Methods for Investigation OF AMINO ACID AND PROTEIN METABOLISM

EDITED BY

Antoine E. El-Khoury

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Methods for Investigation

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Methods in Nutrition Research

Edited by Ronald Ross Watson and Ira Wolinsky

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Methods for Investigation of Amino Acid and Protein Metabolism, Antoine E. El-Khoury

Series Preface

Methods are critical to both good data and their correct interpretation. While there are methods series for biochemistry and other disciplines, nutritional sciences have suffered from the absence of such a resource. Many small but important techniques, procedures, and carefully tested methods make experiments easier and more accurate. This series, *Methods in Nutrition Research*, is designed to gradually fill this gap in research resources. It will provide published and unpublished details of technical procedures used by experts in different areas of nutrition research. It will also describe potential pitfalls to be avoided. The series is designed for the researcher with a focus on laboratory and field nutritional research methods and how to apply them precisely. It will contain books ranging from descriptions of studies of micronutrients in animal models to macronutrients in human studies. The overall aim of the series is to carry out nutritional research as efficiently as possible, without technical errors, from known procedural methods, ones which may not be easily found in the literature. Nutritional researchers will bring together their own as well as others' experiences to provide a single source of detailed, tested methods with complete descriptions on how to apply them.

We welcome *Methods for Investigation of Amino Acid and Protein Metabolism.* This well-conceived and crafted volume will surely contribute to the study of amino acid and protein metabolism.

Ronald Ross Watson
Ira Wolinsky
Series Editors

Preface

Because of the importance of research methods in the field of amino acid and protein nutrition and metabolism, it became clear to me that a book was very much needed to assemble and discuss the relevant aspects of those methods. My thoughts are directed toward an attempt to facilitate the reader's integration of the concepts involved in these investigative research methods and their corollaries. In addition to helping any nutrition investigator design and conduct appropriate research protocols in this area of nutrition, another objective is to assist doctoral students in nutrition who are planning to investigate amino acid and protein metabolism in humans or laboratory animals.

The pivotal role of amino acid transfer across tissue membranes in the compartmentation control of amino acid metabolism makes it essential that Chapter 1 discusses the available methods related to this aspect. The major expansion of the use of stable isotope-based techniques in the investigation of protein nutrition and metabolism highlights Chapters 6 and 7, on the discussion of mass spectrometry methods. Also, it is necessary to consider some specific conditions, in addition to healthy young adult subjects, where these methods can be applied, e.g., the elderly (Chapter 10), the fetus (Chapter 11), burn and trauma patients (Chapter 13), and diabetes mellitus (Chapter 14).

I hope that this book, a product of an excellent collaboration with national and international expert scientists, will permanently remove the shades of uncertainty related to these fundamental methods and inspire further investigation of amino acid and protein nutrition.

Antoine E. El-Khoury, M.D., Editor
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Editor

After earning a Doctor of Medicine's degree from St. Joseph University in Lebanon, Dr. Antoine E. El-Khoury moved to Paris, where he completed four years of clinical training in pediatrics. His interest in nutrition led him to undergo a year's training in Children Parenteral Nutrition at Hopital des Enfants Malades. Later, Dr. El-Khoury trained in Human Nutrition and Metabolism (Research Methods) at the University of Aberdeen and the Rowett Research Institute, Scotland. Since late 1991, Dr. El-Khoury has been a research scientist at the Massachusetts Institute of Technology Clinical Research Center and Laboratory of Human Nutrition, Cambridge, Massachusetts. His research activity with Prof. Vernon R. Young focused on amino acid and protein metabolism and nutrition in healthy young adult humans, with particular reference to the minimum dietary requirements for indispensable amino acids. Dr. El-Khoury has extensively used stable isotopes as tracers for *in vivo* nutritional and metabolic research investigations. He is a member of the American Society for Nutritional Sciences (ASNS), the American Society for Clinical Nutrition (ASCN), and the New York Academy of Sciences. He is an external reviewer for the *American Journal of Physiology (Endocrinology and Metabolism).*

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Dedication

To my wife Maggie and my parents Elie and Vicky

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1 Investigation of Amino Acid Transfer Across Tissue Membranes

