Cataracts in galactosemia

The Jonas S. Friedenwald Memorial Lecture

Jin H. Kinoshita

One purpose of this award to to preserve the name of a great scientist who has contributed so much to the field of ophthalmology. If this were the only purpose, this award would not be necessary, for Dr. Friedenwald is immortalized by his many publications which serve as catalysts for a great number of other investigations. This presentation on the cataracts in galactosemia is an illustration.

Human galactosemia

Congenital galactosemia is now known to be caused by a deficiency of one enzyme, resulting in the inability of the afflicted individual to metabolize galactose. The main clinical findings in infants with this disease are hepatomegaly, malnutrition, cataracts, and galactosemia. Mental retardation becomes apparent with increasing age of the untreated infant. Because cataracts have been observed as early as a few days after birth, they may be the earliest observable symptom.1

Previously this disease was confused with diabetes because of the fact that a sugar was detected in the urine of these patients. However, with more specific tests now available for glucose and galactose, this entity can easily be established. By checking the urine with the glucose or galactose oxidase test, one can rapidly determine which sugar is present in the urine. Positive identification of galactose can be made by paper chromatography.2

This disease can be treated simply by withdrawing galactose from the diet. When galactose is withdrawn, the symptoms seem to correct themselves, provided the disease is detected sufficiently early.3 In most cases cataracts disappear when milk—the principal dietary source of galactose—is eliminated from the infant’s diet. When affected individuals pass their formative stage of life they seem able to tolerate galactose,4 and adults who had been galactosemic are capable of ingesting the sugar without developing any of the symptoms.

Galactosemia is a hereditary disease which is recessive in nature and its occurrence is approximately 1 in 18,000 births.5 The absence of, or at least the depression in the activity of, one particular enzyme involved in galactose metabolism is responsible for the disease. The pathway by which galactose is metabolized usually proceeds in the following sequence of reactions (Fig. 1): First, galactose is phosphorylated by a reaction with ATP* to

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Footnotes:

1. The following are the abbreviations used throughout the text: ATP, adenosine triphosphate; UDPglu, uridine diphosphate glucose; UTP, uridine triphosphate; P.P., pyrophosphate; Gal-1-P, galactose-1-phosphate; CSH, glutathione; Na, sodium ion; K, potassium ion; Cl, chloride ion.

2. Previously this disease was confused with diabetes because of the fact that a sugar was detected in the urine of these patients. However, with more specific tests now available for glucose and galactose, this entity can easily be established. By checking the urine with the glucose or galactose oxidase test, one can rapidly determine which sugar is present in the urine. Positive identification of galactose can be made by paper chromatography.

3. In most cases cataracts disappear when milk—the principal dietary source of galactose—is eliminated from the infant’s diet. When affected individuals pass their formative stage of life they seem able to tolerate galactose, and adults who had been galactosemic are capable of ingesting the sugar without developing any of the symptoms.

4. The absence of, or at least the depression in the activity of, one particular enzyme involved in galactose metabolism is responsible for the disease.

5. The pathway by which galactose is metabolized usually proceeds in the following sequence of reactions (Fig. 1): First, galactose is phosphorylated by a reaction with ATP* to

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Fig. 1. A pathway of galactose metabolism. Conversion of galactose to glucose.

galactose-1-phosphate. This is followed by a transfer type of reaction in which UDP galactose-1-phosphate and glucose-1-phosphate are formed. The third reaction involves the epimerization of UDP galactose to UDP glucose and this compound in turn reacts with pyrophosphate to give glucose-1-phosphate. Thus the three reactions following the galactokinase reaction are required to convert galactose-1-phosphate to glucose-1-phosphate—an intermediate in the pathway of glucose metabolism.

Apparently the transferase enzyme of reaction 2 is the target of the disease process in galactosemia, resulting in a deficiency of the enzyme. Since galactose-1-phosphate cannot be transferred to UDPG, the galactose derivative accumulates in the tissues as demonstrated in the red cells and liver. Diagnosis for galactosemia is made by determining whether there is a depression in the activity of the enzyme transferase in the blood. The galactosemic patients also show an abnormal galactose tolerance curve. The peculiar properties of the galactokinase reaction account for the elevated level of galactose in the blood and, consequently, in the urine. Galactokinase is inhibited by galactose-1-phosphate; therefore the accumulation of the phosphorylated intermediate, because of the enzyme defect, inhibits the first reaction. Moreover, galactokinase is subject to substrate inhibition. Thus, galactokinase is inhibited by galactose-1-phosphate and, in addition, the resulting increase in galactose also tends to decrease the rate of galactose phosphorylation.

