advanced stages were not discarded. In our case the thickness of a rather active zone in the lens was measured and its mean was found significantly reduced already in the very early stage of all types of senile cataract.

In view of these observations the question arises, if in fact the pathological slowing down of fiber formation and proteosynthesis precedes the lens opacities. The answer is affirmative: Goldmann and Favre have indeed shown a thinning of the adult nuclear zone in a certain form of presenile cataract. This anomaly is obviously dependent on the reduction of fibrogenesis; it exists years before the first lens opacities.

On the other hand, Delmarcelle and Luyckx-Bacus measured the depth of the anterior chamber in eyes with transparent lenses, but having a fellow eye with senile cataract. In such cases the still normal lens becomes nearly always opaque sooner or later, but before this change occurs, the anterior chamber is deeper than it is in equally aged nonpredisposed eyes. The lens is smaller, the formation of fibers is depressed before the clinical symptoms of cataract are apparent.

Finally, we can quote our observations on 80-year-old patients with a few small opacities in their lenses. In these cases the rate of fibrogenesis and proteosynthesis is in general considerably reduced for about 10 years and yet it seems, that the opacification process began only recently.

It is concluded that the decrease of fiber formation and proteosynthesis precedes the appearance of lens opacities.

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Key words: human lens, cortex, slit lamp, senile cataract, fiber formation, proteosynthesis.

REFERENCES


Metabolism of 14C palmitic acid by the lens. RUTH VAN HEYNINGEN AND JANE LINKLATER.

14C palmitic acid is oxidised in vitro by the intact human, monkey, bovine, rabbit, and rat lens. 14CO2 and 14C-glutamic acid are formed.

Everett Kinsey discovered that only the epithelial cells of the lens contain cytochrome C and cytochrome oxidase, and that flavoprotein and ATP are at a much higher concentration in these cells than in the rest of the lens. The cytochrome system was detected not only in the bovine and rabbit lens but also in a human lens removed for senile cataract. He postulated that "an important . . . function of the epithelium may be to provide energy for the maintenance of the entire lens" and from measurement of lactate and pyruvate he deduced that the epithelium is functioning "in the oxidation of carbohydrate, and not solely in the oxidation of amino acids and fats."

In spite of their justified assumption that amino acids and fats were substrates for oxidation by the lens epithelium, this is, as far as we know, the only, and belated paper on the subject of fatty acid oxidation by the lens of a variety of species. Werner and Cotlier in a brief abstract, affirm that the rabbit lens oxidizes 14C palmitic acid to 14CO2.

Free fatty acids have been measured in the bovine aqueous humor4 and, after saponification, in the rabbit aqueous humor. In both cases, palmitic acid was the most abundant. Since blood contains free fatty acids it is reasonable to assume their presence in the aqueous humor of other species. In the in vitro experiment of Table I the concentration of palmitic acid in the incubation medium was low, 0.02 µmol per milliliter or less.
Table I. Metabolism of [1-14C] palmitic acid by the lens

<table>
<thead>
<tr>
<th>Experiment No. and species</th>
<th>Lens wet weight (mg.)</th>
<th>Protein (mg.)</th>
<th>CO2</th>
<th>TCA* extract</th>
<th>Protein and lipid (mg)</th>
<th>CO2</th>
<th>TCA* extract</th>
<th>Protein and lipid (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 cow</td>
<td>adult</td>
<td>2,000</td>
<td>666</td>
<td>75</td>
<td>15</td>
<td>76</td>
<td>114</td>
<td>229</td>
</tr>
<tr>
<td>P2 cow</td>
<td>adult</td>
<td>2,000</td>
<td>666</td>
<td>115</td>
<td>15</td>
<td>118</td>
<td>174</td>
<td>227</td>
</tr>
<tr>
<td>P3 monkey</td>
<td>adult</td>
<td>180</td>
<td>60</td>
<td>25</td>
<td>4</td>
<td>85</td>
<td>367</td>
<td>67</td>
</tr>
<tr>
<td>P4 monkey</td>
<td>adult</td>
<td>180</td>
<td>60</td>
<td>12</td>
<td>7</td>
<td>67</td>
<td>150</td>
<td>117</td>
</tr>
<tr>
<td>P5 human (fetal)</td>
<td>16 weeks</td>
<td>15</td>
<td>3</td>
<td>20</td>
<td>—</td>
<td>350</td>
<td>5,000</td>
<td>—</td>
</tr>
<tr>
<td>P6 human (posterior subcapsular cataract)</td>
<td>48</td>
<td>152</td>
<td>68</td>
<td>2</td>
<td>3</td>
<td>406</td>
<td>30</td>
<td>40</td>
</tr>
</tbody>
</table>

