroretinographic results in the patients. For all the subjects, visual loss as determined by two-color, dark-adapted static perimetry fell clearly into one of two patterns: either loss of rod and cone function closely paralleled each other and the disease had a patchy or regional nature (Table 1, P1–P4); or there was a generalized or diffuse loss of rod function across the retina, including areas in which cone function was still good (Table 1, P5–9). Subjects with the latter characteristics generally described themselves as having night blindness for as long as they could remember. In view of the report that in some cases of RP dark adaptation is abnormally slow,[16] the subjects who had previously been classified as belonging to the diffuse type were examined for delayed adaptation; none displayed any evidence for it over the time scale tested (1 hr).

Table 1. Clinical and electroretinographic data on subjects studied

	Age (yrs)	*Sex*	*Nyctalopia onset (yrs)**	*Visual acuity†*	*Rod ERG b-wave amplitude (μV)‡*
P1	46	F	NS	6/7.5	50
P2	31	F	NS	6/6	250
P3	41	M	36	6/6	100
P4	61	M	20	6/6	NA
P5	26	M	C	6/9	ND
P6	24	M	C	6/6	ND
P7	16	F	C	6/6	10
P8	29	F	C	6/6	10
P9	17	M	C	6/6	ND

* Reported age of onset of symptoms.
† Acuity of test eye.
‡ ERG of test eye to blue flash, dark-adapted; normal mean = 319 μV, lower limit (−2 SD) = 141 μV.[24]
NS, no clear symptoms; C, symptoms present since earliest childhood; NA, data not available; ND, nondetectable.

Figure 1 shows kinetic perimetric results from P1 and P5, AD RP patients that are representative of the two different psychophysical subtypes of the disease. P1 (Fig. 1A), a 46-year-old woman, is from a family with the regionalized form, and P5 (Fig. 1B), a 26-year-old man, is a member of a family with the diffuse form. Both P1 and P5 have visual loss throughout the superior hemifield (Fig. 1). In each case the areas where IFR measurements were subsequently carried out are indicated by the dotted circles. In all the cases of AD RP included in this study, the patterns of visual field loss were similar to those shown in Figure 1, ie, scotomatous areas occurred largely in the mid-peripheral upper field.

The rhodopsin double densities measured in P1, shown by the contour map of Figure 2A, were found to vary quite extensively, ranging from essentially undetectable levels to just over 0.1. Superimposed on the contours in Figure 2A are the dark-adapted threshold elevations at 11 test loci to the green stimulus. The values, which are relative to those obtained from normals at the same retinal locations, vary from 0.2 to 1.7 log units above normal, and correlate quite closely with the extent to which measured rhodopsin levels were reduced.

P5, in contrast to P1, had scotopic visual thresholds that were elevated by more than 2.5 log units throughout the field (Fig. 2B) and reported that he had been aware of night blindness since early childhood. As indicated by the contour lines in Figure 2B, the density changes measured in this subject varied between 0.04 and 0.08. These values are between approximately one-third and two-thirds of those obtained from this region in normal subjects,[3,9] ie, much larger than would be expected if loss of pigment were the sole cause of the subject's night blindness.