The only report concerning biochemical changes in the lens of a galactosemic subject was made by Lerman. In this case a 4-week-old boy with galactosemia was found to have a slight opacification of the lens. This patient died of complications, and analyses of the lens revealed that galactose-1-phosphate uridyl transferase was completely lacking.

Even though the enzyme defect is undoubtedly the primary factor in galactosemia, no satisfactory explanation is available of how the absence of the enzyme transferase can account for the symptoms involved. Galactose is an essential component of cerebrosides and mucopolysaccharides, and interference of galactose utilization by a tissue was thought to create a shortage in these vital macromolecules. However, other metabolic pathways are available which could provide sufficient amounts of galactose for this purpose. The accumulation of galactose-1-phosphate was considered to have possible effects on glucose metabolism. It has been shown that galactose-1-phosphate is an inhibitor for the phosphoglucomutase reaction. This is the reaction involving the conversion of glucose-6-phosphate to glucose-1-phosphate in the pathway of glycogen synthesis. However, in this case galactose-1-phosphate is a competitive inhibitor, hence even this reaction is probably not completely blocked in tissues of galactosemic patients. There was one report that galactose-1-phosphate is an inhibitor of glucose-6-phosphate dehydrogenase in the lens, but this report could not be confirmed by others.

Experimental galactosemia and cataracts

There is a good possibility that investigations dealing with lens changes in galactosemia may lead to an understanding of the other symptoms of the disease. Since a cataract can be easily induced by feeding rats diets enriched with galactose, many investigators have used this approach...
in the hope of determining the etiology of this type of cataract.

The morphology of these experimental cataracts seems to differ from those observed in human patients. In young rats the cataracts first appear as vacuoles in the equatorial region of the lens and, as the cataract progresses, the vacuoles become more numerous and eventually a dense nuclear opacity develops. In contrast to these morphological changes in experimental animals, cataracts in human galactosemia are mostly of the nuclear lamellar type. However, in our laboratory we have confirmed the observation that newly born rats, whose mothers were maintained on a galactose diet, develop a nuclear lamella type of cataract closely resembling that observed in the galactosemic infants. Therefore, it seems reasonable to assume that the factors initiating the galactose cataract in young rats are very similar to those involved in the human galactose cataract. The manifestations of the cataract, however, may differ, depending on the developmental stage of the animals. The lens opacities in rats that are fed galactose, like those in human galactosemic subjects, slowly disappear when rats are placed on diets free of galactose.

Studies on the pathogenesis of the cataract of galactose-fed rats have led to the speculation that the overabundance of galactose in the lens in some way interferes with carbohydrate metabolism. But no definite evidence indicating that glucose utilization or lactate production was altered sufficiently to cause the cataract could be provided. Directly bearing on this point is the determination of ATP levels in the lenses of galactose-fed rats when opacities first become visible. At this initial vacuolar stage of cataract, the ATP level was depressed by only 10 per cent of the control, whether the comparison was made on a per lens or a dry weight basis (Table 1). These results are in good agreement with those of Mandel and Klethi who observed only a 12 per cent drop in the lens ATP level when opacities become visible. Sippel showed that in the 50 gram Sprague-Dawley rats the appearance of opacities preceded any change in the ATP level. These findings, showing that no significant change in lens ATP level occurs at the time when opacities first become visible, make it appear doubtful that lack of biological energy is the causative factor in the initiation of the cataractous process. However, since the ATP level is markedly depressed in the later stages of cataract development, a lack of energy may be a contributing factor in producing changes observed later.

Osmotic changes in the lens

A new lead into the pathogenesis of galactose cataract was provided by Dr. Friedenwald in his study on the histopathology of this type of cataract. He observed that the earliest morphologic change was the appearance of hydropic lens fibers. He presented evidence indicating that the accumulation of fluid was intracellular and not extracellular. As the cataract progressed, the hydropic fibers ruptured and dissolved, leading to interfibrillar clefts which were filled with precipitated proteins.

