Transport of alpha aminoisobutyric acid into ocular fluids and lens

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In association with

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The rates of accumulation of C-14–labeled alpha aminoisobutyric acid (α-AIB) in the aqueous and vitreous humors and lenses of rabbit eyes following parenteral administration have been determined. The distribution ratios of α-AIB in the aqueous humor of the posterior and anterior chambers, the vitreous humor, and the lens 24 hours after parenteral administration were found to be 0.75, 0.98, 0.1, and 2.5, respectively. The rate of accumulation and the steady-state distribution of the C-14–labeled α-AIB compound in the aqueous humor and the lens was reduced by the administration of nonlabeled α-AIB 15 minutes prior to administration of the labeled compound. A similar inhibitory effect on the rate of accumulation of the labeled amino acid could be produced by several naturally occurring amino acids. Parenterally administered Diamox increased the rate of accumulation of α-AIB in the posterior and anterior chambers as well as the approximate steady-state ratios in these chambers. The significance of these observations is discussed with respect to the mechanism of transport of α-AIB into the intraocular fluids and lens. It is concluded that α-AIB is actively transported across the ciliary epithelial cells, the lens capsule, and, possibly, the anterior surface of the iris.

The transport of amino acid into cells has been studied extensively[1, 2] since Van Slyke and Meyer[3] first demonstrated that amino acids could be transferred across the membranes of cells against an apparent concentration gradient. Knowledge of the manner in which these compounds move across the blood-aqueous barriers is important since most, if not all, amino acids required for synthesis of lenticular proteins must be derived from the aqueous and vitreous humors. The few investigations which have been made concerning the transport of amino acids from plasma to aqueous humor are limited to the anterior chamber, and none emphasizes the mechanism involved. Davson and others[4] reported that glycine and alanine enter the anterior chamber of cat eyes at about the same rate as urea, i.e., relatively slowly. Langham[5] stated that the penetration of cysteine into the anterior chambers of the same animals was considerably faster than that which Davson and co-workers found for glycine and alanine. Von Sallman and others[6] observed that cysteine and cystine readily penetrated the aqueous humor of rabbit eyes and reached steady state within a few hours.

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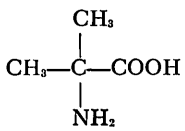
The aforementioned experiments were based on chemical methods of analysis which followed administration of nonlabeled amino acids in substantial quantity. Kinetic studies involving nonocular tissues have shown that mechanisms for active transport can be saturated by concentrations of amino acids not greatly in excess of those normally present in biologic fluids.[7] Thus, any measurement of transfer rate in a system in which active transport occurs, determined by elevating the concentration in the plasma to produce a significant gradient between plasma and aqueous humor, may lead to a maximal rate of transport rather than that prevailing under more physiologic conditions. The deviations from normal introduced by this procedure can be overcome by the use of isotopically labeled amino acids, since, ordinarily, they can be used in such small amounts that they do not contribute significantly to the total amino acid concentration. However, the use of labeled naturally occurring amino acids has the disadvantage that, during the course of the experiment, they may be metabolized in the body or during passage across the blood aqueous barrier, thus making it necessary to isolate the amino acid from its metabolites if the actual quantity of the test substance which is transported is to be determined. Use of the unnatural amino acid, alpha aminoisobutyric acid (hereafter referred to as α-AIB), obviates this difficulty because it is not metabolized, and thus the quantity of amino acid present can be determined by simple measurement of radioactivity without previous isolation of the compound. Although it is an unnatural amino acid, and despite its inert nature,[8, 9] it has been demonstrated that α-AIB is transported into some cells in a manner similar to the naturally occurring amino acids.[10] Its structure is as follows:



This compound differs from alanine in that a methyl group replaces the alpha hydrogen, a substitution which aparently prevents transamination reactions,[11] catabolism,[12] and incorporation into proteins.[13]

The present article, the first of several designed to provide information on the intraocular transport of amino acids, will be concerned solely with α-AIB.

