The metabolism of the lens as related to aging and experimental cataractogenesis

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This paper considers the effect of aging on certain pathways of carbohydrate, protein, and nucleic acid metabolism in the normal lens. These alterations are related to experimental observations on the sugar cataracts and an attempt has been made to correlate the changes in metabolism occurring in the sugar cataracts with the altered activities of certain pathways associated with aging. The relationship between the age of the animal and the production of sugar cataracts may be due to the fact that the initial biochemical alterations involve pathways of glucose metabolism which are significantly affected by the aging process. Specific aspects of protein and nucleic acid metabolism in the normal lens also show a relationship to the aging process. These include changes in the relative concentrations of the soluble and insoluble (albuminoid) protein and RNA fractions and a progressive decrease in the amino acid-RNA and protein incorporating systems. Studies on the metabolism and chemistry of a cold precipitable protein (CPP) in the lens have shown that it consists of an aggregate of the three soluble lens proteins (alpha, beta, and gamma crystallins) in which there is a binding or coprecipitation of approximately 1 to 1.5 per cent RNA. Extensive metabolic and physicochemical studies suggest that this CPP may be an intermediate in the conversion of soluble to albuminoid lens protein.

Certain areas of lenticular metabolism change in activity as the animal ages and there appears to be a direct relationship between these aging changes and the ease with which experimental cataracts can be induced. This discussion will consider specific areas of carbohydrate, protein, and nucleic acid metabolism and attempt to relate the influence of aging on these metabolic pathways with the experimental production of certain types of cataracts.

Carbohydrate metabolism

The lens derives most, if not all, of its metabolic energy from the oxidation of glucose. Almost all of the enzyme systems which are required for the operation of anaerobic glycolysis, the Krebs cycle, and the hexose monophosphate pathway of glucose oxidation have been shown to be present in this organ. The lens also contains a rather unusual pathway for the metabolism of glucose (and other monosaccharides) known as the polyol or sorbitol pathway. Alterations in the activity of the hexose monophosphate and polyol pathways appear to be of importance in initiating the chain of metabolic events.
leading to the development of the experimental sugar cataracts. Prior to considering the evidence relating to these alterations it is important to bear in mind that the age of the experimental animal plays an extremely significant role in the development of these lenticular opacities. Thus the galactose cataract can be easily and rapidly produced in the young rat (5 to 6 weeks of age) but as the animal ages there is a progressive increase in the length of time required for the development of these opacities. In the mature rat (4 to 6 months of age) it is difficult to obtain galactose cataracts by means of the usual cataractogenic galactose diet. The xylose cataract represents an even more extreme example of the effect of aging on cataractogenesis. This type of lenticular opacity, which at least to gross observation appears to regress, can be produced only in the young rat. In the fetal rat lens the hexose monophosphate shunt and the Embden-Meyerhof pathway of glucose metabolism are both present at the seventeenth day of gestation and there is some evidence that the citric acid cycle becomes manifest about two days later.\(^1\)\(^-\)\(^2\) Anaerobic glycolysis is at least as active in the fetal lens as in the mature or old lens.\(^1\)\(^-\)\(^6\) However, the activity of the hexose monophosphate cycle exhibits a marked decline with age. This pathway is at least ten times more active in the fetal and immature rat lens (5-week-old rat) as compared with the lens from a mature (3 to 4 months of age) or old rat.\(^1\)\(^-\)\(^8\),\(^7\)\(^-\)\(^12\) Thus glucose oxidation via the hexose monophosphate pathway exhibits a profound aging effect. In the rapidly developing rat lens this pathway appears to play an active role, but when it reaches maturity very little glucose is metabolized via the direct oxidative pathway. While the activity of the Embden-Meyerhof pathway also declines as the lens reaches its mature state, this fall is nowhere near as precipitous; there is a two- to threefold decrease as compared with a more than tenfold change in the direct oxidative pathway. The altered roles of these two pathways of glucose metabolism as the lens ages take on added significance if one considers the possible part played by the polyol pathway in the genesis of sugar cataracts. The production of all three forms of sugar cataract (galactose, xylose, and diabetic) is directly related to the age of the animal.\(^10\),\(^11\),\(^12\)\(^-\)\(^16\) In the weanling rat these sugar cataracts develop very rapidly; however, as the animal ages it becomes increasingly difficult and requires progressively longer periods of time to produce galactose or diabetic cataracts, while xylose is effective as a cataractogenic agent only in the weanling rat. The formation of sugar alcohols (polyols) in the lens depends to a considerable extent on the availability of TPNH, and the marked decline in the activity of the direct oxidative pathway as the lens matures will result in a progressively decreasing supply of this reduced coenzyme. If the presence of these polyols plays an important role in the formation of vacuoles in the lens fibers by virtue of their osmotic effect,\(^17\)\(^-\)\(^19\) it is not surprising that the ease