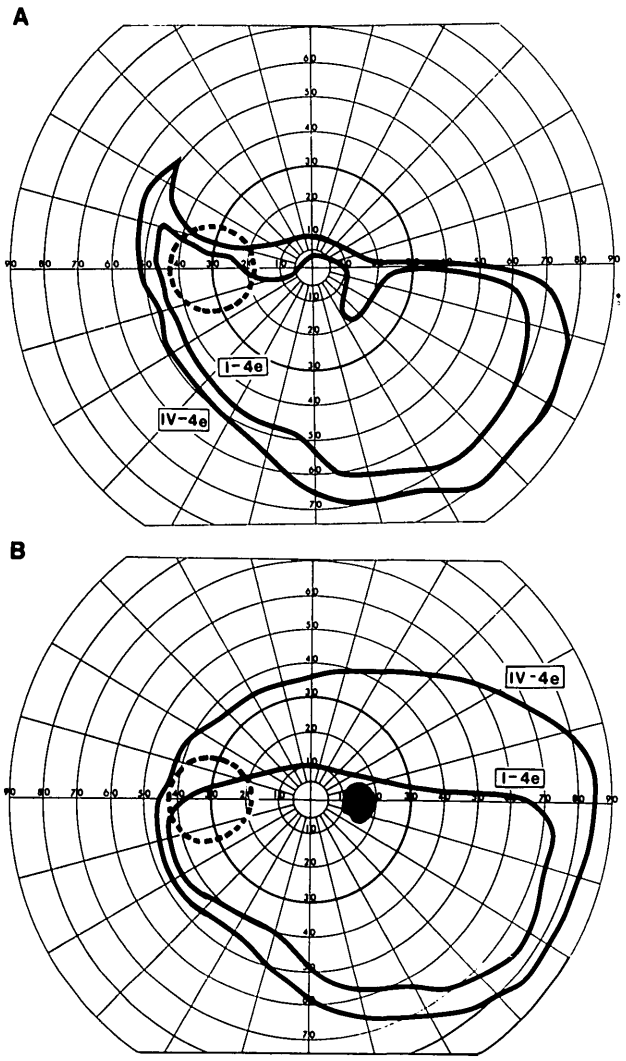

Fig. 1. Goldmann kinetic visual fields using two target sizes (IV-4e and I-4e) for the right eyes of two representative subjects. Subject P1 (A) had the regionalized form whereas subject P5 (B) had the diffuse form of AD RP. The dotted circles indicate the region in the nasal visual field where fundus reflectometric measurements were made.

Figure 3 illustrates the entirely different patterns of relationship between the dark-adapted thresholds to the green stimulus and levels of rhodopsin for the two groups of patients. The log threshold elevation is plotted against the fraction of pigment found in normal subjects from the same retinal areas.[3,9] Data have not been included for those test loci where thresholds were so elevated as to be unmeasurable with the green test stimulus used.[11] Patient P9, for example, whose data are represented by the inverted triangles in Figure 3, could only detect the stimulus reliably at two loci.

The pattern of rhodopsin loss observed in P1 was replicated in the other subjects with the regionalized disease type. Data for these subjects (Fig. 3; filled symbols) show only mild elevation of threshold unless the level of visual pigment is very low. Indeed, they all lie, within experimental error, along the dotted curve which represents the expected relationship between visual loss and pigment deficit if decreased quantal absorption were the sole cause of the dysfunction.[3,6]

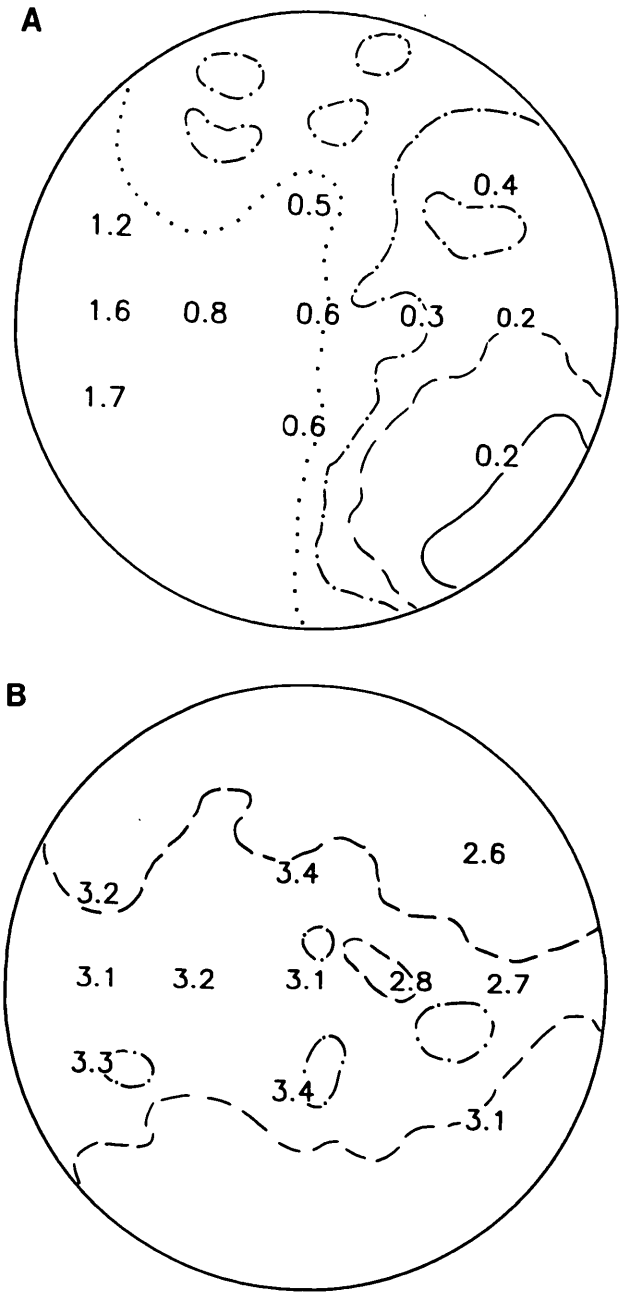

Fig. 2. Contour maps of the visual pigment levels measured in the circular area of temporal retina (diameter, 25°) for subjects P1 and P5. Contours indicate the following double density differences (log units), measured at 500 nm: 0.025, · · · · ·; 0.050, -·-·-·-; 0.075, - - - -; 0.10, ——. Numbers indicate the log threshold elevations for the green test stimulus at the 11 test loci, relative to those obtained from normals.