Results have been brought to an initial radioactivity in the medium of 5.6 x 10⁴ counts per minute. Four lenses were used in P5 and six lenses in P11, but results are given as an average per single lens. The protein content of P1 to P4 has been taken as 33 per cent of the wet weight. In the others, the dry weight of the TCA precipitate was used.

*Tetrachloroacetic acid.

Methods. Lenses were used as soon as possible after death. The incubation medium was tissue culture medium TC199, buffered with HEPES, and with the concentrations of glucose and calcium brought to 10.0 mM and 1.60 mM, respectively. Incubation was in air, in modified Merriam and Kinsey tubes, for 20 hours at 35° C. [1-14C] palmitic acid (57.9 mCi per millimole), in solution in benzene, was from the Radiochemical Centre, Amersham, England. The benzene was removed under a stream of N₂ and the acid was dissolved in ethanol (50 μCi in 0.5 ml.). Five microcuries in 15 ml incubation medium was used for the cow lenses and from 2 to 5 μCi in 5 ml medium for the lenses of other species.

At the end of the experiment radioactivity was measured in CO₂ and in the tetrachloroacetic acid soluble and insoluble fractions of the lens. The methods used have been described previously, except that 14CO₂ was not precipitated in the form of Ba14CO₃ and radioactivity was counted in a Beckman Scintillation Counter LS 100 C. The scintillant fluid was that of Hall and Cocking. A control, with no lens, was done and the small blank 14CO₂ value subtracted from all experimental values.

Results and discussion. Table I shows that bovine, monkey, human, rabbit, and rat lenses all metabolize 14C-palmitic acid to 14CO₂, and that the label is always found in both the trichloroacetic acid soluble and insoluble fractions. The lens extracts from P1 (bovine), P3 (monkey), P5 (fetal human), and P6 (cataract human) were examined by autoradiography or two-way paper electrophoresis and chromatography. In all cases glutamate was found to be labeled, and there were also other fainter spots, two of them in the position of oxidized and reduced glutathione.

The results are far too few for any firm conclusions to be drawn, except that all the lenses metabolized palmitate; the acetyl CoA so formed was metabolized by the citric acid cycle to CO₂ or to glutamic acid.

Fig. 1 shows the following stages in the mitochondrial oxidation of the labeled carbon group of the palmitic acid. (1) β-oxidation, whereby the fatty acid chain is shortened by two carbon atoms, yielding myristoyl coenzyme A and acetyl coenzyme A, which contains the labeled atom. (2) The reaction between acetyl coenzyme A and oxaloacetic acid, the first reaction of the citric acid cycle, which yields citric acid. (3) Several consecutive reactions of the citric acid cycle to yield CO₂ and labelled α-oxoglutaric acid. This can then undergo one of two reactions. (4) Conversion to labeled glutamic acid, by means of a transaminase or of glutamic acid dehydrogenase, or (5) Further reactions in the citric acid cycle to yield unlabeled succinic acid and labeled CO₂.

Thus mitochondrial oxidation of palmitic acid can result in the labeled carbon atom appearing in either CO₂ or glutamic acid.

The amino acid, glutamic acid, is incorporated into lens proteins, a reaction first demonstrated by Merriam and Kinsey. The trichloroacetic acid insoluble fraction contains not only protein, but also lipids, especially those associated with proteins, as in the cell membranes. Radioactivity was presumably incorporated into lipids as well as protein but this was not investigated.