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{A mechanism whereby the sorbitol (polyol) pathway might serve as a transhydrogenase system.}
\end{figure}
Fig. 2. The generation of ATP by means of the alphaglycerophosphate cycle.

with which sugar cataracts are produced will be markedly influenced by the age of the experimental animal. Furthermore, the polyol pathway may also play an important role in lenticular metabolism since it could readily function as a transhydrogenase system, thereby generating oxidized TPN and eventually ATP (Fig. 1). This latter function may prove to be an important one since the relatively large amounts of TPNH formed in the young lens by means of the direct oxidative pathway must be reoxidized in order to enable this pathway to continue functioning. It may also serve as an invaluable source of readily available ATP for the young and rapidly growing lens by means of the alpha glycerophosphate cycle (Fig. 2).

Protein metabolism

Mörner originally classified the soluble lens proteins into the alpha, beta, and gamma crystallins. Studies utilizing more elaborate techniques to separate and characterize lens proteins (including various forms of electrophoresis, immunoelectrophoresis, and ultracentrifugation) indicate that there may be as many as ten sub-units of protein within the lens. The molecular weight of alpha crystallin is approximately one million as compared with the molecular weight of 14,000 to 25,000 for gamma crystallin. Beta crystallin is the most heterogeneous of the three soluble protein fractions and its molecular weight lies somewhere in between the values ascribed to alpha and gamma crystallin. Alpha crystallin consists of an aggregate of smaller protein molecules in which the equilibrium within the lens is toward polymerization. Present theories regarding protein synthesis would rule out the possibility of a protein molecule with a molecular weight of one million being directly synthesized by its appropriate RNA template. There is some evidence that alpha crystallin can be broken down into a series of monomers or subunits with an average molecular weight of 20 to 40,000 and it is possible that the tendency toward polymerization may in part be due to hydrophobic bonding between these monomers, thereby increasing the solubility of the larger molecule.

The most marked aging effect on the concentration of the various lens proteins occurs in the albuminoid and the cold precipitable protein (CPP) fractions. In the newborn rat the albuminoid comprises about 6 per cent of the total lens protein, but it steadily increases in amount as the animal ages until it reaches a level of approximately 57 per cent in the senile (3-year-old) rat. This aging effect is further accentuated by the fact that the major synthesis of soluble lens proteins occurs during the time of rapid growth and development of the lens (weanling rat lens). As the lens matures, the rate of synthesis of soluble lens proteins sharply decreases while the formation of albuminoid proceeds at a relatively steady rate throughout.
Fig. 3. The "cold cataract" in a rat lens (A) and its rapid reversibility on warming (B and C).

Fig. 4. An aqueous extract of lens proteins showing cold precipitation (A) and resolubilization on warming (B and C).

life. A similar situation has been shown to exist in the dogfish lens and it is not unlikely that this phenomenon is present in the human lens. The amino acid (RNA and protein) incorporating systems are quite active in the young lens but decrease progressively with age. The reversal in the relative concentrations of soluble (hydrophilic) proteins and insoluble (hydrophobic) proteins in the lens of the old animal as compared to the young lens is accompanied by a gradual loss in its water content (from 69 to 63 per cent), a decrease in the pliability of many of the lens fibers, an increase in the volume of nuclear fibers, and the development of presbyopia.

