A modified ERG technique and the results obtained in X-linked retinitis pigmentosa

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SUMMARY An electroretinographic (ERG) technique is described in which the relationship between scotopic b wave amplitude and stimulus light intensity is determined. The relative amplitude of scotopic to photopic responses is assessed by means of red light and flicker. The method is applied to the detection of ERG abnormalities in heterozygotes for X-linked retinitis pigmentosa. These have been found in only a proportion of cases. The ERG results can be used to suggest the nature of the retinal abnormality.

It is not clear what relationship may exist between loss of visual function and the change in the electroretinogram (ERG) in retinitis pigmentosa (RP). Karpe¹ found that the ERG was extinguished, but when intense stroboscopic flashes were introduced into clinical practice² it became more usual to obtain some response. It is often considered that the reduction in the response is greater than that found in other conditions, and special explanations have been advanced to account for this.³ To discover whether there is any discrepancy between the ERG and other measures of visual function it is necessary to quantify the ERG result in a way which permits a proper comparison. This may be done by obtaining the stimulus intensity/response amplitude function of the ERG. Such a technique has been proposed,⁴ but its implementation has been difficult until the introduction of comfortable electrodes.⁵ Moreover, a theoretical background has been provided by recent physiological experiments.⁶ For many retinal responses the relationship between response voltage, V , and light intensity, I , has the form

$$V = \frac{V_{\max} \cdot I^n}{I^n + K^n} \quad \text{equation 1}$$

and it has been shown that this simple relationship holds in particular for the b wave of the human ERG⁷ and the photoreceptor response.⁸ In many cases the value of the exponent n is unity, so n may be omitted, providing a further simplification. Thus the response

may be entirely characterised by the values of $V_{\max}$ and 'K'. The range of light intensities over which V alters is determined by the parameter 'K,' which represents the light intensity required to produce a half-maximal response. This simplification permits comparison of the ERGs found in patients and normals, but there are further implications of equation 1 which allow one to use the ERG to investigate the cellular basis of retinal disease. For photoreceptors⁸ the value of 'K' is about 30 quanta absorbed per rod per flash. The reasons why the relationship is obeyed have been analysed.⁸ It has been established that each quantum absorbed liberates a chemical into a restricted region of the cytoplasm of the outer limb. This *internal transmitter* combines with channels in the surface membrane which control the movement of ions across the membrane. The change in the permeability of the membrane causes the photoresponse. The combination between channels and internal transmitter occurs in accordance with the law of mass action. When only a few quanta are absorbed in a flash, each one will be absorbed at a considerable distance from its neighbour, and the patches of membrane affected will be widely spaced and independent of each other. When the flash is more intense, the zone of liberation of each aliquot of internal transmitter will overlap, and the interactions result in a nonlinearity which is predicted by equation 1.

The value of 'K' for the b wave is considerably less than 1 quantum absorbed per rod per flash. Therefore, over most of the range of light intensities over which the b wave grows the postsynaptic cell^{9,10} which

generates the ERG must be stimulated by rods which have each absorbed one quantum. As the light intensity increases, a greater proportion of the group of rods which converge on the postsynaptic cell are excited. This fact explains why, as equation 1 demands, there is a linear relationship between light intensity and b wave amplitude at low light intensities. For higher light intensities this linearity is lost. The fact that equation 1 is obeyed suggests that the neurotransmitters liberated by the rods combine with ionic channels in the postsynaptic membrane, and the non-linearity may be explained in the same way as for the photoreceptor. The analysis is detailed further in the discussion. It is immediately apparent that if in RP the rods all became shorter, but there were no other changes, there would be less rhodopsin, and fewer quanta would be absorbed. The rod signals would be normal in size but reduced in number, so for weak lights the ERG voltage would be reduced. However, $V_{\max}$ would be unaffected and 'K' should increase. But if the b wave generating mechanism were to be abnormal $V_{\max}$ could be reduced, while 'K' remained unchanged.

We have modified our ERG techniques, as described below, so that the parameters of equation 1 can be obtained. Although it is possible to purchase equipment which will allow the investigations described in this paper to be carried out, the apparatus is bulky and expensive. By contrast, the modifications described here are simple, and can be carried out in any clinic. They therefore seemed worth describing in detail. As an illustration of the power of the method of analysis and of the inherent limitations of the ERG we have compared normal subjects to heterozygotes for X-linked retinitis pigmentosa, who are very mildly affected. In later papers we will describe results in other defined modes of RP and give results of familial studies.