Methods

Eight microcuries of carboxyl-labeled C-14 α-AIB were given to albino rabbits which weighed 1.8 to 2.3 kilograms; 25 per cent of the initial dose was given intravenously, and 75 per cent, intraperitoneally. This method of administration kept the plasma level approximately constant for 50 minutes. In experiments of longer duration, an additional 10 per cent of the total dose was given intraperitoneally every 50 minutes following the initial injection. When both labeled and nonlabeled amino acids (5 mmoles per kilogram of body weight) were used, a 5 per cent solution of the latter was administered 15 minutes before injection of the labeled compound. The proportionate amount of nonlabeled amino acid given intraperitoneally and intravenously was the same as that used when the labeled substance was given alone. For determining the approximate steady-state ratios, the procedure of giving repeated injections of 10 per cent of the initial dose was followed over a period of from 23 to 26 hours.

When Diamox* (50 mg. per kilogram) was administered, it was injected intravenously as a 2.5 per cent solution 30 minutes before α-AIB was injected, and, for steady-state experiments, this dose was then given intraperitoneally at 2 hour intervals over a period of from 23 to 26 hours.

Plasma, aqueous humor, and vitreous samples were removed and their radioactivity was determined with a thin window-flow gas counter by methods which have been described.[14, 15] Lenses were weighed

*Supplied by the Lederle Laboratories, a Division of American Cyanamid Company.

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and homogenized in a solution consisting of 1 ml. zinc sulfate and 1 ml. barium hydroxide and the supernatant was assayed for radioactivity. Appropriate corrections were applied to take into account loss of activity due to self-absorption. Plasma was dialyzed to show that all of the α-AIB in the plasma was in a free form. Nelson-Somogyi reagent was used to remove the protein from the homogenates of the lenses, and the radioactivity in the protein was found to be essentially zero. The concentration in the lens was calculated on the basis of the amount of amino acid present in the water in the lens (65 per cent total weight).

Results

Fig. 1 shows the concentration of C-14-labeled α-AIB in the plasma and in the aqueous and vitreous humors in rabbits at various times after parenteral administration. The lines through the data are drawn as best visual fits. At the end of 3 hours the concentrations of α-AIB in the posterior and anterior chambers are, roughly, 35 and 50 per cent, respectively, of the plasma level, while the concentration in the vitreous humor is less than 5 per cent of that in the plasma. Even after 24 hours (Table I), the concentration has risen to only 10 per cent of the plasma level. The failure of α-AIB to accumulate in significant concentration in the vitreous humor is unique among the various substances which we have investigated previously.[15-17] Further experiments showed that significantly large quantities of α-AIB enter the lens. Within 24 hours the concentration rises to a level twice that in the plasma water, 2½ times that in the aqueous humor of the posterior chamber, and approximately 20 times that in the vitreous humor (Fig. 2). The observation that the amino acid concentrates in the lens not only accounts for the low concentration in the vitreous but indicates a mechanism for its active transport which operates across the capsule of the lens.

