NUTRITION REVIEWS

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TRANSPORT OF AMINO ACIDS

Before amino acids nourish the cell they must be transported a) across the intestinal mucosa and b) across the plasma membrane of the cell where they are to be used. In addition, full consideration of their economy in the animal organism must include also their entry into the liver and their resorption from the renal tubule. All of these events appear to have much in common and probably to represent a single type of basic process of the plasma membrane. Very likely, similar transports within the cell are necessary to the ordered metabolism occurring there.

I will try to summarize some of the advances in the understanding of amino acid transport that have been reached in the last few years. First, we may recall that the amino acids fall into at least three transport families, the neutral, the cationic, and the anionic. Competition between amino acids for transport is restricted within these family lines. An exception was proposed a few years ago by C. E. Dent to account for the faulty resorption of cystine, lysine, arginine, and ornithine by the kidney in cystinuria. Dent suggested that cystine might be transported by the same agency serving for the cationic amino acids because it is a diamino acid, even though no cation.

H. R. Rosenberg, S. J. Downing and S. Segal (*J. Biol. Chem.* **237,** *2265 (1962)*) have now tested this proposal using rat kidney slices. Although competitions were observed among arginine, ornithine and lysine, cystine showed no ability to inhibit these transports, nor was its transport inhibited by them. Before we regard the result as conclusive, the demonstration needs to be made for tubular resorption per se. The defect in renal transport of diamino acids of cystinuria applies also for the intestine (M. D. Milne, A. Asatoor, and L. W. Loughbridge, *Lancet* **1,** *51 (1961)*), and these amino acids are reported also to compete with cystine for intestinal transport (*see* T. H. Wilson, *Intestinal Absorption, p. 124 1962*).

Among the neutral amino acids a dichotomy has in the meantime appeared, so that two separate mediators ("carriers") can now be discerned. These show overlapping affinities among the amino acids, so that only quantitative evaluation of the mutual inhibitory actions served to uncover the need for two distinct reactive sites. What actually brought attention to this requirement was the remarkably steep fall-off in transport affinity in the mature red blood cell as the hydrocarbon chain is shortened in the series, leucine, valine, α-aminobutyric acid, alanine, and glycine (C. G. Winter, *Fed. Proc.* **21,** *148 (1962)*). This pattern arises from the almost total loss of an alanine-preferring mediation on the maturation of the red blood cell, so that alanine and glycine enter this cell only very slowly.

For the intestinal transport system H. Akedo and H. N. Christensen (*J. Biol. Chem.* **237,** *113 (1962)*) called attention also to the failure of glycine and α-aminoisobutyric acid to saturate their own transports, and to suffer the degree of competition by valine and methionine that they should from their inferior transport affinity. In the Ehrlich cell, D. L. Oxender (*Fed. Proc.* **21,** *148 (1962)*) by using selected amino acids as differential inhibitors has clearly discerned two distinct mediators: one, a *leucine-preferring* mediator corresponds to the dominant one of the red blood cell and shows a sharp decline of affinity as the hydrocarbon sidechain becomes smaller; the other, an *alanine-preferring* mediator, serves well for glycine and α-

aminoisobutyric acid, but suffers a serious loss of affinity when a branched, isopropyl, isobutyl or tertiary butyl sidechain is present. Therefore, valine, leucine and such artificial amino acids as tertiary leucine (pseudo-leucine) have low affinity for it. If the non-polar chain is straight and long, as in norleucine and methionine, Oxender finds high affinity again.

The consequence is that methionine has good affinity for both mediators. Elevated levels of this amino acid are therefore able to suppress transport of various neutral amino acids.

These two systems must not be supposed to have identical biological significance. Instead they show properties that imply a complementary action; conceivably they are only modified forms of the same mediator. The one designated *alanine-preferring* is able to work steeply uphill and shows a much smaller tendency to mediate exchange between amino acids within the cell and those on the outside. The other mediation has much less uphill action; it serves freely for exchange. If one permits the cell to accumulate methionine, for example, and then places the cell in a leucine-containing solution, leucine will reach a much higher gradient in favor of the cell by exchange for methionine than it would otherwise.

Effects of this type may give dual-affinity amino acids such as methionine a dominant role in determining amino acid distribution. Such amino acids may act not only as inhibitors but also as go-betweens to cause uphill pumping of amino acids that have themselves little affinity for uphill transport. The unusual sensitivity of the organism to elevations of the plasma methionine level may perhaps be explained in this way. Unquestionably the transport process is peculiarly sensitive to imbalance in amino acid nutrition because of the unusually low specificity of the transport carriers or mediators. But to methionine it may be expected to be especially sensitive.