Methods

ELECTRORETINOGRAPHY

Electrodes. Earth and reference electrodes were 5 mm silver-silver chloride standard EEG electrodes, which were placed on the forehead. The corneal electrode was a piece of gold impregnated Mylar which could be placed in the lower fornix without any local anaesthesia.⁵

Light stimuli. We used a common stroboscope (Grass PS22) modified in several very simple ways which improved the performance of the system. Behind the translucent dimpled window of the stroboscope we placed a sheet of tracing paper which diffused the light more evenly. A filter holder was made so that the light intensity could be changed. This was bolted on to the circular cover of the

stroboscope and contained 3 slots. Into these, plastic colour filters could be inserted. Additional filters were made from neutral Cinemoid (Strand Electric Co. Ltd) sandwiched between 2 layers of thin Perspex. The density per sheet is 0.55 for the blue light used to examine rod responses. With these filters, and the front panel controls on the stroboscope unit, we were able to alter the stimulus intensity over a range of approximately 4.4 log units in steps of less than 0.3 log unit. The filter holder also contained baffles so that there was almost no stray light.

Patient viewing. The stroboscope was used to back-illuminate (at 30 cm distance) a 40×25 cm rectangle of 3 mm thick opal Perspex. This screen was placed no more than 4 cm from the patient's nose. It was easy to obtain uniform illumination of the front surface over the smaller area which occupied the patient's visual field. This arrangement provided an effective ganzfeld stimulus very simply.¹¹ It also attenuated the stimulus light in relation to other systems which use the Grass stroboscope. Fixation was obtained by placing a small, hollow, tritium-filled bead on the surface of the opal screen facing the patient. The bead contained a red-light-emitting phosphor. As the screen was so near the eye, normally sighted persons saw 2 dim red fixation points and were asked to look between them. The stroboscope and the screen were mounted on arms made of 1-inch (2.5-cm) tubular steel scaffolding (Kee Klamp), which permitted movement in many planes.

Recording and display. Most of the records were obtained with a Medelec MS6 polygraph system. A few were obtained with a system built around a PDP 11/10 computer. Both sets of equipment produced averaged records and rejected records in which there were large spurious voltage changes, which are due to eye movements (artefact rejection).

Procedures. Patients' pupils were dilated and accommodation paralysed with topical instillation of 1% Mydrilate (cyclopentolate). The patient was then fitted with darkroom goggles, which pass only deep red light and to which we added additional grey Cinemoid filters. We were careful to exclude any light leaks around the nose. The patient could see dimly with cones, but the rods were unstimulated. After 30 minutes the patient was brought into the experimental room, and skin electrodes were attached in a standard manner. The goggles were removed in dim red safe-light and the corneal gold foil electrodes inserted. In many cases the cornea was anaesthetised with oxybuprocaine (benoxinate) 0.4%. The translucent screen and the stroboscope were positioned and all lights turned off. The patient was then allowed to dark-adapt for a few minutes.

A sequence of flashes were given to evoke the ERG with various stimuli. The interval between flashes

Fig. 1 Blue light ERGs obtained: A. After completion of normal dark adaptation with goggles. B. After a further 30 minutes adaptation in total darkness. C. Following stimulation of the eye with maximal blue light flashes. The stimulus is the same for all records, and produces $0.2 V_{max}$. Calibration mark: amplitude scale is $100 \mu V$ per division, time base scale is 40 ms per division.

varied between 1.0 and 10 seconds, depending on the stimulus intensity. The number of responses averaged varied with the stimulus intensity and the ERG amplitude, so an approximately constant signal-to-noise ratio was obtained for the smaller responses. The noise level was $5 \mu V$ peak to peak or less.

Light calibrations. The published figures for the spectral transmission of the blue filter used, and the stroboscope output, together with the spectral sensitivity of the dark-adapted eye, can be multiplied to produce a result which shows that the stroboscope light is equivalent to a monochromatic flash, the wavelength of which is 480 nm . The light intensities were measured with a Tektronix J16 photometer with a direct reading in $\text{cd} \cdot \text{M}^{-2}$. In order to obtain readings for brief flashes, the stroboscope was set to various frequencies and the average output of the photometer read. From graphs of light intensity versus frequency it was possible to determine the light output for a single flash. Care was taken that the single flashes did not saturate the photometer photosensitive surface. This calibration was used to check the range of switches on the Grass stroboscope and the change in the flash tube with time (changes of the order of 50% occurred during the 2-year period of the investigation). By assuming the equivalent wavelength of the light output, and making standard assumptions about the optics of the eye,¹² we can express the number of quanta falling on a unit area of the retina for any of the stimulus flashes. We also determined our own visual thresholds, using the ERG stimulus. This was $1.1\text{--}1.5$ log units below the minimum stimulus used to elicit an ERG. As can be seen below (Fig. 2) the

