Summary. Hexosamine analysis reflecting the content of mucopolysaccharides of the renal papillae of rats in varying states of diuresis and anti-diuresis was carried out. States of anti-diuresis were associated with low levels of hexosamine, while states of diuresis were associated with high levels of hexosamine. Hexosamine contents were correlated with physiologic and histochemical data.

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Lens Cell Membrane Permeability and Cataract Formation.* (29141)

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Theories concerning the mechanism of cataract development following the production of experimental diabetes or the feeding of high galactose diets have been based on data obtained during the cataractogenic period(1-4) and on analyses of lenses with mature cataracts(5). There has been little correlation between morphological and physiological changes. It has been suggested that the vacuoles which appear early in the cataractogenic process are due to osmotic swelling(6) resulting from the high levels of intracellular polyols(7) which, in the lens, follow hyperglycemia. The later and sudden appearance of an opaque lens has not been correlated with a biochemical or physiological change. In this paper is presented evidence that the sudden appearance of cataract is associated with an equally sudden change in the permeability of the lens fiber or cell.

Methods. All experiments were done with diabetic or galactose fed Sprague-Dawley rats. Cataracts were determined with the naked eye by observing the lens daily and noting the day on which the lens became opaque.

The first experiment was run to determine the concentration of lens sorbitol, glucose, fructose, and adenosine triphosphate (ATP) and the values for the lens weight, and extracellular water, before and at the time of diabetic cataract formation. Thirty-eight day old rats were made diabetic by intravenous injection of 50 mg of alloxan monohydrate per kg of body weight. Only rats with average blood sugar over 400 mg percent were used. The median time for cataract formation was 76 days.

The second experiment was designed to provide information similar to that obtained in the first. In this case, however, the cataracts followed the feeding of a 35% galactose diet to rats with an initial age of 27 days. With this regimen, the median time for cataract formation is 18 days.

The lenses were removed and weighed as previously described(8).

Sorbitol was determined by the method of West and Rapoport(9) as modified by Faulkner(10); glucose by the oxidase method(11); fructose by the method of Roe(12); and

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TABLE I. Lens Changes Accompanying the Appearance of Cataracts.

	Without cataract			With cataract		
	No.	Range	Mean ± SD	No.	Range	Mean ± SD
Diabetes						
Wt, mg × 10	6	317- 406	347 ± 31	6	371-479	407 ± 40
Sorbitol, mg/100 g	9	560- 745	650 ± 63	8	100-273	213 ± 60
Glucose, mg/100 g	10	94- 210	131 ± 36	8	153-368	260 ± 85
Fructose, mg/100 g	8	268- 362	321 ± 40	8	111-257	165 ± 61
ATP, mg/100 g	10	74- 127	110 ± 21	6	34- 56	43 ± 9
Extracellular space, g/kg	22	180- 314	251 ± 38	2	596-638	617 ± 3
Galactosemia						
Wt, mg × 10	7	240- 342	297 ± 28	7	286-350	317 ± 28
Dulcitol, mg/100 g	16	700-1315	1200 ± 156	4	166-419	288 ± 133
Extracellular space, g/kg	6	113- 181	136 ± 26	7	334-566	421 ± 71

ATP by the firefly lantern method(13).

The extracellular space of the lens was determined by measuring the distribution of a substance that does not readily penetrate the cell membrane. Tritium labeled sorbitol which meets this requirement(14) was used. The lenses were weighed, incubated for 90 minutes in 0.5 ml of Tyrodes solution containing 50 mg percent of sorbitol with an activity of 136,000 cpm per mg of sorbitol, rinsed in Tyrodes, broken up in a counting bottle, mixed with 1 ml of Hyamine hydroxide (Packard) and then 20 ml of syntillation mixture (5.0 g of 2,5-diphenyloxazole, PPO, and 3.0 ml of 1,4-bis-2,5-phenyloxazolyl benzene, POPOP, in 1 liter of toluene), counted, and calculations made for extracellular water. Time studies with normal lenses indicated that an equilibrium was obtained between the sorbitol of the extracellular water and the incubation medium in less than 60 minutes. Incubations for as long as 4 hours did not change the distribution.

Results. The polyol levels in the lens tend to reach a steady concentration after the first 4-5 days. Therefore, the exact time at which determinations are made prior to the appearance of an opacity is not critical. In the case of diabetes weekly determinations were made during the 5 weeks preceding the 61st day of diabetes, and in the case of galactose fed animals determinations were made at 7, 10, 14 and 17 days. Determinations on cataractous lenses were made during the week following appearance of the cataract. Lens weights were compared on the basis of paired lenses from rats with unilateral cataracts. The results are summarized in the Table. The difference in the lens weights have P values of 0.08 and 0.002 respectively for the galactose fed and diabetic groups. The other differences are highly significant with P values less than 0.001.

With the appearance of cataracts the levels of the polyols, fructose and ATP, which occur in high concentration in the lens as compared with the aqueous, are markedly decreased. On the other hand, the level of glucose, which is relatively high in the aqueous, is markedly increased. These changes in concentrations are associated with a major increase in the extracellular space and a small but definite increase in lens weight.

Discussion. Coincident with the appearance of diabetic and galactose cataract the cell membrane loses its physiological function as a selectively permeable membrane and becomes a porous partition. This conclusion is supported by the observation of a shift of substances across the membrane in accord with the concentration gradient at the time cataracts are formed. Thus glucose enters the cell and raises the concentration in the lens water close to extracellular levels and at the same time fructose and polyols leave the lens. This is supported by the observed increase in the extracellular space of the lens.

The increase in lens weight, with the appearance of cataract, is consistent with a change in the cell membrane from a highly selective organ for active transport to an inert porous partition which is capable of retaining large molecules such as proteins. This limitation of protein diffusion permits

the development of an oncotic pressure which leads to an increase in lens water and lens weight.

During the period before the actual appearance of cataract, the observed biochemical and physiological changes are gradual and it would be difficult to correlate them with any distinct morphological change. There is an excellent correlation, however, between the appearance of a white opacity and the disruption of the cell membrane as determined by a variety of signs.

The demonstration of a change in permeability of the cell membrane as cataracts appear, makes it desirable to review an earlier proposal which stated that the white opacity is due to protein precipitation(15) following an increase in calcium or a loss of solubilizing factors.

The reason for the sudden disruption of the cell membrane is not known. It may be due to the depletion of a structural component of the membrane, or to the depletion of the reserve capacity of an essential functional component, such as ATP or Na-K ATPase, below a critical level. The fact that the polyol levels are elevated for many days before the cell membranes are disrupted argues against a physical and in favor of a chemical mechanism. The fact that cataracts can be delayed or prevented by special diets (4,16) without altering the cataractogenic diet supports this conclusion.

Studies with other types of cataracts indicate a similar loss of cell membrane function. In human senile cataracts the membrane no longer maintains cellular potassium levels(17, 18) and in radiation cataracts cellular proteins are lost(19). Thus, a study of the changes that occur in the cell membranes of the lens may lead to a more general understanding of the etiology of cataracts.

Summary. Analyses of rat lenses just before and just after the appearance of diabetic or galactose cataracts demonstrate that this event is accompanied by an abrupt disruption of the cell membranes of the lens. The concentrations of polyol, glucose and fructose in the lens approach those in the blood; and the extracellular space as determined with radioactive sorbitol is markedly increased. It is suggested that chemical changes in the cell membrane may hold the clue to the etiological mechanism of cataract formation.

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