Variations in ATPase Activity in the Development of Experimental Cataracts[1] (35676)

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When young rats are placed on a high galactose diet, they develop mature cataracts in approximately 3 weeks. Each of the 3 weeks may be considered as a stage in the cataractogenic process inasmuch as the end of each of the weeks is punctuated by an important development.

During the first week, the cataractogenic process is initiated with major changes occurring in the lens. Growth of the lens is gradually stopped. Dulcitol accumulates as a result of the reduction of galactose. The retention of dulcitol leads to lens swelling. These changes reach a maximum at the end of the first week (1).

During the second week, these changes are maintained. Vacuoles, which are first observed on the second or third day in the equatorial region, occupy a steadily increasing area which extends towards the central pole of the lens. During the second week, the process is still reversible. However, at about the end of 2 weeks, it becomes irreversible (2).

During the third week, the effects of galactose feeding persist and the cataractogenic process culminates in the relatively sudden appearance of a mature cataract which is observed as a white opacity that is visible to the naked eye and is located in the central portion of the lens. Termination of the galactose diet during the third week usually will not prevent the development of a mature cataract. The appearance of a mature cataract coincides with major physiological changes in the lens, which are consistent with the destruction of lens fiber membranes and a free exchange of cations and small molecules between the lens and its environment (1, 3, 4).

Similar events occur in the development of mature cataracts as a result of diabetes or the feeding of a high xylose diet. The time required for the development of a mature cataract, however, is variable and seems to correlate with the degree of lens swelling (5). The exact connection between lens swelling, irreversibility, and the final destruction of lens fiber membranes is unknown.

It is possible that lens swelling or some parallel phenomena results in the gradual changes which lead to a sudden destruction of cell membranes when the reserves of compensating mechanisms are depleted. Inasmuch as Na/K ATPase is associated with cell membranes and believed to be responsible for maintaining cellular cation and water balance (6), it is possible that the critical change occurring during the process of cataractogenesis may be associated with changes in this key enzyme.

The present paper reports changes occurring in the levels of Na/K ATPase and Mg ATPase at key periods (0, 6, 15, and 23 days) during the course of cataractogenesis. Na/K ATPase is associated with cell membranes; is dependent on the presence of Na, K, and Mg ions for activity; and is inhibited by ouabain. Mg ATPase is nonspecific, activated by Mg alone, and insensitive to ouabain.

Methods. Male, Sprague-Dawley rats, 4–5-weeks-old and weighing 80–85 g, were placed on an unlimited diet consisting of 35% galactose and 65% ground chow for an experimental period of 23 days. Control groups were fed laboratory chow. Animals were sacrificed by decapitation after 0, 6, 15, and 23

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days of the experiment. The eyes were enucleated as previously described (7). The lenses were examined under a dissecting microscope; and any adhering ciliary body was dissected away with a fine pair of forceps. Any damaged lenses were discarded. When desired, the epithelium and capsule were removed from the rest of the lens by making an incision through the posterior capsule and peeling them away from the cortex with a pair of fine forceps.

Whole lenses and decapsulated lenses were individually homogenized in 1.5 ml of a buffer containing in final concentration 0.031 *M* glycylglycine, 0.029 *M* imidazole, and 0.003 *M* magnesium chloride adusted to a pH of 7.4. Aliquots of 0.1 ml were placed in small test tubes and frozen by placing them in a bath containing dry ice and acetone. Samples were stored at —20° until analysis were made at a later time.

ATPase activity was determined by measuring the amount of P_i released from ATP by the enzyme under standard conditions. The value for total ATPase activity was obtained when Mg, Na, and K ions were all present in the medium. The value for Mg ATPase activity was obtained when Mg ions were present and Na and K ions were absent from the medium. To insure against any activation of the Na/K ATPase by cations from the lens, ouabain was added to the medium that did not contain Na or K. The activity of the Na/K ATPase was calculated by subtracting the Mg portion from the total ATPase activity.