calculation of quanta per flash gives threshold values in good agreement with those obtained for absolute visual threshold in other physiological experiments, and suggests the calibrations are accurate within a factor of 2. Neutral filters were calibrated with the photometer in a standard manner.

OTHER EXAMINATIONS ON PATIENTS

All patients were interviewed in the genetic clinic, and members of the families who were symptom-free were also examined to verify the mode of inheritance. Full clinical examination included fundus photography and sometimes fundus angiography. The visual fields were carefully recorded by a limited number of practised persons, always using the same calibrated Goldmann perimeter.

Results

Rod responses of normal subjects in dark adaptation. By using flashes of blue light in the dark-adapted eye a range of b waves can be recorded which are produced by the scotopic system. Fig. 1 shows a control experiment. Record A was obtained after our standard dark adaptation procedure, record B after a further 30 minutes of adaptation in total darkness, and record C within 2 minutes of exposing the patient to blue light flashes of maximal intensity. In each case the stimulus intensity was the same and produced a b wave of about 20% maximum. The 3 ERGs are of almost identical amplitude and waveform, showing that our standard procedure is sufficient to dark-adapt the eye completely, and the stimulating flashes do not disturb the state of adaptation.

Fig. 2 shows the relationship between b wave amplitude and the intensity of the stimulating blue light flashes. The points are average values for 10 normal subjects, of ages ranging from 18 to 49 years of age. The line joining the points is the theoretical relationship as shown in equation 1 with $n=1$, and this fits the data well. A similar result has already been reported.⁷ The agreement between data and the theoretical curve in Fig. 2 supports the assumption that we have achieved ganzfeld stimulation. If more intense flashes are used, ERG amplitudes larger than those obtained in Fig. 2 can be elicited; these have prominent a waves, and wavelets appear on the ascending limb of the b wave. Such appearances are often considered as evidence of photopic activity.^{13,14} In records from individual patients or subjects it is often possible to see that there is a discontinuity in the amplitude/intensity function at this point. The ERG grows more rapidly than expected, since photopic responses begin to contribute to the b wave voltage. In order to determine the value of 'K' (equation 1) it is necessary to estimate the value of V_{max} for the rods.

Fig. 2 Scotopic responses of the normal subjects. The curve drawn is the relationship of equation 1, with $n=1$, and 'K' chosen as 0.2 quantum incident per rod per flash. Note the value of 'K' for photoreceptors is about 35 quanta absorbed per rod per flash. A criterion voltage (e.g., 10 μV or 5% of V_{max}) is often considered as an ERG 'threshold'. The light intensity corresponding to this level is a flash for which 3 rods in 1000 absorb a quantum. Abscissa: relative light intensity (log units). Ordinate: V/V_{max} .

Because of the intrusion of photopic mechanisms, this involves some assumptions. Usually we have plotted graphs on log-log co-ordinates and have extrapolated a smooth curve through the points to estimate a value for V_{max} . Another method is to inspect the curve, identify the discontinuity, and assume the maximum b wave amplitude obtained with a flash less intense than that which causes the cone intrusion. We have compared the values of 'K' which can be derived by measuring the results in these 2 ways. There is remarkably little difference. One reason is that the contribution from the photopic mechanism is small. In addition, if all values of V are divided by the same value of V_{max} , the value of 'K' is not very sensitive to quite considerable changes in the estimate of V_{max} . The imprecisions due to such causes affect the 'K' values obtained by less than 0.1 log unit. In Fig. 2 the value of 'K' is 0.2 quantum incident per rod per flash. This is in contrast to the value for all known photoreceptors of approximately 30 quanta absorbed per rod per flash. The standard errors of the points in Fig. 2 vary, the variation depending on light intensity, but for the point nearest to half V_{max} the standard errors are contained within the symbol on the graph.

