Transport of amino acids into intraocular fluids and lens in diabetic rabbits

D. V. N. Reddy and V. Everett Kinsey

The transport of amino acids into the posterior and anterior chambers and lenses of rabbits which have alloxan diabetes was studied by measuring the rates of accumulation of non-metabolized amino acids, cycloleucine, and α-AIB, and by determining the concentration of amino acids ordinarily present in intraocular fluids and lenses of normal and diabetic animals. The rate of accumulation of cycloleucine in both aqueous humors and lenses in vivo is decreased significantly in diabetic animals. A similar reduction was found in the concentration of most naturally occurring amino acids in both the anterior aqueous humor and lenses. In cultured lenses removed from animals 48 hours or more after injection of alloxan, the accumulation of α-AIB is decreased to 50 per cent of normal.

The observed reduction in the transport of amino acids into intraocular fluids and lenses in diabetic animals is attributed to an impairment of active transport into both the aqueous humors and lens.

The steady state concentration of cycloleucine in the aqueous of the anterior chamber of normal animals was above that in both the plasma and posterior aqueous; the concentration in the vitreous humor was much lower than that in either of these fluids. These observations suggest that amino acids are actively transported into the anterior chamber across the anterior surface of the iris and unidirectionally moved from the vitreous humor into the blood.

Recent studies have shown that amino acids in rabbits are actively transported into both the posterior chamber and lens against concentration gradients.[1-5] The existance of two transport systems acting stepwise to move these compounds into the lens suggests that relatively high concentrations of free amino acids may be required for maintenance of normal protein metabolism, impairment of which might result in opacity of the lens. On the basis of this reasoning, transport of amino acids would be expected to diminish in eyes developing cataracts. To learn whether this is true, we measured the rate of accumulation and steady state distribution of amino acids in the aqueous humors and lenses of animals which have alloxan diabetes, a disease which usually results in cataract formation.

The transport of sugars in eyes of animals having diabetes has been investi-

From the Kresge Eye Institute, Detroit, Mich.

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gated,[5, 7] but we know of no analogous studies on amino acid transport.

Methods

Alloxan diabetes was induced in rabbits by a procedure which resulted in an unusually low mortality rate. Albino rabbits, weighing 1.8 to 2.3 kilograms, were fasted overnight but were given free access to drinking water. The animals were anesthetized lightly with ether and given a single intravenous injection of freshly prepared 5 per cent alloxan solution (100 mg. per kilogram body weight), followed immediately and every 4 hours thereafter for 48 hours by an intraperitoneal injection of 20 ml. of sterile 10 per cent glucose solution. An intravenous injection of 5 ml. of glucose solution was also given 24 and 34 hours following injection of alloxan and whenever animals showed signs of convulsion. A 5 per cent glucose solution was substituted for drinking water during the same 48 hour period. More than 90 per cent of the animals survived for a week and almost all had hyperglycemia with blood sugar levels between 350 and 800 mg. per cent as determined by a method employing glucose oxidase.[8]

C^{14}-labeled amino acids, 1-amino cyclopentane carboxylic acid (cycloleucine) and alpha amino isobutyric acid (α-AIB) were used to measure the rate of accumulation in intraocular fluids and lens according to methods previously described.[1, 2] These compounds are not found in nature, and since they are not metabolized, measurement of radioactivity from biologic samples gives a direct indication of the amount of amino acid present. Cycloleucine is absorbed so efficiently by the kidney tubules[9] that a constant plasma level can be maintained for several days following a single parenteral injection, which makes it possible without repeated injection to follow its uptake in intraocular fluids and lens over prolonged periods.

The uptake of labeled amino acids in the lens in vitro was studied by culturing them for 24 hours in a medium which had a composition the same as KEI-3,[10] but without other amino acids. The methods employed are described in detail elsewhere.[3]

The concentration of free amino acids in samples of plasma, anterior aqueous humor, and lenses taken from the same normal or diabetic rabbit was determined chromatographically with an automatic amino acid analyzer.[5, 11] Pooled samples, used for these analyses, were prepared by withdrawing an amount of plasma from each animal proportionate to the quantity of aqueous humor obtained from the same rabbit. This procedure was employed so that valid comparisons of the relative concentration of amino acids in the different fluids could be made.