Table I. Changes in ATP levels in the initial vacuolar stage of galactose cataract

<table>
<thead>
<tr>
<th>No.</th>
<th>Glucose-fed rats</th>
<th></th>
<th>Galactose-fed rats</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ATP content of lens (mumoles/mg. dry weight lens)</td>
<td>ATP content of lens (mumoles/lens)</td>
<td>ATP content of lens (mumoles/mg. dry weight lens)</td>
<td>ATP content of lens (mumoles/lens)</td>
</tr>
<tr>
<td>26</td>
<td>7.3 ± 0.6</td>
<td>72 ± 0.7</td>
<td>24</td>
<td>6.8 ± 0.7</td>
</tr>
</tbody>
</table>

Analyses were made on lenses from 100 gram rats which were kept on a high galactose diet until lens opacities first became visible. For controls, rats were kept on a glucose diet for the same period. The firefly luminescence assay for ATP was used.

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The problem was, then, to find an explanation of how these hydropic lens fibers develop. It would seem that there are two main reasons for the swelling of a cell. Osmotic changes can result either from the accumulation of abnormal metabolites or of electrolytes. In the galactose cataract an obvious abnormal metabolite is galactose-1-phosphate. Although galactose-1-phosphate accumulates in the lens of a galactose-fed rat, it does this slowly, and the level attained would probably exert only a minimal osmotic effect. A more likely abnormal metabolite to effect an osmotic change was uncovered by Van Heyningen. By chromatographic methods she was the first to demonstrate the presence of dulcitol, the sugar alcohol form of galactose, in the lens of a galactose-fed rat. The nature and factors concerning the synthesis and accumulation of this compound strongly suggest that the early osmotic changes can be explained by the retention of dulcitol. First of all, the level of dulcitol found in the lens of galactose-fed rats is about equal to that of the K ion—the normal lens constituent which is present in the highest concentration. The retention of dulcitol in the lens to such a level must obviously have some osmotic consequence, for it creates a hypertonicity and the obligatory response is the movement of water into the fibers. These osmotic changes were shown to occur both in vivo and in vitro. In both cases, as shown in Figs. 2 and 3, the accumulation of dulcitol in the lens is paralleled by an increase in lens water.

The study of the enzymatic mechanism involved in the reduction of galactose to dulcitol has revealed that the lens is a particularly favorable site for the accumulation and production of dulcitol. Galactose concentration must be fairly high before the enzyme aldose reductase can convert significant amounts of the sugar to the alcohol form. In other tissues, even though the organ may be exposed to high levels of galactose, the phosphorylation mechanism is sufficiently active to keep the sugar at low levels. On the other hand, since the lens has a sluggish system to phosphorylate galactose, there is a tendency for the sugar to increase when high levels of it are made available to this ocular tissue. Furthermore, the lens has an active shunt mechanism which provides TPNH—the co-enzyme necessary for the

![Fig. 2. Increases in dulcitol and in water in the lens of galactose-fed rats. From Kinoshita et al.: Nature 194: 1085, 1962.](image1)

![Fig. 3. Increases in dulcitol and in water in the rabbit lens incubated in high levels of galactose. The incubation medium contained either 30 mM fructose + 5 mM glucose (control), or 30 mM galactose + 5 mM glucose. Paired lenses were used, one lens placed in the control medium and the other in the aldose medium. Data republished from Kinoshita, Hayman, and Merola: J. Biol. Chem. 240: 310, 1965.](image2)
reduction of galactose. Thus because of the distribution of enzymes characterized by a low activity of galactokinase, and a high shunt activity, coupled with a relatively active aldose reductase, the lens is a favorable site for dulcitol synthesis. Furthermore, in contrast with other sugar alcohols, dulcitol is not a suitable substrate for polyol dehydrogenase—the enzyme responsible for the further oxidation of polyols. Sorbitol and xylitol are actively oxidized to their keto sugar form, but dulcitol is not.30 Another important factor is that sugar alcohols do not easily penetrate the lens membrane. Since dulcitol does not readily leak out of the lens nor is it metabolized, its continued synthesis can lead to unusually high levels within the lens fibers.

We believe that the sequence of events in galactose cataract leading to the appearance of vacuoles is as follows: The initiating factor is the increased level of galactose in the aqueous humor which, in turn, increases the concentration of this sugar in the lens. The increased availability of galactose stimulates the synthesis of dulcitol. A hypertonic condition is created as dulcitol accumulates, but this is immediately corrected by movement of water into the lens fibers. The resulting osmotic swelling of the lens fibers probably explains the histological appearance of the hydrops. As the swelling continues, some of the fibers rupture, and vacuoles probably represent areas in the lens where considerable osmotic dissolution of fibers has occurred.