A 0.1-ml sample of lens homogenate was incubated in a final volume of 0.4 ml at 44° for 30 min. Except when indicated, the incubation medium contained in final concentration 2.7 m*M* ATP, 90 m*M* NaCl, 22 m*M* KCl, and 1 m*M* oaubain. The reaction was stopped by the addition of 0.1 ml of 0.24 *M* silicotungstic acid containing 2.2% perchloric acid. The tubes were mixed and placed on ice. All further work was done in an ice bath until the final stage of color development, which was done at room temperature.

The inorganic phosphorus was determined by an extraction procedure developed by Wahler and Wollenberger (8) which was modified by Post (9). This method was further modified in this laboratory to a semimicrotechnique.

One-half ml of sodium molybdate reagent was added to the solution containing 0.4 ml of the incubation mixture plus 0.1 ml of the silicotungstic acid reagent. The sodium molybdate reagent contains 245 ml of 60% $HClO_4$; 90 g of NaOH, adusted to pH 7.4; and 14.5 g of Na_2MoO_4/liter. One ml of butyl acetate was added to the same tube and then mixed thoroughly on a Vortex Jr. mixer. The tubes were centrifuged at 1800 rpm for 2 min to separate the organic layer from the aqueous layer. A 0.5-ml aliquot from the

TABLE I. Activity of ATPase in Whole Lenses from Normal and Galactose-Fed Rats (μM P_i per lens per hour ± SD).

In diet (days)	*n*	ATPase activity		
		Total	Mg	Na/K
		Normal		
0	10	0.93 ± 0.04	0.40 ± 0.05	0.53 ± 0.05
6	10	1.16 ± 0.06	0.47 ± 0.06	0.59 ± 0.04
15	10	1.21 ± 0.04	0.56 ± 0.06	0.65 ± 0.04
23	10	1.19 ± 0.04	0.58 ± 0.04	0.61 ± 0.03
		Galactose fed		
0	10	0.93 ± 0.04	0.40 ± 0.05	0.53 ± 0.05
6	10	0.92 ± 0.03	0.38 ± 0.03	0.54 ± 0.02
15	15	0.93 ± 0.05	0.43 ± 0.06	0.50 ± 0.05
23	10	0.77 ± 0.03	0.38 ± 0.03	0.39 ± 0.03
23[a]	10	0.54 ± 0.05	0.36 ± 0.04	0.18 ± 0.02

[a] Mature cataract.

TABLE II. Activity of ATPase in Decapsulated Lenses from Normal and Galactose-Fed Rats (μM P_i per lens per hour ± SD).

On diet (days)	n	ATPase activity		
		Total	Mg	Na/K
		Normal		
0	16	0.65 ± 0.03	0.25 ± 0.03	0.40 ± 0.04
6	10	0.77 ± 0.04	0.32 ± 0.03	0.45 ± 0.04
15	18	0.88 ± 0.04	0.39 ± 0.06	0.49 ± 0.04
23	14	0.85 ± 0.03	0.41 ± 0.05	0.44 ± 0.01
		Galactose fed		
0	16	0.65 ± 0.03	0.25 ± 0.03	0.40 ± 0.04
6	10	0.67 ± 0.04	0.32 ± 0.03	0.35 ± 0.04
15	13	0.56 ± 0.05	0.34 ± 0.04	0.22 ± 0.03
23	10	0.54 ± 0.04	0.36 ± 0.03	0.18 ± 0.03
23[a]	10	0.41 ± 0.04	0.31 ± 0.03	0.10 ± 0.02

[a] Mature cataract.

top, organic layer, containing the phosphomolybdate complex was removed and placed in a clean test tube. To this aliquot, 0.5 ml of an isopropanol reagent (500 ml of isopropanol containing 5.1 mg of $CuCl_2 \cdot 2H_2O$ and 7.5 ml of concentrated H_2SO_4) was added. One drop of mercaptoethanol, a reducing agent, was added; and the contents were mixed well on a Vortex Jr. mixer. The color was allowed to develop for 30 min. The optical density was read at 725 mμ on a spectrophotometer and compared with a standard containing inorganic phosphate. Activity is expressed in terms of the amount of inorganic phosphate released per unit per hour.

