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Studies on the crystalline lens
X. Transport of amino acids

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A study has been made of the accumulation of the amino acid C^{14}-labeled alpha amino isobutyric acid (α-AIB) in rabbit lenses cultured under varying conditions. In a medium containing no other amino acids, this compound accumulates at approximately a linear rate for the maximum period studied (48 hours), at which time the ratio of concentration in lens water to concentration present initially in the medium, is 22. Addition of nonlabeled α-AIB to culture medium saturates the transport system, the uptake of the tracer amino acid decreasing asymptotically with the total concentration of amino acid present. The apparent Michaelis-Menten constant k_m is 2.5 mmoles per liter and the maximum velocity is 0.25 μmoles per lens per hour. Neutral, but not basic or acidic, amino acids inhibit the transport of labeled α-AIB into the lens in varying degree. The gamma isomer of α-AIB does not inhibit transport and the D *forms of neutral amino acids are less effective inhibitors than the* L *forms. At least three separate systems, perhaps carriers or carrier sites, are involved in transporting amino acids in the lens. Accumulation of α-AIB requires energy, about three quarters of which can be supplied anaerobically through consumption of glucose, is highly dependent upon temperature, and can be inhibited by various metabolic poisons. Mechanisms responsible for active transport of amino acids are associated with the capsule and epithelium. Binding is not responsible for the concentration gradient between amino acids in the lens and its environment. The lens acts like a "pump-leak system," the steady state concentration being determined by a balance between accumulation of amino acids through active transport and loss by diffusion. The system responsible for active transport of neutral amino acids in the lens in vivo appears to have a large reserve capacity for moving these compounds against appreciable concentration gradients.*

A century and a half ago, Berzelius[1] found that more than a third of the ocular lens consists of proteins. By measuring the turnover of glycine in rabbit lenses, Merriam and Kinsey[2] showed that as much as one twentieth of this protein may turn over each day. Proteins in the lens, like those elsewhere in the body, are in a state of dynamic equilibrium, i.e., they are continually being autolyzed and resynthesized. Maintenance of normal levels of proteins in the lens is dependent upon an adequate supply of amino acids which is favored by efficient mechanisms for their transport from blood to the posterior chamber and from there into the lens interior.

The present authors investigated transport of amino acids into the intraocular fluids and lens[3, 4] and observed that active transport contributes to accumulation of amino acids in both posterior chamber and

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lens. We showed also that the concentration of most naturally occurring amino acids in aqueous humor of the rabbit is above that in plasma, and the concentration of each amino acid in the lens is higher than that in the aqueous humors, and still higher than that in vitreous humor.[5]

Kern[6] reported that the combined concentration of free amino acids and peptides in beef lens is also greater than that in aqueous humor, and he showed that during culture for 20 hours several amino acids in the lens accumulate to concentrations significantly higher than those in the medium.

The present study was designed to obtain further knowledge about the entrance of various amino acids into the lens, and some of the factors which influence their entrance and accumulation.

Methods

C^{14}-labeled alpha amino isobutyric acid (α-AIB) was employed as a tracer in all of the experiments. Lenses from albino rabbits (1.8 to 2.3 kilograms) were cultured by methods described earlier[7] with the use of 5 ml. of a medium having the same composition as KEI-3, but without amino acids unless otherwise noted. When lenses were cultured for 48 hours, the medium was replaced at the end of 24.

The radioactivity accumulated by lenses used in the experiments involving kinetics was determined by Kinoshita's[8] technique with the use of a scintillation counter. Lenses were homogenized in 3 ml. 10 per cent TCA, and centrifuged, and 0.1 ml. of supernatant was added to 20 ml. of toluene-ethanol mixture containing PPO and dimethyl POPOP. Before a scintillation counter became available to us, lenses were homogenized in 2 ml. of Nelson-Somogyi reagent (1 ml. $ZnSO_4$ and 1 ml. $Ba(OH)_2$), and the radioactivity in the supernatant was determined after plating on copper planchettes with a thin window flow gas counter. It was observed that the Nelson-Somogyi precipitate adsorbs some radioactivity (35 per cent). Appropriate corrections were applied to adjust for loss of activity on the precipitate and for differences in self-absorption when lens filtrates and media were counted.

The uptake of amino acid is expressed as the ratio of concentration in lens water (65 per cent) to the initial concentration in the medium. The values are approximately 25 per cent lower than they would have been had they been calculated on the basis of the average between the initial and final concentrations.

