normal lenses than in cataractous lenses indicates that it does represent a real change in protein structure. Conceivably, such protein in the intact lens may be composed of even larger macromolecular aggregates than the HMWP fraction and would, therefore, also contribute to the scattering of light. The transformation to insoluble protein may be accelerated in the developing cataract and thus partially account for the observation of only modest increments in HMWP in such lenses.

It is of interest to note that in the cortical region the light scattering cannot be explained simply on the basis of the appearance of HMWP. However, recent experiments of Kuwabara’s (T. Kuwabara: private communication) suggest a possible explanation for such scattering. Scanning electron microscopy of the cortical region of normal lens indicates highly irregular interdigiting fiber surfaces which may cause light scatter. The increase in scattering from this region with aging may be due principally to the increase in size of the cortical region. In contrast, the nuclear region contains highly regular interdigitating fiber boundaries which would not be expected to cause significant scattering. Thus in the aging process, molecular changes may cause most nuclear scattering and normal morphological structure, cortical scattering.

The skilled technical assistance of Stojmen Djalazov is appreciatively noted. We thank Dr. Emil Wirostko for permitting us to use his Zeiss slit lamp camera.

From the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, New York, N. Y. 10032. Supported by grants from the National Eye Institute. Submitted for publication April 26, 1974. *Dedicated to David G. Cogan, who created a free, stimulating intellectual atmosphere at the Howe Laboratory, Harvard University, which led to an unprecedented era of scientific accomplishment. **Fight for Sight Fellow.

Key words: lens, high molecular weight protein, aging, light back-scatter, nucleus, cortex.

REFERENCES


Retinol in retinitis pigmentosa: evidence that retinol is in normal concentration in serum and the retinol-binding protein complex displays unaltered fluorescence properties. SIDNEY FUTTERMAN, DAVID SWANSON, AND ROBERT E. KALINA.

The concentration of retinol in serum in retinitis pigmentosa was determined by a reliable, conventional fluorometric procedure involving extraction and column chromatography, and by a new procedure that measures directly the fluorescence of the retinol-binding protein complex in serum. The mean values for 15 serum samples analyzed by the two methods were, respectively, 66 and 67.

Downloaded From: https://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/932880/ on 11/19/2018
Defective absorption or transport of retinol as in a β-apoproteinemia1 or cystic fibrosis,7,8 or impaired cleavage of β-carotene occurring with an exclusively vegetarian diet4 can lead to night blindness and characteristic retinal changes.5 These disorders occurring in man as well as experimentally produced vitamin A deficiency states in animals8 share certain physiologic and morphologic features with retinitis pigmentosa,7 a prevalent retinal degeneration of man.

The retina, unlike other tissues requiring vitamin A, cannot utilize retinoic acid (vitamin A aldehyde) to retinoic acid,9 it is reasonable to postulate that one variety of retinitis pigmentosa might stem from a defect in the transport of retinol from liver to target tissues. While the photoreceptors were experiencing a selective deficiency, the systemic vitamin A requirements could be met adequately by circulating retinoic acid derived by the transformation into retinoic acid of a small fraction of the retinol formed during the course of intestinal cleavage of dietary β-carotene. It is, therefore, of great interest that a low concentration of retinol in blood has been found in 91 per cent of one group of adult patients with retinitis pigmentosa.10 However, this observation has not been confirmed in other laboratories.11-13 More recently, it has been reported that the concentration of circulating retinol-binding protein is reduced in retinitis pigmentosa.14

One possible mechanism that has not yet been investigated is that the retinol transport complex might be present in normal concentration but have altered properties that reduce its effectiveness. A significantly altered retinol-binding protein might be expected to display altered fluorescence excitation or emission maxima or altered fluorescence enhancement.15 The present study was undertaken both to explore these ideas and to re-examine the question of the concentration of retinol in blood in retinitis pigmentosa with a comparison of results obtained by two different assay methods,15 one giving a measure of the native retinol-binding protein, and the other determining retinol recovered after extraction and chromatographic separation.

**Methods.** Subjects were selected because of a history of decreased night vision, progressive loss of visual field, and a previously established diagnosis of retinitis pigmentosa. The diagnosis was confirmed in all cases by a complete eye examination yielding dilated fundus examination evidence of advanced pigmentary retinopathy and narrowed retinal blood vessels. Some, but not all, of the subjects also demonstrated immature cataracts, waxy optic discs, or pigmentary macular changes. Goldmann perimetry confirmed characteristic peripheral visual field loss in patients with retained vision. Affected relatives of subjects were identified by history but were not examined. Subjects were questioned in order to exclude recent supplemental vitamin A intake.