Friedenwald20 has shown that the fibers at the equator which are immediately superficial are not first affected, but that those deeper in the lens are the most susceptible. No experimental evidence is available to explain these observations.

**Defect in the amino acid-concentrating mechanism**

There are other changes which occur in the development of galactose cataract which must be related directly either to dulcitol formation or to the resultant osmotic changes. One change which occurs early in the course of cataract development is the depression of the amino acid level in the lens.32 Since many of the early changes which occur in the lens of a galactose-fed rat can be reproduced by simply incubating the lens in a medium containing high levels of galactose, the amino acid problem was studied in vitro. As shown in Table II, there is a 40 per cent depression of total amino acids in these lenses incubated in a high galactose medium compared with those incubated in a control medium.

**Table II. Changes in the level of amino acids in the rabbit lens incubated in the presence of galactose**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Amino acids in control medium (μmoles/lens)</th>
<th>Amino acids in galactose medium (μmoles/lens)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.84</td>
<td>2.39</td>
<td>-38</td>
</tr>
<tr>
<td>2</td>
<td>3.57</td>
<td>2.25</td>
<td>-37</td>
</tr>
<tr>
<td>3</td>
<td>3.85</td>
<td>2.43</td>
<td>-37</td>
</tr>
<tr>
<td>4</td>
<td>3.73</td>
<td>2.24</td>
<td>-40</td>
</tr>
<tr>
<td>5</td>
<td>3.58</td>
<td>1.89</td>
<td>-47</td>
</tr>
<tr>
<td>6</td>
<td>4.35</td>
<td>2.75</td>
<td>-38</td>
</tr>
</tbody>
</table>

Average increase in lens weight = 20.9 mg.

Paired lenses from rabbits were used, one lens incubated in a control medium, 30 mM. fructose plus 5 mM. glucose, and the other in a medium containing 30 mM. galactose and 5 mM. glucose. After 21 hours the lens was homogenized in 10 per cent trichloroacetic acid. The values given are for the total ninhydrin-reactive material measured in the trichloroacetic acid filtrates.

**Table III. Accumulation of AIB in media containing various aldoses**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Increase in sugar alcohol (μmoles/lens)</th>
<th>Increase in water (mg/lens)</th>
<th>(AIB) Lens/(AIB) Medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>---</td>
<td>---</td>
<td>24.6 ± 3.5 (128)</td>
</tr>
<tr>
<td>Galactose</td>
<td>6.9 ± 0.5</td>
<td>19.5 ± 1.5</td>
<td>7.6 ± 1.2 (28)</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.9 ± 0.7</td>
<td>12.5 ± 1.2</td>
<td>10.5 ± 2.0 (37)</td>
</tr>
<tr>
<td>Mannose</td>
<td>1.2 ± 0.2</td>
<td>4.2 ± 0.9</td>
<td>17.2 ± 1.6 (7)</td>
</tr>
</tbody>
</table>

In all experiments, paired lenses from a rabbit were used, one lens placed in the control medium, 30 mM. fructose plus 5 mM. glucose, and the other in a medium containing 30 mM. aldose in addition to 5 mM. glucose. Each medium contained 0.1 mM. [14C]-AIB and had an initial tonicity of 250 to 253 milliosmolar. The changes in sugar alcohol level and water content of the lens incubated for 21 hours in the aldose solution are given as the increases above those of the lens incubated in the control medium. The results are given as the mean ± the standard deviation. The number of experiments are indicated in parentheses.
medium. As shown by Kinsey and Reddy, further insight into this problem can be gained by studying the uptake of \(\alpha\)-aminoisobutyric acid (AIB)—a nonmetabolizable acid. AIB is taken up by an active process which concentrates it in the lens to a level much higher than that of the medium. In Table III, we see that a lens incubated for 21 hours concentrates AIB 25 times above the concentration in the medium.\(^{33}\) In the presence of galactose, however, the lens is able to concentrate AIB to about 30 per cent of normal. Other aldoses, glucose and mannose, have a similar effect. The extent of the inhibition by these aldoses on AIB uptake seems to have an inverse relationship to the amount of sugar alcohol and gain of water in the lens. For example, the lens exposed to galactose accumulates the highest level of sugar alcohol, has the greatest increase in water, and is least effective in concentrating AIB. On the other hand, in the presence of mannose the lens accumulates very little sugar alcohol, has the slightest gain in water, and also produces the slightest inhibition of AIB uptake. The effect of glucose is intermediate between that of galactose and mannose. Apparently, either the sugar alcohol itself or the concomitant osmotic change seems to have an adverse effect on the ability of the lens to concentrate amino acids. The possibility that the effect was osmotic was first studied. Changes in the state of hydration can also be achieved by another means, that is, by incubating the lens either in a hypo- or hypertonic condition. The lens behaves as an osmometer, in that if the lens is placed in a medium which is hypotonic it tends to take up water, and when placed in a medium which is hypertonic it loses water. As shown in Fig. 4, one can see that in a range from 64 milliosmolal hypotonic to about 32 milliosmolal hypertonic to the normal medium there is a linear relationship between the change in water and that of the tonicity of the medium. Thus, in this limited range of tonicity the lens behaves as an osmometer. Furthermore, the lens is a sensitive osmometer in that it responds to a 5 per cent change in the tonicity of the medium. The effect of changes in tonicity on the ability of the lens to concentrate AIB is shown in Fig. 5. When exposed to hypotonic media the lens is less able to concentrate AIB, and, up to a point, the more hypertonic the solution, the more effectively is the lens able to concentrate the amino acid. Thus, tonicity plays a very important part in determining the effectiveness of the amino acid-concentrating mech-

