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Amino
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Interpretation of amino acid patterns in mammalian species

Review Article

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Quantitative methods for the assays of amino acids in biological samples were introduced in the late fifties. Methodological work in the succeeding years has greatly improved the quality of results and more than twenty amino acids can now be rapidly analysed routinely and simultaneously in a small sample with a high degree of accuracy and precision. No doubt new techniques will continue to be introduced for the analysis of amino acids, singly or in combinations, offering advantages in terms of sensitivity, economics or speed.

One remaining problem is the presentation and interpretation of the results. Usually the concentrations are simply listed in the order of appearance of the amino acids on the chromatogram, together with some reference values. It is then often left to the end-user, e.g. the clinician or dietician, to attempt an interpretation. It is more than likely that important information is lost with this primitive way of looking at the outcome of a sophisticated investigation.

There have been attempts in the past to improve this situation by simple means, e.g. by splitting the result into non-essential and essential amino acids (Arrogave, 1970), by grouping them or by expressing them in a graphical form, indicating their distance from the reference mean (Briddon and Oberholzer, 1987). Such procedures are time-consuming if they are carried out manually. Manufacturers of amino acid analysers could however, as a standard feature, include software in their equipment for the editing of amino acid profiles, both on screen and in print-out.

Long-term, there is a need for developing more sophisticated strategies for interpreting amino acid chromatograms, incorporating the vast biochemical and physiological knowledge of amino acids in healthy subjects and in disease, that has emerged as a result of three decades of research. Such strategies should involve so-called "knowledge-based" computer systems, which would allow the development of a range of suitable algorithms for interpretation and presentation of results. They would be complementary to the use of modelling for characterising dynamic aspects of amino acid metabolism (Hjelm and Seakins, 1992).

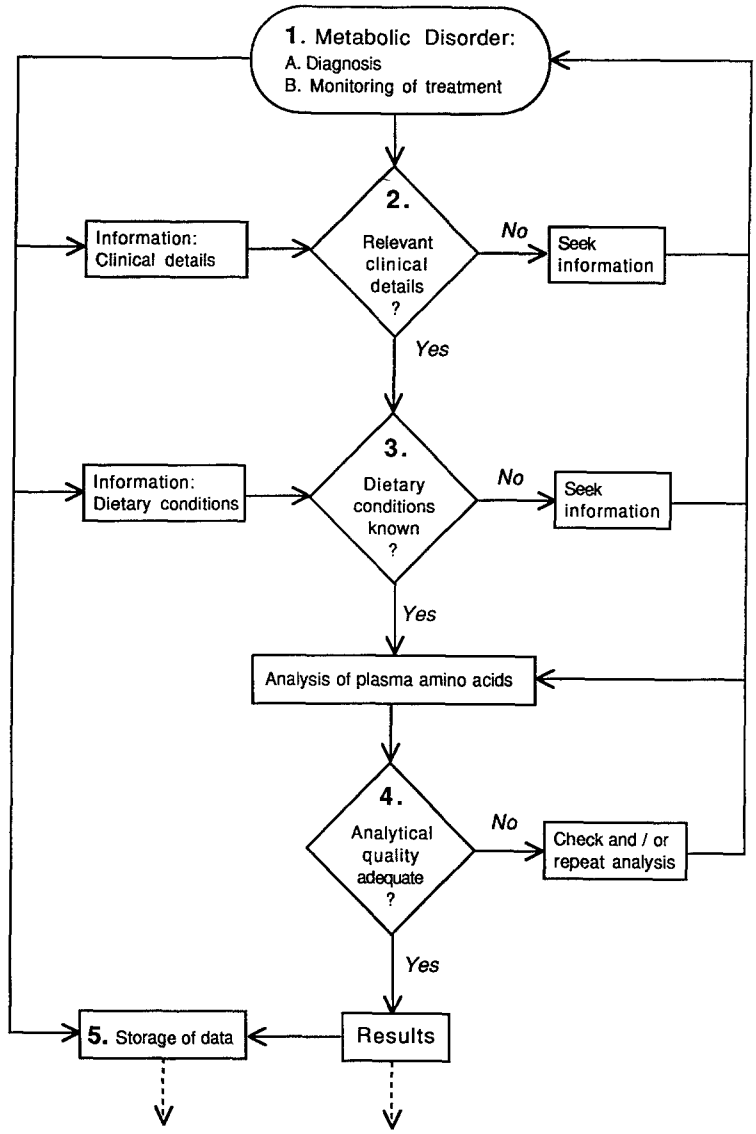

Fig. 1. First part of algorithm for the interpretation of amino acid profiles, continued in Figs. 2 and 3