When the lens from a young animal is placed in an ice bath, it rapidly becomes opaque. This so-called "cold cataract" can usually be reversed by warming the lens (Fig. 3). Opacification of the lens in the cold appears to be due to the presence of a soluble protein fraction which becomes insoluble at temperatures below 10°C; (Fig. 4). The CPP can be easily obtained by homogenizing the lenses in distilled
water, centrifuging off (at 18 to 20°C) the insoluble protein at 600 x g for 15 minutes, removing the microsomes by centrifugation at 20,000 x g for one hour at 18 to 20°C, and subjecting the supernatant to a temperature below 10°C. The precipitate that subsequently forms represents the CPP. The results of electrophoretic (Fig. 5), immunochemical analyses, (Fig. 6), and sephadex column chromatography (Fig. 7) demonstrate that the CPP fraction consists of portions of the three major soluble protein fractions (alpha, beta, and gamma crystallin) of the lens and twice as much CPP is present in the cortex as compared with the nucleus. Ultracentrifugal analysis of the CPP and the total soluble minus cold precipitable protein (56,100 r.p.m., 20°C, pH 7.5) indicates that the CPP consists of a single component with a sedimentation rate of 4s as compared to the three components found in the total soluble minus cold fraction with sedimentation rates of 3, 13, and 19s representing the alpha, beta, and gamma crystallin fractions, respectively (Fig. 8). However, when the CPP fraction derived from the rat lens is dissolved in an acid solution (pH 3) two peaks become apparent in the ultracentrifugation pattern with sedimentation rates of 3 and 17s, respectively. At pH 10 only a single peak is apparent with a sedimentation rate of 4s (Fig. 9). Urea in a concentration of 0.25 to 0.5M appears to be capable of preventing the cold precipitation phenomenon in aqueous extracts of rat lens protein and in the dogfish lens. Normally the dogfish lens does not show the cold cataract phenomenon. This appears to be due to the presence of a relatively high concentration of urea (0.25M) in the lens. When urea is removed by dialysis, the cold precipitation phenomenon can be demonstrated. The ul-

Fig. 5. Acrylamide gel electrophoresis on the cold precipitable protein (CPP) and the total soluble minus cold precipitable protein (TMCP) showing the similarity in the number of bands obtained.

Fig. 6. Immunochemical analysis (Ouchterlony technique) of the soluble minus cold precipitable protein (T) showing at least three identical precipitation bands associated with both fractions.
Fig. 7. Chromatographic separation (Sephadex G-100) of the CPP and TMCP fraction in the rat lens cortex and nucleus.

Fig. 8. Ultracentrifugation pattern (56,100 r.p.m., 20° C, pH 7.5) of the CPP (upper) and TMCP (lower) fractions derived from the rat lens.

tracentrifugation pattern of the dogfish lens CPP at pH 7.5 is identical with that obtained on the rat lens fraction (Fig. 10).

Metabolic studies on the CPP derived from the rat lens indicate that the level of this fraction decreases markedly with age; from approximately 35 per cent of the total soluble protein in the 21-day-old fetus to about 7 per cent in the 2-year-old rat (Figs. 11 and 12). There is a concurrent loss of precipitability of the lens which occurs at 7 to 8 weeks of age at which time the concentration of CPP has leveled off to a relatively constant amount. Thus the characteristic cold cataract is most often seen in the young mammalian lenses. A similar aging effect is also present in the dogfish lens. However, aqueous extracts from lenses of all age groups continue to show precipitation at temperatures below 10° C. provided that the concentration of CPP is greater than 3 mg. per milliliter.

Studies on the incorporation of 14C-labeled leucine into rat lens CPP and TMCP indicate that this isotope is incorporated into the alpha, beta, and gamma portions of both fractions.

Dische has previously reported on the presence of an oxidizable protein fraction in the rat lens and he noted that this fraction decreased in amount as the animal became older. The rate of oxidation of the rat lens CPP by potassium ferricyanide as compared to the total soluble minus cold precipitable fraction (TMCP) is shown in Fig. 13. While there is no effect in the TMCP fraction over a 2½ hour period, the CPP fraction shows a marked effect at 20° C. and this is enhanced at 37° C. It is possible that the CPP fraction may be similar to the oxidizable fraction originally reported by Dische.

A small, but significant, amount of RNA (approximately 1.5 per cent of the weight...
of the CPP) is associated with the CPP fraction, suggesting that there is a binding or coprecipitation of RNA which occurs when the cold protein fraction precipitates. This RNA fraction has an absorption spectrum characteristic of RNA and shows a significant incorporation of $^1$C-adenine (Table I).

The foregoing experiments suggest the possibility that the CPP fraction may be an intermediate in the conversion of soluble to insoluble (albuminoid) protein in the lens. One can also speculate that at least some of the soluble lens proteins consist of an aggregate of subunits or monomers (molecular weight 20 to 40,000) held together by means of hydrophobic bonding. This would in effect increase the solubility of the polymers constituting the alpha and beta fractions. The cold precipitable protein which is composed of all three classes of soluble lens protein (alpha, beta, and gamma) actually contains a much higher concentration of the gamma fraction as compared to the other two classes. The latter fraction which appears to be made up of the smaller subunits or monomers would therefore have more hydrophobic groups exposed which would in effect render them more insoluble as the temperature was lowered. The effect of urea which prevents the cold precipitation phenomenon may be due to the fact that urea is capable of increasing the water solubility of hydrocarbons and other compounds with exposed hydrophobic groups.