Analytic methods used for the determination of retinol in serum are described in the preceding paper.15

**Results.** The concentration of retinol in serum samples from retinitis pigmentosa patients (Table 1) fell largely (Fig. 1), although not entirely within the range of samples from normal subjects previously analyzed by these methods. The mean values were not significantly in excess of the mean values of 60.1 and 60.8 µg per 100 ml found for normal subjects.15 These findings indicate that the range of fluorescence enhancement that is observed for retinol in its native complex in serum samples from retinitis pigmentosa patients does not differ significantly from the range of fluorescence enhancement found for normal subjects. Fluorescence spectra were obtained for each of the diluted serum samples. All gave excitation and emission maxima at 335 nm and 458 nm for the retinol peak, and showed no deviations from normal spectra.15 The height ratios of the retinol (335 nm) to "protein" (285 nm) peaks also were all within the normal range.
**Reports**

**October 1974**

Table I. Retinol content (micrograms per 100 ml.) of serum in retinitis pigmentosa patients as determined by a new, direct fluorometric method and by conventional method involving extraction and chromatography

<table>
<thead>
<tr>
<th>Retinitis pigmentosa serum sample</th>
<th>Age and sex</th>
<th>Retinol determination</th>
<th>Carotenoids (μg/100 ml.)</th>
<th>Protein (Gm./100 ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>New method</td>
<td>Conventional method</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>21, M</td>
<td>83</td>
<td>63</td>
<td>203</td>
</tr>
<tr>
<td>2</td>
<td>24, M</td>
<td>62</td>
<td>56</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>26, F</td>
<td>47</td>
<td>53</td>
<td>140</td>
</tr>
<tr>
<td>4</td>
<td>28, M</td>
<td>43</td>
<td>41</td>
<td>128</td>
</tr>
<tr>
<td>5</td>
<td>30, M</td>
<td>83</td>
<td>70</td>
<td>126</td>
</tr>
<tr>
<td>6</td>
<td>35, F</td>
<td>46</td>
<td>46</td>
<td>93</td>
</tr>
<tr>
<td>7</td>
<td>30, M</td>
<td>55</td>
<td>65</td>
<td>54</td>
</tr>
<tr>
<td>8</td>
<td>40, M</td>
<td>67</td>
<td>75</td>
<td>64</td>
</tr>
<tr>
<td>9</td>
<td>48, F</td>
<td>56</td>
<td>66</td>
<td>72</td>
</tr>
<tr>
<td>10</td>
<td>50, M</td>
<td>87</td>
<td>92</td>
<td>77</td>
</tr>
<tr>
<td>11</td>
<td>52, M</td>
<td>52</td>
<td>55</td>
<td>86</td>
</tr>
<tr>
<td>12</td>
<td>52, M</td>
<td>89</td>
<td>104</td>
<td>108</td>
</tr>
<tr>
<td>13</td>
<td>58, F</td>
<td>97</td>
<td>95</td>
<td>145</td>
</tr>
<tr>
<td>14</td>
<td>64, M</td>
<td>56</td>
<td>58</td>
<td>106</td>
</tr>
<tr>
<td>15</td>
<td>76, F</td>
<td>66</td>
<td>67</td>
<td>92</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td><strong>65.9</strong></td>
<td><strong>67.1</strong></td>
<td><strong>106.1</strong></td>
</tr>
<tr>
<td><strong>S.E.</strong></td>
<td></td>
<td><strong>4.3</strong></td>
<td><strong>4.6</strong></td>
<td><strong>9.6</strong></td>
</tr>
<tr>
<td><strong>P</strong></td>
<td></td>
<td><strong>NS</strong></td>
<td><strong>NS</strong></td>
<td><strong>NS</strong></td>
</tr>
</tbody>
</table>

*Significance of difference from normal subjects.*

Table II. Distribution (per cent) among component fractions of total fluorescence of serum extract recovered after chromatography on alumina

<table>
<thead>
<tr>
<th>Serum samples</th>
<th>Hydrocarbon fraction</th>
<th>Retinyl esters</th>
<th>Retinol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinitis pigmentosa (15)</td>
<td>17.9 ± 2.6</td>
<td>9.1 ± 0.9</td>
<td>73.0 ± 3.2</td>
</tr>
<tr>
<td>Control subjects (21)</td>
<td>24.2 ± 1.4</td>
<td>7.3 ± 0.4</td>
<td>68.5 ± 1.5</td>
</tr>
<tr>
<td>Significance of difference</td>
<td>P &lt; 0.05</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± S.E. for data obtained from analyses of total fluorescence in the column fractions was the same.

