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Low b-wave amplitudes in a strain of rabbits with a pigment epithelium defect

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Abstract

When preparing isolated rabbit retinas we found in some animals fundi which were not uniformly dark but had abnormal areas of red coloration. The in situ electroretinograms (ERG) of 82 rabbits recorded after 1 h of dark adaptation were checked for abnormalities indicative of a degenerative disorder. The ERGs of eight rabbits with small dark adapted b-waves (≤ 250 μV) were re-recorded and their b-waves found to decline with time. The greatest reduction, in three rabbits, was ≥ 150 μV over 2.5 years. After 1 year, however, the light adapted b-waves were similar to those of rabbits with normal dark adapted b-waves. The majority of the progeny of these rabbits also had small b-waves, which became still smaller in 2 years. Ultrastructural studies of two rabbit retinas of the first generation showed pathological changes of the pigment epithelium (Wrigstad, Hanitzsch & Nilsson, Ultrastructural and electrophysiological studies of the retina and the retinal pigment epithelium in rabbits with low b-wave amplitudes, in preparation). Evidently there is an inheritable defect in the pigment epithelium which first impairs the rod pathway.

Keywords: b-wave; Electroretinogram (ERG); Rabbit; Degeneration; Pigment epithelium

1. Introduction

When preparing isolated rabbit retinas for other studies we noticed that the fundus of some animals was not homogenously black, but showed patches of red coloration (Fig. 1). A similar condition has been described previously (Reichenbach & Baar, 1985), with the suggestion that it could be indicative of a progressive retinal degeneration. If so, an abnormality in the electroretinogram (ERG) could be expected. We therefore recorded the in situ ERGs of the rabbits in our animal house to see if there were abnormalities which could be indicative of retinal defects in general, and of retinal pigment epithelium (RPE) defects and photoreceptor degeneration in particular. Rabbits with naturally-occurring retinal and/or RPE defects could provide a useful animal model for the study of human retinal degenerative diseases and in the search for possible treatments.

Structural and functional defects have also been found in mice (Carter-Dawson, LaVail & Sidman, 1978; Sanyal & Zeilmaker, 1984; Heckenlively, Winston & Roderick, 1989) and rats (Bourne, Campbell & Tansley, 1938; Dowling & Sidman 1962; Bok & Hall, 1971; Lin, Fan, Sheedlo, Aschenbrenner & Turner, 1996), Abyssinian cats (Narfström, 1985; Narfström & Nilsson, 1985, 1989) and Briard dogs (Narfström, Wrigstad & Nilsson, 1989; Nilsson, Wrigstad & Narfström, 1992; Wrigstad, Narfström & Nilsson, 1994). Rabbit eyes are of a size which may make them more suitable than those of mice and rats for experimental techniques such as the transplantation of cells or the development of experimental techniques such as the implantation of light-sensitive microchips for communication with secondary neurones, while dogs and cats are expensive experimental animals.

With the objective of finding a rabbit model of human retinal diseases in mind, we first recorded the in

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Fig. 1. Photograph of a rabbit eye fundus, which was not homogenously black but had an abnormal central area of reddish colour, which appears in the photograph in darker red (marked by black arrows) than the surrounding area.

situ ERGs of 82 conscious rabbits which were already in our animal house. Further recordings were made of the ERGs of ten of these rabbits (eight rabbits with small b-wave amplitudes and two control rabbits) at intervals during 1 year. The ERGs of three of the rabbits with small b-waves showed a decline of the dark adapted b-wave of ≥ 125 μV over the year, so the surveillance of the ERGs of these three rabbits was continued over a longer period. The light adapted b-wave, however, remained after 1 year indistinguishable from those of rabbits showing no evidence of ERG abnormality. This could be indicative of a defect in the rod pathway. The majority of the progeny of rabbits with small b-waves also had small b-waves, which likewise declined further over a 2-year period. The preliminary electron microscopic studies of the retina and RPE of two rabbits of the first generation which had abnormal red coloration of the fundus has revealed severe pathological changes of the RPE (Wrigstad, Hanitzsch & Nilsson, in preparation). The results suggest that an inheritable defect in the RPE involves impairment of the rod pathway.