The possibility that α-AIB is actively transported across the blood-aqueous bar-

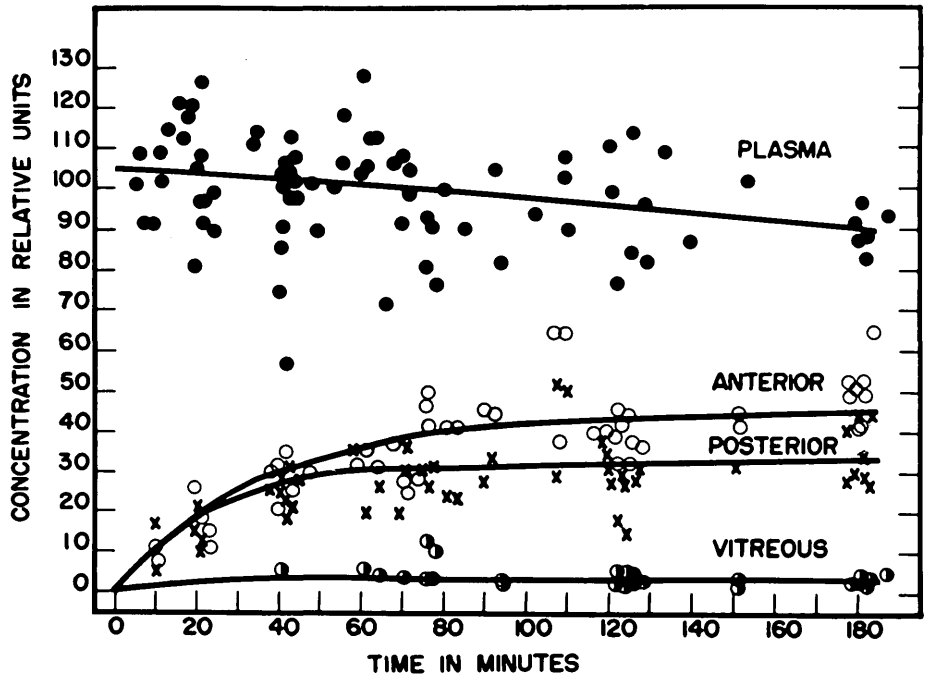

Fig. 1. The concentration of radioactive α-AIB in aqueous and vitreous humors and plasma of rabbits at various times following parenteral administration of C-14-labeled amino acid.

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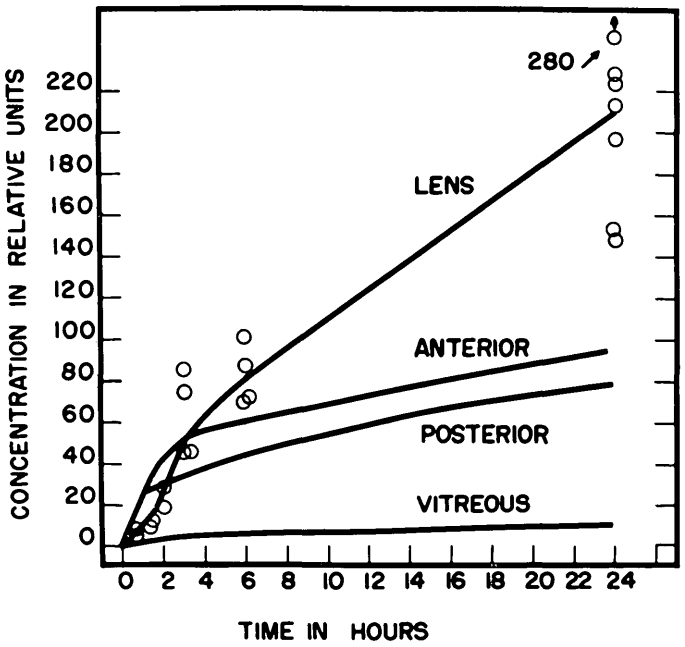

Fig. 2. The concentration of radioactive α-AIB in the lenses of rabbits compared with that in aqueous and vitreous humors at various times following parenteral administration of C-14–labeled amino acid. The concentration of α-AIB in the plasma was essentially constant at a level of 100 relative units throughout the whole time course. The experimental values for the intraocular fluids are taken from Fig. 1 and Table I. (The number *280* in the diagram represents an experimental value too high to fit the scale.)

