Dulcitol and water concentrations in galactose cataracts of the hamster

Albert Hofeldt, Robert P. Burns,* Fredrick T. Fraunfelder,** and Virginia Weimar

The rat has been the only laboratory animal in which galactose cataracts have been produced in vivo. Syrian golden hamsters, when fed a diet of 45 to 85 per cent galactose, also develop cataracts. With increasing concentrations of galactose, weight loss and death frequently occurred. The biomicroscopic lens changes were similar to those reported in the rat, and the cataracts were partially reversible. The data showed a quadratic relationship between dulcitol accumulation and (1) days on the galactose diet with a 98 per cent degree of fit and (2) stage of cataract formation with a 79 per cent degree of fit. A linear relationship was demonstrated when the water content was compared with (1) days on the galactose diet with a 77 per cent degree of fit and (2) stage of cataract formation with a 71 per cent degree of fit. Therefore, in the hamster's galactose cataract, the increase in dulcitol and increase in water were closely related but not exactly parallel.

Key words: galactose cataract, dulcitol, water, concentration, crystalline lens, experimental production, galactose, paper chromatography, gas liquid chromatography, hamsters.

It has been stated that the rat is the only common laboratory animal in which galactose cataracts have been regularly produced in vivo. The lenses of rabbits incubated in vitro with galactose accumulate the sugar alcohol of d-galactose and d-galactitol (dulcitol), but not galactose, to the high levels found in galactosemic rats. After we noted that the Syrian golden hamster developed cataracts when given a high-galactose diet, we repeated some of the observations of Kinoshita, Patterson and Bunting, and Cotlier, to determine if the mechanisms of cataract development in the hamster were the same as those in the rat.

Materials and methods

Thirty-nine young Syrian golden hamsters, weighing 38 to 60 grams, were divided into 8 galactose-fed experimental groups, each containing 4 animals, and a control group of 7 animals. The control diet was Purina Lab Chow. Experimental animals were fed 75 per cent d-galactose (Eastman Organic Chemicals) and 25 per cent Purina Lab Chow, for the following time intervals: 6 hours, 1 day, 2 days, 4 days, 6 days, 8 days, 10 days, and 13 days.

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Dulcitol and water concentrations in hamster

Cataracts were graded with a Haag-Streit biomicroscope after pupillary dilatation. The stage of cataract formation was recorded on a scale of 1 to 5. In a Stage I cataract, there was only equatorial haziness. Stage II showed vacuoles at the equator. In Stage III, an increase in vacuoles developed, with extension to the anterior and posterior cortices. In Stage IV, the nucleus was opaque. In Stage V, nuclear and over-all anterior cortical opacity occurred. Intermediate stages were indicated by plus signs.

Animals were killed by cervical fracture, and the eyes were enucleated immediately. The posterior pole of the eye was opened and the lens removed by gentle pressure. One lens was rolled on weighing paper, weighed, dried in a drying oven for 48 hours at 100°C, placed in a desiccator at room temperature for 48 hours, and reweighed. The opposite lens from each hamster was homogenized in a glass homogenizer with 2 ml. of 5 per cent trichloracetic acid (TCA). An additional 2 ml. of 5 per cent TCA was used to rinse the plunger of the homogenizer. The lens homogenate was centrifuged at 1,000 g for 20 minutes and stored in a refrigerator until all the lenses were harvested.

Water content was determined by comparison of the wet and dry weights of the lens. The only carbohydrate found in sufficient quantity to measure by paper chromatography and gas-liquid chromatography was dulcitol.

Descending paper chromatography was done for 48 hours on a portion of 5 per cent TCA supernatant fluid with an ethyl acetate-pyridine-water system in the proportion of 14:5:1. Chromatographic detection was performed by the method of Bean and Porter.

Gas-liquid chromatography was done on the TCA lens supernatant fluid following methylation and acetylation, and the supernatant fluid was dried by rotary vacuum evaporation. d-Arabinose, 1.20 μg (Nutritional Biochemicals Corp.), was added to the residue as internal standard with 4 ml. of 1.25N HCl in absolute methanol. The solution was allowed to react at room temperature for 12 hours, and again dried by rotary vacuum evaporation and vacuum desiccation. Under these conditions the aldoses reacted to form methyl glycosides. To the residue resulting from methylation, 250 μl of acetic anhydride-pyridine-acetylating reagent (Applied Science Laboratories, Inc.) were added. The solution was warmed in a water bath at 37°C for 15 minutes with occasional mixing in a parafilm-sealed test tube. This resulted in acetylation of the hydroxyl groups of the methyl glycosides and of the sugar alcohols.

