Lens assays on diabetic and galactosemic rats receiving diets that modify cataract development

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Cataract formation is delayed or prevented in diabetes and galactosemia by diets rich in fat and protein. This is accomplished in the presence of high levels of blood sugar and lens sugar alcohols. Diabetic rats on a high-fat diet for 235 days had lenses that were clear to the naked eye, whereas control animals developed cataracts in a median time of 65 days. The lens protein level of treated animals was about 90 per cent of normal. There was no evidence of lens swelling; however, the concentration of lens amino acids was low.

A theory for explaining the etiology of "sugar" cataracts is now gaining acceptance. Diabetes, galactosemia, and xylosemia are a group of conditions with common characteristics. They act synergistically in the production of cataracts[1]; they produce cataracts of similar appearance[2]; they develop cataracts in response to hyperglycemia[3, 4]; and they benefit in the prevention of cataracts by a lowering of the blood sugar.[5, 6]

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The proposed but not necessarily proved steps in this theory, following hyperglycemia, are as follows: aqueous sugar is increased[7]; transport of sugar into the lens is increased; the excess lens sugar is reduced by aldose reductase to give high levels of sugar alcohols[8]; the lens swells in response to the osmotic effects of these sugar alcohols[9]; the active transport that concentrates free amino acids in the lens is decreased by the swelling[10]; the lens free amino acid level drops[11]; protein synthesis is arrested[12, 13]; the fiber membranes lose their selective permeability and become porous partitions as a result of a deficiency of structural proteins or proteins associated with transport[14]; the remaining lens proteins are precipitated by the loss of a solubilizing factor or by the entrance of a precipitating factor[15]; and the lens thus appears white and opaque.

These steps are accompanied by certain morphologic changes. At first, vacuoles are observed around the periphery of the lens and this finding has been correlated with lens swelling.[9] Later, the lens suddenly (24 hours) becomes opaque and this change has been correlated with a rupture of the fiber membranes.[14] Vacuoles will disappear if the cataractogenic process is reversed. The appearance of the white opaque lens, however, represents the formation of a mature cataract and this change is irreversible. In this paper, the term "cataract" is used to designate a mature cataract.

A major piece of evidence contrary to the proposed theory is the observation that cataracts are delayed or prevented, in the presence of hyperglycemia, by diets that have a high content of protein and fat.[16-18]

It is the purpose of this paper to present new data which will allow further consideration of the mechanism involved in the prevention or delaying of sugar cataracts by these diets.

Methods

Male Sprague-Dawley rats weighing 125 ± 10 grams were used in all experiments. Cataracts were determined by observing the lenses with the naked eye each day for the appearance of a white opacity.

Diabetes was produced by the intravenous injection of 50 mg. of alloxan monohydrate per kilogram of body weight. Animals were given unlimited amounts of water and food. All rats were fed a dog chow (Purina) diet for the first 38 days after the injection of alloxan, and blood sugars were determined by the oxidase method.[19] Only animals with blood sugar averages above 400 mg. per cent were used. On the thirty-eighth day the animals were divided into two groups. One group (10 rats) remained on a chow diet and the other (22 rats) was placed on a high-fat diet consisting of a mixture of 65 per cent ground chow and 35 per cent corn oil. In the latter group 45 per cent of the animals died without developing cataracts. These are not included in the results.

The methods for assaying lens amino acids,[11] sugar alcohols,[14] fructose,[14] and glucose[14] have been described in previous reports.

Galactose-fed animals were fed a mixture of 65 per cent ground chow and 35 per cent galactose 4 days of each week. The amount given was adjusted to the rats that ate the least so that all the rats received the same total amount of galactose. On Mondays, Wednesdays, and Fridays the animals were either starved or received limited amounts of dietary supplements—glucose, 8.0 Gm.; casein, 8.0 Gm.; corn oil, 4.0 Gm. (a smaller amount of fat to approximate caloric equivalence); or two component mixtures with half the above amounts. Where levels of dulcitol were determined, they represented the average values obtained from analyzing four lenses from rats that were sacrificed 24 hours after they were given the dietary supplement on Wednesdays or the usual diet containing galactose on Thursdays.

Results

Diabetic rats on a chow diet developed cataracts in a median time of 65 days and a maximum time of 87 days. Diabetic rats given a high-fat diet after 38 days of diabetes did not develop cataracts (Table I).