The results from which Fig. 2 is constructed take about 25 mins to collect in each patient, and in addition it is usually necessary to record the ERGs evoked by flicker, or red light stimulation, etc. (see below). In order to reduce the examination time in patients who may have no change in the value of 'K'

Fig. 3 ERG waveforms in normal subjects. The numbers to the left refer to the sequence of the stimuli in the protocol. Only some of the responses are shown. The blue light records (4 and 7) illustrate responses slightly less than or greater than the half maximal response. (V_{max} was determined by averaging records 11 and 12). Note the change of amplifier gain for record 4. Records 17 and 18 illustrate the cone and rod responses obtained with 2 different intensities of red light. The white light responses (note the change in time base) show mixed rod and cone activity. Record 22 is filtered to show the oscillatory potentials, and record 31 shows a response to a light flashing at 30 times a second. The figure illustrates the results obtained with the 'short protocol', which is used to examine most patients who do not have RP. Some of the RP patients were examined in this way (or with a few additional light intensities), but most were examined with the 33 combinations of colour, intensity, and repetition rate specified in the appendix. Calibration mark: for records 4, 17, and 18 amplitude scale is 100 μV per division, time base scale is 40 ms per division. Records 7, 11, and 12, amplitude scale is 200 μV per division, time base scale is 40 ms per division. Records 19, 20, 21, and 31, amplitude scale is 200 μV per division, time base scale is 20 ms per division. Record 22, amplitude scale is 20 μV per division, time base scale is 20 ms per division.

we have devised a shortened protocol, which includes only those blue light stimuli calculated to produce responses of 0.2, 0.6, and 1.0 V_{max} . It is easy to estimate from these whether in any patient 'K' has in fact altered, and if so the entire $V/\log I$ relationship may be determined. (Note that Fig. 1 shows the results are not affected if weak stimulus flashes follow more intense ones.) In some patients there is no point in attempting to determine the value 'K.' For example, in some cases of RP the most intense blue flash evokes no rod b wave at all. Fig. 3 shows a series of ERGs which form the basis of a short protocol which is used routinely on every patient, unless the initial results indicate that more detailed testing is required. The ERG normal results are given as Appendix A. In all, 33 different stimuli can be used, with various combinations of stimulus intensity, dominant wavelength, repetition rate. The numbers on the left hand side of the trace in Fig. 3 refer to the conditions listed in Appendix A (the long protocol).

Photopic and flicker ERGs in normal subjects. In our standard clinical routine (the short protocol), illustrated in Fig. 3, red light flashes follow the blue light stimuli. In our conditions the weakest red stimuli produce 2 distinct b waves. The earliest (sometimes called b_1 or the x wave¹⁵⁻¹⁷) is produced by cone mechanisms. The red light also weakly stimulates the rods and produces a delayed response similar to that evoked by a dim blue light. Thus such flashes of red light produces responses in which cone and rod activity is separated in time and the ratio of the amplitudes of cone to rod responses is obtained. In normal observers there is little variability in the measure. Various intensities of red light were employed (see Appendix A) in the long protocol, but if the intensity is raised by more than 0.6 log unit the time to peak of the rod b wave is decreased, so that cone and rod responses overlap in time.

Flashes of white light were used, so that our results could be compared with those of other clinics. Such responses, obtained without neutral filters, are larger than those which can be evoked by the coloured lights in our system. The a waves are more prominent, and additional minor components appear on the leading edge of the b wave. When the stimulus repetition rate is raised, so that the light flickers, rod responses are selectively reduced. This is in part due to the light adaptation caused by the repetitive stimulus, and in part due to the fact that the scotopic mechanism produces slowly developing, prolonged b waves which cannot follow a rapid stimulus repetition rate. Thus the reduction in the ERG amplitude associated with flicker is also an index of the relative activity of photopic and scotopic systems. The peak of the flicker ERG is quite sharp, and the time to peak does not vary more than 5 ms in normals, though it may be

increased in retinal disease. If the ERG amplifier bandpass is modified to attenuate all frequencies below 100 Hz, the b wave is greatly reduced, and with intense white light stimulation the wavelets on its rising phase can be amplified to appear more prominent. Such a record is shown in Fig. 3. It has been claimed that such 'oscillatory potentials' are reduced in retinal disease such as diabetic retinopathy,¹⁹ and therefore such recordings were included in the short protocol.