Only a few well documented examples of the diffuse form of the disease were available for study. Of the five included here, four were members of one family (P5–P8). By contrast to the data from patients with the regionalized form, the data from this group with diffuse rod dysfunction (Fig. 3; open symbols and *) all lie much above the solid line, indicating that for them the level of pigment does not determine visual threshold. There appears to be no good correlation between the elevation of threshold and the loss of rhodopsin. However, the psychophysical data must be interpreted with caution, since although they were obtained by using a green stimulus which in normal circumstances elicits responses unambiguously from rods, some of the elevations observed here were so large that the thresholds were probably set by cones.

The question arises as to whether the results obtained from the subjects with diffuse disease could be artefactual and are caused by changes in stray light levels between experiments. However, if such artefacts had occurred in these subjects, they would tend to minimize the difference between the diffuse and regional forms of the disease, rather than overemphasize it.[4,13,14,17,18]

In addition to determining how the levels of rhodopsin varied in the two forms of AD RP, attempts were made to measure the rates at which it regenerated following light adaptation. Two examples of the observed time courses are shown in Figure 4. The data from P2, who has the regional form, (Fig. 4A) were obtained from a region of relatively normal function, with virtually no sensitivity loss. Also shown is a single exponential regeneration curve, for which the time of half recovery ($t_{1/2}$) is 6.0 min, a value within the normal range.[3] While it would have been of value to determine whether there were abnormalities in the rates of regeneration in relatively severely affected retinal regions in these subjects, density changes there were too small for any quantitative resolution of the time course of their occurrence to be possible.

The data of Figure 4B, obtained from two different experiments on P5, who has diffuse rod sensitivity loss, were of course measured in dysfunctional retina. Also shown is an exponential recovery curve with a $t_{1/2}$ of 5.4 min. The level of visual pigment reached during the measurement period was only about 60% of the normal level, but the data suggest that recovery had essentially ceased.

Discussion

The classification of AD RP into at least two subtypes on the basis of the pattern of their visual loss

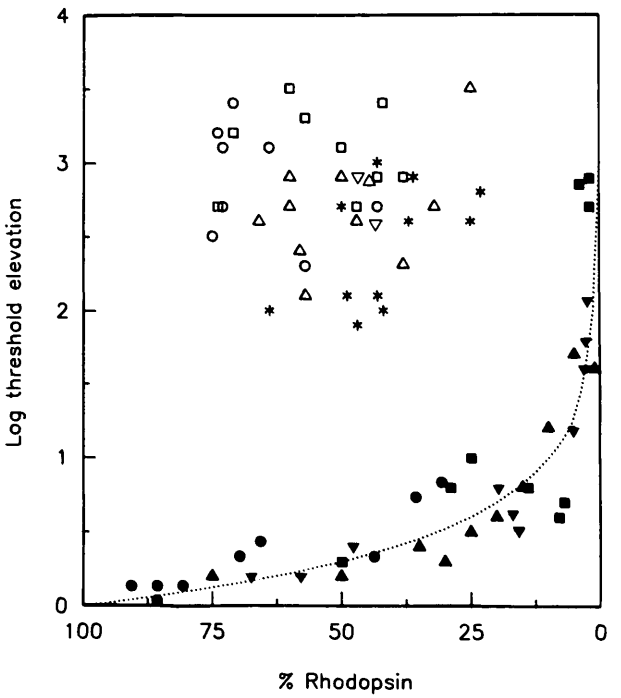

Fig. 3. Relationship between log threshold elevation and rhodopsin levels for four subjects with the regional form of AD RP (filled symbols) and five subjects with the diffuse form (unfilled symbols and *). The dotted curve is the relation which would be observed if threshold were determined by the probability of quantal absorption.