The gas chromatograph was an F M Model 410 with 6 foot long glass columns, packed with 0.75 per cent diethylene glycol succinate (DEGS) /0.25 per cent ethylene glycol succinate (EGCSS-X) as the liquid phase on 60 to 80 mesh Gas Chron Q (Applied Science Laboratories, Inc.). The results were graphically recorded. Better separation of glucose and galactose from sugar alcohols was obtained by methylating the sugars before acetylation, which decreased the chromatographic retention time of the sugars. Chromatographic parameters included injection sample of 10 μl; nitrogen carrier gas at a flow rate of 38 ml. per minute; compressed air purge at a flow rate of 450 ml. per minute; hydrogen flame at a flow rate of 38 ml. per minute; an injection port temperature of 200°C; an oven temperature of 180 to 190°C; and a detector temperature of 230°C.

Standard solutions of d-glucose, d-galactose, d-arabinose, dulcitol, and d-sorbitol were subjected to gas-liquid chromatography after methylation and acetylation to determine the chromatographic sequence and the relationship between concentration and area under the curve (measured with a planimeter) on the graph paper. d-Arabinose was used as an internal standard to correct for chromatographic variation. Dulcitol was the only measured carbohydrate constituent present in the galactose-fed hamster lens in quanties sufficient to measure. From the area under the dulcitol curve, the micrograms and micromoles of dulcitol per lens were determined and converted to micrograms and micromoles per 10 mg. of dry lens.

The data presented in Table I were statistically analyzed by the analysis of variance multiple comparison test. The curves shown in Figs. 1, 2, and 3 were obtained by statistical analysis, using the method of stepwise multivariate analysis. Each curve obtained was analyzed for "lack of fit," and only those curves were selected which showed an insignificant degree of "lack of fit." These statistical methods of curve fitting are especially valuable in the handling of biological data because of the inherent variations in such data. The use of such methods in the analysis of biological data has become feasible only with the advent of the digital computer. In such analyses usually more than one equation can be found which will describe a given curve. The significance of the given equations in this discussion is in the shapes of the curves represented by them, i.e., straight line or curve.

Results

Syrian golden hamsters fed a diet of 45, 55, 65, 75, and 85 per cent galactose developed cataracts. Young hamsters were used because they developed cataracts more readily than did older animals. The biomicroscopic appearance of these cata-
## Table I. Tabulated summary of mean (with standard deviation) lens weight, water content, and dulcitol content for hamsters fed 75 per cent galactose and 25 per cent Purina Lab Chow