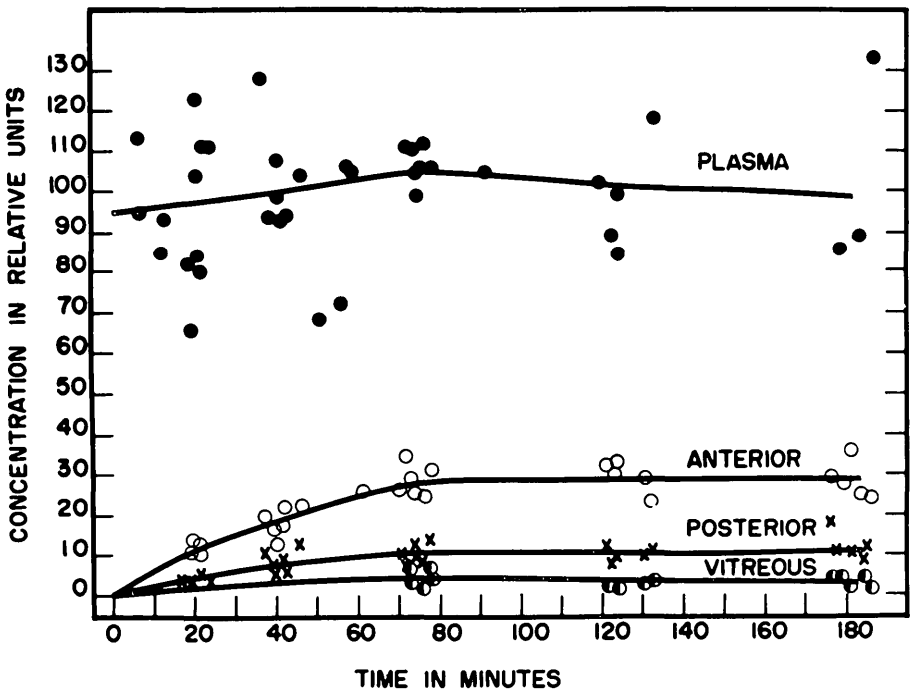

Fig. 3. The concentration of radioactive α-AIB in aqueous and vitreous humors and plasma of rabbits at various times following parenteral administration of C-14–labeled amino acid and 5 mmoles per kilogram of nonlabeled amino acid.

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Table I. Relative concentration of α-AIB* in the aqueous and vitreous humors and in the lenses of rabbits compared with plasma under various conditions 24 hours after the administration of C-14–labeled compound

Posterior aqueous humor: plasma			*Anterior aqueous humor: plasma*			*Vitreous humor: plasma*			*Lens: plasma*	
α-AIB† only	*α-AIB† with non-labeled compound*	*α-AIB† with Diamox*	*α-AIB† only*	*α-AIB† with non-labeled compound*	*α-AIB† with Diamox*	*α-AIB† only*	*α-AIB† with non-labeled compound*	*α-AIB† with Diamox*	*α-AIB† only*	*α-AIB† with non-labeled compound*
0.81	0.26	1.03	1.13	0.68	1.02	0.13	0.05	0.11	1.95	0.95
0.93	0.18	1.00	1.17	0.52	1.07	0.11	0.02	0.11	2.80	0.33
0.58	0.17	0.91	0.90	0.42	1.11	0.06	0.15	0.11	2.23	0.27
0.62	0.23	0.89	0.79	0.42	1.11	0.08	0.08	0.10	2.54	0.24
0.46	0.30	0.85	0.72	0.43	0.92	0.10	0.03	0.25	1.54	0.32
0.41	0.25	0.85	0.62	0.46	0.96	0.09	0.04	0.10	1.48	0.32
0.92	0.17		1.23	0.43			0.00		2.13	0.49
0.96	0.17		1.17	0.43			0.00		2.27	0.42
0.72	0.12		1.02	0.45			0.01			
0.79	0.15		0.99	0.45			0.02			
0.86	0.13		1.10	0.36			0.02			
0.83	0.13		1.04	0.35			0.02			
0.75	0.23		0.95	0.56			0.02			
0.79	0.29		0.92	0.58			0.11			
0.75	0.19	0.92	0.98	0.47	1.03	0.10	0.04	0.13	2.12	0.42
±0.17	±0.06	±0.07	±0.17	±0.09	±0.07	±0.02	±0.04	±0.05	±0.46	±0.21

Mean
S.D.‡

*α-AIB, alpha aminoisobutyric acid.
†α-AIB-C-14–labeled compound.
‡S.D., standard deviation.

rier was investigated by administering 5 mmoles per kilogram of nonlabeled amino acid just prior to giving the labeled compound to determine whether the amount of amino acid transported depends upon the concentration in the plasma.*

Comparison of Figs. 1 and 3 shows that the rate of accumulation of labeled α-AIB in the posterior chamber following administration of the nonlabeled substance is significantly lower than when only tracer amounts of the amino acid are employed. For instance, the concentration in the posterior aqueous humor after 3 hours is 12 per cent of the plasma level when the nonlabeled compound is given compared with 33 per cent when tracer α-AIB only is used. The rate of accumulation in the anterior chamber is likewise reduced in the presence of nonlabeled amino acid (30 per cent versus 50 per cent after 3 hours).

*This experiment was suggested by Dr. Ernst Bárány of the University of Uppsala.

