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# Studies on retinitis pigmentosa in man.

## II. Erythrocyte osmotic fragility

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**SUMMARY** The osmotic fragility of erythrocytes from patients with genetically classified forms of retinitis pigmentosa (RP) has been studied. The mean fragility was increased in autosomal dominantly inherited RP, where the dystrophy was expressed regionally in the retina, with both rods and cones affected. In contrast it was normal in patients with the dominantly inherited disease, which leads to a diffusely distributed dystrophy of, predominantly, rod photoreceptor cells. Raised osmotic fragility of erythrocytes has also been observed in female patients with multiplex (recessive) RP and in female carriers of the X-linked form of the disease.

Retinitis pigmentosa (RP) is the name given to a group of inherited diseases, generally of unknown aetiology, which lead to the degeneration of rod photoreceptor cells; cones may also be affected. In our studies on RP in man we have adopted the premise that there might be a generalised metabolic lesion that is expressed overtly in photoreceptors because of their unique composition, function, and/or environment.<sup>1</sup>

The response of erythrocytes to hypotonic insult provides a simple monitor of general body homeostasis, since it is influenced not only by the metabolic status of the cells but also by the composition of plasma.<sup>2</sup> We have therefore routinely included the test in our studies on patients with RP, and here we report our initial findings. Erythrocyte osmotic fragility varies with the age of the donor and the turnover time of the cell population.<sup>3–5</sup> The latter is reflected in heterogeneity of cell fragilities within individual samples.<sup>6</sup> In the course of the study we also detected sex-related differences.<sup>5</sup> We have therefore considered these variables in the evaluation of our results.

Several distinct inheritance patterns of RP are recognised. They include autosomal dominant, X-chromosome-linked (X-hemizygote), and autosomal recessive. The last is most clearly distinguished when brothers and sisters express the disease but there is no other affected relative, a category referred to by Jay<sup>7</sup> as mixed multiplex. As in our previous study,<sup>8</sup> we

have used this terminology, referring also to RP patients with no known affected relative as simplex.

### Materials and methods

The patients reported on here attended the Genetic and Electrodiagnostic Clinics of Moorfields Eye Hospital, London, where the category of retinal dystrophy was defined genetically, clinically, and where possible by psychophysical and electrophysiological tests.<sup>9–11</sup> In particular, patients with autosomal dominantly inherited disease were divided into those with a diffuse loss of, principally, rod vision (D type), and those with more regionalised retinal lesions and significantly affected cone as well as rod vision (R type) (Ernst WJK, Lyness AL, personal communication).

Osmotic fragility was determined with hypotonic, phosphate-buffered saline, pH 7.4, as described by Dacie and Lewis.<sup>12</sup> Heparinized blood (50  $\mu$ l) was mixed with 5.0 ml buffered saline (12 tubes with dilutions ranging from 1.0 to 9.0 g/l NaCl), and after 30 min at room temperature the absorbance at 540 nm of the centrifuged solutions was measured.

Haemolysis curves were constructed from the experimental data, and a linearising transform<sup>3</sup> was applied to permit linear regression statistics to characterise the osmotic fragility curve by its mean cellular fragility, X-50 (that is, the molar NaCl concentration at 50% haemolysis) and the fragilities distribution parameter,  $\beta$ . A broadening of the range of fragilities would be quantified with a decrease in  $\beta$ . Results are expressed as the mean  $\pm$  SEM.

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Table 1 Osmotic fragility of erythrocytes from patients with retinitis pigmentosa

Category	Males			Females		
	Number	Age	X-50×10 <sup>3</sup> †	Number	Age	X-50×10 <sup>3</sup> †
(1) 'Normal'	36	40±2	73.5±0.4	42	47±3	72.0±0.4
(2) Autosomal dominant (R type)	6	46±6	76.0±0.9*	11	43±5	75.3±0.8***
(3) Autosomal dominant (D type)	5	26±2*	71.0±1.6	8	37±4	72.2±0.5
(4) X-linked hemizygote	16	33±3	72.1±0.7			
(5) X-linked heterozygote				13	36±4	73.6±0.5*
(6) Multiplex	6	46±6	74.6±0.3	5	51±6	74.5±0.6**
(7) Simplex	11	40±4	73.4±0.7	5	40±10	72.8±0.8

\*p<0.05, \*\*p<0.02, \*\*\*p<0.001 when compared with the normal group.