Some basic features of such algorithms are illustrated in Fig. 1–3, focussed on the diagnosis and monitoring of metabolic disorders.

The following comments can be made:

Clinical, dietary and analytical information

Steps 1, 2 and 3. It is essential to provide information about a minimum number of clinical details and the dietary situation preceeding the collection of samples, including e.g. information about clinical presentation, family history and fasting or non-fasting state at sampling (e.g. on intravenous or tube feeding).

Step 4. The analytical quality of the results needs to be assured. It is likely that the quality of individual amino acids results varies, depending on their

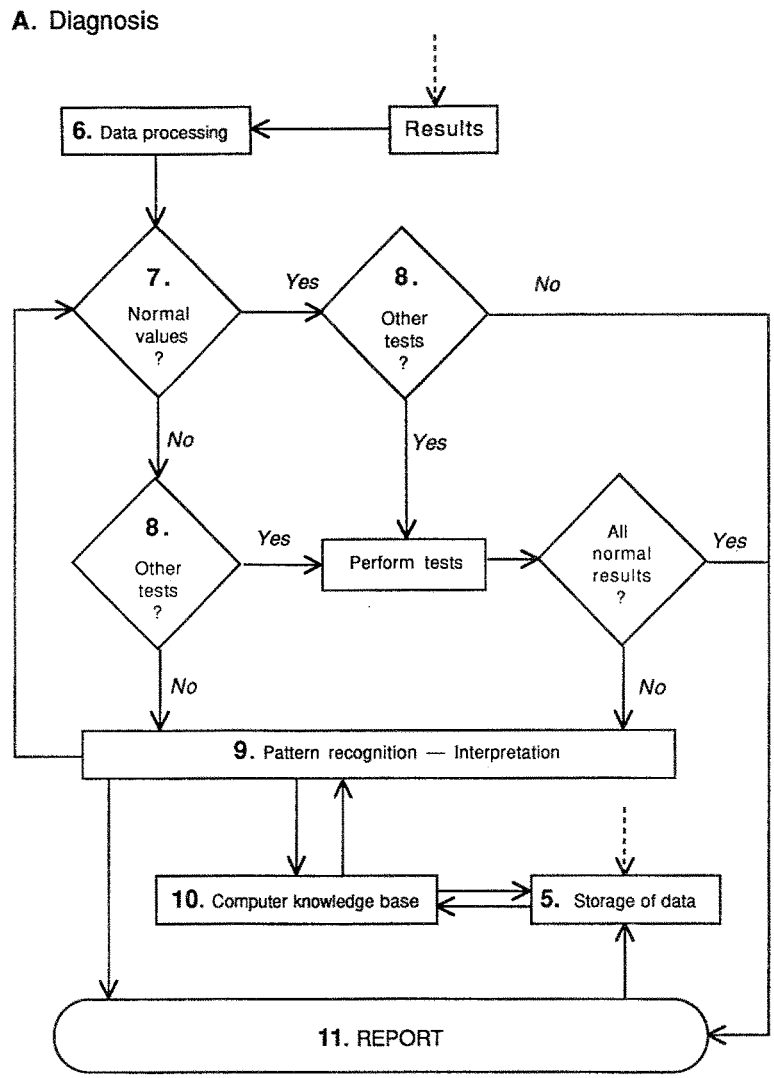

Fig. 2. Data processing of results for diagnostic tests, continued from Fig. 1

relative concentration (though a systematic investigation of this aspect seems to be missing). It would be important to improve the quality control procedures, especially for amino acids normally present at low concentrations, e.g. for phenylalanine and tyrosine used to monitor phenylketonuria, as both falsely decreased and increased levels from the normal may have detrimental clinical effects. Such control procedures could involve the use of a series of internal calibration materials and control materials and computerised processing of the data, included as a feature in the equipment.