![Fig. 4. The effect of varying tonicity of the medium on the hydration of the lens. Rabbit lenses were incubated for 21 hours. Equivalent amounts of NaCl were added to or subtracted from the control medium to vary the tonicity. The results are given as the mean ± the standard deviation of the mean. Data republished from Kinoshita, Hayman, and Merola: J. Biol. Chem. 240: 310, 1965.](http://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/932953/)
anism in the lens. In view of these results, the osmotic change may be the explanation of what occurs in the lens when exposed to galactose. In this case the dulcitol-induced osmotic change would simulate the swelling induced by a hypotonic medium. The osmotic swelling may be the reason for the lowered efficiency of the AIB-concentrating mechanism in the galactose-exposed lens. If this is the case, then preventing the swelling from occurring, despite the accumulation of dulcitol, should allow the lens to concentrate AIB to normal levels. To investigate this possibility, the lens was incubated in a medium containing galactose. However, during the course of the experiment the tonicity of the medium was gradually increased so that even though dulcitol accumulated, the movement of water into the lens was prevented. The incubation of the lens which was exposed to galactose in the osmotically compensated medium for 24 hours maintained the normal water volume of the lens (Table IV). Furthermore, under these conditions, the lens was able to concentrate AIB as well as the lens in the normal medium. To illustrate that this effect is not limited to a specific amino acid, the lenses exposed to galactose but incubated in an osmotically compensated medium were able to retain normal levels of amino acids (Table V). Thus, from these experiments it would appear that the loss of amino acids in the lens when exposed to galactose is primarily due to the osmotic swelling of the lens brought about by the retention of dulcitol.

In human galactosemia one of the interesting clinical findings is that these patients have an amino aciduria. All of the amino acids are found in excessive quantities in the urine. This problem was studied in the rat by Rosenberg and associates, who found that maintaining rats on a high

Table IV. Accumulation of AIB by rabbit lens in osmotically compensated medium

<table>
<thead>
<tr>
<th>Medium</th>
<th>Distribution of AIB (AIB)/L.</th>
<th>H₂O (mg/lens)</th>
<th>Sugar alcohol (umoles/ lens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.3 ± 2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galactose</td>
<td>22.7 ± 1.5</td>
<td>-1.3 ± 1.2 mg.</td>
<td>+7.8 ± 0.2</td>
</tr>
</tbody>
</table>

Table V. Changes in the level of amino acids in the rabbit lens incubated in an osmotically compensated medium containing galactose

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>In control medium</th>
<th>In osmotically compensated galactose medium</th>
<th>Change in lens weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amino acids (umoles/lens)</td>
<td>Amino acids (umoles/lens)</td>
<td>(mg.)</td>
</tr>
<tr>
<td>1</td>
<td>3.73</td>
<td>3.64</td>
<td>+1.6</td>
</tr>
<tr>
<td>2</td>
<td>3.90</td>
<td>3.43</td>
<td>+1.4</td>
</tr>
<tr>
<td>3</td>
<td>3.25</td>
<td>3.36</td>
<td>+1.9</td>
</tr>
<tr>
<td>4</td>
<td>2.63</td>
<td>2.50</td>
<td>+0.7</td>
</tr>
<tr>
<td>5</td>
<td>3.05</td>
<td>3.04</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>2.87</td>
<td>3.16</td>
<td>-0.2</td>
</tr>
</tbody>
</table>