Fig. 4 ERGs from an X-linked heterozygote, aged 78, clinic no. 9227. Note the ERG is very small, the weaker blue lights evoke almost no rod response, and the cone responses are reduced. The dotted line is the average normal response to the same scale. The change in 'K' is 0.93 log unit, with a V_{max} for blue light of 40 μV . However, if the light required to produce a b wave of the criterion amplitude is considered, the loss of sensitivity is 2.3 log units. Calibration mark: for records 4 and 7, amplitude scale is 12.5 μV per division, time base scale is 40 ms per division. Records 11, 12, and 18, amplitude scale is 25 μV per division, time base scale is 40 ms per division. Record 31, amplitude scale is 12.5 μV per division, time base scale is 20 ms per division. The small bar above record 31 indicates the limit of normal time to peak for 30 Hz flicker responses.

ERGS FROM X-LINKED HETEROZYGOTES

FOR RP

We tested a group of 36 women, aged between 11 and 78 years, from families whose histories provided evidence for X-linked inheritance of RP. Both obligate heterozygotes (father and/or son affected) and presumed heterozygotes (compatible pedigree with identified fundus changes) were included in the patient group. Figs. 4, 5 and 6 show the ERGs elicited from this group. It can be seen that in some cases (Fig. 4) the ERG is obviously abnormal, weak blue light eliciting only very small delayed rod responses, so that the working range of the ERG is shifted to higher

Fig. 5 ERGs from an X-linked heterozygote, aged 32, clinic no. 9400. The responses indicate that the patient is moderately affected. The change in 'K' is 0.39 log unit. V_{max} for blue light was 192 μV . The dotted line on record 12 indicates the normal response drawn to the same scale. Calibration mark: Record 4, amplitude scale is 25 μV per division, time base scale is 40 ms per division. Record 7 and 11, amplitude scale is 50 μV per division, time base scale is 40 ms per division. Record 12, amplitude scale is 200 μV per division, time base scale is 40 ms per division. Record 18, amplitude scale is 100 μV per division, time base scale is 40 ms per division. Record 22, amplitude scale is 200 μV per division, time base scale is 20 ms per division. Record 31, amplitude scale is 50 μV per division, time base scale is 20 ms per division.

light levels. Red light produced smaller responses in which the relative cone contribution was reduced, indicating that in these cases the condition is not confined to scotopic mechanisms. However, in the cases illustrated in Figs. 5 and 6 the ERG amplitude is within normal limits, and the only abnormality illustrated in Fig. 5 consists of a delay in the peak time of the flicker response. In Fig. 6 no ERG abnormality is present.

SCOTOPIC RESPONSES

Fig. 7 shows the relationship between amplitude and light intensity in the patients, plotted in the same manner as for normals (Fig. 3). The full line is the theoretical relationship (equation 1), with $n=1$. To obtain this graph, the results from the patients were

Fig. 6 ERGs from an X-linked heterozygote patient aged 38, clinic no. 9441. The responses are within the normal limits. The change in 'K' is 0.1 log unit. V_{max} for blue light was 310 μV . The dotted line on record 12 indicates the normal response drawn to the same scale. Calibration mark: Record 4, amplitude scale is 50 μV per division, time base scale is 40 ms per division. Records 7, 11, 12, and 18, amplitude scale is 200 μV per division, time base scale is 40 ms per division. Record 19, amplitude scale is 200 μV per division, time base scale is 20 ms per division. Record 31, amplitude scale is 50 μV per division, time base scale is 20 ms per division.

Fig. 7 Scotopic responses of X-linked heterozygotic patients. The curve is the one drawn for the normals (Fig. 2), and the patients' curves have been displaced horizontally along the x axis to obtain the best fit. The average shift for the group is 0.01 log units. Abscissa: relative light intensity (log units). Ordinate $V/V_{\max}$. For further details see text.

slid horizontally to obtain the best fit by eye to this curve. The greatest shift required represented a decrease in the value of 'K' by 0.93 log unit, while the

mean result was a loss of 0.1 log unit, with a standard deviation of 0.3. Thus for the group there is no significant change in the value of 'K'. However, $V_{\max}$ is 270 μV ($\text{SD} \pm 126 \mu\text{V}$) significantly less than the normal mean (398 μV), $0.001 < p < 0.01$. Fig. 8 shows the distribution of amplitude in the patients and the normal limits.

We analysed the results as a function of age (Table 1). Below age 20 we have only 3 cases, and these all have scotopic b waves within the normal range of amplitude. Only in women over 41 is there a marked reduction in scotopic b wave amplitude and a small loss in sensitivity as measured by 'K'.