When nonlabeled α-AIB or one of the other amino acids was added to the medium, the osmolarity was maintained constant by varying the concentration of sodium chloride.

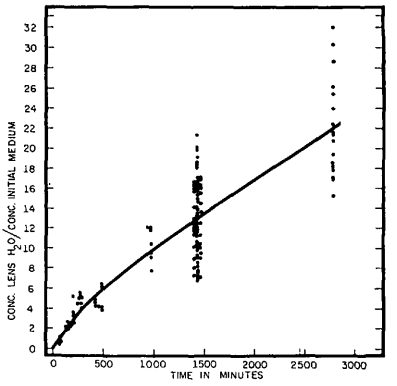

Fig. 1. Accumulation of C^{14}-labeled α-AIB by rabbit lenses cultured in a medium containing no other amino acids.

Results

The data of Fig. 1 show that the concentration of tracer α-AIB in lens water after 48 hours of culture is approximately 22 times that present initially in the medium, the uptake being linear with time after the first 6 to 8 hours.* Other experiments were performed in which lenses were cultured and the concentration of α-AIB in only the capsule and epithelium combination was measured for different periods and compared with that in the remainder of the lens. The concentration in the surface membranes is at first greater, but after about 8 hours it is indistinguish-

*Since the concentration of the tracer in the medium (5 ml.) decreases with time to reach about 45 per cent of the initial level by the end of 24 hours, the actual rate of accumulation per unit concentration in the medium increases slightly with time. This increased efficiency of uptake could be due to decreased saturation of the transport mechanism, as the amino acids normally present in the lens are eluted into the medium.

able from that in the cortex and nucleus, indicating that by this time a quasi steady state exists in which the rates of uptake and penetration to deeper layers of the lens are approximately equal.

The effect of the presence of increasing concentrations of nonlabeled α-AIB in the media on the rate of accumulation of the tracer amino acid is illustrated in Fig. 2. The period of culture is 24 hours. The average concentration ratio (7 lenses) for tracer α-AIB only (zero concentration of nonlabeled compound), corresponds closely to the average for 67 lenses shown in Fig. 1. The other points represent the mean value for 6 lenses each. The graph shows that the ratio of concentration of tracer α-AIB in the lens to that in the medium decreases asymptotically with increasing concentration of nonlabeled compound present.

The total amount of α-AIB accumulated by lenses cultured in different concentrations of α-AIB can be calculated from the data shown in Fig. 2, assuming that lenses do not distinguish between labeled and nonlabeled amino acid, i.e., that the concentration ratio shown on the ordinate (Fig. 2) applies to the nonlabeled compound as well. The quantity of α-AIB in the lens is equal to the product of the concentration ratio and the concentration in the medium times the volume of water in the lens (average 0.21 ml.). The total quantity of α-AIB present in lenses at the end of 24 hours is shown in Fig. 3.

Fig. 4 shows a Lineweaver-Burk plot of the data in Fig. 3. The straight line relationship between the reciprocal of the concentration in the media and that of the amount of α-AIB accumulated by the lens is in agreement with Michaelis-Menten kinetics. The half saturation level is 2.5 mmoles per liter and the maximum velocity is 0.25 μmoles per lens per hour.

To obtain a better understanding of the nature of the mechanisms involved in moving naturally occurring amino acids into the lens, the comparative efficacy of 21 L- and 3 D-amino acids in saturating

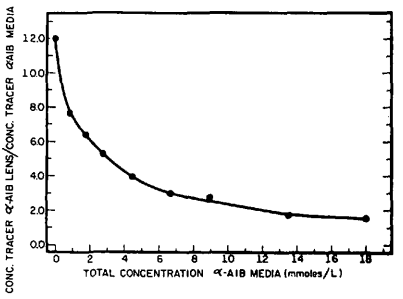

Fig. 2. Effect of nonlabeled α-AIB in the media on the accumulation of labeled compound in the lenses cultured for 24 hours.

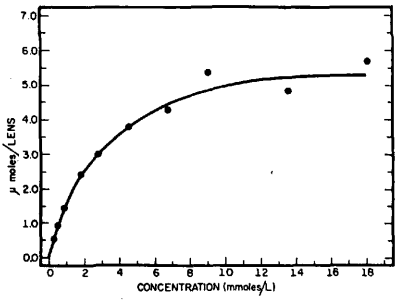

Fig. 3. Effect of nonlabeled α-AIB on the total amount of amino acid accumulated by lenses in 24 hours.