Our supposition is that on maturation the erythrocyte may no longer generate enough energy to activate the alanine-preferring uphill transport that was still active at the reticulocyte stage. Hence, the leucine-preferring mediation predominates. It will be important to determine whether the former arises at the expense of the latter if sufficient energy is made available.

Model amino acids resistant to metabolic alteration provide special assistance in discerning these mediators with overlapping affinity. α-Aminoisobutyric acid may serve to block the alanine-preferring mediation without detectable influence on the leucine-preferring one. 1-Aminocyclopentanecarboxylic acid, strangely, serves as a model for methionine since it also has dual affinity. For the separate suppression or observation of the leucine-preferring mediation, tertiary leucine ($(CH_3)_3C\ CH(NH_3^+)COO^-$) is recommended.

The above separation of the neutral amino acids into two overlapping transport classes promises to help account for the peculiar pattern of sensitivity of transport to insulin. Glycine, α-aminoisobutyric acid, 1-aminocyclopentane-carboxylic acid, methionine, and proline all have their uptake by the diaphragm accelerated by insulin (Akedo and Christensen, *J. Biol. Chem.* **237,** *118 (1962)*), whereas many other neutral amino acids do not. The action of insulin may well be restricted to one mediation. Akedo showed that the action of insulin was to increase the apparent affinity of amino acids for the transport mediation; no change in the diffusion constant could be detected.

The impressive multiplication of examples of hormone action on biological transport is perhaps somewhat beyond the scope of this summary. A special feature lies in the indications that general modifications of membrane behavior are often produced, including changes in transport not only of amino acids or of potassium ion, but of a variety of substances. For the disulfide hormones these modifications may be perhaps

initiated by a sulfhydryl-disulfide exchange (C. T. O. Fong, L. Silver, D. R. Christman and I. L. Schwartz, *Proc. Nat. Acad. Sci.* **46,** *1273 (1960)*). Subcellular membranes as well as the plasma membrane may perhaps also respond to this process (D. Neubert and A. L. Lehninger, *J. Biol. Chem.*, **237,** *952 (1962)*).

In this connection, the association between alkali metal migration and amino acid transport should be mentioned. T. R. Riggs, L. M. Walker, and Christensen (*J. Biol. Chem.* **233,** *1479 (1958)*) showed that depletion of cellular potassium (with sodium ion replacement) was the common denominator linking together a great variety of toxic actions suppressing amino acid transport. Removing the poison failed to restore transport until the alkali metal ions were returned to their normal intracellular location. The energy represented by the gradients of the two alkali metals may serve for amino acid transport or, alternatively, the alkali metal ions may have a prior demand for transport energy over the organic molecules. Related sensitivities of sugar transport, especially to the sodium ion, have recently been shown by T. Z. Csáky and M. Thale (*J. Physiol.* **151,** *59 (1960)*) and by Bihler and Crane (*Biochim. Biophys. Acta* **59,** *78 (1962)*).

No final answer is available as to the nature of the association of the natural economy of vitamin B_6 and amino acid transport, although intestinal transport has also been shown sensitive to the B_6 supply (F. A. Jacobs and R. S. L. Hillman, *J. Biol. Chem.* **232,** *445 (1958)*; Akedo, Sugawa, Yoshikawa, and Suda, *J. Biochem. (Japan)* **47,** *124 (1960)*). The action of free pyridoxal phosphate in steepening amino acid accumulation by the Ehrlich cell apparently arises from an inhibition of the leucine-preferring mediation, by which many amino acids escape from the cell after having been "pumped in" by the other, alanine-preferring system (Oxender, *unpublished results*). This action of the aromatic aldehydes is probably not related to the question of the natural role of vitamin B_6.

The principal questions as to how the amino acid is grasped and how energy is utilized to propel it into the cell, remain to be explained, as well as the means by which hormones modify this behavior.

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FLUORIDATION IN GREAT BRITAIN

Fluoridation of the water supplies in three communities in Great Britain for five years resulted in major reductions in the dental caries incidence of deciduous teeth of young children.

In 1952, upon recommendation of the Medical Research Council, the British government sent a group of qualified specialists in various fields to the United States and Canada to study fluoridation in operation and the results achieved. This group reported that fluoridation was a valuable health measure in the reduction of dental caries and that there was no scientific evidence of danger to health from prolonged consumption of fluoridated water, and recommended that fluoride should be added to water supplies under appropriate circumstances in selected communities in Great Britain (*The United Kingdom Mission on the Fluoridation of Domestic Water Supplies in North America*, Her Majesty's Stationery Office (*1953*)).

As a sequel to these recommendations, the communal water supplies in Watford and part of the county of Anglesey in England and Kilmarnock in Scotland were fluori-