The time to peak of the scotopic b wave in the patients is nearly identical to that found in our group of normals (Fig. 9), over a wide range of light intensities. There was no evident change in the waveform of the ERGs detectable by eye.

PHOTOPIC MECHANISMS

A sensitive ERG measure of photopic activity is the time to peak of the 30 Hz flicker response. This is significantly increased in the patient group. Fig. 10 shows the distribution in comparison with the normal range. Other ERG measures have a larger variability, but relative changes in photopic to scotopic response amplitudes are apparent when red light or white light flicker is used as a stimulus. In most patients (Table 1) the ratio b_1/b_2 was reduced, implying that the fast acting cones were selectively more affected by the disease. The exceptions were in patients over 40 years of age (see above), in whom there is a marked loss of the rod b wave, and the ratio is more normal. Thus it appears from Table 1 that in the younger patients there may be a selectively greater loss of the cone ERG.

Fig. 8 Maximum rod b wave amplitudes in X-linked heterozygotes. The range of the normal is indicated. Abscissa: maximal rod b wave. Ordinate: number of patients.

Fig. 9 Time to peak as a function of light intensity. Full line and dots, normal values. Crosses, average results from X-linked heterozygotes. Abscissa: relative light intensity (log units). Ordinate: time to peak of scotopic b wave, ms.

Diagnosis in the individual patient. Only a proportion of the patients have responses which can be considered abnormal (Table 2). The most common abnormality is an increase in the time to peak for white light flicker. Of 36 patients investigated 18 showed this sign. In 17 of these the scotopic b wave was significantly reduced in amplitude, for both weak and strong flashes. In one patient the blue light b wave was small and outside the normal range, but the flicker time to peak was not abnormally long. In this report we have accepted the clinical diagnoses which

are sometimes made on the basis of minimal clinical signs, such as the presence of a tapetal reflex or small areas of pigmentary disturbance. Among all the RP patients on our lists, the incidence of X-linked RP²⁰ is high. It might be considered that a proportion of the normal ERGs reflected mistaken diagnoses. We accordingly split off a subgroup of obligate heterozygotes. It can be seen that the percentage of abnormalities is slightly but insignificantly higher. Thus it is unlikely we have included normal women in this series.

Discussion

The technique of clinical electroretinography described above is relatively simple to use and in our experience is not unduly time-consuming. It is easily tolerated by patients. No complex stimulating equipment is required. Ultimately the acceptance of such a technique depends on whether it adds information about the nature of the disease which helps the management of the individual patient or the understanding of the underlying pathology. In this paper we consider only one type of RP, and the results are moderately encouraging.

Table 1 Statistical analysis of the ERG data from X-linked heterozygotes as a function of age

Age	No.	Blue light b wave V _{max} μV	change in 'K' q/rod	Red light b ₁ /b ₂ ratio	Flicker TTP ms
Under 20	3	394	0.02	1.07	28.6
21-30	13	275**	0.1	0.72*	33.2**
31-40	10	311*	0.07	0.75**	30.8
Over 41	10	172*	0.31	0.95	34.4

The probability p, that the observed difference from normal, is due to chance is given by *0.05<p<0.02, **0.01<p<0.001.

Table 2 Percentage of X-linked heterozygotes with ERG abnormalities. The final column shows the average number of abnormalities per patient. Note this is significantly increased in the older age groups

Age	No.	Blue light		White light Flicker 30 Hz	Red light		Average no. of criteria failed per person
		V _{max}	'K'		b ₁	b ₂	
Under 20	3	0%	0%	0%	0%	0%	0.0
21-30	13	30%	8%	61%	33%	18%	1.6
31-40	10	30%	10%	55%	0%	0%	1.6
Over 41	10	75%	50%	50%	50%	37%	2.7
Total all ages	36	37%	18%	57%	25%	13%	1.4
Obligate heterozygotes only	22	50%	19%	57%	35%	15%	1.8

PATHOLOGICAL BASIS OF X-LINKED RP

It is known that the outer limbs of receptors are shortened and abnormal in X-linked RP.^{21,22} This cannot be the only or the major pathological process which occurs in this group of heterozygotes because

Fig. 10 Time to peak of the 30 Hz flicker responses in X-linked heterozygotes. The normal range is indicated. Abscissa: 30 Hz flicker time to peak (ms). Ordinate: number of patients.