Preliminary experiments have shown that the transport of labeled α-AIB into the ocular chambers can also be inhibited by administration of 5 mmoles per kilogram of some, but not all, of the naturally occurring amino acids. We have some evidence, too, of reciprocal inhibition, i.e., α-AIB can inhibit the transport of other amino acids into the posterior chamber.

The effect of Diamox on the relative concentrations of α-AIB in the aqueous and vitreous humors and plasma is shown in Fig. 4. Again the lines through the data are drawn as best visual fits.

In most instances, the experiments involving Diamox were performed on the same day, and on animals from the group that was used for determining the rate in untreated rabbits. Despite attempts to make the experimental conditions as similar as possible in the two sets of experiments, the animal-to-animal variation was considerably greater when Diamox was given.

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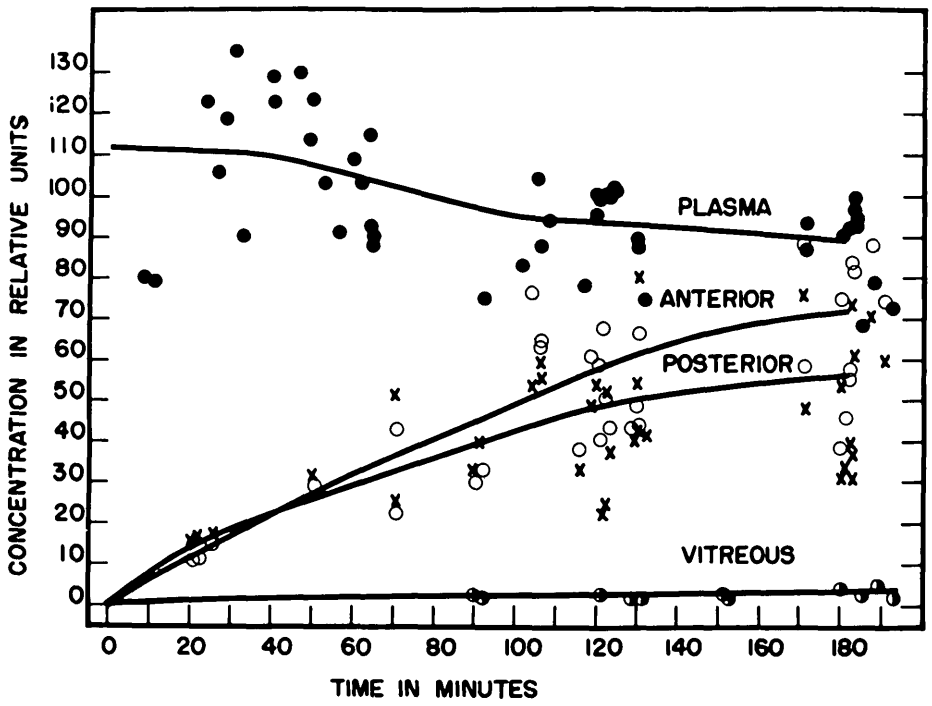

Fig. 4. The concentration of radioactive α-AIB in aqueous and vitreous humors and plasma of Diamox-treated rabbits at various times following parenteral administration of C-14–labeled amino acid.

Notwithstanding the spread in the data, when Figs. 1 and 4 are compared there seems to be some justification for the conclusion that accumulation of the amino acid in both the posterior and anterior chambers is appreciably greater in rabbits receiving Diamox.

Table I shows the relative concentration of α-AIB in the ocular fluids and lens with respect to plasma after 24 hours. Because α-AIB is still being removed by the lens at this time (Fig. 2), the values shown for this compound do not correspond as closely to the steady-state conditions as for those substances studied heretofore at the same period. This lack of saturation, despite relatively high lens-to-plasma ratios, has the effect of retarding the rate at which both the aqueous humor of the posterior chamber and the vitreous humor reach steady state.