Epithelial cell counts were made as follows: The epithelia and capsules were removed and immediately placed in tubes containing 0.5 ml of 2% citric acid. The tubes were periodically agitated. (The citric acid plus the agitation lyses the cells, freeing the nuclei from the cell and from the capsule.) Each tube was checked under an inverted microscope to determine if all the cells were lysed and freed from the capsule. If they were, 0.1 ml of crystal violet (0.1% crystal violet in 0.1 *M* citric acid) was added to stain the nuclei blue for easier counting. An aliquot was then taken for enumeration in a hemocytometer. If all the cells were not lysed, the tube was agitated until they were, then stained, and counted.

Dry weights were determined by removing the lenses and cleaning them as described above, weighing them quickly and placing them in an oven at 90° and drying them to constant weight. The standard time period was 16 hr.

Results. The level of total ATPase activity, Mg ATPase activity, and, by difference, Na/K ATPase activity for whole lenses from normal control and galactose fed rats at 0, 6, 15, and 23 days is shown in Table I. Similar data for decapsulated lenses which represents the activity of the fibers is shown in Table II, and as calculated for the epithelium by subtracting the values for decap-

TABLE III. The Calculated Activity of ATPase in Epithelium plus Capsule from Normal and Galactose-Fed Rats (μM P_i per lens per hour).

On diet (days)	ATPase activity		
	Total	Mg	Na/K
	Normal		
0	0.28	0.15	0.13
6	0.29	0.15	0.14
15	0.33	0.17	0.15
23	0.34	0.17	0.17
	Galactose fed		
0	0.28	1.15	0.13
6	0.25	0.06	0.19
15	0.37	0.09	0.28
23	0.23	0.02	0.21
23[a]	0.13	0.05	0.08

[a] Mature cataract.

TABLE IV. Activity of ATPase in Isolated Epithelia plus Capsules from Normal and Galactose-Fed Rats (μM P_i per lens per hour).

On diet (days)	n	ATPase activity		
		Total	Mg	Na/K
		Normal		
0	5	0.25	0.14	0.11
6	3	0.27	0.15	0.12
15	3	0.31	0.16	0.15
23	4	0.33	0.17	0.16
		Galactose fed		
0	5	0.25	0.14	0.11
6	4	0.22	0.05	0.17
15	3	0.33	0.09	0.24
23	5	0.21	0.02	0.19
23[a]	4	0.12	0.05	0.07

[a] Mature cataract.

sulated lenses from comparable values for total lenses in Table III. The values for Na/K ATPase activity were described in a preliminary report (10) and are repeated here for comparison with the activity of Mg ATPase and for the calculation of specific activity. The level of activity of Na/K ATPase in the decapsulated lens is about 75% of that found in the whole lens. This is in agreement with the ratio reported by Yuge for calf lens (11). The values calculated for activity in the epithelium plus capsule shown in Table III were confirmed by direct analysis of activity in isolated epithelia plus capsules. These results are shown in Table IV.

Changes in the epithelial cell population in lenses from normal and galactose-fed rats during the experimental period are shown in Table V. The epithelial cell population for lenses from rats fed a normal diet are in agreement with those reported by VonSallman (12) for rats of a similar age. The observation of a cell population almost three times as high as normal in rats fed a galactose diet is in keeping with histological studies that demonstrate a proliferation and stratification of epithelial cells during the cataractogenic period (13).

The ATPase activities per epithelial cell were calculated by dividing the average activity of the epithelium plus capsule, as shown in Table III, by the average number of epithelial cells in comparable lenses as shown in Table IV. The calculated results are shown in Table VI. The Mg ATPase and the Na/K ATPase activities per epithelial cell are essentially the same throughout the experimental period for lenses from rats receiving a normal diet. The levels of activity per epithelial cell are lower in the lenses obtained from rats on a galactose diet.