Peter M. Taylor and Sylvia Y. Low

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1.1 INTRODUCTION: FUNCTIONS OF AMINO ACID TRANSFERS

Plasma membrane of cells is the principal physical barrier limiting protein and amino acid (AA) movement between different metabolic compartments in an organism. Control of these movements is now recognised to be important for overall control of whole-body protein metabolism.[1-5] AA transport processes fulfill a number of specialised but essential body functions alongside their basic role in supplying cellular AAs for protein synthesis and cell metabolism. These include absorption/reabsorption

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of AAs (from intestinal/renal lumen, respectively),[6-8] control of neurotransmission (re-uptake of AA transmitters from synaptic cleft),[8-10] and inter-organ exchange of carbon and nitrogen.[1,2] The most quantitatively important sites of AA transfer in the human body are likely to be skeletal muscle, the kidneys, and tissues bathed by the splanchnic circulation (notably the liver and small intestine).[1,2,11] Dietary protein is hydrolysed to small peptides and AAs within the intestinal lumen; although peptide transport represents a considerable proportion of the total amino-N uptake across the brush-border membrane, intracellular peptide hydrolysis means that the overall trans-epithelial movement of amino-N is almost entirely in the form of AAs.[1,2,12] In this article, we provide a brief overview of the mechanisms involved in AA movements across cell membranes before describing methods available for their study, focusing on techniques applicable to *in vivo* or intact tissue/organ investigations.

1.2 MECHANISMS OF AMINO ACID TRANSFER

1.2.1 Passive Diffusion

The simplest mechanism by which AAs cross cell membranes is by passive diffusion. Diffusional fluxes of solutes are proportional to the concentration difference across a permeable barrier (e.g., lipid bilayer of the cell membrane) and the concentration gradient lies within the barrier itself.[13] This relationship can be described quantitatively by a form of **Fick's First Law of Diffusion**:

$$J_{1\rightarrow2} = D.A.(c_1 - c_2)/x \qquad (1)$$

where J = net diffusional flux (mol/sec), D = diffusion coefficient of substance across the barrier (cm^2/sec), A = barrier area (cm^2), c = concentration of substance (mol/cm^3), and x = barrier thickness (cm). The value of D is specific for both barrier *and* diffusing molecules. The flux J is directly proportional to this value and also to exchange area and solute concentration difference (Δc), whilst it is inversely proportional to membrane thickness. Both D and x are difficult to measure experimentally, so in practice the term permeability (P; cm/sec; where P = D/x) is generally used.[13] Thus:

$$J_{1\rightarrow2} = P.A.(\Delta c_{1\rightarrow2}) \qquad (2)$$

Passive diffusion across the lipid bilayer is favoured for small lipophilic molecules. The selective permeability of the membrane depends on the relative tendency of a given solute to dissolve in lipid/water, given by the solvent-water partition coefficient (K_p), with molecular size playing a secondary role.[13] AAs are hydrophilic molecules with K_p values much lower than 1, and passive diffusion is usually much too slow for the required metabolic fluxes of AAs across membranes of living cells.[1,2] Cells have evolved "pores" (channels and transporters) which enable the membrane barrier to be bypassed for effective transmembrane exchange of polar solutes.[2,13] Important characteristics of these pores are:

(a) markedly greater solute flux than predicted by passive diffusion; (b) specificity for single or small structurally related group of substrates; (c) saturability (at least in theory); and (d) susceptiblility to specific inhibitors/inactivators.