The rats receiving the high-fat diet were kept for a total of 235 days. The average blood sugar fell to 346 mg. per cent at 231 days. No cataracts were present. On the two hundred thirty-fourth day the ani-

Table I. Effect of high-fat diet* on the development of diabetic cataracts

Days of diabetes	*Average blood sugar for group of 10 control rats*		*Cumulative per cent cataracts*	*Average blood sugar for group of 12 high-fat diet rats*		*Cumulative per cent cataracts*
	Range	*Median*		*Range*	*Median*	
8	417 to 567	495	0	350 to 510	436	0
19	460 to 595	429	0	390 to 560	482	0
27	460 to 550	512	0	390 to 545	480	0
33	432 to 590	518	0	390 to 595	495	0
56	368 to 595	450	0	372 to 487	420	0
61			30	355 to 532	439	0
76	500 to 540†	508	70	380 to 490	452	0
83			90	334 to 510	415	0
87			100			0

*Diet started after 38 days of diabetes.
†Three rats only.

Table II. Assays on lenses of diabetic rats fed a high-fat diet for 235 days

	Range	*Median*
Wet weight (mg.)	43.1 to 45.1	43.7
Dry weight (mg.)	17.3 to 18.9	18.3
Water (per cent)	57 to 60	59
Glucose (mg./100 Gm. of lens)	46 to 53	49
Fructose (mg./100 Gm. of lens)	243 to 260	253
Sorbitol (mg./100 Gm. of lens)	578 to 645	620

mals were transported about 500 miles in the back seat of a car, kept overnight, and sacrificed the following morning. The right lenses of 6 rats were assayed for water content, and those of 6 additional rats for glucose, fructose, and sorbitol (Table II). The 12 lenses from the left eyes were pooled and assayed for amino acids (Table III). The ratios of the concentration of free amino acids in the lens water to the concentration in the plasma were calculated and compared with values calculated

Table III. Free amino acid levels in lenses of diabetic rats fed high-fat diet for 235 days and control normal rats (mM./Kg. lens water)

	Lens		*Plasma*	
	Normal	*Diabetic*	*Normal*	*Diabetic*
Aspartic acid	0.17	0.06	Trace	Trace
Threonine	0.46	0.22	0.27	0.23
Serine	0.82	0.32	0.31	0.32
Proline	Trace	Trace	0.27	0.19
Glutamic acid	2.70	0.94	0.14	0.13
Glycine	0.38	0.31	0.46	0.50
Alanine	0.84	0.21	0.50	0.27
Valine	0.44	0.16	0.21	0.20
Methionine	0.10	0.05	0.06	0.05
Isoleucine	0.25	0.11	0.10	0.11
Leucine	0.48	0.20	0.18	0.16
Tyrosine	1.02	0.33	0.12	*
Phenylalanine	0.50	0.19	0.04	*

*Peaks low and poorly separated.

Table IV. Lens water to plasma ratio of free amino acids for diabetic rats fed a high-fat diet for 235 days and control normal rats* compared with similar data for 6 day diabetic and normal rabbits†

	Normal		*Diabetic*	
	Rats	*Rabbits*	*Rats*	*Rabbits*
Threonine	1.7	6.5	0.9	0.5
Serine	2.6	3.0	1.0	0.5
Glutamic acid	19.3	115.0	7.2	10.7
Glycine	0.8	1.1	0.6	1.3
Alanine	1.7	5.9	0.8	1.3
Valine	2.1	2.8	0.8	0.2
Methionine	1.7	5.4	1.0	1.0
Isoleucine	2.5	2.2	1.0	0.3
Leucine	2.7	2.4	1.3	0.3
Tyrosine	8.5	8.9		2.0
Phenylalanine	12.5	5.7		0.8

*Calculated from Table III.
†Calculated from Reddy and Kinsey.[11]

from data in the literature[11] for normal and diabetic rabbits (Table IV). At the time of sacrifice all of the lenses were clear to the naked eye and had the appearance of normal lenses. The sorbitol, fructose, and glucose levels were 620, 253, and 49 mg. per 100 Gm. of lens, respectively. These values compare with levels of 903, 240, and 129 mg. per 100 Gm. obtained on 27 rats with 21 to 36 days of diabetes and an average blood sugar of 494 mg. per cent. Six rats with a similar duration and severity of diabetes that were starved for 40 hours gave values of 628, 214, and 32 mg. per 100 mg. of lens for sorbitol, fructose, and glucose. The level of glucose is most responsive to starvation. Therefore, the relatively low level of glucose in the long-term diabetic rats plus the fact that the average blood sugar at the time of sacrifice had dropped to 306 mg. per cent indicates that the rats may have eaten a little less than usual during their transportation from one laboratory to another.

Swelling of the lens is best determined in relation to the dry weight, inasmuch as the total wet weight of lenses from experimental animals may be less than normal. The ratio of the wet weight to the dry weight has been used as a hydration index.[20] In normal animals this index is about 2.45 and in untreated diabetic animals it is 2.70.[20] This agrees with other data[21] which indicate a hydration index of 2.47 for normal 50 mg. rat lenses. In the rats that received the high-fat diet it averaged 2.45.