The results demonstrate clearly the effect of the nonlabeled amino acid in lowering the approximate steady-state distribution ratio of α-AIB in the aqueous humor and lens. The inhibitory effect on transport into the posterior aqueous humor and lens under approximate steady-state conditions is particularly striking, a result similar to that found for shorter time periods. The administration of nonlabeled amino acid reduces the average distribution ratio at 24 hours in the posterior chamber from a value of 0.75 to 0.19; this reduction in distribution ratio corresponds to an inhibition of 75 per cent. The analogous inhibition in the lens is 80 per cent, while the ratio in the anterior aqueous humor is lowered by 50 per cent. In contrast, administration of Diamox increased the distribution ratio in the posterior chamber by 23 per cent. All of these alterations in distribution ratio are significant ($p < 0.01$). A slight increase in the value for the anterior aqueous humor was also noted in animals treated with Diamox.

Discussion

In previous papers[15-17] the rate of accumulation of various test substances in the posterior chamber was expressed by the

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$$\frac{dC_h}{dt} = k_{fh}\,(C_s - C_h) + k_{d.ph}\,(C_p - \alpha C_h) - \left[\frac{DA}{\Delta X V_h}\,(C_h - C_v)\right]_{x\,=\,0}$$

A Area posterior chamber-vitreous interface
α Donnan factor
C_h Concentration of aqueous humor, posterior chamber
C_p Concentration of plasma
C_s Concentration of secreted fluid
C_v Concentration of vitreous humor
D Diffusion constant
$k_{d.ph}$ Transfer coefficient by diffusion plasma to posterior chamber
k_{fh} Transfer coefficient by flow into and out of posterior chamber
V_h Volume of posterior chamber
x Space variable

equation at the top of the page. The last term of the equation was introduced to take into account loss of the substance from the posterior chamber to the "lumped" environment (vitreous and lens). The loss of sodium and chloride to the lens was only about 10 per cent of that to the vitreous. To simplify the mathematical treatment, but at the same time to compensate partially for loss to the lens, the value for A (area of the posterior chamber-vitreous interface) was increased slightly, and loss of the test substance to the environment was thereafter considered as occurring only to the vitreous. In the present study, however, the loss to the lens was so large (Fig. 2) that it could not be included with the loss to the vitreous without introducing serious errors in calculating the loss term. Thus, in the absence of information concerning the distribution of the amino acid as a function of time and space in both the lens and vitreous, it has not been possible to calculate the proportionate amounts of α-AIB entering the posterior chamber by secretion and by diffusion, as we have done previously for other substances. Qualitatively, however, certain inferences can be made concerning the mode of transport of this amino acid into the posterior chamber.

Comparison of the data presented in Figs. 1 and 3 shows that the presence of nonlabeled α-AIB produces a reduction in the rate of accumulation of the labeled compound in the aqueous humor of the posterior chamber. Table I indicates that the use of the nonlabeled amino acid also decreases the approximate steady-state ratio of labeled α-AIB. These results suggest that the mechanism of transport becomes progressively saturated as the absolute concentration of the amino acid in the plasma rises. Thus, the amount of amino acid transported appears to be limited by some factor other than the quantity present in the blood. From similar evidence, one of us (V.E.K.) demonstrated that the mechanism concerned with secretion of ascorbic acid into the rabbit eye could likewise be saturated.[18] The same investigator reported that the approximate steady-state ratio of 20:1 in the aqueous humor of the anterior chamber[18] of the rabbit could be maintained only as long as the concentration of ascorbic acid in the plasma did not exceed 3 mg. per cent. Subsequently, similar results were found for the cat[5] and the guinea pig.[19] In the latter animal a concentration ratio of 30:1 was maintained only when the plasma concentrations were below 2 mg. per cent. Although all of the former studies were concerned with aqueous humor of the anterior chamber, it was concluded that the inhibition took place in the ciliary processes. Later, direct analysis for ascorbic acid in the posterior chamber of the rabbits whose plasma concentration was raised showed that this conclusion was valid.[20]

The existence of an active mechanism of transport *out of the eye* has been reported by Forbes and Becker[21] for para-amino hippurate and iodopyracet and for I-131 in our discussion of the paper by Forbes and Becker. In all instances, evidence that active transport occurred consisted in the observation that transport of tracer amounts of these substances could be reduced by the presence of appreciable amounts of nonlabeled compounds.