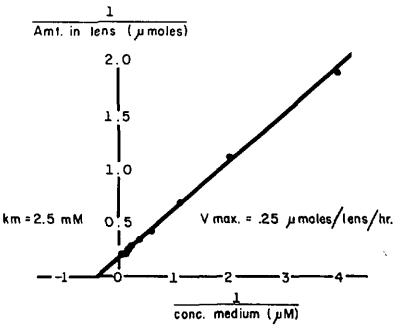

Fig. 4. Lineweaver-Burk plot of the accumulation of C^{14}-labeled α-AIB in lenses cultured for 24 hours. *Km*, apparent Michaelis-Menten constant. *Vmax.*, the maximum velocity of transport.

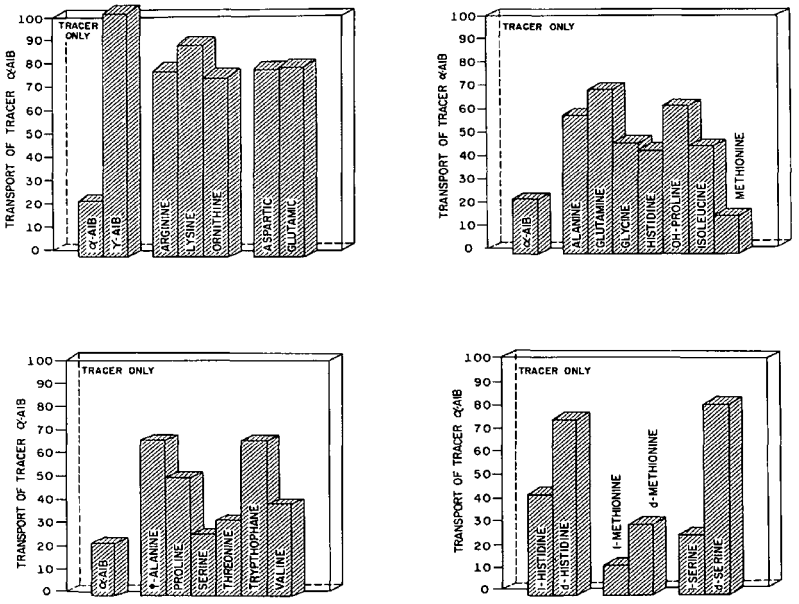

Fig. 5. Effect of various amino acids (4.5mM.) on the transport of C^{14}-labeled α-AIB into lenses cultured for 24 hours.

the transport system for α-AIB was studied. Table I shows the effect of the separate addition of these amino acids at a concentration of 4.5 mM. on the amount of tracer α-AIB accumulated by lenses during 24 or 48 hours of culture. To simplify comparison of the relative effectiveness of the different amino acids in saturating the transport system for α-AIB, the data for the 24 hour culture period have also been expressed graphically on a percentage basis in the block diagrams of Fig. 5.

The degree of saturation for the two time periods (Table I) is in reasonable agreement, considering the variability of the data, although there appears to be a slight tendency toward greater inhibition after 48 hours. The upper left-hand chart (Fig. 5) shows that the gamma isomer of AIB and both the basic and acidic amino acids have little saturating effect on the transport of labeled α-AIB into the lens. Neutral amino acids, however, in varying degrees decrease accumulation, methionine (as in other cell systems) being a more effective inhibitor than α-AIB. Of the three D-amino acids tested, all were less effective than the corresponding L-form.

Influence of temperature, energy source (glucose), oxygen, and various poisons on accumulation of α-AIB in cultured lenses is shown in Table II. Accumulation of α-AIB is highly dependent on temperature, and has an apparent Q_{10} of approximately 4. Transport proceeds at approximately three quarters of the normal rate under anaerobic conditions, but is greatly impaired when glucose is absent from the medium. All of the metabolic poisons tested, DNP, iodoacetate, ouabain, and cyanide, depress transport in varying degrees.

Discussion

The dependence of the amount of C^{14}-labeled α-AIB accumulated by lenses on the total concentration of α-AIB in the media suggested that the processes responsible for uptake would obey saturation kinetics as described by the Michaelis-Menten equations, and is in accord with the previously held view that active transport is involved. The observation that the lens can accumulate α-AIB against a large concentration gradient and that this process requires energy and is affected by poisons, is also consistent with the idea that active transport is involved.