Data processing and storage

Step 5. In some instances no specific interpretation of the amino acid chromatogram can be offered. Such patterns with the corresponding clinical and

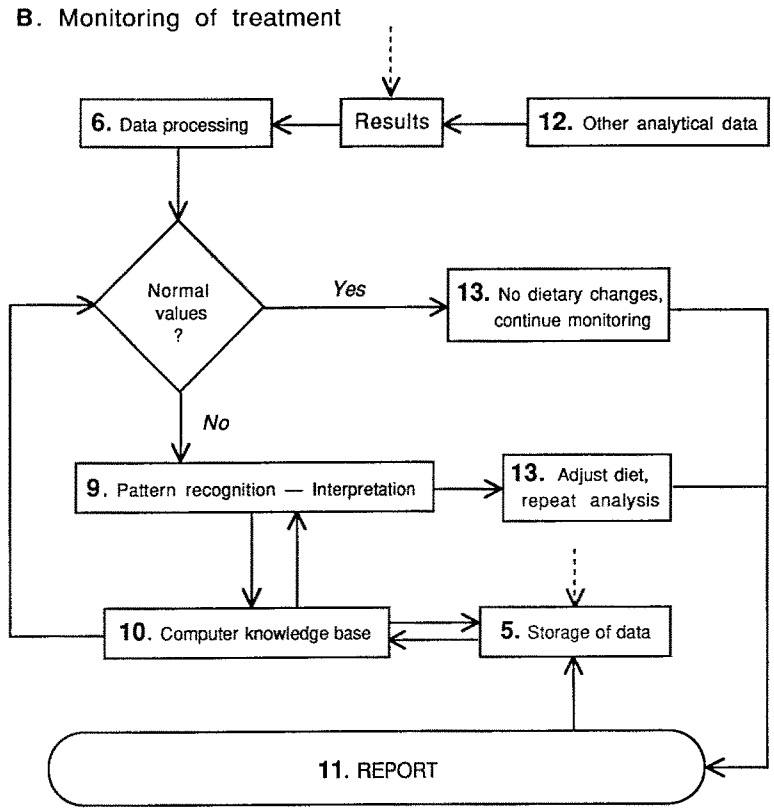

Figure 3. Data processing of results for dietary monitoring, from Fig. 1

dietetic information should still be kept in the computerised knowledge base for future reference, should similar profiles occur again.

Step 6. Data processing of the results could involve sorting the amino acids in appropriate groups, e.g. essential and non-essential amino acids (Arrogave, 1970; Holmgren, 1974; Whitehead and Dean, 1964), calculating ratios, e.g. phenylalanine/tyrosine ratios for the diagnosis of phenylketonuria (Perry et al., 1967) or glutamine/valine ratios for OCT deficiencies, or providing a graphical representation of the statistical measures of the distances of the individual amino acid metabolism to their reference mean (Oberholzer and Briddon, 1991).

Step 11. The report would consist of the individual amino acid results, relevant reference values, and could include statistical interpretation in numerical or graphical form, ratios for selected amino acids and an interpretative summary.

Diagnostic significance of results

Step 7. Independent of a normal amino acid chromatogram outcome, the case history may motivate other investigations to be carried out, for example urinary amino acids.

Step 8. Suggestions for such additional tests could be obtained from the computerised knowledge base.