of a central finding, that the value of V_{max} is reduced while the value of 'K' and n is unchanged (except in elderly women). This conclusion follows directly from the finding that the stimuli required for the scotopic b wave are extremely weak, so that for almost all the range investigated the probability that any individual rod will absorb a quantum is less than 1, and the probability of multiple absorptions is remote. Thus the cells the activity of which ultimately generates the b wave will receive a greater or lesser number of minimal rod stimuli, and the size of the b wave depends on the number of the convergent inputs and on the amplitude of the separate signals. If the photoreceptors contained less pigment, they would absorb less light, and in order to obtain the same number of minimal rod signals the stimulus intensity would have to be raised. This would cause an increase in the value of 'K'. Since V_{max} is produced by a light which produces very small signals in each rod, once the stimulus light intensity has been raised to compensate for the decreased light absorption, V_{max} should be unchanged.

The argument can be extended to consider what would happen if the transduction of light in the outer limb or the formation of neurotransmitter was made less effective by RP. In both cases the size of the rod signal would decrease, but this would increase the value of 'K' without affecting V_{max} . This is not what is observed, and this implies that some photoreceptors, at least, must function totally normally.

It might be thought that an explanation of the findings was that small patches of retina were functionless, in accordance perhaps with the Lyon hypothesis,²³ and the reduction in the b wave simply reflects a 50–60% decrease in the area of active retina. But the ERG results imply a further constraint. The b wave integrates rod activity over a distance of 2 mm or more.²⁴ If, for example, half of the photoreceptors chosen at random ceased to function, bipolar or other retinal neurones would continue to sum the signals from the remaining rods and produce an ERG in the usual way: 'K' would increase by 0.3 log unit, but V_{max} would remain unchanged. If half the photoreceptors contributing to the response had a 10-fold decrease in sensitivity, V_{max} would remain unchanged, 'K' would increase slightly, and the value of n would fall to 0.8.

What is required is that essentially all the receptors in large patches of retina cease to function. If the diseased area was confined to a single patch of retina, which our results imply would have to be about 50% of the total, then some of the b wave generators would be completely inactive and V_{max} reduced without a change in 'K'. It has been suggested that in X-linked heterozygotes patchy disease may occur^{25–27} and nonuniform loss of photoreceptors has been reported in pathological material from an elderly

patient.²² Such lesions would account for the ERG result in patients with definite field loss, but it should be noted that in the younger patients cone ERGs are also reduced in amplitude, so the abnormality affects both scotopic and photopic systems. The non-functioning patches of cone retina in young X-linked heterozygotes should produce numbers of small scotomata at the posterior pole, and these have not been described (or excluded).

The only simple remaining explanations are either that the condition directly affects the b wave generators, to reduce the current they produce, or alternatively that the current is produced but the current pathways within the eye are changed by RP, so that a reduced corneal voltage is recorded. Because of selective loss of cone components the latter possibility is the less likely, but it cannot be excluded. What is demanded by both explanations is that cells which are not photoreceptors must be damaged by the condition, and the damage is more pronounced than for the photoreceptors. Thus, the use of equation 1 leads to a clarification of the possible types of pathological processes which occur in this group of patients.

DIAGNOSIS IN THE INDIVIDUAL PATIENT

We have not been very successful in making ERG diagnoses of abnormality in our individual X-linked heterozygotes. Only about 50% of such patients have abnormal ERGs, although it has been claimed that the ERG changes are diagnostic in nearly all cases. The discrepancy between this finding and that of Berson *et al.*^{18 27 28} is unlikely to be due to differences in testing methods, since we have tried to reproduce his technique. Alteration in stimulus intensity, length of dark adaptation, and other details of method have been eliminated as significant variables. Perhaps we have seen a different patient population from what others have, since the incidence of X-linked disease is higher among our RP patients than has been reported from North America. We feel that it is unlikely that improvement in technique would increase the detection rate. The number of ways in which patients' ERGs fail to meet the criterion levels increases with age. Thus in older women the ERG is smaller, the peak time delayed, the flicker responses abnormal, and so on, while in younger women the only detectable ERG abnormality may be a delay in the

