ABSORPTION OF PROTEINS AND AMINO ACIDS

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Abstract

Although the absorption of proteins and amino acids is an important issue in nutrition, its measurement is not common because of the methodological difficulties. Complications are attributable in particular to the magnitude of endogenous protein secretion and to the diversity of absorption mechanisms for amino acids either as individual units or as peptides. Methods for studying absorption include balance techniques, tolerance tests, tracer techniques using proteins or amino acids labelled with ^{131}I, ^{3}H, or ^{15}N, intestinal perfusion studies, and others; they must be selected according to the nature of the information sought. Improvements over the current methods would be useful.

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1. INTRODUCTION

Proteins are nutrients of fundamental importance and malabsorption and/or gastrointestinal loss of protein are important causes of wasting, oedema and weakness in many gastrointestinal diseases. It would therefore be logical to measure protein absorption in such diseases to elucidate the severity of the disease and the pathogenesis of symptoms, such as oedema, and to advise on dietetic treatment. For example, if the patient malabsorbs only fat and not protein, then it would be logical to advise a low-fat, high-protein diet. Finally, such studies would help to clarify the degree of improvement brought about by specific treatment.

While the above theoretical considerations look attractive, in practice protein-absorption studies in their simplest forms are difficult to interpret and have not become popular as a standard test for malabsorption[1] mainly because in their most basic forms they do not add significantly to knowledge already gained by a fat-balance or fat-excretion study. In consequence, widely accepted tests of amino-acid or protein absorption have not been forthcoming. In this paper an attempt will be made firstly to outline the physiology of the digestion and absorption of protein as it is currently understood, secondly to outline the techniques available to measure absorption, thirdly to see how these techniques may be applied to the known physiological processes to answer questions about malabsorption, and finally to speculate about possible future developments.

2. PROTEIN DIGESTION AND AMINO-ACID AND PEPTIDE ABSORPTION

2.1 Digestion

Proteins are digested by gastric pepsin to polypeptides. The polypeptides are then subjected to digestion by pancreatic enzymes - trypsin, chymotrypsin, elastase and carboxypeptidases A and B[2]. The result is a mixture of small peptides mainly containing glycine, threonine, glutamic acid and proline, together with free amino acids[3,4].

2.2 Amino acid transport

Most neutral amino acids are transported actively by a system coupled to the sodium pump. They are transported in an order of priority with methionine having the highest affinity and threonine the lowest[5]. The basic amino acids share a separate and active absorptive transport system which with the exception of lysine[6] is coupled to sodium transport[7]. The N-substituted amino acids and glycine have a separate system that does not require Na^+ for transport[8]. The acidic amino acids are also actively transported by a mechanism that is partially Na^+ dependent[9]. The transport of glutamic acid is inhibited by neutral amino acids[10]. Within each group there is competition for transport and therefore in a mixture of free amino acids their relative proportions would determine the rate at which they are absorbed. For example, a mixture simulating egg albumin resulted in the greatest simultaneous absorption when compared to mixtures with other ratios such as in casein or zein[11].

2.3 Peptide transport

Recently the importance of protein absorption as peptides has become evident. It has been recognized that proteins are absorbed faster than they can be broken down to amino acids[12]. Human studies using liquid meals[3,4] have shown that intraluminal hydrolysis is too slow to account for the absorption of glycine, threonine, proline, hydroxyproline, aspartic and glutamic acids. Furthermore, when free amino acids are perfused the rates at which they are transported depend upon relative affinities[13], while in the above study the proportions of amino acids absorbed were similar to each other and bore no relationship to the affinities. These findings were confirmed by a study of dipeptide transport in man[14,15], which has shown that it is independent of amino acid transport, is greater for a peptide than for the equimolecular mixture of amino acids, and is not inhibited by genetic defects in the transport of the dipeptide's constituent amino acids. For example, histidine

cannot be transported by the intestine of a patient with Hartnup's disease but the same patient can absorb histidine as the dipeptide carnosine, thus circumventing the amino-acid transport block[14]. Since patients with Hartnup's disease do not become protein deficient despite extensive block of amino-acid transport, the peptide mechanism must be of great physiological importance.

