before and after the addition of beta-glucuronidase. In circumstances where the actual plasma free fluorescein has not been measured, the term "plasma-free fluorescence" should be quoted. It is then better to measure overall fluorescence (fluorescein and the glucuronide metabolite) in protein-free plasma ultrafiltrate and the fluorescence appearing in the ocular compartments using the same excitor and emission filters.

Fluorescein glucuronide is a potential source of variability in studies of blood-ocular dynamics using fluorescein. Its exact role has yet to be established.

Key words: Blood-ocular barriers, diabetes, plasma ultrafiltrate, fluorescein glucuronide, fluorescence, protein-binding

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References

Diosynrhesis of Neopterin, Sepiapterin, and Diopterin in Rat and Human Ocular Tissues

Gadiparithi N. Rao and Edward Cotlier

Neopterin, sepiapterin, and biopterin synthesis by lens, retina, and ciliary body–iris of rat and human indicates pteridine formation from their precursor, GTP. The pteridine biosynthesis was higher in the retina (neopterin 422 ± 27, 260 ± 24; sepiapterin 135 ± 12, 118 ± 14; biopterin 76 ± 10, 68 ± 8 nanomoles/g soluble protein/hr, in rat and human, respectively) than in the ciliary body–iris and lens. The light-sensitive pteridines may protect eye tissues against the effects of sunlight in addition to their role in the hydroxylation of aromatic amino acids. Invest Ophthalmol Vis Sci 26:768–770, 1985

Yellow and red eye pigments of Drosophila melanogaster were believed to be due to the presence of pteridines, sepiapterin, and drosopterin, respectively.1 Studies on fluorescent compounds in the mammalian ocular lens have suggested the occurrence of glucoside of 3-hydroxykynurenine,2 β-carbolines, and pteridinelike substances.3,4 Protective role of pteridines against light-induced effects has been proposed in lens and retina,4 however, evidence on the presence of these compounds in ocular tissues is lacking. Since the discovery of tetrahydrobiopterin, a reduced form of dihydrobiopterin, as a natural cofactor for aromatic amino acid hydroxylases,5,6 a number of reports on the biosynthesis of pteridines in the tissues of higher animals have been published,8,9 and a pathway shown in Figure 1 has been proposed.8 The aim of the present investigation, indeed, is to know whether lenticular tissues possess a biosynthetic pathway to synthesize pteridines.

Materials and Methods. Neopterin, sepiapterin, and biopterin were purchased from Dr. Schrick’s laboratory, Switzerland. NADPH, Tris and Dowex-50H+ were obtained from Sigma Chemical Company (St. Louis, MO). (U-14C)-GTP was procured from Amersham (Arlington Heights, IL). All other reagents were of analytic grade. Albino rats weighing 200–300 g body weight were used. Human eyes were supplied by the Connecticut Eye Bank & Visual Research Foundation, Inc. (New Britain, CT). Ciliary body–iris, lens, and retina were dissected out carefully from the eyeballs. Tissues were homogenized in homogenizing medium consisting of 10 mM Tris-HCl buffer–40 mM KCl (pH 8.0) using Potter-Elvehjem homogenizer. The homogenates were centrifuged at 17,000 x g for 1 hr in Sorvall R. C. 2B centrifuge at 0°C, and the supernatants were used for pteridine biosynthesis. All studies utilizing experimental animals conformed with the ARVO Resolution on the Use of Animals in Research.