The investigations do not, however, provide information about where in the lens the active transport takes place, e.g., whether it is across the surface membranes or some other structure, for instance the fiber walls, or both. To clarify this point, lenses without capsules and epithelia were cultured for various periods in media containing: (1) tracer α-AIB only, (2) tracer and 5 mmoles per liter of nonlabeled α-AIB, and (3) tracer with 0.3 mmoles per liter of iodoacetate. The results shown by the filled circles, Fig. 6, indicate that tracer α-AIB accumulates rapidly in decapsulated lenses and reaches a maximum concentration within about an hour. Accumulation appears to be unaffected by the presence of either nonlabeled α-AIB or iodoacetate, both of which depress transport into intact lenses. These results show that the mechanisms responsible for accumulation by active transport are associated with surface membranes, and, therefore, it seems unlikely that underlying struc-

Table I. Influence of addition of various amino acids (4.5 mM) on the accumulation of C^{14}-labeled α-AIB in rabbit lenses cultured for 24 or 48 hours in a medium containing no other amino acids

Amino acid (α-AIB, tracer only)	*Conc. Lens water / Medium*	
	*24 Hours 13.0 ± 3.66 (67)**	*48 Hours 22.2 ± 4.8 (17)**
α-AIB	2.7 (20)	6.5 (6)
γ-AIB	12.7 (2)	23.2 (2)
Alanine	7.3 (6)	13.6 (2)
Arginine	9.9 (4)	13.5 (2)
Aspartic	9.8 (6)	16.4 (4)
Glutamic	9.9 (4)	14.0 (4)
Glutamine	8.7 (4)	10.4 (2)
Glycine	5.9 (4)	6.8 (4)
Histidine	5.4 (6)	6.0 (6)
Hydroxyproline	7.8 (4)	10.1 (4)
Isoleucine	5.6 (5)	7.0 (2)
Leucine	6.7 (2)	7.9 (2)
Lysine	11.2 (6)	24.3 (2)
Methionine	1.9 (4)	2.3 (2)
Ornithine	9.5 (4)	16.4 (4)
Phenylalanine	8.4 (4)	16.1 (2)
Proline	6.4 (4)	7.3 (4)
Serine	3.3 (4)	4.8 (2)
Threonine	4.0 (4)	11.9 (6)
Tryptophane	9.1 (2)	9.1 (2)
Valine	4.8 (4)	8.7 (4)
D-histidine	8.4 (5)	8.4 (8)
D-methionine	3.7 (2)	10.2 (2)
D-serine	10.1 (2)	13.3 (2)

*Number of lenses in parentheses.

Table II. Influence of various factors on the accumulation of C^{14}-labeled α-AIB on rabbit lenses cultured for 24 hours in a medium containing no other amino acids.

Condition or poison	*Concentration*	*Conc. α-AIB Lens water / Medium*	*Control (%)*
Control 37°	—	13.0 ± 3.66 (67)*	100
Reduced temp. 23°	—	2.2 ± 0.47 (12)	18
Glucose absent	—	1.7 ± 0.57 (7)	15
Anaerobiosis	—	8.8 ± 2.42 (12)	73
Dinitrophenol	3×10^{-3} M	7.4 ± 1.07 (6)	62
Iodoacetate	3×10^{-3} M	0.74 ± 0.19 (5)	6
Ouabain	3×10^{-5} M	3.4 ± 0.88 (9)	28
Sodium cyanide	5×10^{-3} M	1.55 ± 0.40 (13)	13

*Number of lenses in parentheses.

tures in the lens can transport or actively accumulate amino acids.

The ratio of concentration of tracer α-AIB in lens water to that in the medium is approximately 0.25 (Fig. 6). This value is similar to the steady state ratio for chloride in intact lenses[9] and suggests that the "amino acid space" and chloride space may be the same (approximately 15 to 20 per cent of lens volume). The concentration gradient between lens and medium, if calculated on the basis of only the water in the amino acid space, would be much higher than that reported in this paper based on total lens water.

Finally, these experiments provide evidence that the relatively high concentration of amino acids in the lens[10] is not due to binding by any of its constituents, a conclusion also reached by Kern[6] for calf lenses.