Recent studies employing sephadex and DEAE-cellulose column chromatography have shown that the relative concentrations of alpha, beta, and gamma crystallin in the CPP fraction are 23, 13, and 64 per cent, respectively. The corresponding total soluble minus cold protein fraction (TMCP) contains alpha, beta, and gamma crystallins in a ratio of 3:3:4 (31, 28, and 41 per cent, respectively). The gamma crystallins isolated from the CPP or the TMCP are capable of cold precipitation while the alpha and beta crystallins from either fraction do not precipitate in the cold. This can be taken as further (presumptive) evidence that hydrophobic groups may be responsible for the cold precipitable phenomenon.

Cold precipitation of either purified gamma fraction (from the CPP or TMCP) can be prevented by the addition of a sufficient amount of purified alpha or beta crystallin or a combination of both. If the concentration of alpha or beta (or the
combined alpha and beta crystallins) is less than the amount of gamma crystallin present, then cold precipitation can still occur. This suggests that the alpha and beta crystallins (if present in sufficient concentration) may inhibit cold precipitation by hydrophobic bonding with the exposed apolar groups in the gamma crystallin (masking of the hydrophobic groups). Studies are currently in progress to define this phenomenon further.

**Nucleic acid metabolism**

The lens contains a relatively small number of nucleated cells, hence the concentration of lenticular DNA is very low. There are only several micrograms of DNA compared to approximately 50 μg of total RNA in the lens from a 5-week-old rat. The concentration of RNA in the lenses of many species including man is shown in Table II. The specific activity of soluble, ribosomal, and albuminoid RNA, and the specific activity of

### Table I. The incorporation of $^{14}$C-adenine into the various RNA fractions of the rat lens

<table>
<thead>
<tr>
<th>RNA fraction</th>
<th>RNA (c.p.m./mg.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soluble RNA</td>
<td>114,333 ± 3600</td>
</tr>
<tr>
<td>Microsomal RNA</td>
<td>7625 ± 813</td>
</tr>
<tr>
<td>Albuminoid RNA</td>
<td>4125 ± 333</td>
</tr>
<tr>
<td>RNA associated with cold precipitable protein fraction</td>
<td>6025 ± 500</td>
</tr>
</tbody>
</table>

### Table II. The RNA content in the lenses of various species

<table>
<thead>
<tr>
<th>Species</th>
<th>Total RNA (μg/100 mg. wet weight)</th>
<th>Total protein (mg./100 mg. wet weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>285.0</td>
<td>34.5</td>
</tr>
<tr>
<td>Rabbit</td>
<td>147.3</td>
<td>33.5</td>
</tr>
<tr>
<td>Human</td>
<td>57.5</td>
<td>35.5</td>
</tr>
<tr>
<td>Bovine</td>
<td>39.7</td>
<td>29.5</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>29.0</td>
<td>30.5</td>
</tr>
<tr>
<td>Chicken</td>
<td>42.5</td>
<td>26.5</td>
</tr>
<tr>
<td>Pigeon</td>
<td>38.5</td>
<td>26.5</td>
</tr>
<tr>
<td>Carp</td>
<td>42.5</td>
<td>26.5</td>
</tr>
<tr>
<td>Dogfish</td>
<td>177.3</td>
<td>43.1</td>
</tr>
</tbody>
</table>

Fig. 12. Per cent of CPP (C) and TMCP (T) in the rat lens.

Fig. 13. The oxidation of rat lens CPP (solid and open circles) and TMCP (solid and open triangles).

Table I. The incorporation of $^{14}$C-adenine into the various RNA fractions of the rat lens

Table II. The RNA content in the lenses of various species
the nucleotides of the various RNA fractions derived from rat lenses incubated with $^{32}$P is shown in Table IV.\textsuperscript{55} The presence of messenger RNA has also been demonstrated in the dogfish lens by means of pulse labeling experiments.\textsuperscript{50} Although there appears to be a significant turnover of RNA, especially in the lenses of young animals, there is little if any evidence of RNAase activity in these organs (Table V).\textsuperscript{57} There is, however, some indication that RNAase activity becomes manifest at the time that visible opacities become apparent in the experimentally induced cataractous lenses.