2. Method

The eyes of conscious rabbits were locally anaesthetised with Oxbarukain®, and the pupils dilated with Mydrum® and Neosynephrin®. The ERG was recorded using AC (alternating current)-coupling (0.1–500 Hz) and ERG-jet® electrodes (Universo) as corneal electrodes. The reference was a silver electrode on the skin above the eye. The ERG of four rabbits were recorded under light general anaesthesia (10 mg Ketanest® and 2 mg Rompun®/kg body weight). This allowed longer ERG recordings with AC-coupling. The ERGs of two animals were recorded with DC (direct current)-coupling during deeper general anaesthesia (50 mg Ketanest® and 4 mg Rompun®/kg body weight). The corneal electrode was connected to an Ag/AgCl-wire via a Ringer filled polyethylene tube. The reference electrode was an Ag/AgCl-wire placed under the lower lid.

For the amplification and recording of the ERG a differential input-stage amplifier (World Precision Instruments), and an amplifier and recorder Dash IV (Astro-Med) were used. The white light stimuli of 1 s duration were provided by a Xenon arc lamp. The stimulus interval was more than 30 s between different intensities. Each intensity was applied three times with a stimulus interval of approximately 10 s. Log 0 intensity corresponded to 19 Wm^{-2} measured at the level of the cornea. For scotopic ERG recordings the animals were dark adapted for at least 1 h. For photopic ERG recordings preadaptation was by normal day-light and, during recordings there was a background illumination of 300 mWm^{-2}. No data were collected until it had been established that, with the technique employed, the variation of repeated records of b-waves was not greater than about 50 μV. Table 1 shows such data obtained from five rabbits by repeated records within 1 week.

Table 1
Repeated recordings of the maximal b-wave amplitude of the in situ ERG of five rabbits within 8 days under identical conditions

Rabbit	Record 1		Record 2		Record 3	
	b-wave amplitude (μV)	Time of day	b-wave amplitude (μV)	Time of day	b-wave amplitude (μV)	Time of day
27	225	13.50	200	10.50	175	13.30
35	150	10.00	150	11.30	125	14.00
49	150	13.30	225	10.00	175	11.15
64	225	14.45	200	9.00	225	15.00
59	150	14.15	175	11.30	200	14.15

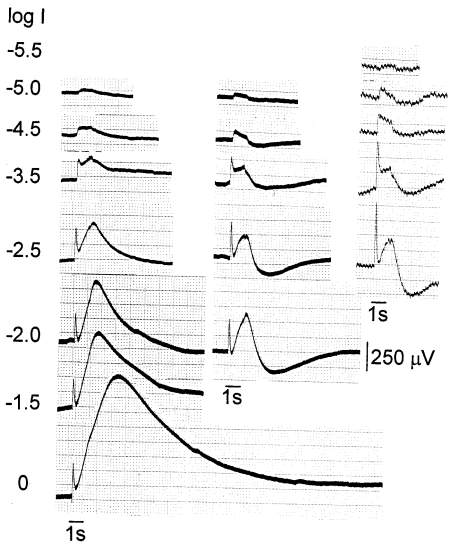

Fig. 2. ERG recordings of a healthy rabbit (rabbit No. 7) response to 1 s light stimuli of increasing intensity (as indicated). First column, ERGs recorded in general anaesthesia with DC-coupling; second column, with AC-coupling; Third column, ERGs with AC-coupling from the unanaesthetised animal.