2.4 Transamination

Glutamic and aspartic acids are transaminated during absorption[16]. At low levels of input all the glutamic acid is converted to alanine but when the concentration of glutamic acid presented to the lumen increases above 2% most is absorbed intact as the capacity for transamination is saturated. The oxoglutarate so produced is metabolized by the intestine, and pyruvate for this reaction also appears to be provided from this source since blood levels of these two metabolites during absorption of glutamic acid do not change.

2.5 Endogenous protein

The exogenous intestinal contents are diluted by endogenous protein from enzyme and other proteins in secretions, shed cells, and bacteria. The extent of endogenous protein admixture was initially believed to be greater[17] than the ingested protein but is now shown to comprise only 13% - 53%[3,4] of the protein ingested. This endogenous protein is probably digested more slowly than dietary protein and is absorbed further along the alimentary tract[18].

3. METHODS OF MEASURING ABSORPTION IN MAN

3.1 Balance techniques

This method depends upon feeding a known amount of dietary protein and measuring excretion of nitrogen in the faeces. The intake minus excretion should represent absorption. Unfortunately a faecal nitrogen, while indicating the degree of nitrogen excretion, does not strictly indicate the amount of dietary protein absorbed because of admixture with endogenous protein and bacteria. In support of this conclusion are the findings that faecal nitrogen is independent of diet in quantity and quality[19,20]. Furthermore, isotope-dilution studies with ^{15}N have shown that at least 50% of faecal nitrogen was endogenous[21]. Hence in contrast to faecal fat, faecal nitrogen is not a good index of the actual absorption of dietary protein. Although a high faecal nitrogen would indicate malabsorption, it does not differentiate between exogenous protein on the one hand and endogenous protein and bacteria on the other.

3.2 Tolerance tests

In these tests a dose of inactive or radioactive protein or amino acid is ingested and the levels of amino acids or radioactivity in the blood are measured as a function of time thereafter.

3.2.1 Rise in plasma levels after an oral load of inactive amino acids[22,23]

This test is performed by administering an oral load of amino acid, the most commonly used being glycine, and measuring the rise in peripheral-blood level, the peak concentration being noted after about 1 hour. Unfortunately, the rise in plasma is partly due to absorption of amino acids not fed (i.e. endogenous protein) or from release of tissue amino acid. Also, liver disease results in an enhanced level. While this test in qualitative terms can differentiate normals from malabsorbers of the amino acid tested, it has the following potential difficulties:

(1) It is unreliable in the presence of liver disease.

(2) It represents the absorption only of the sole amino acid tested and cannot be extrapolated to the absorption of an amino acid mixture, as amino acids influence the absorption of each other.

(3) It does not reflect the absorption of protein-bound amino acid. In the latter situation many amino acids are absorbed as peptides. An important example is glycine. This amino acid is absorbed relatively poorly as such but much better as glycyl-glycine[15]. In this instance, glycine in protein would be absorbed better than indicated by the tolerance test. In like manner, histidine is poorly absorbed as an amino acid in Hartnup's disease and a histidine tolerance test would suggest that such patients should become deficient in amino acids[14]. This is not true clinically and the discrepancy was resolved when it was shown that peptide-bound histidine is well absorbed in patients with Hartnup's disease. In this case the tolerance test does not correlate with the clinical status.

(4) Many other factors influence the rise in plasma amino-acid level, such as the hormonal status, the rate of tissue uptake and presence or absence of kidney lesions causing aminoaciduria.

3.2.2 Rise in plasma amino acids after an oral load of protein[22]

The peak level of amino acids in plasma has been observed at about 2 hours after ingestion and the rise in the different amino acids did not reflect their respective concentrations in the ingested protein. This is to be expected from the current status of our knowledge of protein absorption. Here again the levels reflect not the composition of the ingested protein, but rather the absorption of

the intestinal mixture together with the effects of the meal on blood hormone levels and intestinal metabolism.