Although the concentration of DNA remains relatively constant during most of the life span of the lens, there is a continuous change in the concentration of RNA which appears to be related to the aging process. There is a close correlation between the changes in the relative concentration of soluble and insoluble lens proteins with the effect of aging on the levels of soluble, ribosomal, and albuminoid RNA in the rat lens.\textsuperscript{1, 2, 45, 58, 59} (Table VI). The relative concentrations of albuminoid RNA and protein increase progressively as the animal ages until they constitute more than one half of the total RNA and protein fractions, respectively. The amount of soluble RNA remains relatively constant but there is a marked fall in the concentration of ribosomal RNA as the lens matures. There is also a fall in the concentration of soluble lens protein which becomes most marked after the animal has reached maturity. The turnover rates of $^{32}$PO\textsubscript{4} and $^{14}$C-labeled purines into lenticular RNA also show a progressive decline with aging.\textsuperscript{57, 60} Studies on albuminoid and soluble RNA derived from the dogfish lens indicate that these two fractions have a similar molecular weight as well as composition and it is possible that the albumin-

![Fig. 14. The effect of formaldehyde and heating on the change in optical density in albuminoid RNA from normal and irradiated rat lenses. (From Lerman, S., and Zigman, S.: Invest. Ophth. 2: 626, 1963.)](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932951/)
Table IV. Incorporation of $^{32}$P into the nucleotides of three RNA fractions of the rat lens

<table>
<thead>
<tr>
<th>Nucleotide</th>
<th>Specific activities (c.p.m./mg of nucleotide)</th>
<th>Ratios of specific activities (%)</th>
<th>Ratios of nucleotides (moles %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microsomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guanylic</td>
<td>1,604</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>Adenyllic</td>
<td>2,850</td>
<td>22.1</td>
</tr>
<tr>
<td></td>
<td>Cytidylic</td>
<td>3,700</td>
<td>32.6</td>
</tr>
<tr>
<td></td>
<td>Uridylic</td>
<td>3,200</td>
<td>28.2</td>
</tr>
<tr>
<td></td>
<td>Soluble</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guanylic</td>
<td>3,600</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td>Adenyllic</td>
<td>5,500</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>Cytidylic</td>
<td>5,500</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>Uridylic</td>
<td>4,200</td>
<td>22.7</td>
</tr>
<tr>
<td></td>
<td>Albuminoid</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Guanylic</td>
<td>6,560</td>
<td>15.8</td>
</tr>
<tr>
<td></td>
<td>Adenyllic</td>
<td>11,167</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td>Cytidylic</td>
<td>14,215</td>
<td>34.1</td>
</tr>
<tr>
<td></td>
<td>Uridylic</td>
<td>9,900</td>
<td>23.4</td>
</tr>
</tbody>
</table>

oid fraction may derive from the RNA which is associated with the cold precipitable protein fraction.56

Aside from the association of RNAase activity with the appearance of visible opacification in the sugar cataracts, very few changes in RNA metabolism have been noted and correlated with experimental cataractogenesis. However, an early alteration in the albuminoid RNA fraction has been noted within several hours after the rat lenses have been exposed to a cataractogenic dose of ionizing radiation.42, 43 These changes include a marked increase in the incorporation of $^{14}$C-adenine and $^{32}$PO$_4$ into the albuminoid fraction (Table VII) and an apparent change in the physical characteristics of this fraction, which may be due to breakage in the main chain (Fig. 14).

Galactose cataract

Two pathways of carbohydrate metabolism are initially affected by the accumulation of galactose in the lens. They are the hexose monophosphate shunt and the polyol pathway.51, 52, 53, 54, 55, 56, 57 An inhibition of the hexose monophosphate