3. Results

The use of anaesthetic agents to facilitate in situ recordings of the ERG had unexpected influences on the recordings which needed to be considered. Fig. 2 shows ERG-recordings from a healthy rabbit (rabbit No. 7) in response to light stimuli of increasing intensity: (i) under general anaesthesia and with DC-coupling (column 1); (ii) under general anaesthesia and AC-coupling (column 2); and (iii) without anaesthesia and AC-coupling (column 3). The third column is incomplete because closure of the eyes of the conscious rabbit in response to high light intensities invalidated the ERG recordings. It was, however, possible to use the light intensities needed to evoke the maximal amplitude of the b-wave. The DC-recordings under general anaesthesia showed the well known features of the a-, b- and c-wave, the c-wave being very pronounced in response to high light intensities. The c-wave was smaller when AC-coupling was employed. In the absence of anaesthesia the b-wave was larger, although this varied from animal to animal. With a reduced level of anaesthesia there was still a reduction in the b-wave. The effect of anaesthesia on the b-wave is shown in Fig. 3, in which the upper curve shows the b-wave amplitudes from rabbits without general anaesthesia, whereas the lower curve shows the b-wave amplitudes from rabbits under general anaesthesia. Values marked with an asterisk are statistically different (Wilcoxon test, $P = 0.021$). Because the anaesthetic agents reduced the b-wave, the inter-rabbit comparison of the b-wave amplitude when the animals were unanaesthetised was preferred, despite the greater difficulty in obtaining stable ERG records, especially when the rabbits were unaccustomed to the procedure. All data presented in the following figures and tables were recorded from the conscious, locally anaesthetised rabbit with AC-coupling of the amplifier.

The maximum ERG b-waves recorded from the rabbits of our animal house varied greatly, so the population was divided into two groups. Group 1 consisted of those rabbits with maximum b-wave amplitudes ≥ 275 μV (upper part of Table 2a). Group 2 consisted of those rabbits with amplitudes ≤ 250 μV (lower part of Table 2a and upper part of Table 2b). The latter group, with the smaller b-waves, was investigated further. We also recorded the ERGs from another group of rabbits reared elsewhere. All these animals had large b-waves (275–400 μV), and are included in group 1 (upper part of Table 2a). These imported rabbits had previously been fed only fresh vegetation, but after acquisition they were fed a standard dry pellet diet during several weeks. The rabbits which had been in our animal house for much longer had always received the standard dry pellet diet. In consideration of the possibility that the diet could influence the ERG, fresh vegetation was added to the diet of all animals being studied. Following this change there were significant elevations of b-waves. In group 1 there was an increase from 354 ±

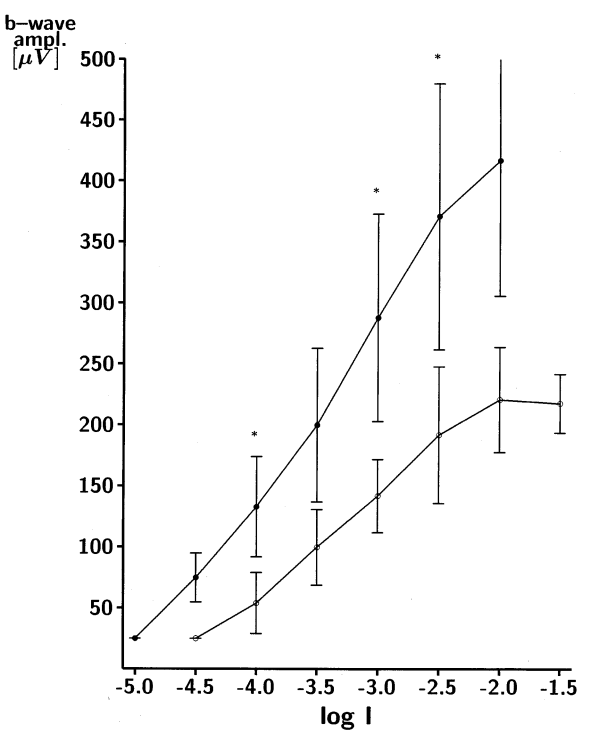

Fig. 3. The influence of anaesthetic drugs on the ERG b-wave. Results from unanaesthetised rabbits (filled symbols) compared to those from anaesthetised rabbits (open symbols) [mean values ± S.D.]. Those values indicated by an asterisk are significantly different (Wilcoxon test $P = 0.021$; $n = 6$).