1.2.2 Carrier-Mediated Transport

Metabolically important AA movements across cell membranes involve transporters (or "carriers") rather than channels (aqueous pores).[5,8,13,14] Transporters offer the required degree of substrate selectivity related to molecular interactions between solute "substrate" and binding site on the transporter protein, whereas channel selectivity is largely limited to discrimination between size and charge of potential substrates.[2,13,14] Transport mechanisms involve adsorption of solute from bulk fluid phase onto the binding site and a conformational change of the transport protein to move solute across the membrane. The binding site is alternately exposed to the two sides of the membrane during a transport cycle, in tandem with association or disassociation of the carrier-substrate complex.[13,14] The simplest carriers (facilitative transporters) act to "accelerate" (or facilitate) the process of diffusion down an (electro-) chemical gradient of solute. More complex carriers include co-transporters and counter-transporters, in which there is rigid coupling of the movement of two solutes either in the same or opposite directions.[13,14] Important factors influencing unidirectional flux through a transporter will include[13,14] the stoichiometry of a co-transport or counter-transport process, affinity of the binding site for each substrate, dependence on the membrane potential (particularly important if a net charge movement is involved), and the availability of substrates both at the *cis* and *trans* sides of the membrane (see Section 1.3). The physiological significance of coupling is that it allows the gradient of one solute to drive another solute uphill against an electrochemical gradient. Frequently, the gradient of Na^+ is used to drive accumulation of metabolically active substrates (including AAs) into cells. Transport stoichiometry is physiologically important.[1,2,13,14] For Na^+-coupled transport of solutes (e.g., AA), the equilibrium distribution ratio ($[S]_i/[S]_o$,where$[S]_i/[S]_o$ denote intra-/extracellular concentrations) or "concentrating power" of the transporter is related to both the Na^+- gradient (chemical + electrical) and coupling ratio (n); any increase in n markedly increases the achievable $[S]_i/[S]_o$.[13,14] Equilibrium$[S]_i/[S]_o$ values are not achieved *in vivo* because of energy losses, transporter "slippage" and dissipation of [S] gradient by other mechanisms.[13,14] The Na^+ gradient is generated largely by the Na^+ pump (Na^++K^+ATPase, a primary active transport process), and Na^+-coupled transporters utilising this gradient represent secondary active transport processes.[2,13,14] Solute gradients generated by the latter process can in turn be used to generate uphill transport of a different substrate by heteroexchanging carriers, although with low concentrating power (this is termed tertiary active transport).[1,2,12] Detailed descriptions of models of a variety of transport processes can be found elsewhere.[13,14] Although we focus exclusively on the plasma membrane in this article, it should be recognised that transport across intracellular membranes such as those of the mitochondria, lysosome and the endoplasmic reticulum also represent potentially important steps for control of AA metabolism.[2,12]

1.2.3 Amino Acid Transport Systems

The pioneering work of Christensen and colleagues in the early 1960s led to the identification of a wide variety of amino acid transport mechanisms (or "systems") based on criteria of function[1,2,12] and, latterly, of structure after molecular cloning.[5,8] These transporter mechanisms differ in their substrate range, dependence on co-substrates such as ions (particularly Na^+), and their regulatory properties such as sensitivity to hormones, e.g., insulin.[2,5,12] AA transporters for the 20 common AAs are generally selective for a particular type of substrate. This selectivity is related to side-chain features, such as **charge** (anionic, cationic, zwitterionic or "neutral"), **size** (small or large neutral) and **structure** (e.g., extra N in glutamine, asparagine and histidine).[2,5,12] AA transporters may be functionally subdivided as follows:[2,8,9,12]

- Na^+-dependent: Systems A, ASC (ubiquitous), Systems B^0, $B^{0,+}$ (epithelial brush borders, embryonic tissue), System N (liver, muscle)
- Na^+ and K^+-dependent: System X^-_{ag} (widespread, especially neural and epithelial tissues)
- Na^+ and Cl^--dependent: GABA, taurine and glycine transporters (concentrated in neural tissue)
- Na^+-independent: Systems L, T, y^+, $b^{0,+}$

Suffixes + or - denote carriage of appropriately charged amino acid substrates. The "system"-based nomenclature is largely derived from **model** or **paradigm** substrates, and kinetic discrimination between systems has been aided by design and synthesis of "system-specific" non-metabolisable amino acid analogues, e.g., N-methylaminoisobutyric acid (MeAIB) for System A and 2-aminobicyclo [2,2,1] heptane-2-carboxylic acid (BCH) for System L.[1,2,12] A number of amino acid transporter proteins have now been cloned.[5,8,9]

1.3 KINETIC PROPERTIES OF AMINO ACID TRANSPORTERS

Kinetic properties of transport processes can be conveniently subdivided into those occurring at *cis*- or *trans*-faces of the membrane (i.e., at the same or opposite sides of the membrane, respectively, relative to the substrate under study).

The major *cis*-effects are (a) saturability and (b) stereospecificity.

Saturability results from competition between substrate molecules for a finite number of binding sites on transporter proteins. Saturable transport may be described by the **Michaelis-Menten** equation:

$$v = \frac{\mathbf{V}_{max}[\mathbf{S}]}{\mathbf{K}_m + [\mathbf{S}]} \quad (3)$$