The plasma amino acid levels of the long-term diabetic animals were similar to those found in normal rats of the same age (Table III). The lens levels, however, were markedly reduced in the diabetic rats. This reduction is similar to that reported for rabbits, as indicated by a comparison of the ratios of concentration of amino acids in the lens water to the concentration in the plasma (Table IV). The specific differences in the normal groups for threonine, glutamic acid, alanine, and methionine may be due to a species or age difference. In the diabetic groups, with the exception of glutamic acid, the ratios were approximately one or less and were similar for young diabetic rabbits and old rats on a high-fat diet.

Table V. Effect of dietary supplements on the development of galactose cataracts

Treatment on Monday, Wednesday, and Friday	*No. of rats*	*Median time for cataract appearance (days)*	*Cataracts at 100 days (per cent)*
Starved	17	27	100
Glucose	38	37	84
Corn oil	19	41	89
Casein	19	56	79
Corn oil plus glucose	43	45	75
Glucose plus casein	33	51	82
Corn oil plus casein	49	60	61

The dry weight of the lens is nearly all accounted for by the protein content. Normal lenses from rats the same age as the experimental diabetic group had a wet weight of 51.3 mg. and an estimated dry weight of 21 mg. The dry weight (18.4 mg.) for the diabetic group was 88 per cent of normal. Five diabetic rats that were starved for 40 hours each week were observed at the same time as the rats on a high-fat diet. At the time these animals were sacrificed (237 days), one of the rats had a cataract. The average weight of the 9 noncataractous lenses were 38.6 mg. The dry weight may be estimated as being between 14.3 and 15.8 mg., depending on the amount of swelling. This would be 68 to 75 per cent of the normal dry weight. The dry weight of lenses from galactose-fed rats just prior to cataract formation is 74 per cent of normal.[20] Thus, there appears to be a correlation between the prevention of cataracts and the maintenance of a high protein level in the lens.

Galactose-fed rats receiving dietary supplements developed cataracts at different times, depending on the supplement (Table V). Casein, of the single component supplements, and casein plus corn oil, of the double component supplements, were

Table VI. Dulcitol in lenses of galactose-fed rats receiving dietary supplements for 3 days each week

	On day of supplement feeding				*On day of galactose feeding*			
	Casein-fat group		*Glucose group*		*Casein-fat group*		*Glucose group*	
Week	*Weight (mg.)*	*Dulcitol (mg./100 Gm.)*	*Weight (mg.)*	*Dulcitol (mg./100 Gm.)*	*Weight (mg.)*	*Dulcitol (mg./100 Gm.)*	*Weight (mg.)*	*Dulcitol (mg./100 Gm.)*
1	19.2*	465	19.8	518	21.0	1,160	20.6	1,012
2	21.8	702	22.1	784	21.8	910	22.4	1,020
3	24.1	816	25.1	708	25.6	1,050	28.2	1,210
4	26.0	910	30.5	970	24.5	1,190	28.5	951
5	27.2	864	26.5	863	28.6	1,065	29.2	1,152
Average	23.7	751	24.8	769	24.3	1,075	25.8	1,069

*All numbers represent the average value for four lenses.

most effective in delaying and preventing cataracts. This is in general agreement with previous results.[17]

Two groups of galactose-fed rats receiving a supplement of glucose in one group and casein plus corn oil in another had the same levels of dulcitol for the first 5 weeks (Table VI).

Discussion

The prevention of cataracts by a given procedure provides data which may be helpful in defining the process of cataractogenesis. Prevention may be associated with two different mechanisms. First, it may prevent the "insult" to the lens and, second, it may overcome the effect of the "insult."

With diets containing a high level of fat and protein, the preventive action is associated with overcoming the effects of the "insult." In the experiment concerned with diabetes, the blood sugar levels during the cataractogenic period overlapped those of the control group, yet the control group had a 100 per cent incidence of cataracts while the incidence in the group on the high-fat diet was zero. In the experiment in which rats received the galactose diet supplemented with either glucose or casein plus fat, all had equivalent concentrations of dulcitol in their lenses and yet the median time required for cataract formation was 50 per cent longer and the per cent without cataracts at 100 days was more than double for the group which received the casein plus fat supplement. Thus, in both instances, the "insult" was adequate to produce cataracts, but appropriate dietary supplements effectively overcame the "insult" by delaying or preventing cataract formation.

After 235 days on a high-fat diet, lenses taken from diabetic rats are clear and the lens protein level is relatively high. The concentration of amino acids, however, is low. Thus, cataracts can be prevented without raising the concentration of amino acids to normal levels.

The finding of a normal hydration index associated with a low level of lens amino acids indicates that some process other than swelling must play a role in lowering the concentration of lens amino acids.

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