Descriptions are the same as those given in Table II and Table IV.
galactose diet does produce an amino aci-
duria. In addition, it has recently been re-
ported that dulcitol was found in the urine
of human galactosemic patients and in
the kidney of galactose-fed rats. These
findings indicate that studies to determine
whether the same factors are affecting
both the lens and kidney in the galacto-
semic patients may prove informative.

Changes in lens hydration during the
course of galactose cataract

The discussion thus far has dealt with
changes which occur prior to the appear-
ance of vacuoles in the lens of a galactose-
fed rat. As the cataract progresses, these
vacuoles which first appear at the equa-
torial region become more numerous and
spread over the anterior surface of the
lens. This continues for a period of about
14 to 15 days after the onset of the galac-
tose diet. Any time after this period,
usually within a few hours, a dense nu-
clear opacity develops. Along with the
appearance of nuclear cataract there is an-
other and a most dramatic osmotic change.
To illustrate this point, the changes in hy-
dration during various stages of galactose
cataract are shown in Fig. 6. The initial
vacuolar stage is marked by a 25 to 30
per cent increase in lens water. Between
the initial and the late vacuolar stage—the
period just prior to the dense nuclear opac-
ity—there is essentially no increase in lens
water. However, the animals are 2 weeks
older than those at the initial vacuolar
stage and consequently the lenses normally
would have a lower water content. Thus,
in comparison with the lens of a control
rat, the late vacuolar stage cataracts show
a 50 per cent increase in lens water. The
major change in lens water occurs, how-
ever, when the lens develops the nuclear
opacity (mature cataract). A three- to four-
fold increase in lens water is observed at
this stage. These changes in hydration dur-
ing the development of a galactose cataract
are very striking and, although a complete
explanation is not available, there are some
findings which undoubtedly bear upon this
problem.

Electrolyte changes

It is generally accepted that inorganic
electrolytes are the constituents of the lens
or of any cell which regulate the normal
cell volume. Thus, it seemed important to
follow the changes in the Na, K, and Cl
contents during cataract development. As
shown in Fig. 7, there is a dilution of cation
ions in the initial vacuolar stage. The total
cation (Na+K) concentration on a water
basis of the lens from a galactose-fed rat
is lower than that of the normal lens. How-
ever, this does not mean there is a decrease
in the total Na and K ion, for actually the
total amount remains the same, as shown
by the results expressed on a dry weight
basis. The dilution of the cation is ex-
plained by the fact that the dulcitol reten-
tion is responsible for drawing water into the lens. Apparently the movement of water is not accompanied by electrolytes at this stage of cataract development. The difference in the total cation concentration expressed on a water basis between the early cataracts and control lenses indicates that a 24 per cent dilution of the cations had occurred. This value is in fair agreement, considering the different techniques employed, with the actual measurement of the net increase in water. In these lenses the increase in water was 5.3 mg. per 10 mg. dry weight of the lens, representing an increase in hydration of about 30 per cent over that of the normal lens. It is also of interest to compare the amount of dulcitol found in the lens with the increase in water observed. At this stage of cataract the amount of dulcitol recovered was 2.5 mg. per 10 mg. dry weight of the rat lens. Thus, the ratio of the amount of dulcitol found to the increase in water was 0.472 µmole per milligram water. In other words, with the amount of dulcitol found, a larger increase in lens water would be expected. Part of this discrepancy is explained by the loss in amino acids and glutathione. In addition, even though some dulcitol accumulates in the nucleus of the lens, nevertheless, because of existing hydrostatic forces, this area of the lens may not take up theoretical amounts of water. In contrast to the rat lens, incubation of rabbit lens in a high galactose medium has led to a more favorable ratio of the amount of dulcitol found to the increase in water. During a three-day incubation, this ratio ranged from 307 to 373 µmoles per milligram of water increase.