Table 2
Maximal b-wave amplitudes of four rabbit groups followed up over at least 1 year[a]

Rabbit No.	Born	Sex	Dry food	Dry+natural food								
				July 1996	October 1996	December 1996	March 1997	May 1997	June 1997	July 1997	March 1998	December 1998
b-wave ≥275 μV (at beginning of investigations)												
4			400	Breeds	450	Died						
6			375	550	Acute experiment							
7		f	350	525	500		425	375	475	450	425	400
30			400	Acute experiment								
36		m	275	475	450	Died						
37	2 May 1994	m	325	350	Acute experiment							
42			350	Acute experiment								
50	22 November 1995			475	Acute experiment							
58	1 January 1996	m		475			425	375	450	450	375	400
Rabbit No.	Born	Sex	Dry food	Dry+natural food								
				July 1996	October 1996	December 1996	March 1997	May 1997	June 1997	July 1997		
b-wave ≤250 μV (at beginning of investigations)												
[8	26 June 1994	f	150	Breeds	275	275	200	325	300	250]	EM	
[28	26 May 1995	f	250	350	250	225	325	275	275	250]	EM	
[35	**19 September 1995**	**m**	**250**	**225**	**200**	**200**	**200**	**200**	**150**	**175]**	**pigment epithelium defect**	
[47	**17 December 1995**	**f**	**150**	**375**	**200**	**250]**	**pigment epithelium defect**					
(48	17 December 1995	f	175	325	225	225	250	225	200	225)		
(49	17 December 1995	f	250	200	200	200	250	150	175	250)		

Table 2 (continued)

Rabbit No.	Born	Sex	Dry food	Dry+natural food								
				July 1996	October 1996	December 1996	March 1997	May 1997	June 1997	July 1997	March 1998	December 1998
b-wave reduction ≥150 μV (from beginning of investigations)												
27	26 May 1995	m	225	375	250	225	225	175	200	225	225	150
34	**19 September 1995**	**f**	**225**	**350**	**300**	**200**	**250**	**250**	**200**	**225**	**200**	
59	1 January 1996	f	475	300		150	150	175	175	150	200	
Rabbit No.	Born	Sex	Litter of	Dry+natural food								
					October 1996	December 1996	March 1997	May 1997	June 1997	July 1997	September 1997	December 1998
b-wave (μV) of second generation												
64	2 July 1996	m	8+27		250		175	225	250	150	175	
67	2 July 1996	m	8+27		325	300	250	250		275		100
71	**10 August 1996**	**f**	**28+35**			**200**	**225**	**250**	**175**	**150**		**150**
72	**10 August 1996**	**m**	**28+35**		**225**	**225**	**300**		**200**		**175**	
75	**10 August 1996**	**f**	**28+35**		**250**	**225**	**200**	**150**	**150**		**150**	
[68	**10 August 1996**		**28+35**		**325**	**250**	**225**			**175] → EM**		

[a] First group: rabbits with b-waves ≥275 μV; 2nd group: rabbits with b-waves ≤250 μV at beginning of investigations; 3rd group: rabbits with a decline ≥150 μV in 1 year; 4th group: rabbits of second generation with small b-waves. Change of food for all rabbits indicated; f, female; m, male. Bold letters indicate rabbits with so far identified RPE defect or siblings or offsprings of them.