<table>
<thead>
<tr>
<th>Days on galactose diet</th>
<th>Control</th>
<th>6 hr.</th>
<th>1 day</th>
<th>2 days</th>
<th>4 days</th>
<th>6 days</th>
<th>8 days</th>
<th>10 days</th>
<th>13 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh lens* weight (mg.)</td>
<td>12.67</td>
<td>12.15</td>
<td>14.13†</td>
<td>15.28</td>
<td>13.96</td>
<td>14.37</td>
<td>15.68</td>
<td>16.25</td>
<td>17.03</td>
</tr>
<tr>
<td>±1.81</td>
<td>±0.30</td>
<td>±0.91</td>
<td>±0.88</td>
<td>±1.04</td>
<td>±0.92</td>
<td>±0.58</td>
<td>±1.01</td>
<td>±0.57</td>
<td></td>
</tr>
<tr>
<td>Dry lens* weight (mg.)</td>
<td>4.52</td>
<td>4.23</td>
<td>4.36</td>
<td>4.78</td>
<td>4.21</td>
<td>4.35</td>
<td>4.38</td>
<td>4.31</td>
<td>4.36</td>
</tr>
<tr>
<td>±0.50</td>
<td>±0.24</td>
<td>±0.07</td>
<td>±0.13</td>
<td>±0.31</td>
<td>±0.07</td>
<td>±0.17</td>
<td>±0.35</td>
<td>±0.20</td>
<td></td>
</tr>
<tr>
<td>Water per lens* (mg.)</td>
<td>8.16</td>
<td>7.95</td>
<td>9.75§</td>
<td>10.50</td>
<td>9.75</td>
<td>10.03</td>
<td>11.30</td>
<td>11.95</td>
<td>12.68</td>
</tr>
<tr>
<td>±1.51</td>
<td>±0.19</td>
<td>±0.86</td>
<td>±0.73</td>
<td>±0.79</td>
<td>±0.86</td>
<td>±0.40</td>
<td>±0.66</td>
<td>±0.54</td>
<td></td>
</tr>
<tr>
<td>mg H₂O per* 10 mg. dry lens</td>
<td>18.08</td>
<td>18.76</td>
<td>22.42§</td>
<td>21.97</td>
<td>23.19</td>
<td>23.08</td>
<td>25.82</td>
<td>27.80</td>
<td>29.10</td>
</tr>
<tr>
<td>±2.81</td>
<td>±1.28</td>
<td>±1.75</td>
<td>±1.21</td>
<td>±0.84</td>
<td>±1.64</td>
<td>±0.36</td>
<td>±0.92</td>
<td>±2.08</td>
<td></td>
</tr>
<tr>
<td>Per cent H₂O of* total lens weight</td>
<td>64.05</td>
<td>65.19</td>
<td>69.08</td>
<td>68.68</td>
<td>69.82</td>
<td>69.71</td>
<td>72.08</td>
<td>73.53</td>
<td>73.38</td>
</tr>
<tr>
<td>±3.05</td>
<td>±1.48</td>
<td>±1.71</td>
<td>±1.22</td>
<td>±0.79</td>
<td>±1.44</td>
<td>±0.29</td>
<td>±0.65</td>
<td>±1.32</td>
<td></td>
</tr>
<tr>
<td>μg dulcitol per* lens</td>
<td>0</td>
<td>24.60</td>
<td>157.00§</td>
<td>319.00§</td>
<td>422.00§</td>
<td>424.67</td>
<td>440.75</td>
<td>443.00</td>
<td>414.33</td>
</tr>
<tr>
<td>±3.74</td>
<td>±14.07</td>
<td>±67.62</td>
<td>±25.06</td>
<td>±27.57</td>
<td>±32.10</td>
<td>±100.92</td>
<td>±37.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>μmoles dulcitol* per lens</td>
<td>0</td>
<td>0.14</td>
<td>0.87§</td>
<td>1.75§</td>
<td>2.33</td>
<td>2.33</td>
<td>2.42</td>
<td>2.43</td>
<td>2.28</td>
</tr>
<tr>
<td>±0.02</td>
<td>±0.08</td>
<td>±0.37</td>
<td>±0.14</td>
<td>±0.15</td>
<td>±0.18</td>
<td>±1.05</td>
<td>±0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>μmoles dulcitol* per 10 mg. dry lens weight</td>
<td>0</td>
<td>0.33</td>
<td>1.97</td>
<td></td>
<td>3.65</td>
<td></td>
<td>5.40</td>
<td></td>
<td>5.39</td>
</tr>
<tr>
<td>±0.06</td>
<td>±0.16</td>
<td>±0.67</td>
<td>±0.40</td>
<td>±0.41</td>
<td>±0.35</td>
<td>±2.15</td>
<td>±0.43</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Each time period is compared with the immediately preceding time period.
†P < 0.05.
‡P < 0.025.
§P < 0.01.
||P < 0.005.
||P < 0.001.
Fig. 1. Dulcitol and water accumulation in the lenses of hamsters fed a diet of 75 per cent galactose and 25 per cent lab chow.

Each curve represents the best fitting curve obtained by computer analysis, using the method of stepwise multivariate analysis. For dulcitol:

\[ \ln Y = 0.7109 + 0.9965 \ln X - 0.2463 (\ln X)^2 \]

where \( Y \) = \( \mu \) moles of dulcitol per 10 mg. dry lens and \( X \) = days on galactose diet.

\( R^2 \), the degree of fit = 0.98, indicating that 98 per cent of the experimental data is expressed by this equation (\( P < 0.001 \)).

A fairly good fit can also be obtained with the quadratic equation:

\[ Y = 0.4983 + 1.3399X - 0.0782X^2 \]

but a slight degree of "lack of fit" was obtained. The data cannot be fit, however, with a linear equation.

For water:

\[ Y = 19.368 + 0.7894X \]

where \( Y \) = mg. H\(_2\)O per 10 mg. of dry lens and \( X \) = days on galactose diet.

\( R^2 = 0.77 \); i.e., 77 per cent of the experimental data is expressed by this equation (\( P < 0.001 \)).
racts was the same as those in the rat. With the higher concentrations, weight loss and death frequently occurred. Young hamsters fed 75 per cent d-galactose with 25 per cent Purina Lab Chow developed Stage I cataracts in 2 days, Stage II cataracts in 4 to 8 days, Stage III in 7 to 10 days, Stage IV in 7 to 20 days, and Stage V in 14 to 32 days. Cataracts up to Stage III were reversible upon discontinuance of galactose diet.\textsuperscript{10, 11}

Glucose, galactose, and sorbitol did not appear in significant quantities in the lenses of galactose-fed hamsters. Dulcitol was identified by both descending paper and gas-liquid chromatography. Dulcitol was measurable after 6 hours on the galactose diet. There were significant progressive increases in the micrograms of dulcitol per lens from 6 hours to 1 day ( $P < 0.01$ ), from 1 day to 2 days ( $P < 0.001$ ), and from 2 days to 4 days ( $P < 0.05$ ). From 4

![Stage of Cataract Formation](image)

Fig. 2. Dulcitol and water accumulation in progressive stage of cataract formation in hamsters fed 75 per cent galactose and 25 per cent Purina Lab Chow.