In the late vacuolar stage there seems to be a dilution of the total cation concentration on a water basis but, in contrast with the initial stage cataract, there is also a slight increase in total cations (Fig. 7). Therefore, it appears at this stage that the increase in hydration is due in part to dulcitol retention as well as to electrolyte increase. Furthermore, there seems to be some obvious change in the distribution of Na and K, in that the concentration of Na and of K is decreasing. This change is also taking place in the cataract at the initial stage, but is most apparent in the late vacuolar stage. The cation changes are paralleled by the changes in concentration of chloride ion. In the initial stage there is a dilution of chloride without a net change in the total amount of this anion. In the late vacuolar stage the increase in chloride content corresponds roughly to that in total cations. Up to the appearance of the dense nuclear cataract the increase in electrolytes is not quantitatively striking and amounts to approximately an 18 per cent increase in total electrolytes. However, with the onset of the nuclear cataract a tremendous increase in the amount of salt results (Fig. 7). This marked change in electrolyte content is characterized by extremely high levels of Na and Cl, with low levels of K. The large increase in salt can account for all of the water gained by these cataracts. At this stage the lens has lost its ability to maintain normal permeability characteristics, in that Na and Cl, which are normally excluded, find ready access into the intralenticular spaces, and dulcitol is no longer retained. The accumulation of salt is accompanied by a movement of water so that the situation becomes a classical demonstration of colloidal osmotic or Donnan
swelling. It has been shown in other cells that this type of osmotic swelling is an unstable condition in which equilibrium is never established unless an external force, such as a hydrostatic pressure, is imposed. Otherwise, the cell continues to swell until it eventually bursts. Since most cell membranes are permeable to electrolytes and impermeable to proteins, the question may be raised why cells do not generally undergo Donnan swelling. The explanation is that all cells are apparently endowed with a cation pump. It is by this mechanism that a cell prevents the free movement of cations by excluding Na and retaining K. Poisoning the pump with ouabain or interfering with the metabolism so that energy is not adequate to maintain the pump are two examples of conditions which would eventually lead to Donnan swelling.

The effect of galactose on cation pump mechanism

One reason for the striking osmotic swelling observed in the nuclear cataract stage may be that the cation pump fails to function. This possible explanation was tested by studying in vitro the uptake of $^4$K and $^{21}$Na by the rat lens at the various stages of cataract development. In the initial stage there is a slight drop in the $^4$K uptake and a further drop in the late vacuolar stage (Fig. 8). However, it is interesting to note that the nuclear cataract is equally effective in concentrating $^4$K as is the cataract of the late vacuolar stage, so there is no sudden decrease in the activity of the K pump to account for the marked Donnan swelling. The data obtained on the $^{21}$Na uptake mechanism illustrate the ability of the normal lens to exclude Na (Fig. 9). Only a small fraction of $^{21}$Na is taken by the rat lens during the incubation. The cataract in the initial vacuolar stage appears to exclude Na as well as the normal lens. However, in the cataract of the late vacuolar stage there is a definite uptake of $^{21}$Na. As the cataract progresses to the nuclear opacity stage, the lens seems unable to exclude Na, as evidenced by the marked uptake of $^{21}$Na.

The results of the cation uptake studies are expressed as ratios of the concentration of $^4$K or $^{21}$Na in the lens against that in the medium. The values may be somewhat misleading. For example, the uptake of $^4$K by the initial vacuolar stage cataract appears to be significantly lower than that of the control lens. However, if the increase in hydration of the cataract is taken into consideration, the amount of $^4$K actually taken up is very close to that of the control. A more meaningful analysis of changes in cation pump activity is to measure the rate of exchange of cations or the turnover rate. This type of flux measurements for K in the lens has been accom-
plished by Thoft\textsuperscript{16} and is now being made to determine changes in the K-concentrating mechanism in the lenses of galactose-fed rats, as well as in rabbit lenses incubated in a high galactose-containing medium. The results have shown that the uptake of K by the lenses in the early cataract stage was at normal rate or slightly higher.\textsuperscript{16} Even in the nuclear cataract stage the K pump is 50 per cent of normal; therefore, the changes in the cation pump do not appear to be responsible for the major alteration occurring in the nuclear cataract stage.\textsuperscript{16} In the rabbit lens exposed to high levels of galactose for 20 hours, the rate of K exchange was found to be higher than that of the control lens.\textsuperscript{16} The increase in K flux observed in the lens incubated in galactose indicated that the rate of both uptake and leakout of K is increased. In all probability the increase in K flux is initiated by an increase in the leakout of K. This fact would be consistent with the AIB uptake studies. The lens exposed to galactose undergoes osmotic swelling, the result of which is an increase in permeability.