The active transport of α-AIB by other cells (ascites tumor) has been demonstrated by Christensen and Riggs,[10] who

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also found that it can be inhibited by several naturally occurring amino acids.[22]

In a number of instances the distribution ratio in the anterior chamber at the end of 24 hours is greater than 1.0 (Table I). Without invoking active secretion across the anterior surface of the iris, it is difficult to explain how the concentration in the aqueous humor of the anterior chamber can exceed that in the plasma, since the concentration ratio of the aqueous humor flowing from the posterior chamber to the anterior chamber is at all times below 1.0. It is possible that the concentration in the plasma some hours prior to withdrawal of aqueous humor was significantly higher than at the time the samples were taken, an effect which might result in an overshooting in the concentration in the aqueous humor. In the animal in which distribution ratios were the highest (1.23 and 1.17), the radioactivity in the plasma at 7, 14, 24, and 26 hours was 96, 105, 91, and 98 counts per minute per 0.1 Gm. of plasma water, respectively. The concentrations in the anterior aqueous humor of this animal at 26 hours were 117 and 111 counts per minute per 0.1 Gm. of fluid in the left and right eyes, respectively. The ratios, 1.23 and 1.17, were calculated on the assumption that average plasma concentration during the last few hours of the experiment was 96 counts per minute per 0.1 Gm. of plasma water. While it is not certain that the concentration in the plasma during the period just before the 24 hour sample was taken was not greatly in excess of 96 counts per minute, examination of data from all the animals showed that repeated intraperitoneal administration of the test compound resulted in essentially constant plasma levels in all instances. It seems doubtful, therefore, that the steady-state ratios above 1 can be accounted for by lack of constancy of the concentration of the amino acid in the plasma.

Another possibility is that the correction applied to take into account self-absorption in the aqueous humor and plasma samples was in error. Accordingly, another series of experiments were performed to redetermine the correction factors, but the factors were found to be essentially identical with those previously employed.

Because of lack of evidence that active transport occurs for other substances, we are reluctant to accept the idea that α-AIB is actively transported across the anterior surface of the iris. Inability to explain high ratios on the basis of faulty methodology leaves no alternative. We feel that more information over longer periods of time should be obtained and that better mathematical methods should be employed in treating the data before definite conclusions are drawn.

The evidence for active transport into the lens, as shown by the concentration gradient existing between the lens and the ocular fluids, indicates that the mechanism in the eye for active transport of α-AIB is not limited to the ciliary processes. Moreover, the mechanism for active transport across both the ciliary processes and the lens is not limited to the transport of α-AIB but must operate in the transport of naturally occurring amino acids and probably other compounds required to meet the nutritional needs of the avascular tissues of the eye.

The observation of an apparent increase in the steady-state concentration following administration of Diamox, especially in the posterior chamber, is similar to that found for urea.[15] In the latter instance the increased concentration could be accounted for by a twofold elevation in the quantity of urea entering the posterior chamber and by a 40 per cent increase in the amount entering the anterior chamber by diffusion. Whether Diamox similarly increases the diffusion rate of α-AIB, or whether it affects the proportion of this compound which is transported actively but is not directly associated with flow cannot be determined until improved quantitative methods are available for treating the data.

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Discussion

Dr. Bernard Becker, St. Louis, Mo. The data reported in this paper add greatly to our knowledge of ocular transport problems and raise many important questions.

Clear-cut evidence is presented that there is an active transport of α-AIB into the lens. This raises questions as to the nature and requirements of this transport process. Does it occur in lenses maintained in vitro? Does such an in vitro system demonstrate saturation kinetics with α-AIB and

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with L-amino acids? What are the rate characteristics of lens accumulation in vivo and in vitro? Does Diamox alter the accumulation?

The evidence presented for the transport of α-AIB into the aqueous humor is largely dependent upon the finding that, although this substituted amino acid is present in deficit in the aqueous humor, the 25 per cent deficit in the posterior chamber at 24 hours is increased to an 81 per cent deficit following the administration of 5 mmoles of α-AIB. The question naturally arises as to why the deficit occurs in the nonsaturated animal. This could result in part from continued uptake by the lens and failure to reach steady state at 24 hours, as postulated by the authors. Is there any evidence for similar uptake by the retina? One may also raise the question of whether the transport system in the untreated animal is already partially saturated. From this point of view it would be important to learn the plasma level of related amino acids in the untreated animal and how much this is altered by the administration of 5 mmoles of α-AIB. Essentially, one needs a saturation curve for the true steady-state ratio at various plasma concentrations. From such a curve and its Lineweaver-Burk plot, extrapolation might suggest the aqueous/plasma ratio for very small amounts of amino acid presented to the secretory site. Under such circumstances one might well find concentrations in the aqueous in excess of plasma.