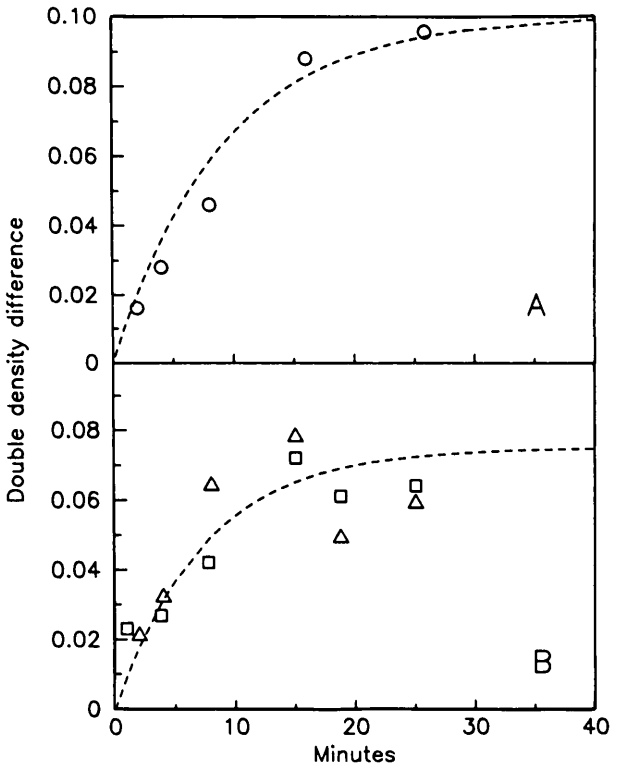

Fig. 4. Regeneration of visual pigment for subjects P2 and P5. Data for P2 were obtained from a region of maximum pigment density within the measurement area.

raises the question whether they represent different forms of retinal disease. The present study strongly suggests that this is the case. As has previously been suggested,[3,4] in the group with regionalized retinal dysfunction, the loss of vision is most likely to be due either to an abnormally low amount of pigment in all the rods (probably resulting from a generalized reduction in the length of the outer limbs), or to a partial loss of rods, so that the receptor mosaic is imperfect.

The results in the AD RP patients with diffuse rod dysfunction are similar to those reported by Perlman and Auerbach[4] for other genetic types of RP. In this form of the disease, rhodopsin levels remain substantial (and therefore presumably the rod outer segments have not been lost) in areas of the retina where scotopic function is grossly impaired. It follows that the lesion arises either in the transduction apparatus of the rods, or elsewhere in the retina. Since rod ERG data for the subjects described here[8,19] show that they have virtually no measurable a-wave (and b-wave), it appears that the site of the dysfunction is likely to lie in the rods themselves. A similar lack of ability of the receptors to signal quantal absorption as in the diffuse type of AD RP has been reported in subjects with autosomal dominant stationary night blindness.[20] However, unlike these subjects with stationary night blindness, the levels of pigment in the RP patients were all substantially lower than normal. For the RP patients, caution is also needed in interpreting the ERG results, as these are mass responses, and their absence could be primarily caused by large regions of scotomatous retina. Thus, it is possible that there may be regions of retina (such as where the present measurements were made) in which outer segment function and rhodopsin levels are reasonably intact, and the loss of sensitivity is due to a postreceptor abnormality.

The relationship between the threshold elevation and level of pigment observed for the AD RP patients with diffuse rod dysfunction is qualitatively similar to that observed in night blindness resulting from systemic vitamin A deficiency.[3,6,21] However, there are significant differences which argue against that as the basis for the dysfunction. In relatively mild vitamin A deficiency, where some rod function is retained, the most striking effect is the reduced rate of dark adaptation,[22] which is parallelled by a prolongation in the time course of rhodopsin regeneration.[23] In the diffuse AD cases, dark adaptation appeared to proceed at normal rates, as did the regeneration of rhodopsin. Furthermore, serum vitamin A levels were determined in three of them and were found to lie well within the normal range, far above that normally associated with ocular disturbance.[22] This does not, of course, rule out some abnormality in the carrier protein or in the transport mechanisms for the vitamin within the eye, either of which could lead to a local deficiency at the photoreceptors. However, the differences in the time courses of adaptation in the two forms of dysfunction do not support such a notion.

It should be noted that data were obtained only from two families with the diffuse form of RP. These were the only representatives available for study, which reflects the relative rarity of this subtype within autosomal dominant RP patients. It is not safe, therefore, to assume that all members of the group will follow the same pattern. This applies equally to the regional subtype, where there are already indications that further subdivision of the group is warranted on psychophysical grounds.[16,24]

Key words: fundus reflectometry, retinitis pigmentosa, rod, visual pigment, visual threshold

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