Results

Table I shows the distribution of C^{14}-labeled cycloleucine in aqueous and vitreous humors and lenses of normal and diabetic rabbits 48 and 72 hours following parenteral administration. The concentration of the labeled compound in the posterior and anterior aqueous and lens, but not in the vitreous humor, of diabetic rabbits is significantly less than that in normal animals. For ease of comparison, the data for the 48 hour interval are shown graphically in Fig. 1.

The accumulation of α-AIB is also reduced in the aqueous humors of diabetic animals, the average concentration of tracer amino acid in the posterior and anterior chambers (6 eyes) of diabetic rabbits 50 minutes after parenteral administration being 69 per cent and 43 per cent, respectively, of normal.

The effect of the presence of diabetes on the distribution of naturally occurring amino acids in the aqueous humor and plasma was determined by comparing the steady state ratio of concentration of 18 amino acids in the anterior aqueous humor and plasma of diabetic and normal rabbits. Table II shows that in diabetic animals the

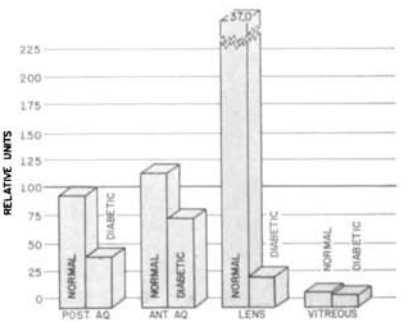

Fig. 1. Relative concentration of C^{14}-labeled cycloleucine in ocular fluids and lenses of normal and diabetic rabbits 48 hours after parenteral administration. All values are expressed as percentage of concentration in plasma water.

Table I. Distribution of C^{14}-labeled cycloleucine in ocular fluids and lenses in normal and diabetic rabbits. (Cycloleucine was administered to the diabetic animals 6 days after they received alloxan. All values are expressed as percentage of plasma water)

	Interval of distribution (hrs.)	*Normal*	*Diabetic*
Posterior aqueous	48	100 (7) 81-117	48 (6) 45-50
	72	88 (2) 83-93	64 (5) 59-71
Anterior aqueous	48	121 (6) 117-128	77 (6) 66-89
	72	130 (2) 130-130	88 (6) 71-103
Lens water	48	370 (6) 295-440	28 (6) 22-29
	72	530 (2) 524-535	45 (6) 37-57
Vitreous humor	48	14 (7) 9-16	11 (6) 8-12
	72	14 (2) 14-14	12 (6) 10-16

steady state ratio of concentration is reduced for all but three amino acids, where no change was observed. Thus, the accumulation of most naturally occuring amino acids in the aqueous humor is also reduced when the animals are diabetic.

The relative amount of tracer α-AIB accumulated during 24 hours of culture by lenses removed from rabbits at various intervals after injection of alloxan is shown by the shaded blocks (Fig. 2) in comparison with that found in lenses of normal animals (open parallelepiped used as background). The accumulation of α-AIB in all lenses from animals that had received alloxan 48 hours or more previously was reduced to approximately 50 per cent of normal. The reduction in uptake was still greater in four lenses removed from animals which had been diabetic for much longer periods of time (83 days). These lenses were opaque, and, when they were cultured, accumulated only 2 per cent of the normal amount of tracer α-AIB.

Fig. 3 shows the concentration of some naturally occurring amino acids and related compounds in lenses removed from animals given alloxan 6 days previously, expressed as percentage of that in lenses from normal rabbits. The data show the profound effect of the presence of diabetes on the amino acid composition of the lens. Except for arginine and lysine, the concentration of all compounds in the lens is depressed when the disease is present.