In the assay of retinol by the conventional procedure, retinyl esters, and a fluorescent hydrocarbon fraction are separated chromatographically from retinol. The relative proportion of retinyl esters in serum samples in retinitis pigmentosa did not differ significantly from the proportion in normal subjects (Table II). The fraction of the total fluorescence attributable to the hydrocarbon fraction was somewhat reduced in retinitis pigmentosa, but in view of the known dietary origin of phytofluene as the major component of this fraction, it is unlikely that this difference is attributable to the disease. The carotenoid concentration did not differ significantly from the value obtained for normal subjects.

The mean serum retinol and carotenoid levels of several subgroups of patients were compared with the other patients and with normal subjects. Seven patients with waxy optic discs and two patients with late onset of symptoms had serum values not significantly different from the other patients or from normal subjects. Three patients with family histories suggesting an x-linked recessive inheritance pattern also had normal serum values as did two patients from probable autosomal dominant pedigrees. Eleven patients had either no family history or a history of retinitis pigmentosa suggesting autosomal recessive inheritance. Those eleven patients had a mean serum retinol level greater than the other five patients (p < 0.05) and greater than the normal subjects (p < 0.01). However, the significance of these results is reduced both by the small number of patients and the older average age of the eleven patients.

**Discussion.** The concentration of retinol in blood below which night blindness becomes manifest in human volunteer subjects can be calculated to be about 15 μg per 100 ml. All retinitis pigmentosa serum samples examined in this study had retinol concentrations higher at least by about threefold.

Both the concentration of retinol in serum and the fluorescence properties of the retinol-binding protein complex in retinitis pigmentosa patients are indistinguishable from those of normal sub-
jects. These findings constitute new evidence in support of the view that the transport of vitamin A in retinitis pigmentosa is unimpaired, but do not rule out the possibility that the mechanism of release of retinol from retinol-binding protein may be abnormal.

The well-known tendency of the retinol concentration in blood to increase with age probably reflects a gradual diet-related increase in the storage of retinyl esters in the liver that commonly continues throughout life, but can be greatly accelerated by vitamin supplementation. It seems likely that the presence of a high concentration of retinyl esters in the liver might increase production and release of retinol-binding protein into the circulation. There was some indication that the retinol concentration in blood increased with age (Table 1) in the retinitis pigmentosa patients in this study as well as in the normal subjects previously examined. Although the retinitis pigmentosa patients and the normal subjects were not precisely matched by age, statistical analysis reveals no significant difference between the groups with respect to the concentration of retinol in the circulation. It would appear that some uncontrolled factor of age, diet, or methodology may have accounted for the reduced concentrations of retinol in blood that have been reported in retinitis pigmentosa.

Excellent technical assistance was provided by Mrs. Martha H. Bollins and advice concerning statistical analysis of the data was obtained from Ms. Barbara Campbell.

From the Department of Ophthalmology, University of Washington School of Medicine, Seattle. This study was supported by United States Public Health Service Research Grant No. EY 00343 and Training Grant No. EY 00050 from the National Eye Institute. Submitted for publication April 29, 1974. Reprint requests: Dr. S. Futterman, Department of Ophthalmology, University of Washington School of Medicine, Seattle, Wash. 98195.

Key words: retinol, retinol-binding protein, retinitis pigmentosa, vitamin A, retina, carotene.

REFERENCES


A possible cause of decreased vision in cryptococcal meningitis. CARL KUPFER AND EDNA MCCRANE.

The optic nerves, chiasm, optic tracts, and lateral geniculate nuclei of six patients having fatal cryptococcal meningitis were examined histopathologically using the Smith-Quigley stain for myelin and periodic acid-Schiff stain for cryptococcal organisms. A correlation was made between the invasion of visual pathways by the cryptococcal organism, the presence or absence of papilledema, and the preservation or loss of visual acuity. In three patients with papilledema and loss of visual acuity, multiple cryptococcal abscesses were present in the optic