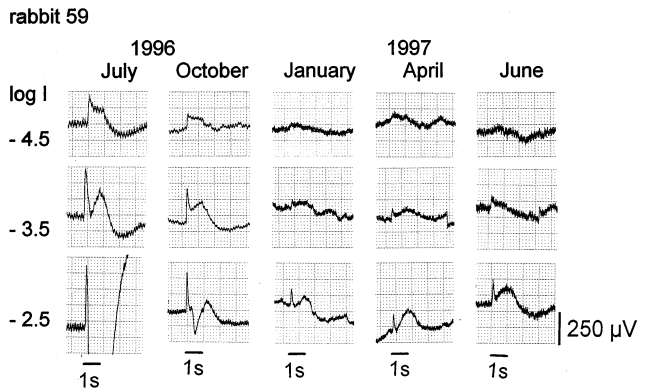

Fig. 4. Progressive reduction of the b-wave in rabbit No. 59. Light intensities and stimulus duration as indicated.

44 µV (S.D., $n = 7$) to 475 ± 69 µV (S.D., $n = 6$), in group 2 there was an increase from 209 ± 44 µV (S.D., $n = 8$) to 328 ± 78 µV (S.D., $n = 8$). These changes in the b-wave occurred between March and July 1996, and while they could be due to the dietary change, a seasonal influence is also possible.

The retina and the RPE of two animals from group 2 (Nos. 35 and 47) which had small b-wave amplitudes were investigated ultrastructurally by Nilsson and Wrigstad and found to have severe pathological changes of the RPE. Additional eyes of the first and second rabbit generation, together with eyes of control animals are now under further investigation (Wrigstad, Hanitzsch & Nilsson, in preparation).

Ophthalmoscopic examination of the fundus of rabbits with small dark-adapted b-waves has not revealed visible abnormalities.

Because of uncontrollable variations in the conditions of the conscious rabbits and the possibility that naturally occurring seasonal influences could underlie the observed changes in ERG, we monitored the ERG of eight rabbits with low b-waves (Nos. 8, 28, 35, 48, 49, 27, 34, 59) and two rabbits with normal b-waves as controls (Nos. 4 and 58) over an 1-year period (Table 2a and Table 2b/upper part). The reduction of the b-wave was largest (≥ 125 µV) in rabbit Nos. 27, 34 and 59 (Table 2b). In these three animals recordings of the ERG at different light intensities were obtained. Fig. 4 shows the reduction of the b-wave amplitude of rabbit No. 59 between summer 1996 and June 1997. There was a strong decline of the maximal b-wave amplitude in the first year from 475 to 175 µV. For these three rabbits (Nos. 27, 34, 59) the period of observation was extended by 18 months until December 1998. During this period there was a continuing decline in the b-wave at all intensities tested (Fig. 5).

Rabbits Nos. 48 and 49 had dental anomalies and they were eliminated from the study because of the possibility that, as a result of these anomalies, malnutrition could reduce the ERG b-wave in a way that would be indistinguishable from a b-wave reduction due to pigment epithelial and photoreceptor defects.

The ERGs of litters of rabbits born to Nos. 28 and 35 mainly showed small b-waves. The ERGs of five of this second generation rabbits (Table 2b: Nos. 64, 67, 71, 72 and 75) were checked over a 2-year period during which time the b-wave declined significantly from 245 ± 37 µV to 150 ± 31 µV (mean value $\pm$ S.D., $n = 5$, Wilcoxon signed-rank test/single sided, $P = 0.03$).

Additionally ERG recordings were made under conditions of light adaptation in spring and summer 1997. Fig. 6 shows both dark- and light-adapted ERG records from rabbits Nos. 7, 59 and 35. No. 59 had the strongest reduction in the dark adapted b-wave in the first year and No. 35 is the one with the RPE defect. During dark adaptation the b-waves of rabbit Nos. 59 and 35 were much smaller than that of rabbit No. 7 for which the ERG record was normal. With light-adaptation, however, the b-waves of rabbit Nos. 59 and 35 were practically indistinguishable from those recorded when the eye was dark adapted. By contrast, the b-wave of rabbit 7, which had a normal ERG, differed markedly between the two states of adaptation, that