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The method for pteridine biosynthesis followed here is essentially that of Yoshioka et al. with minor modifications. For neopterin and bioperin biosynthesis, reaction mixture (total 1.0 ml) containing 100 \( \mu \)mol Tris-HCl buffer (pH 7.2), 5 \( \mu \)mol MgCl_2 \( \cdot \)6H_2O, 1 \( \mu \)mol NADPH, 0.2 \( \mu \)mol (U-\( ^{14} \)C)-GTP (SA 4.51 \( \mu \)Ci/\( \mu \)mol), and 0.3 ml of the supernatant was incubated for 3 hr at 37°C in the dark. The reaction was terminated by the addition of 150 \( \mu \)l of 20% (W/V) TCA and centrifuged at 9,000 \( \times \)g for 30 min to remove protein. To 300 \( \mu \)l of the clear supernatant, 50 \( \mu \)l of 30 \( \mu \)M each of neopterin and biopterin were added as internal carriers, and the entire mixture was treated with equal volume of iodine solution for 1 hr (2 g KI and 1 g iodine in 100 ml of double-distilled water). Excessive iodine was destroyed by the addition of 1% (W/V) ascorbic acid. The mixture (pH 1.0) was then passed through 0.6 \( \times \) 20 mm of Dowex-50 W(H\(^+\), 12X, 200-400 mesh) and washed in the column with double-distilled water. The water wash was discarded. Pteridines were eluted with 3.0 ml of 1.0 M NH_4OH, and the eluate was evaporated to dryness under nitrogen. The residue was dissolved in known amount of water. Ten microliters were then spotted on Whatman No. 1 paper and developed in the following solvent systems: (1) 3% NH_4Cl; (2) 1-propanol:1% NH_4OH (2:1); (3) 1-propanol:ethyl acetate:water (7:1:2), and (4) 1-butanol:acetic acid:water (4:1:2). After identification of the spots under UV light, they were cut and placed in scintillant vials containing 10 ml of scintillant fluid, aqualyte. The radioactivity was measured in Nuclear-Chicago Mark II liquid scintillation system. For the estimation of sepiapterin formed, the reaction mixture as described above was incubated for 3 hr in the dark at 37°C, and the reaction was terminated by the addition of 4 volumes of ice-cold ethanol. The protein was removed by centrifugation, and the supernatant was evaporated to dryness. The residue was dissolved in known amount of distilled water. Ten microliters of this was spotted on Whatman No. 1 paper and developed in different solvent systems as mentioned above with carrier sepiapterin. The rest of the procedure is the same as above.

Protein content was determined according to Lowry et al. with the use of bovine serum albumin as standards.

Results and Discussion. From our results it is clear that the ciliary body--iris, lens, and retina possess the biosynthetic pathway to synthesize pteridines. The pteridine-synthesizing activity in the ocular tissues was found to be high in retina as compared with ciliary body--iris and lens (Table 1). The pteridine-synthesizing activity in rat and human lenses was only 14% and 21%, respectively, of that of retina. The ciliary body--iris and retina of rat possess the high activity of neopterin, sepiapterin, and biopterin synthesis as compared with the ciliary body--iris and retina of human.

![Fig. 1. A proposed pathway of dihydrobiopterin biosynthesis. I: GTP cyclohydrolase; II: sepiapterin-synthesizing enzyme 1; III: sepiapterin-synthesizing enzyme 2; IV: sepiapterin reductase.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933354/)

Table 1. Neopterin-, sepiapterin-, and biopterin-synthesizing activities in ocular tissues (nanomoles/g soluble protein/hr)

<table>
<thead>
<tr>
<th></th>
<th>Neopterin</th>
<th>Sepiapterin</th>
<th>Biopterin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciliary body--iris</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>274 ± 16 (6)</td>
<td>91 ± 15 (6)</td>
<td>53 ± 6 (6)</td>
</tr>
<tr>
<td>Human</td>
<td>182 ± 31 (5)</td>
<td>34 ± 3 (5)</td>
<td>38 ± 4 (5)</td>
</tr>
<tr>
<td>Lens</td>
<td>20 ± 5 (6)</td>
<td>24 ± 4 (6)</td>
<td>30 ± 6 (6)</td>
</tr>
<tr>
<td>Retina</td>
<td>45 ± 12 (5)</td>
<td>22 ± 3 (5)</td>
<td>27 ± 4 (5)</td>
</tr>
<tr>
<td>Human</td>
<td>422 ± 27 (6)</td>
<td>135 ± 12 (6)</td>
<td>76 ± 10 (6)</td>
</tr>
<tr>
<td></td>
<td>260 ± 24 (5)</td>
<td>118 ± 14 (5)</td>
<td>68 ± 8 (5)</td>
</tr>
</tbody>
</table>