The dry weights of lenses obtained from rats on normal and galactose diets of comparable age and experimental conditions to those

TABLE V. Cell Counts in Lens Epithelia from Normal and Galactose-Fed Rats (in thousands).

On diet (days)	Normal		Galactose fed	
	n	Cells/lens	n	Cells/lens
0	10	92 ± 0.5	10	92 ± 0.5
6	10	102 ± 0.7	11	165 ± 0.7
15	10	119 ± 0.6	12	262 ± 0.7
23	10	120 ± 0.8	12	316 ± 1.0
23[a]			15	316 ± 1.0

[a] Mature cataract.

TABLE VI. The Activity of ATPase per Lens Epithelial Cell from Normal and Galactose-Fed Rats ($\mu\mu M$ P_i cell per hour).

On diet (days)	ATPase activity	
	Mg	Na/K
	Normal	
0	1.6	1.4
6	1.5	1.3
15	1.4	1.3
23	1.4	1.5
	Galactose fed	
0	1.6	1.4
6	0.4	1.2
15	0.4	1.1
23	0.2	0.7
23[a]	0.2	0.3

[a] Mature cataract.

reported in other experiments in this series are shown in Table VII. The specific activities for Mg ATPase and Na/K ATPase per unit of dry weight are depicted in Figs. 1 and 2.

The effects of galactose feeding on the levels of ATPase activity in the epithelial and fiber portions of the lens may be studied by comparing the activities found in lenses from galactose-fed rats with those in the lenses of comparable rats that have been on a normal diet. This type of comparison has been made in Table VIII by calculating the activity of the experimental group as a percentage of the control. The most pronounced effects of galactose feeding appear to be an early and pronounced lowering of Mg ATPase activity in the epithelium, and, at 15 days, a marked lowering of the Na/K ATPase activity in the fibers.

Discussion. The changes occurring in lens epithelia during the cataractogenic process take place under circumstances of cell proliferation, therefore, changes in ATPase activity are the possible resultant of many simultaneous processes. The significance of a lowered activity of Mg ATPase in lenses from galactose-fed rats is not known and it is difficult to postulate inasmuch as the role of this type of enzyme activity in cell physiology remains unknown.

The fiber mass of the lens remains relatively stable during the cataractogenic process. Therefore, the changes in ATPase activity are more likely to be the resultant of effects on the enzyme without the complications of cell division and new cell formation. Viewed in this light, the decrease in Na/K ATPase activity in the decapsulated lens or lens fibers after 15 days of galactose feeding might be the result of enzyme destruction or inactivation. The fact that Mg ATPase activity is not lowered suggests that this enzyme is not being destroyed and that the decrease in Na/K ATPase activity may be related to inactivation of the enzyme. Inasmuch as this is a sulfhydryl enzyme, inactivation might be related to oxidation or to chemical combination. In red blood cells, the activity of Na/K ATPase is known to be decreased by oxidized glutathione (14) and preliminary experi-

TABLE VII. The Dry Weight of Lenses from Normal and Galactose-Fed Rats (mg ± SD).

On diet (days)	Total lens		Decapsulated lens		Epithelium and capsule	
	n	wt	*n*	wt	*n*	wt
			Normal			
0	15	7.9 ± 0.2	15	7.6 ± 0.1	5	0.4 ± 0.1
6	13	9.0 ± 0.1	15	8.6 ± 0.2	4	0.4 ± 0.2
15	18	10.4 ± 0.3	12	9.9 ± 0.2	4	0.6 ± 0.1
23	15	11.7 ± 0.2	14	11.0 ± 0.3	5	0.6 ± 0.2
			Galactose fed			
0	15	7.9 ± 0.2	15	7.6 ± 0.1	5	0.4 ± 0.1
6	12	8.5 ± 0.3	10	7.9 ± 0.2	5	0.6 ± 0.3
15	12	9.3 ± 0.5	13	8.5 ± 0.3	5	0.9 ± 0.2
23	13	9.6 ± 0.4	12	8.5 ± 0.3	4	1.3 ± 0.2
23[a]						

[a] Mature cataract.