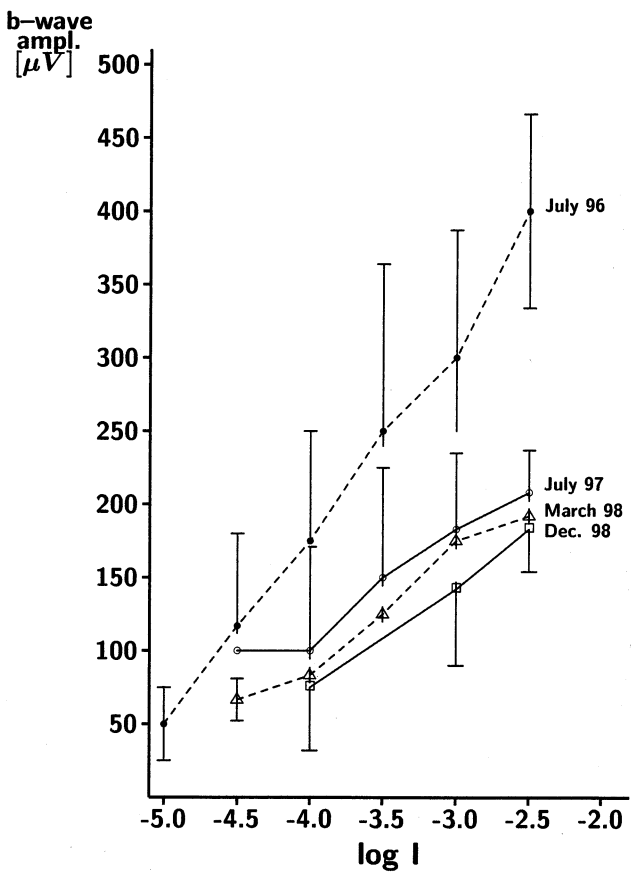

Fig. 5. b-wave amplitude (mean values and S.D.) as a function of increasing light intensity from rabbits No. 27, 34 and 59 in July 1996 (dots), in July 1997 (open circles), in March 1998 (triangles) and in December 1998 (squares).

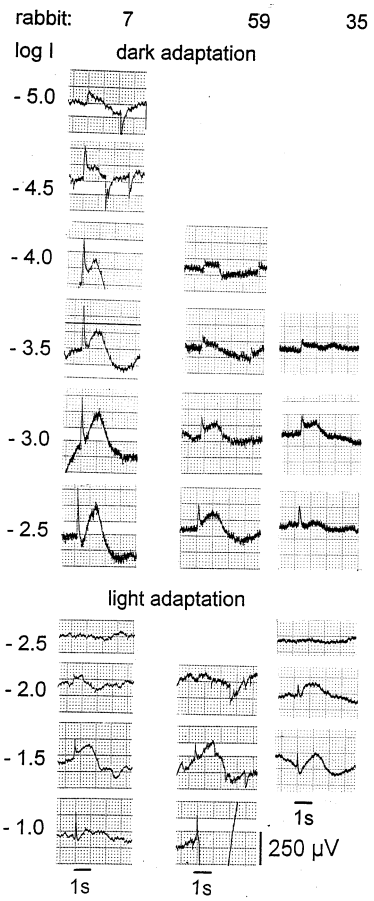

Fig. 6. ERGs of a healthy rabbit (No. 7, left column) and two rabbits with suspected disease (No. 59, middle column, No. 35, right column) under dark adaptation (upper part of the picture) and light adaptation (lower part of the picture). Light intensities and stimulus duration as indicated.

under dark adaptation being much larger than that under light adaptation.

