Nucleic acid metabolism in the lens
I. Isolation and composition of RNA

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Ribose nucleic acids of rat and dogfish lenses were extracted by the Duponol method. The nucleotide composition of albuminoid, ribosomal, and soluble RNA was determined by alkaline hydrolysis of the various RNA fractions followed by paper chromatography. The ultraviolet absorbing spots were eluted from the chromatograms with HCl and the resulting solutions were subjected to spectral analyses and microorcinol determinations. The results of these determinations, including ultraviolet absorption spectra of the individual nucleotides, are presented. The nucleotide composition of lens RNA is similar to that of other organs (liver and kidney) which have been reported.

A derangement in protein metabolism probably occurs during the development of every form of cataract. The exact mechanisms involved in protein synthesis are still somewhat speculative. There is, however, general agreement that ribose nucleic acid (RNA) serves as the template for protein synthesis in all cells. The lens has been shown to contain at least three forms of RNA: soluble, ribosomal, and albuminoid. In the rat and dogfish lens there is a definitive relationship between the relative concentration of the three RNA fractions in the lens and the age of the animal.

This report is the first in a series of studies of nucleic acid metabolism in the lens, and deals with the nucleotide composition of rat lens and dogfish lens RNA.

Methods

Fresh lenses from 6-week-old white male Holtzman rats and from adult dogfish were used exclusively in these studies. Groups of lenses, from 12 to 100 (depending on the species being studied), were homogenized in distilled water and were separated into the albuminoid, ribosomal, and soluble RNA fractions as previously reported. The RNA was extracted from these fractions with 0.41 per cent sodium dodecyl sulfate (Duponol) in 0.9 per cent NaCl at 60° C, and the protein was precipitated out by making the solution up to 1 N NaCl. After centrifugation, the RNA in the supernatant was precipitated with two volumes of 0.5 per cent ethanol in the cold for 18 hours. The orcinol reaction was employed to estimate the concentration of RNA obtained. The RNA was then hydrolyzed with 0.3 N KOH at 37° C for 18 hours and neutralized with HCl. The supernatant containing the ribonucleotide solutions was evaporated to dryness and then

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resuspended in distilled water to obtain a final concentration of 50 to 75 μg of nucleotide per 10 μl of water. Descending chromatograms were run on Whatman No. 1 filter paper strips in a solvent of isopropanol:HCl:H2O (650:165:185) for 20 hours at room temperature. The ultraviolet absorbing spots were eluted for 18 hours in 0.1 N HCl and the ultraviolet absorption curves of the ribonucleotides were run on a Perkin-Elmer automatic recording spectrophotometer to identify them. The amount of nucleotide present in each spot was estimated spectrophotometrically at the peak absorption found for each nucleotide.

Results

Fig. 1 demonstrates the chromatographic separation of 75 μg of alkaline-hydrolyzed ribonucleotides extracted from rat lenses as described above. It demonstrates the relative mobilities of the four nucleotides, with uridylic acid showing the greatest migration followed by cytidylic, adenylic, and guanylic acids in a decreasing order of mobility. An identical pattern is present for mixtures of known nucleotides and alkalihydrolyzed purified yeast ribonucleotides.

Fig. 2 is a tracing of the ultraviolet absorption curves of 75 μg of rat lens ribosomal ribonucleotides eluted from the four ultraviolet absorbing areas shown in Fig. 1. The nucleotides were identified by their positions along the strips, by their ultraviolet absorption spectra, and by rechromatographing the eluted spots mixed with known nucleotides. The absorption maxima for the four nucleotides were as follows: 260 mμ for uridylic acid, 277 mμ for cytidylic acid, 258 mμ for adenylic acid, and 257 mμ for guanylic acid.

The nucleotide ratios of albuminoid, ribosomal, and soluble RNA of the rat lens and of albuminoid and ribosomal RNA of the dogfish lens are shown in Table I.
Table I. Nucleotide ratios of lens RNA subcellular fractions as compared with rat liver RNA

<table>
<thead>
<tr>
<th>Preparation</th>
<th>No. of determinations</th>
<th>Guanylic acid</th>
<th>Adenylic acid</th>
<th>Cytidylic acid</th>
<th>Uridylic acid</th>
<th>Purines</th>
<th>Pyrimidines</th>
<th>6-Aminos</th>
<th>6-Ketos</th>
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<tbody>
<tr>
<td>Rat RNA</td>
<td></td>
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<td></td>
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<tr>
<td>Lens albuminoid</td>
<td>4</td>
<td>32 ± 2.2</td>
<td>17 ± 1.4</td>
<td>32 ± 1.4</td>
<td>19 ± 2.4</td>
<td>0.98</td>
<td></td>
<td></td>
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<tr>
<td>Lens microsomal</td>
<td>4</td>
<td>29 ± 1.3</td>
<td>20 ± 1.1</td>
<td>35 ± 0.9</td>
<td>18 ± 0.7</td>
<td>0.93</td>
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<tr>
<td>Lens soluble</td>
<td>4</td>
<td>26 ± 0.6</td>
<td>17 ± 1.1</td>
<td>35 ± 1.7</td>
<td>23 ± 1.4</td>
<td>0.75</td>
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<tr>
<td>Liver</td>
<td></td>
<td>32 ± 2.2</td>
<td>17 ± 1.4</td>
<td>32 ± 1.4</td>
<td>19 ± 2.4</td>
<td>0.98</td>
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<tr>
<td>Dogfish RNA</td>
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<tr>
<td>Lens albuminoid</td>
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<td>32 ± 1.8</td>
<td>22 ± 1.3</td>
<td>29 ± 0.6</td>
<td>19 ± 1.4</td>
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<tr>
<td>Lens microsomal</td>
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<td>21 ± 1.0</td>
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<td>23 ± 0.9</td>
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</tr>
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</table>

*Data obtained as indicated in Methods.
1 Standard error.

Discussion

It is interesting, but not surprising, that the composition of the various RNA fractions in the dogfish lens is quite similar to that of the rat lens. Other experiments from this laboratory have indicated that the concentration of the three RNA fractions in the dogfish lens follow an aging pattern similar to that reported on the rat lens. Furthermore, the turnover of the three RNA fractions in both species, as measured by P-32 and C-14 adenine incorporation experiments, also appears to be related to the age of the lens.

The nucleotide composition of rat and dogfish lens RNA reported in this article appeared to differ somewhat from the results obtained by van Heyningen and Waley for calf, cattle, and rabbit lens RNA. It is difficult to ascribe these differences solely to species variation since different procedures were employed in determining the composition of RNA.

REFERENCES