Accumulation of tracer α-AIB in intact lenses is decreased significantly by all of the neutral amino acids, but not by basic and acidic ones, suggesting that a single mechanism, perhaps one site on a carrier, is involved in the active transport of all neutral amino acids. Other experiments, to be reported elsewhere, show that basic and acidic amino acids are transported by separate systems. Thus, as in most cells,[11] including those in the ciliary epithelium,[4]

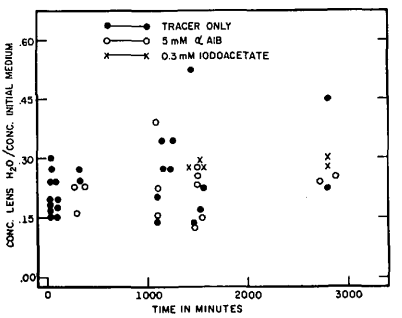

Fig. 6. Accumulation of C^{14}-labeled α-AIB by decapsulated lenses cultured in a medium containing no other amino acids.

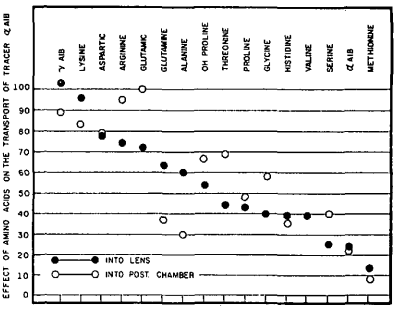

Fig. 7. Relative effectiveness of different amino acids in saturating the systems involved in transport of labeled α-AIB into lens and posterior chamber.

there appear to be at least three separate systems which transport amino acids into the lens.

The relative effectiveness of different amino acids in saturating the system involved in transporting α-AIB into the lens (Table I) compared with that responsible for transporting α-AIB into the posterior chamber, is shown in Fig. 7. Data on the posterior chamber reported previously[4] were obtained from experiments in which rabbits were injected with equal quantities of different amino acids 15 minutes prior to administration of tracer α-AIB. The depression in the amount of labeled α-AIB accumulating in the posterior chamber by active transport in 45 minutes was used as a criterion of the degree of saturation. The inhibitory effect of different amino acids on accumulation of α-AIB in the lens and posterior chamber (except for arginine, glutamic acid, glutamine, and alanine) is reasonably similar, considering the differences in techniques employed and the possibility that concentration of the injected amino acids in the plasma may be different, even though similar quantities of each were given.

The data in Table II show that anaerobic processes provide the chief source of energy for amino acid transport and that transport is depressed by low concentrations

of ouabain.* These data are in agreement with results obtained by Kern[6] on calf lenses. Unlike Kern, however, we observed that dinitrophenol, or lack of oxygen, decreased uptake by about a third. These observations suggest that in rabbit lenses the process responsible for amino acid transport derives part of its energy from respiration. The reduction in the amount of α-AIB accumulated by the lens in the presence of sodium cyanide is much greater than would be expected from even complete inhibition of respiration, the explanation for which may be that cyanide increases the rate of leakage out of the lens.

These studies support the conclusion that α-AIB and at least some of the naturally occurring amino acids are actively transported into the lens where they are found in concentrations higher than those in the intraocular fluids.[10] It is now possible to speculate about the effectiveness of the systems in maintaining the elevated concentration in the lens.

The lens appears to behave like a "pump-leak" system in which the concentration of any amino acid is determined by the balance between rate of entrance through active transport and exit by diffusion. The former depends not only on the total potential capacity for transport, but on the degree of saturation of the system involved in moving a particular amino acid, the greater the saturation the larger the amount transported.

An experiment was designed to obtain some knowledge of the degree of saturation of the system which transports α-AIB, and thus neutral amino acids generally under conditions as they occur in life. The accumulation of tracer α-AIB was determined in a medium containing concentrations of amino acids intermediate between those found in aqueous and vitreous humors of rabbits under fasting conditions.[5]

The average ratio of concentration of tracer α-AIB in lens water to that in the medium for 14 lenses after 24 hours of culture was 7.75 ± 1.2, a value corresponding to the saturation produced by about 1 mmole per liter of nonlabeled α-AIB (Fig. 2). The data in Fig. 3 show that at this concentration the system is approximately one third saturated, and thus has a reserve capacity for transporting α-AIB equal to approximately twice that being used. To the extent that neutral amino acids share the same transport system and are transported like α-AIB, the conclusion seems justified that, in vivo, the carrier system for these compounds can transport at least twice the amount of amino acid moved under fasting conditions. Thus, the system is capable of increasing the rate of transport of amino acids into the lens should their concentration in the intraocular fluids became elevated, i.e., it can maintain the concentration of amino acids in the lens relatively constant despite wide fluctuations in their concentration in the plasma.

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*The inhibition of α-AIB uptake by ouabain occurs only after several hours of culture.

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