Table 3 shows differences between the maximum light adapted b-wave and the maximum dark adapted b-wave in 23 rabbits. The amplitude of the light-adapted b-wave was expressed as a percentage of the amplitude of the dark-adapted b-wave. The animals with percentages of less than 62% were placed in one group, and those with percentages of 62% or greater were placed in another group. The mean value of the first group was $42 \pm 9\%$. That of the second group was $71 \pm 10\%$. In this latter group the amplitudes of the light adapted b-waves were within the range obtained from apparently normal retinas, but the dark-adapted b-waves were consistently below the range for apparently normal retinas. This group included rabbit No. 35, which had the identified RPE defect. Rabbit No. 34 is a sibling of rabbit No. 35, and rabbit No. 72 an offspring of rabbit No. 35. Rabbit No. 48 is a sibling of rabbit No. 47 which had an identical RPE defect. The three animals with the strongest b-wave reduction (Nos. 59, 27, 34) were also included in this second group.

4. Discussion

In humans anaesthesia can influence the ERG (Marmor & Zrenner, 1995). In rabbits also, even at low levels, anaesthetic agents have significant effects on the ERG. We decided, therefore, to make ERG recordings from conscious animals in this study so that the effects of applied drugs did not distort those due to pathological conditions. This decision, however, introduced the problem that the ERG records made on unanaesthetised rabbits are more variable than are those obtained under general anaesthesia. This is evident from the larger standard deviation in Fig. 3. To overcome this problem we made frequent ERG recordings in eight rabbits of the first generation over a 1 year period and in three of these rabbits over 2.5 years. By this means it was hoped to exclude uncontrolled influences on the ERG of diet and season which may have happened between March and July 1996, and which could not be studied further. Most of the eight rabbits showed a reduction in the b-wave amplitude with time, with those of rabbits Nos. 27, 34, and 59 being most severe: from 400 ± 66 μV to 183 ± 29 μV. In sharp contrast, the b-wave amplitudes of the control rabbits Nos. 7 and 58, which had normal b-waves, showed no such reduction with time.

The progressive reduction over more than 2 years of the dark adapted ERG b-wave of three rabbits, and the stability of the light adapted ERG b-wave at a level close to that of rabbits with apparently normal retinas during at least the first year, indicates that the defect is primarily affecting the pathway through the retina from the rods. This proposition is consistent with the evidence of the severe pathological state of the RPE revealed by the EM study of the retinas of rabbit No. 47 and 35 (Nilsson & Wrigstad, in preparation). This defect is probably inherited since the dark-adapted ERG of at least some of the offspring of the affected rabbits have also been found to exhibit small and declining dark-adapted b-waves.

We (Nilsson, Wrigstad and Hanitzsch) are continuing the ultrastructural studies and the recordings of dark- and light-adapted ERGs, including DC-recordings from the dark adapted eye, to see whether the c-wave is markedly affected. These studies will be also extended to the just born third generation (offsprings of Nos.72 and 71; 72 and 75). A rabbit strain with inherited pigment epithelium defect causing photoreceptor disease could prove a useful tool in the study of human retinal degenerations.

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Table 3
Maximal dark adapted b-wave compared to light adapted b-wave (background light: 300 mWm^{-2}) and ratio of the light adapted b-wave to the dark adapted for one of the rabbits with a normal light/dark ratio (upper part of table) and for rabbits with a noticeable high light/dark ratio (lower part of table)[a]

Rabbit	Age (years)	Maximum b-wave (μV)		b-wave relation light/dark (%)
		Dark adapted	Light adapted	
7	~2	475	200	42
58	~1.5	450	175	39
Selection out of 16 cases with a mean value of 42 ± 9%				
b-wave relation light/dark ≥62%				
35	**~2**	**200**	**125**	**62**
34	**~2**	**200**	**125**	**62**
72	**~1**	**150**	**125**	**83**
48	**~1.5**	**200**	**150**	**75**
27	~2	200	125	62
59	~1.5	175	150	86
64	~1	225	150	67
				$\bar{x} = 71 \pm 10\%$

[a] For the number and age of rabbits at time of measurements see columns 1 and 2. Bold letters indicate rabbits with so far identified RPE defect or siblings or offsprings of them.

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