Parentheses indicate number of determinations.
Values are mean ± SE.
Table 2. Biopterin-synthesizing activity in rat lens and retina extracts in various incubation conditions (nanomoles/g soluble protein/hr)

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>Lens</th>
<th>Retina</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete system</td>
<td>26.48 ± 4.16</td>
<td>84.62 ± 12.47</td>
</tr>
<tr>
<td>−NADPH</td>
<td>8.05 ± 2.24</td>
<td>22.14 ± 4.35</td>
</tr>
<tr>
<td>−MgCl₂·6H₂O</td>
<td>10.36 ± 2.68</td>
<td>28.10 ± 3.80</td>
</tr>
<tr>
<td>−(U-¹⁴C)-GTP</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Boiled homogenate</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Parentheses indicate number of determinations. Values are mean ± SE.
ND: not detectable.

iris and retina of human (Table 1). Control experiments run without GTP or supernatant of the tissue homogenates failed to generate neopterin, sepiapterin, and biopterin. Addition of boiled homogenate to the assay mixture did not produce pteridines. In the absence of either NADPH or magnesium chloride from the incubation mixture, the biopterin synthesis was depleted by 60 to 70% in lens and retina of rat (Table 2). The remaining biopterin-synthesizing activity in the lens and retina of rat in absence of either NADPH or magnesium chloride from the incubation mixture could be due to the tissue levels of the latter compounds. Further, in the absence of (U-¹⁴C)-GTP in the assay mixture, no radioactivity was observed in neopterin, sepiapterin, or biopterin. From these results it is evident that GTP is the precursor for pteridine biosynthesis and that the pathway is NADPH and magnesium ion-dependent.

Sepiapterin is a naturally occurring yellow pteridine eye pigment in Drosophila melanogaster and was isolated from the same by Forrest and Mitchell for the first time. These pteridines in insect eye were thought to provide light protective pigmentation.12 Cremer-Bartels isolated a light-sensitive fluorescent substance from the rabbit and bovine lenses and thought that it may be pteridine.4 Cremer-Bartels further suggested that these pteridine-like substances may have a role in the protection of these tissues from the light-induced effects. Though it is evident from the present study that ocular tissues synthesize pteridines, the role of these substances in the mammalian eye tissues towards pigment formation is not yet known. It may be mentioned that neopterin, sepiapterin, and biopterin are intermediates towards the synthesis of tetrahydrobipterin, a natural cofactor for aromatic amino acid hydroxylation reactions. The cofactor activity of tetrahydrobipterin, a reduced form of dihydrobipterin, in the hydroxylation of aromatic amino acids is well-established.5,7 The synthesis of pteridines in retina and ciliary body–iris may provide a pteridine cofactor for tyrosine and tryptophan hydroxylase activities, ie, the initial enzymes in the production of catecholamines and serotonin, respectively. Lens is devoid of nerve supply and, as such, the existence of tyrosine and tryptophan hydroxylase activities in lens is doubtful. The other possibility is that the conversion of phenylalanine to tyrosine may be beneficial in the lens, and, hence, the pteridines in lens may serve as cofactor for phenylalanine hydroxylation activity. The sepiapterin- and biopterin-synthesizing activities in rat retina were 30 and 120% higher than the values of rat brain reported by Yoshioka et al.9 The ability of ciliary body–iris to synthesize pteridines is the same as found in rat brain by Yoshioka et al.9 The higher activity of pteridine synthesis in retina as compared with that of brain deserves further studies on these compounds to understand their physiologic function in ocular tissues.

Key words: Neopterin, pteridine, sepiapterin, lens, retina, ciliary body–iris, rat, human

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