In view of the importance placed on the saturation of a transport system resulting in reduction of steady-state concentration of α-AIB, one must raise questions about nonspecific effects of this substance on the eye and its secretory processes. Does the administration of 5 mmoles of α-AIB alter the concentration in the aqueous humor of such anions as ascorbate or bicarbonate? Does α-AIB alter the tonogram in the rabbit?

It is of related interest to point out that we have been unable to demonstrate accumulation of α-AIB in ciliary body–iris preparations in vitro. Furthermore, the tissue medium ratio is not altered by increasing the concentration of nonlabeled α-AIB in the medium.

In a transport system as exciting as this one, one immediately wonders about methods for the alteration of this secretion and the effects of such alterations. In some tissues the accumulation of α-AIB is decreased by alloxan diabetes and increased to above normal by insulin. It would be interesting to know whether these effects hold for the transport into the aqueous humor and also for the accumulation by the lens.

Vitamin B_6 deficiency in the rat (induced in 10 hours by the administration of L-penicillamine) decreases the absorption of L-amino acids from inverted intestinal sacs in vitro. The effect is reversed by the administration of vitamin B_6. Interestingly enough, vitamin B_6 deficiency does not alter the absorption of D-amino acids. Is any comparable information available on the transport into aqueous humor and lens?

Drs. Reddy and Kinsey have pointed out that administration of Diamox to the rabbit results in a more rapid and greater accumulation of α-AIB. As they indicated, this effect is impossible to understand without more information. The effect could be explained by a lowering of the plasma amino acid level, and I wonder whether any plasma measurements have been made. The Diamox effect could also be explained by a decreased uptake by the lens, retina, and other ocular tissues. What is the effect of Diamox on the uptake of α-AIB by the lens? What happens when saturating doses of α-AIB are administered to the Diamox-treated animals? Does one get a different set of saturation kinetics as has been demonstrated for ascorbate in Diamox-treated rabbits? In addition to these more remote possibilities, one must also consider the direct effects of Diamox, or the acidosis induced by it, on the transport system for amino acids as well as differential effects on the rate of flow of aqueous humor and the transport of its constituents.

As I indicated initially, this is a most stimulating paper and, as such, raises innumerable questions. I anticipate that the authors already have answers for many of these.

Dr. Reddy (closing). Dr. Becker's comments clearly illustrate his insight into the numerous questions posed by the observation that α-AIB appears to be actively transported from the plasma across the ciliary epithelium into the posterior chamber and from the posterior chamber into the lens.

Some of the problems Dr. Becker poses concerning the nature and requirements of this transport process have been the subject of intensive study in our laboratory since completion of the present paper.

While answers to many of Dr. Becker's questions must await further experimentation, it appears clear at this time that most of the naturally occurring amino acids are also actively transported both into the posterior chamber and into the lens. Moreover, the systems responsible for their transport are capable of being saturated in varying degrees by the amino acids normally present in plasma and/or posterior chamber aqueous. The possible interrelationship between amino acid transport systems and other mechanisms by which substances gain entrance to the posterior chamber and lens have yet to be established although we believe that ascorbic acid does not saturate the system by which α-AIB is transported into the eye

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nor does administration of 5 mmoles of α-AIB alter the concentration of ascorbate in the aqueous humor.

It is hoped that by the time of the next meeting of the Association for Research we shall be able to clarify many of the interesting points which Dr. Becker has raised. Again, we wish to thank him for his stimulating discussion.

With regard to Dr. Lerman's question, viz., whether α-AIB is in the free form within the lens, all evidence thus far indicates that the amino acid is not bound to the protein. Precipitation of lens proteins with picric acid or Nelson Somogyi reagent shows that there is no detectable radioactivity associated with the proteins. These procedures are commonly employed for the determination of free amino acids in tissues.

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