shunt develops two days after the start of the cataractogenic galactose diet. This inhibition is indicated by a marked decline in the degree of recovery of $^{14}$CO$_2$ from lenses incubated with glucose-$1^{-14}$C (Fig. 15), a concurrent inhibition of the activity of glucose-6-phosphate dehydrogenase which catalyzes the first step of the hexose
monophosphate pathway (Fig. 16), and a marked decline in the TPNH:TPN ratio while the DPNH:DPN ratio does not appear to be affected during the first 9 or 10 days on the diet. Anaerobic glycolysis does not appear to be initially involved until the ninth day on the diet at which time permanent lenticular opacification becomes apparent. During the initial experimental period prior to the ninth day of the diet, the degree of recovery of $^{14}$CO$_2$ from lenses incubated with glucose-6-$^{14}$CO$_2$ remains unchanged, the DPNH:DPN ratio remains normal, and the concentration of lactic acid in such lenses is maintained at normal levels. Aside from the impairment of the hexose monophosphate pathway, there is an accumulation of free galactose in these lenses leading to a marked build-up of the corresponding sugar alcohol (dulcitol) which is found in a concentration of approximately 20 mg. per gram wet weight after the animal has been maintained on the galactose diet for 2 days. The lens is not freely permeable to any of the sugar alcohols and dulcitol cannot be rapidly metabolized by this organ. There is a close correlation between the amount of dulcitol and water accumulating in the experimental galactose lenses and this can be accounted for by the marked increase in osmolarity caused by the high dulcitol content. The hydropic degeneration of the lens fibers which is the earliest histological evidence of galactose cataract may be due to the sudden increase in lenticular hydration. However, it should be pointed out that these initial opacities, which appear within 2 days following galactose feeding, are completely reversible if the galactose diet is stopped after 6 or 8 days. The permanent opacification which develops in

Table VI. Changes in the concentration of rat lens RNA and protein with aging

<table>
<thead>
<tr>
<th>Age</th>
<th>Total protein (mg./lens)</th>
<th>Soluble protein (% of total)</th>
<th>Insoluble protein (% of total)</th>
<th>Total RNA (pg./lens)</th>
<th>Albuminoid RNA (% of total)</th>
<th>Ribosomal RNA (% of total)</th>
<th>Soluble RNA (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>At birth</td>
<td>0.25</td>
<td>95</td>
<td>5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>28 days</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>35 days</td>
<td>7.80</td>
<td>88</td>
<td>12</td>
<td>52</td>
<td>12-14</td>
<td>75</td>
<td>12-14</td>
</tr>
<tr>
<td>42-45 days</td>
<td>8.10</td>
<td>87</td>
<td>13</td>
<td>64</td>
<td>25-28</td>
<td>54</td>
<td>18-20</td>
</tr>
<tr>
<td>70 days</td>
<td>12.60</td>
<td>80</td>
<td>20</td>
<td>112</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>365 days</td>
<td>24.30</td>
<td>64</td>
<td>36</td>
<td>105</td>
<td>54</td>
<td>39</td>
<td>17</td>
</tr>
<tr>
<td>3 years</td>
<td>25.40</td>
<td>43</td>
<td>57</td>
<td>70</td>
<td>48</td>
<td>37</td>
<td>15</td>
</tr>
</tbody>
</table>

Table VII. The in vitro uptake of $^{32}$P and $^{14}$C adenine by three RNA fractions from normal and irradiated rat lenses*

<table>
<thead>
<tr>
<th>Tissue fraction</th>
<th>Isotope</th>
<th>Specific activity of normal rat lens RNA (c.p.m./mg. RNA)</th>
<th>Specific activity of x-irradiated rat lens RNA (c.p.m./mg. RNA)</th>
<th>% change from normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albuminoid</td>
<td>$^{32}$PO$_4$</td>
<td>6,680 ± 560*</td>
<td>9,707 ± 950</td>
<td>+ 45.3%</td>
</tr>
<tr>
<td>Microsomes</td>
<td>$^{32}$PO$_4$</td>
<td>4,324 ± 970</td>
<td>4,157 ± 324</td>
<td>+ 4.0%</td>
</tr>
<tr>
<td>Soluble</td>
<td>$^{32}$PO$_4$</td>
<td>366,200 ± 54,100</td>
<td>354,000 ± 39,300</td>
<td>− 3.4%</td>
</tr>
<tr>
<td>Albuminoid</td>
<td>$^{14}$C-Ad.</td>
<td>933</td>
<td>2,650</td>
<td>+190</td>
</tr>
<tr>
<td>Microsomes</td>
<td>$^{14}$C-Ad.</td>
<td>625</td>
<td>807</td>
<td>− 2.5%</td>
</tr>
<tr>
<td>Soluble</td>
<td>$^{14}$C-Ad.</td>
<td>45,140</td>
<td>45,050</td>
<td>− 0.2%</td>
</tr>
<tr>
<td>Albuminoid§ (no capsules)</td>
<td>$^{32}$PO$_4$</td>
<td>9,900</td>
<td>12,000</td>
<td>+ 21.2%</td>
</tr>
<tr>
<td>Whole capsule‡ RNA</td>
<td>$^{32}$PO$_4$</td>
<td>34,700</td>
<td>34,400</td>
<td>− 0.9%</td>
</tr>
</tbody>
</table>