Table 3

Run no.	Strobe setting	Filters		Aperture size	Frequency Hz	High-pass filter, Hz	Low-pass filter, Hz	Analysis time, ms
		ND	Colour					
1	1	I+II	Blue	Small	1.0	0.16	1.6k	200
2	2	I+II	Blue	Small	1.0	0.16	1.6k	200
3	4	I+II	Blue	Small	1.0	0.16	1.6k	200
4*	8	I+II	Blue	Small	1.0	0.16	1.6k	200
5	2	I+II	Blue	Large	1.0	0.16	1.6k	200
6	4	I+II	Blue	Large	1.0	0.16	1.6k	200
7*	8	I+II	Blue	Large	1.0	0.16	1.6k	200
8	16	I+II	Blue	Large	0.5	0.16	1.6k	200
9	2	I	Blue	Large	0.5	0.16	1.6k	200
10	4	I	Blue	Large	0.5	0.16	1.6k	200
11*	8	I	Blue	Large	0.5	0.16	1.6k	200
12*	16	I	Blue	Large	0.5	0.16	1.6k	200
13	2	None	Blue	Large	0.2	0.16	1.6k	200
14	4	None	Blue	Large	0.2	0.16	1.6k	200
15	8	None	Blue	Large	0.2	0.16	1.6k	200
16	16	None	Blue	Large	0.2	0.16	1.6k	200
17*	8	None	Red	Large	0.2	0.16	1.6k	200
18*	16	None	Red	Large	0.2	0.16	1.6k	200
19*	1	None	None	Large	0.2	0.16	1.6k	100
20*	2	None	None	Large	0.2	0.16	1.6k	100
21*	16	None	None	Large	0.2	0.16	1.6k	100
22*	16	None	None	Large	0.2	100	1.6k	100
23	1	None	None	Large	1.0	0.16	1.6k	100
24	1	None	None	Large	1.5	0.16	1.6k	100
25	1	None	None	Large	2.0	0.16	1.6k	100
26	1	None	None	Large	3.0	0.16	1.6k	100
27	1	None	None	Large	4.0	0.16	1.6k	100
28	1	None	None	Large	5.0	0.16	1.6k	100
29	1	None	None	Large	10.0	0.16	1.6k	100
30	1	None	None	Large	20.0	0.16	1.6k	100
31*	1	None	None	Large	30.0	0.16	1.6k	100
32	1	None	None	Large	40.0	0.16	1.6k	100
33	1	None	None	Large	60.0	0.16	1.6k	100

Table 4 ERG normal values

Blue light				Red light		White light			
V_{max} μV		'K' Quanta inc. %		b_1/b_2 %		30 Hz/1 Hz %		TTP 30 Hz ms	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
398	82	0.2	0.062	1.045	0.506	12.4	4.3	29	1.5

Table 5 Lower limits for normals*

Run no.	Amplitude				Latency			
	a wave		b wave†		a wave		b wave	
	Mean μV	Lower limit μV	Mean μV	Lower limit μV	Mean ms	Upper limit ms	Mean ms	Upper limit ms
4	—	—	68	0	—	—	116	141
7	—	—	199	79	—	—	100	126
11	43	4	316	157	31	36	65	75
12	68	18	319	141	29	19	57	69
17								
Cone	29	3	91	16	22	36	48	56
Rod	30	0	155	0	65	76	114	144
18								
Cone	39	1	153	59	21	14	47	57
Rod	30	10	200	52	59	70	100	124
19	98	36	383	232	23	28	46	59
20	132	41	384	208	22	25	45	49
21	262	133	404	227	15	20	50	56
22	—	—	24	10.5	—	—	23	26
31	—	—	43	10	—	—	29	32

*Limits are ± 2 SD as appropriate from mean. Number of patients is 38. Age range is 7 to 71 years old.

†Measurements on a wave made from baseline to trough. Measurements on b wave made from trough of a wave (when present) to peak of b wave. Measurements on flicker responses are trough to peak.

time to peak of the 30 Hz flicker. Thus it is much easier to identify the more abnormal ERGs in the older patients. However, as Table 2 shows, the proportion of older patients with abnormal ERGs is not significantly different from the proportion in younger age groups. This is not what we would find if our technique detected only gross abnormalities in the ERG. It is possible that there are 2 populations of heterozygotes. In only one of these are ERG abnormalities prominent, and this is not determined by age.

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Appendix A

Table 3 gives the various filter and aperture combinations which, in combination with the stroboscope settings, provide variations in light intensity. The 2 neutral filters each were of 1.1 log units density. The 2 diaphragms were of 3 and 9.5 cm diameter. The stimulus repetition rates and amplifier settings are listed, and the averaged timebase asterisks in column 1 show the stimuli employed on all patients (the short protocol).

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