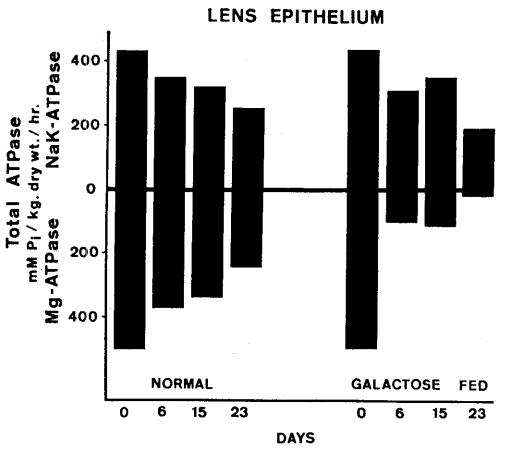

FIG. 1. Specific activity of ATPase in the epithelium of lenses from normal and galactose-fed rats during the cataractogenic period.

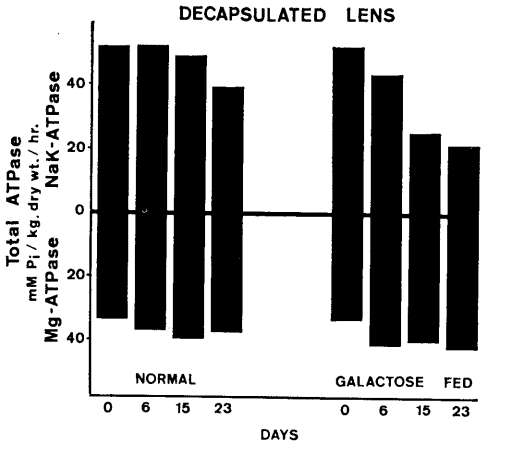

FIG. 2. Specific activity of ATPase in the fiber portion of lenses from normal and galactose-fed rats during the cataractogenic period.

ments in this laboratory indicate that the activity of this enzyme in whole lens may be decreased to less than 50% of normal activity in the presence of 10^{-3} *M* oxidized glutathione. The known decrease in glutathione levels early in the cataractogenic process (15) may result in a shift of the oxidation–reduction environment of the fiber with a loss of enzyme activity.

The observed results are consistent with the view that the final destruction of fiber membranes may be the result of the inability of the cation pump in the fiber membranes to maintain salt and water balance.

TABLE VIII. Comparison of Lenses [of the Same Age] from Galactose-Fed Rats with Those on a Normal Diet (percentage of normal value).

	On diet (days)		
	6	15	23
Epithelium			
Na/K ATPase activity per			
lens	136	175	123
unit of dry wt	91	109	75
cell	84	80	47
Mg ATPase activity per			
lens	40	53	12
unit of dry wt	27	33	7
cell	24	24	4
Fibers			
Na/K ATPase activity per			
lens	78	37	23
unit of dry wt	85	48	52
Mg ATPase activity per			
lens	100	87	88
unit of dry wt	110	103	113

Summary. Determinations of Mg ATPase and Na/K ATPase activity in lens epithelium and in decapsulated lenses or lens fibers were made in lenses obtained from rats on normal and galactose diets at 0, 6, 15, and 23 days of the experimental period. Galactose feeding resulted in a marked lowering of Mg ATPase activity in the epithelium after 6 days on the diet. The level of Na/K ATPase activity was markedly decreased in the fibers after 15 days on the diet. The lowering of Na/K ATPase activities in the fibers may provide a possible link between the known decrease in the glutathione early in the cataractogenic process and the known destruction of fiber membranes that is associated with the appearance of mature cataracts.

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