Table 1. Some examples of significant plasma amino acid findings

Amino acid	Finding[a]	Suggested action
Alanine (Ala)	1a. Increased in disorders associated with accumulation of lactate and pyruvate	Measure lactate and pyruvate and other organic acids in plasma and urine, etc
	1b. Increased with Gln in urea cycle defects	Measure ammonium, etc
	2. If increased with Gln in urea cycle defects	Check protein intake (and Arg supplement), and other plasma amino acids
Arginine (Arg) (semi-essential)	1a. Increased in arginase deficiency	Confirm
	1b. Decreased in other urea cycle defects	Confirmatory tests
	2. If abnormal in urea cycle defects	Check Arg intake
Glutamine (Gln)	1a. Increased (with Ala) in urea cycle defects	Measure ammonium, etc
	1b. Decreased in phenylketonuria (PKU)	[Confirm]
	1c. Decreased in patients on low protein intakes	Check protein intake
	2. Low in treated PKU	Use concentration of other amino acids to monitor intake
	3. Ready conversion to glutamate and pyroglutamate	Associated with elevated glutamate, check quality of sample
Glycine (Gly)	1a. Increased in non-ketotic hyperglycinaemia	Measure Gly in CSF
	1b. Increased in several organic acidurias	Urinary organic acids
	1c. Increased in long-term starvation	Check protein intake etc
	1d. Increased during Valproate (Epilim) therapy	[None]
Leucine (Leu) Isoleucine (Ileu) Valine (Val) (all essential amino acids)	1. Increased in maple syrup urine disease (MSUD)	
Methionine (Met) (essential)	1a. Increased in hypermethioninaemia	[?Low protein diet]
	1b. Increased in homocystinuria	Confirmatory tests
	1c. Increased in tyrosinaemia I	Confirmatory tests
	1d. Increased in liver disease	[Other clinical tests]
	2a. Increased in non-responsive pyridoxine homocystinuric patients receiving choline or betaine	Check plasma homocystine to ascertain whether or not choline or betaine supplements are adequate
	2b. If increased in pyridoxine-responsive homocystinuria	Check pyridoxine and protein intake
Phenylalanine (Phe) (essential)	1a. Increased in PKU and variant forms	Confirm diagnosis
	1b. May be increased in tyrosinaemia I	Check plasma tyrosine
	1c. May be increased in galactosaemia	Measure urinary galactose etc
	2. If increased in PKU	Adjust Phe intake until P-Phe in upper normal range

[a] 1. In the diagnostic situation; 2. Monitoring diet; 3. Artefactual changes

Step 9, 10. In many instances, typical abnormalities involving one or several amino acids are linked to specific metabolic disorders. Some examples of significant results of amino acid analysis and their interpretation are given in Table 1. Such patterns can be recognised visually (Briddon and Oberholzer, 1987), but the identification could be aided by using a computerised knowledge base.

This approach has been tried successfully for classifying dysmorphic syndromes (Baraitser and Winter, 1984) and inherited metabolic disorders (Seakins and Kay, 1990). A computerised knowledge base has the great advantage that it can easily be updated as new information emerges, continuously improving the predictive value of the pattern recognition analysis.

Monitoring of treatment

Step 12. Additional information may be required when using amino acids for monitoring of treatment. Such data could include assays of organic acids in plasma and urine, e.g. orotic acid in urea cycle defects or methylmalonic acid in methylmalonic aciduria.

Step 13. Both in cases of no dietary change or an adjustment of the diet, the optimal time until the next follow-up needs to be decided. The computer knowledge base could serve as a source of such information.

The algorithm, presented as an example, may seem complicated but nevertheless represents the thought processes applied to the interpretation of plasma amino acid profiles. The computerisation of the algorithm would simplify the handling of thought processes and should ensure that the data provides optimal information to both the general end user and the specialist.

Workshop

A workshop has been arranged at the 3rd International Congress on Amino Acids, Peptides and Analogues in Vienna, Austria, on August 23–27, 1993, with the aim of addressing all aspects of these matters. The workshop will start with a short overview of the situation, proceed with a poster session, where individual contributions will be verbally presented, and close with a discussion aimed at putting forward recommendations to be published in "Amino Acids". We invite the widest possible audience, including biochemists, pharmacologists, physicians, physiologists, neurochemists, nutritionists and others, with a professional or industrial background, with or without experience of animal experiments, to participate in this workshop by submitting their posters and taking part in the discussions.

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