Discussion

The rate of accumulation of cycloleucine and α-AIB in the aqueous humors, and the steady state levels of most of the naturally occurring amino acids in the

Table II. Relative concentration of free amino acids in anterior aqueous humor and plasma of normal and diabetic rabbits. (Samples from diabetic animals were removed 6 days after injection of alloxan)

Concentration $\frac{\text{Aqueous humor}}{\text{Plasma water}}$	*Normal*	*Diabetic*
Alanine	1.5	1.0
Arginine	3.1	2.4
Aspartic acid	2.0	2.0
Cystathionine	1.0	1.0
Glutamic acid	3.6	1.6
Glycine	0.48	0.43
Histidine	1.3	1.0
Isoleucine	0.90	0.52
Leucine	1.0	0.54
Lysine	2.4	1.8
Methionine	0.67	0.40
Ornithine	1.7	1.4
Phenylalanine	1.4	0.75
Proline	1.1	0.68
Serine	1.5	1.5
Threonine	0.92	0.54
Tyrosine	1.5	1.0
Valine	1.0	0.46

aqueous humor of the anterior chamber were shown to be reduced in diabetic rabbits. The rate of accumulation of a substance in the aqueous humors, as well as its steady state concentration, is determined by the rates of diffusion, flow, and active transport.[12] An increase in rate of diffusion would tend to reduce the steady state ratio for a substance that normally has a value in excess of one, and raise the ratio for a substance that has a value less than one. Table II shows that the steady state ratios of amino acids in normal rabbits lie both above and below one. It shows also that the values for all but three of the steady state ratios were reduced when diabetes was present, and in no case was there an increase. Moreover, Becker[13] has shown that the flow rate in diabetic animals is unchanged. Therefore, it is likely that the reduced steady state ratio of amino acids in the anterior aqueous is due to a direct effect of some factor associated with diabetes on active transport into the posterior chamber, and possibly, too, a direct effect on any active transport taking place across the iris.

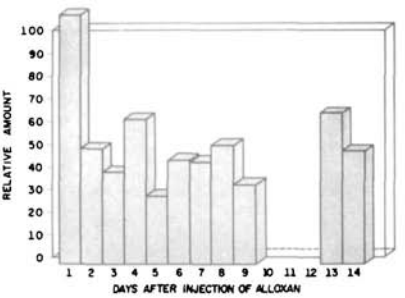

Fig. 2. Effect of injection of alloxan on the accumulation of C^{14}-labeled α-AIB in the lenses cultured for 24 hours. The value given by each block is an average for 6 lenses. The amount of amino acid accumulated by normal lenses per unit initial concentration of radioactivity in the medium is arbitrarily set to a value of 100 which is shown by open block in the background.

The accumulation of most amino acids in the lenses of diabetic animals is also

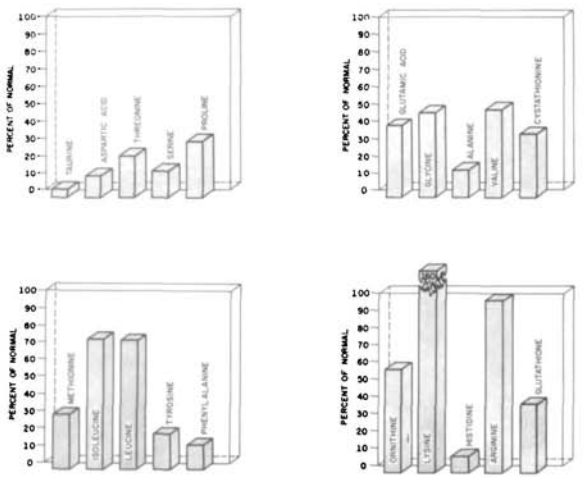

Fig. 3. Concentration of free amino acids and related compounds in the lenses of diabetic animals expressed as a percentage of that in the normal lenses. Lenses were removed from rabbits 6 days after injection of alloxan.

reduced (Figs. 1, 2, 3), possibly because of a direct effect on the processes involved in movement of amino acids into and out of the lens, as well as from their reduced availability in the aqueous humors. Fig. 1 shows that the reduction in the concentration of C^{14}-labeled cycloleucine in lenses of diabetic animals is much greater than could be accounted for on the basis of reduced concentration in the aqueous humor alone. The concentration of cycloleucine in the posterior and anterior chambers is reduced to 50 and 65 per cent, whereas the concentration in the lens is reduced to 10 per cent of normal. Thus, in addition to the reduction in the movement of the cycloleucine into aqueous humor noted in the diabetic animals, the presence of this disease must have a direct effect on its transport into or out of the lens.