Each curve represents the best fitting curve obtained by computer analysis using the method of stepwise multivariate analysis.\textsuperscript{9}

For dulcitol:

$$Y = 0.6270 + 1.9467X - 0.1980X^2$$

where $Y =$ moles of dulcitol per 10 mg. of dry lens and $X =$ stage of cataract formation.

Seventy-nine per cent ($R^2 = 0.79$) of the experimental data is expressed by this quadratic equation ($P < 0.001$).

For water:

$$Y = 19.5051 + 1.4439X$$

where $Y =$ mg. H$_2$O per 10 mg. of dry lens and $X =$ stage of cataract formation

$R^2 = 0.71$; $P < 0.001$. 

\textsuperscript{9} Downloaded from iovs.arvojournals.org on 03/22/2019
to 13 days there were no significant increases or decreases in micrograms of dulcitol per lens (Table I). Dulcitol expressed in micromoles per 10 mg. of dry lens showed progressive significant increases from 6 hours to 1 day (P < 0.01), from 1 day to 2 days (P < 0.001), and from 2 days to 4 days (P < 0.05). From 4 days to 13 days, no further significant increases were observed (Table I). Progressive significant increases in the milligrams of water per 10 mg. of dry lens occurred from 0 to 1 day (P < 0.001), from 1 to 8 days (P < 0.025), and from 8 to 13 days (P < 0.025).

Micromoles of dulcitol per 10 mg. of dry lens, when compared with days on the galactose diet, can be expressed by a quadratic equation (Fig. 1). The data cannot be fit with a linear equation. Milligrams of water per 10 mg. of dry lens, however, has a linear relationship to the number of days on the galactose diet and is represented by a linear equation (Fig. 1).

Micromoles of dulcitol per 10 mg. of dry lens increased as the stage of cataract formation increased, with a plateau at Stages II to III (Fig. 2). This relationship may be expressed by a quadratic equation.

![Graph](image)

Fig. 3. Lens water compared with fresh lens weight of galactose-fed hamsters.

The curve represents the best fitting curve obtained by computer analysis using the method of stepwise multivariate analysis. The points represent the actual experimental data. The equation of the line is:

\[ Y = 4.0472 + 1.0346X \]

where \( Y \) = weight of fresh lens (mg.) and \( X \) = mg. H₂O per lens (mg.)

Note that the constant 4.0472 represents the dry weight of the lens which was found to be constant (see Table I).

\[ R^2 = 0.97 \text{ with } P < 0.001. \]
On the other hand, water (milligrams) per 10 mg. of dry weight of the lens shows a linear relationship to the stage of cataract formation (Fig. 2).

These data show that in the hamster's lens the increase in dulcitol and the increase in water, when compared with either the number of days on the galactose diet or the stage of cataract formation, are related, but are not perfectly parallel. Possible reasons for this disparity have been noted by Kinoshita in showing a decrease in amino acids and ATP, as well as increased sodium and decreased potassium in the galactose cataract.

The weight of the fresh lens progressively increased with the increasing number of days on the diet (Table I). This increase in fresh lens weight was due to an increasing water content since the dry weight of the lens remained constant (Table I). Fig. 3 shows a linear relationship between the weight of the fresh lens and the water (milligrams) per lens. It is to be noted that the dulcitol and water determinations were carried through Stage III cataracts only.

Comment

The Syrian golden hamster can develop galactose cataracts when fed a diet with a high concentration of galactose. The rat is the only other experimental animal in which in vivo galactose cataracts have been regularly produced. Regarding the galactose cataract of the hamster and rat, several points are comparable: (1) young animals develop cataracts more readily than do older animals; (2) the sequential cataractous morphologic changes are similar as viewed through the biomicroscope; (3) the galactose cataract in the hamster may be reversible on resumption of a normal diet; (4) both animals accumulate dulcitol to significant quantities in the lens; and (5) in the hamster the accumulation of dulcitol and water in the lens is comparable but not exactly parallel.

REFERENCES