†Results expressed as moles/litre NaCl: see text for explanation.

Categories: (1) Apparently healthy volunteers. (2) Males and/or females affected in more than one generation: regionalised retinal lesions. (3) As (2) but diffuse retinal lesions. (4) X-chromosome-linked RP: affected males. (5) Female 'carriers' of (4). (6) Siblings express the disease; no other known affected relative. (7) No known relative with the disease.

## Results and discussion

The mean osmotic fragilities of erythrocytes obtained from patients with retinitis pigmentosa are shown in Table 1. The most striking result is the raised fragility of cells from patients with the dominantly inherited disease that leads to regionalised lesions and to the degeneration of both rod and cone photoreceptors.  $\beta$  Values did not significantly differ from normal (control males,  $323 \pm 10$ ,  $n=36$ ; R type,  $265 \pm 28$ ,  $n=6$ ; control females,  $317 \pm 8$ ,  $n=42$ ; R type,  $301 \pm 21$ ,  $n=11$ ). This suggests that erythrocyte turnover was not impaired and that the change is caused not by age variation in the cell population but by metabolic or extracellular factors—for example, plasma composition. The result is made more intriguing because of the normal fragility seen in the group of patients who also had autosomal dominantly inherited RP but who were characterised by a more generalised loss of rod function and early onset of night blindness (Ernst WJK, Lyness AL, personal communication). Erythrocyte osmotic fragility increases with age of the donor (3–5). We must therefore also consider the significantly lower mean age in males with the D-type lesion (Table 1). However, an approximate correction, based on the rate of increase in X-50 in normal males over the age range 26 to 40 years,<sup>5</sup> predicted a mean X-50 for D types of only  $72 \pm 2$  at 40 years of age.

Increased fragility was also evident in erythrocytes from females with multiplex RP and from carriers of the X-linked form of the disease. Although the latter do express a milder form of RP,<sup>10</sup> it is not possible from current knowledge to explain why they and not the more affected males show a change in the erythrocyte response.  $\beta$  Values were normal for both 'multiplex' females ( $304 \pm 23$ ,  $n=5$ ) and X-linked 'heterozygotes' ( $324 \pm 35$ ,  $n=13$ ).

Diverse factors interact to determine the membrane composition of circulating erythrocytes.

Systems of potential relevance to the onset or progression of RP are lipid status<sup>1,2</sup> and the levels of free radical potentiating and scavenging agents. As regards the latter, erythrocytes and photoreceptors subserve functions that may expose them to internally generated free radicals with the subsequent production of activated oxygen. In erythrocytes this may arise from breakdown products of haemoglobin<sup>13</sup> and in photoreceptors from byproducts of the absorption of light by visual pigments or their photoproducts.<sup>14,15</sup> Vitamin E is the principal free radical scavenger and protectant for both systems,<sup>13,16</sup> and lack of the vitamin will lead to photoreceptor degeneration<sup>17</sup> and an increase in erythrocyte fragility.<sup>18</sup> However, other dysfunctions or deficiencies might have similar outcomes, and the actual cause(s) of the increased erythrocyte fragilities observed in RP patients in the present investigations must form the subject of subsequent research.

This study needed patients classified into RP subgroups, and for these we are indebted to our colleagues who passed on their unpublished results. We sincerely thank those who have volunteered or taken blood for us and, in particular, we are grateful for the encouragement and time given by Professor Geoffrey Arden, Professor Alan Bird, Dr Wojtek Ernst, and Miss A Lou Lyness.

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