The data in Table III show that the ratio of concentration of all but 4 amino acids in lens water to that in the aqueous humor was reduced, indicating that for naturally occurring amino acids, too, the concentration in the lens is decreased to an extent greater than could be accounted for on the basis of lowered concentration in the aqueous humors.

The apparent direct effect of some factor associated with diabetes on the mechanisms responsible for movement of amino acids into or out of the lens could be due to inhibition of active transport or to an increase in diffusion out of the lens. The results of preliminary studies based on measurements of transport of amino acids across a single surface seem to rule out the possibility that diabetic lenses become more "leaky," suggesting that it is active transport of amino acids into the lens, as well as into the aqueous humors, which is impaired in diabetic rabbits. Whatever the mechanism of action, it is tempting to speculate that the marked reduction in concentration of free amino acids in lenses of these animals may be a contributing factor in the etiology of diabetic cataract.

Two observations made in the course of this investigation, and in a previous one,[4] which have no special relevance to the main theme of this paper, appear to have important physiologic implications. While studying the distribution of naturally occuring amino acids in the eye, we observed that the concentration of several compounds in the anterior aqueous humor was slightly above that in the aqueous of the posterior chamber and the plasma.[4] This apparent movement against a concentration gradient suggested the possibility that some active transport may occur across the anterior surface of the iris. In the same study, the concentration of all of the amino acids in the vitreous humor was found to be much below that in both the aqueous humor of the posterior chamber and the plasma. This observation could be explained on the assumption that amino acids in the vitreous are depleted through utilization by the retina, an explanation usually given to account for the deficiency of glucose in the vitreous.[14] However, the explanation might lie in the existence of still another pump in the eye, one capable

Table III. Relative concentration of free amino acids in lens and anterior aqueous humor of normal and diabetic rabbits. (Samples from diabetic animals were removed 6 days after injection of alloxan)

Concentration $\frac{\text{Lens water}}{\text{Aqueous humor}}$		
	Normal	*Diabetic*
Alanine	4.2	1.3
Arginine	1.1	1.5
Aspartic acid	3.0	3.0
Cystathionine	1.9	0.70
Glutamic acid	32.0	6.7
Glycine	2.2	3.0
Histidine	2.3	0.43
Isoleucine	2.4	0.50
Leucine	2.4	0.50
Lysine	1.6	1.5
Methionine	8.0	2.5
Ornithine	3.0	1.6
Phenylalanine	4.1	1.0
Proline	5.2	2.3
Serine	2.7	0.79
Threonine	4.3	0.93
Tyrosine	5.9	2.0
Valine	2.8	0.43

of actively transporting amino acids across the retina. From data previously available, it was not possible to determine the portion of the relative deficiency of amino acids in vitreous humor attributable to utilization by the retina and the portion due to any active transport out of this body.

The data obtained in the present study suggest that utilization cannot be the sole explanation for deficiencies of amino acids. The results presented in Table I and Fig. 1 show that even after 72 hours the concentration of cycloleucine in the vitreous was only 10 per cent of that in either the aqueous of the posterior chamber or plasma. Since this amino acid is not metabolized, it appears that its deficiency must be due to loss from the vitreous by transport across the retina into the blood, perhaps as part of the meridional flow system recently described by Fowlks.[15]

Since the deficiency of the naturally occurring amino acids is, in general, no greater than that for cycloleucine, it is probable that these compounds may also be moved unidirectionally from vitreous to plasma.

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