When exposed to a high level of galactose, the lens appears to be much more effective in preserving the normal distribution of cations than of the amino acids. This is illustrated in the initial vacuolar stage cataract where there is a slight loss of K and gain of Na, but the 40 to 50 per cent decrease in the amino acid content is much more striking.\textsuperscript{18} Furthermore, the rabbit lens incubated in galactose lost 40 per cent of its free amino acids but incurred only a slight change in the distribution of cations. The AIB uptake in vitro by the rabbit lens is depressed by 70 per cent in the presence of galactose, while the K flux is increased. The cation pump appears much more effective than the amino acid pump, in that its rate can be accelerated to compensate for the increase in lens permeability, and thus is less vulnerable to the toxic effects of galactose.

Since the cation pump is sufficiently active in the nuclear cataract stage, it is probably not the factor responsible for the marked loss in K, gain in Na and Cl and water which characterize the changes of the terminal stages of the cataract. A probable explanation for these changes is a continual increase in lens permeability during the entire course of the cataractous process. The early swelling caused by dulcitol retention initiates the changes in lens permeability. It accounts for loss in amino acids and also begins to affect the cation distribution, resulting in a Na increase and K decrease. The shift in cations does not necessarily cause an increase in hydration. As long as the rate of Na entry is matched by the rate of K loss, no gain in cations occurs and consequently no increase in tonicity. This is illustrated in the experiments in which the lens was exposed to cold temperatures. Under these conditions substantial changes in Na and K occurred.
without an increase in lens water. However, when a stage is reached where the gain in Na is no longer compensated for by the loss in K, then an increase in hydration occurs. Not only is there a net increase in Na but, since electroneutrality must be maintained, Cl also gains access into the lens. The increase in osmotically active components would have to be compensated for by an increase in water. This kind of situation might be involved at the nuclear cataract stage. At the onset of this stage of cataract, additional changes, such as the loss in ATP, may affect the permeability still further.

The object here was to point out the factors involved in the osmotic swelling that occurs during the development of galactose cataract. I have neglected to delineate the metabolic changes which are undoubtedly important, especially as they affect the later stages of the cataract. The recent finding of Becker and Cotlier that the lens permeability is dependent upon metabolism is particularly pertinent. Another finding related to metabolism which requires further study is the observation of Patterson and co-workers, who found that a diet rich in fats and proteins delays the nuclear cataract stage. Although some studies on protein metabolism in galactose cataract have been reported, an attempt must be made to integrate these findings in order to account for the appearance of the dense nuclear opacity. Obviously, additional work is needed before we can have a more definitive picture concerning the pathogenesis of galactose cataract.

Summary
A summary of the events occurring in galactose cataract is presented in Fig. 10. The primary factor initiating this type of cataract is the high concentration of galactose in the aqueous humor. The abnormal level of galactose in the lens triggers the enzyme aldose reductase to convert galactose to dulcitol. Since the lens membranes are relatively impermeable to sugar alcohols, dulcitol once formed begins to accumulate, creating a hypertonic condition. To maintain osmotic equilibrium, water is drawn into the lens fibers. Unless, at this point, the swelling is checked by withdrawing galactose, the viability of the lens steadily declines. Even before any lens changes are grossly visible—the prevacuole stage—the resulting osmotic swelling has deleterious effects on the lens. The increase in hydration markedly affects the amino acid transport mechanism, accounting for the decrease in amino acid content. In the prevacuole stage, osmotic changes probably are also responsible for the appearance of hydrops, which later, with further increase in water, disintegrates to form vacuoles.

In the late vacuolar stage the dulcitol content is maintained at the level observed in the initial stage. This probably does not mean its synthesis has stopped, but since the permeability properties are sufficiently altered the rate of exit is equal to that of

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Fig. 10. Changes that occur during development of a galactose cataract.
its formation. There is only a slight increase in water, but changes in cations reveal a dangerously abnormal condition. The Na content is approaching the level of K, indicating that the lens is having difficulty excluding Na. The significant lowering of ATP may be contributing to the marked changes in cation. In spite of these changes there is no major change in hydration during the period just prior to the appearance of the nuclear opacity.

The nuclear cataract stage is characterized by another and more pronounced increase in lens hydration. The swelling is marked changes in cation. In spite of these changes there is no major change in hydration during the period just prior to the appearance of the nuclear opacity.

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