†Standard error.  
§Whole lenses incubated; then capsules removed.  
¶These were not separated into tissue subfractions.
the galactose cataract may be due to an alteration in the physicochemical characteristics of the lens proteins. There is an apparent cessation of soluble protein synthesis in the experimental galactose cataract after the animal has been on the diet for 4 to 6 days, which may be correlated with the significant decline in the concentration of ATP (Fig. 17). There is also a specific alteration in the concentration of the cold precipitable protein fraction (Table VIII) which does not appear to be affected by galactose feeding until permanent opacification develops, at which time there is a very marked decline (approximately two-thirds) in the concentration of this fraction. The foregoing discussion has indicated that the initial cata-

Table VIII. The effect of a cataractogenic galactose diet on the CPP fraction in the rat lens

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of experiments</th>
<th>No. of lenses per group</th>
<th>Average wet weight of lenses (mg.)</th>
<th>Polyol (µg/lens)</th>
<th>% of total protein</th>
<th>Lens protein fraction</th>
<th>TMCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow-fed control lenses</td>
<td>5</td>
<td>15</td>
<td>31.7</td>
<td>1.8 ±0.4</td>
<td>26.5</td>
<td>Insoluble</td>
<td>6.3 ±0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>61.8</td>
</tr>
<tr>
<td>Calactose-fed non-cataractous lenses</td>
<td>3</td>
<td>17</td>
<td>36.5</td>
<td>1.9±0.1</td>
<td>24.4</td>
<td>CPP</td>
<td>4.8 ±0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>61.5</td>
</tr>
<tr>
<td>Calactose-fed cataractous lenses</td>
<td>6</td>
<td>15-16</td>
<td>35.4</td>
<td>1.5±0.2</td>
<td>30.0</td>
<td>% of total protein</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% of total protein</td>
<td>3.3 ±0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>65.9</td>
</tr>
</tbody>
</table>

The cataractogenic action of galactose is mainly directed toward specific aspects of glucose metabolism. This may explain the effect of aging on galactose cataractogenesis. In the young and rapidly growing lens the direct oxidative pathway is much more active and galactose may exert its cataractogenic action on this pathway as well as on the polyol pathway. The normally diminished activity of the hexose monophosphate shunt in the lens of the mature and older animal may be related to the difficulty of inducing galactose cataracts and this might also play a role in decreasing the efficacy of the polyol pathway since it depends on the availability of adequate amounts of TPNH.

Xylose cataract

Xylose and galactose differ quite markedly in their cataractogenic action. When young rats (4 to 5 weeks of age) are placed on a 50 to 70 per cent galactose diet, they develop permanent dense cataracts in 8 to 12 days, but the lens opacities which develop with a 30 per cent
xylose diet (in 8 to 14 days) are neither progressive nor permanent with respect to lenticular transparency and function in spite of the continued ingestion of xylose. The entire animal as well as the lens appears to be capable of recovering from the initial effects of xylose. In the lens, new fibers form around the original opacities (which represent hydropic degeneration) and eventually the lens appears to be almost completely transparent.

In older rats (3 to 4 months of age or more) the prolonged feeding of 35 percent xylose diet does not lead to any lenticular changes.

If a weanling rat is placed on a 30 percent xylose diet for 2 to 3 days, xylitol accumulates to a level of 2 to 4 mg. per gram of lens and there is a small but significant amount of sorbitol also present. It is possible that the accumulation of these sugar alcohols also renders the lens fibers somewhat hypertonic, resulting in an imbibition of water. The histological appearance of the early changes in xylose cataract resemble those found in galactose cataract. The hydropic degeneration persists to a varying degree in spite of the fact that the lenses appear to be grossly clear after the animals have been on the diet for 3 to 4 weeks. The areas of hydropic degeneration are now found in the deeper cortical zones of the lens. The sugar alcohol level in these lenses remains elevated for the duration of the experimental period of 60 days (Fig. 18). If adult rats are maintained on a cataractogenic xylose diet (35 percent xylose) for a prolonged period of time, there is a

Fig. 18. The level of total polyols and free glucose, fructose, and xylose in the lenses of rats maintained on a cataractogenic xylose diet for 60 days.