The results in Table I are expressed in two ways—on the basis of counts recovered from the metabolism of the whole lens, and also on the basis of counts recovered per milligram of dry weight of the lens. It is interesting that, in single experiments, the human lens (fetal, P5 and adult cataractous, P6) incorporates more label into protein plus lipid than any of the other lenses.
As expected, the radioactivity recovered in CO₂ and in protein plus lipid (on a dry weight basis) is lower in the adult rabbit lens (P8 and P10) than in the young rabbit lens (P9 and P12).

However, this study is of an exploratory nature and only serves to show that palmitic acid, like several of the amino acids, is oxidized in vitro by the intact lens.

Oxidation of fatty acids by the lens could supply a considerable proportion of its energy requirements. Complete oxidation of one molecule of palmitic acid yields about 131 molecules of ATP, compared with the two molecules of ATP derived from the glycolytic breakdown of one molecule of glucose with the formation of two of lactic acid.

We thank Dr. Paul Trayhurn for helpful advice.


Key words: lens, oxidation of fatty acids, palmitic acid, citric acid cycle.

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Incorporation of glutamic acid into soluble protein as a function of age. JEAN KLETI.

The incorporation of \(^{14}\)C glutamic acid into soluble bovine and human lens protein fractions has been studied as a function of age. Culture technique and DEAE cellulose fractionation has been used. In bovine lenses the incorporation of glutamic acid into \(\alpha\) and \(\gamma\) crystallins decreased with age whereas in the \(\beta\) crystallin group the specific activities remained constant. All protein fractions of the human lenses showed a gradual reduction of the incorporation of the radioactive amino acid between 40 and 74 years of age. The highest specific activity was found to correspond to Spector's HL protein and its existence is confirmed throughout the whole human life-span.

In preliminary experiments concerning the metabolism in the aging human lens there were only a few changes observed in the level of several important compounds implicated in carbohydrate metabolism. ATP was not significantly diminished and lactic acid seemed not to be reduced whereas the glucose level increased with age after 40 years. In order to obtain more information on the evolution of glucose breakdown in human lens culture technique was employed to study the incorporation of glucose \(^{14}\)C into lactic acid. The study of this metabolic parameter showed no significant differences between lenses of humans of 34 to 40 years and those of 65 to 72 years of age. These results prompted us to investigate other aspects of human lens metabolism, namely, the synthesis of lens protein as a function of age.

The first study done on this subject was the incorporation of glycine and serine into lens protein by Merriam and Kinsey in 1950. Since that report, many papers have confirmed the capability of the lens to synthesize protein. Most studies have emphasized the incorporation of amino acids either in the whole lens protein or in the total soluble protein of the lens. In the present work we have measured the incorporation of \(^{14}\)C glutamic acid into different fractions of soluble protein which can be easily separated by DEAE cellulose. This technique was described by Spector and Papacostantinou and co-workers during the sixties. Spector and Kinoshita demonstrated the incorporation of amino acids into soluble protein fractions, pointing out the existence of a highly labeled (HL) protein in the calf and rabbit lens. More recently Spector, Stauffer, and Sigelman have reported similar results with human lenses. Our aim was to compare the incorporation of an amino acid as a function of age in the different soluble protein fractions in bovine and human lenses. The amino acid chosen was glutamic acid which despite the fact that it is well metabolized, is the most abundant amino acid in the three crystallins and is comparable in proportion in each of the crystallins.

Initially, we studied the incorporation into whole bovine lenses to get the overall change as a function of age and once worked out the same procedures were applied to the whole human lenses. This approach was necessary because of the lack of sufficient human lens material.

Materials and methods. The lenses were cultured for 6 hours in TC 199 medium containing neutralized \(^{14}\)C glutamic acid. The medium was gassed with air and \(\text{CO}_2\) (95:5) and standard antibiotics were added. The bovine lenses were cultured, within one hour after slaughter, in 5 ml. of medium for each calf lens and 10 ml. for each bovine lens. Medium contained in each case 75 \(\mu\)Ci \(^{14}\)C glutamic acid per milliliter. Pairs of human lenses, transparent by slit lamp examination, from the same donor were cultured in 4 ml. of medium containing 125 \(\mu\)Ci labeled glutamic...