Fig. 19. The recovery of $^{14}$CO$_2$ from normal and xylose-fed rat lenses incubated with glucose-$^{14}$C. (From Lerman, S., and Heggeness, F. M.: Biochem. J. 79: 224, 1961.)
similar accumulation of polyols but these lenses never develop any visible opacities. There does not appear to be any accumulation of sugar intermediates such as xylulose, sedoheptulose, etc., which could possibly exert an initial effect on the hexose monophosphate pathway, nor is there any evidence of an inhibition of any of the enzyme systems in the hexose monophosphate shunt or in anaerobic glycolysis. There is also no alteration in the TPN or DPN xylitol (L-xylo- D-xylulose) dehydrogenase enzymes catalyzing the oxidation of xylitol in the lens.13

Aside from the accumulation of polyols, the only other metabolic changes which have been shown to develop in the xylose-fed rat lenses include a temporary inhibition of the hexose monophosphate pathway of glucose oxidation as evidenced by a fall in the recovery of \(^{14}\)C from lenses incubated with glucose-1-\(^{14}\)C (Fig. 19) and by a temporary decline in the TPN: TPN ratio while the DPNH:DPN ratio remains unimpaired (Fig. 20). The level of ATP in these lenses falls by 20 to 30 per cent after the animal has been on the diet for about 3 to 4 days but returns to a normal concentration at the time that the animal and the lens appear to be recovering from the initial deleterious effects of xylose.

If the polyol pathway plays a significant role as a transhydrogenase system in the young lens, it is possible that xylose could exert its effect on such lenses by partially inhibiting the hexose monophosphate shunt and by competing with glucose for the aldose reductase enzyme which catalyzes the first step in the polyol pathway. This would decrease the supply of DPNH that might be formed by means of the polyol transhydrogenase system and lead to a lowering in the concentration of ATP. As the lens adapts to the xylose load (after 2 to 3 weeks on the diet) the activity of the direct oxidative pathway returns to normal levels and there is a concomitant marked increase in the accumulation of sorbitol.64 This would indicate that the activity of the sorbitol pathway has at least returned to normal levels and the increased generation of DPNH by this pathway could explain the recovery in the concentration of ATP which occurs at approximately the same time. Such a mechanism would also explain the fact that the mature and old rat lenses are not affected by xylose diet since the hexose monophosphate pathway in the lenses of these animals is much less active and much less significant.

**Diabetic cataract**

The sorbitol pathway appears to play a role in the formation of the hydropic degenerative changes in the diabetic cataract. The hexose monophosphate shunt also shows signs of early impairment in the experimental (alloxan) diabetic cataract; there is a decline in total ATP in such lenses, and there is a marked diminution in the concentration of free amino acids which becomes apparent shortly after the administration of alloxan.57-58

**Summary**

The foregoing discussion of the sugar cataracts has indicated many of the similarities between these three forms of len-

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**Fig. 20.** The TPNH:TPN and DPNH:DPN ratios in normal and xylose-fed rat lenses. TPNH/TPN ratio in the xylose-fed (A) rat lens; TPNH/TPN ratio in the control (△) rat lens; DPNH/DPN ratio in the xylose-fed (●) rat lens; DPNH/DPN ratio in the control (○) rat lens. Each plotted point represents a single determination performed on two lenses. (From Lerman, S., and Heggeness, F. M.: Biochem. J. 79: 224, 1961.)
ticular opacification in the young animal. They all have a relatively rapid mode of onset with changes developing initially in the superficial cortex. These changes which consist of hydropic degeneration are probably due to the accumulation of polyols within the lens fibers with the retention of water leading to the characteristic histological picture. This type of opacity appears to be completely reversible, by stopping the galactose, or by giving insulin in the case of the diabetic cataract. In the case of the xylotic cataract these changes are spontaneously reversible. The permanent opacification which develops relatively suddenly in the galactose and diabetic cataracts is probably due to a severe metabolic upset leading to a marked alteration in the physicochemical state of the lens proteins. The ease with which the experimental sugar cataracts are produced is directly related to the age of the animal. The hexose monophosphate pathway is quickly impaired in all three types of sugar cataract, while anaerobic glycolysis does not become affected until the later stages of the disease processes. There is also a significant decline in ATP content and an impairment in amino acid incorporation.

REFERENCES
62. Lerman, S., and Ishida, B. K.: Pathogenetic