3.2.3 Gelatin tolerance test[24]

This test consists of measuring total urinary excretion of hydroxyproline (free + peptide-bound) after an oral load of gelatin. It has been shown that after gelatin is eaten hydroxyproline peptides are absorbed and excreted because they are relatively inert metabolically. Clearly this method is not subject to the errors resulting from the operation of factors controlling the utilization of the absorbed amino acid. However, this method is not suitable for the investigation of conditions with specific amino-acid transport defects.

3.3 Labelled-protein absorption

3.3.1 ^{131}I-albumin and ^{131}I-casein[22,25,26]

These substances are fed by mouth and the rise in blood ^{131}I radioactivity is observed, with a peak concentration of ^{131}I at about 2 hours. This method can differentiate between patients with pancreatic insufficiency and normals, but there is no difference between controls and patients with coeliac disease. The criticism of this method is the finding of free iodide in intestinal contents during digestion, suggesting that part of the absorption measured is of free iodide[27]. This problem may not apply to ^{131}I in unhydrolysed protein, and hence with this method patients having maldigestion of protein (pancreatic insufficiency) do not absorb the tracer. However, once the ^{131}I-labelled protein is digested and free mono- or di-^{131}I-tyrosine is liberated, then deiodination may invalidate the test by separating ^{131}I from amino acid, making it unreliable in patients with normal digestion but malabsorption.

3.3.2 ^{3}H-human albumin[27]

In this procedure the amino acids are labelled randomly and no significant exchange of ^{3}H with stool water is noted. With this method both poor absorption and loss of endogenous material could be detected using ^{51}Cr as a second marker. The test dose was fed along with ^{51}Cr, and ^{3}H activity appearing after ^{51}Cr activity ceased to be excreted was due to excretion of endogenously labelled material. In this way, it was shown that malabsorption of exogenous and/or endogenous protein occurred in different conditions. The use of this technique for measuring absorption of whole undigested protein has recently been validated showing stability of the label *in vivo*[28].

3.3.3 ^{15}N-yeast protein[21,29]

This method has been used recently to measure the absorption of proteins and amino acids in patients with various diseases and in controls. The oral

dose should be mixed with sufficient carrier to differentiate normals from subjects with disease. For example, patients with pancreatic insufficiency appear to absorb normally unless given a 20 g load of inactive protein along with the labelled protein[21]. It is desirable to measure blood from the hepatic vein to bring out delay in absorption, since peripheral blood does not indicate differences clearly[21]. Faecal excretion of ^{15}N in pancreatic diseases showed[21] that there was marked excretion of endogenous ^{15}N in patients with pancreatic insufficiency, confirming the previous observations with ^{3}H-albumin[27].

The above studies with labelled proteins are useful in that they have demonstrated the role of endogenous protein as a source of nitrogen in stools of patients with malabsorption, but are investigative rather than diagnostic tools at the present time. They do not indicate the exact mechanism of malabsorption.

3.4 Intestinal perfusion studies

Marker perfusion techniques that use a double- or triple-lumen tube[30] and that perfuse a length of intestine with a single amino acid or mixtures can be used to demonstrate transport defects of the amino acid concerned. The technique is capable of defining the site of the defect and its nature; however it does not reflect the uptake of dietary protein, which is absorbed both as amino acids and as peptides. Furthermore, it has now been shown that tryptic digests of casein[31] and equimolar mixtures of amino acids are absorbed maximally at different sites. Additionally, different amino acids are transported at different rates from a free amino acid mixture, while with a protein meal the results are proportional to the concentrations of the amino acids. This difference suggests different mechanisms of absorption,[13,4] and clearly invalidates the extrapolation of data on free amino-acid transport as obtained from perfusion studies to the *in vivo* state. However, a similar perfusion technique can be used to study peptide transport[15].

3.5 Sampling of intestinal contents after a test meal

This method consists of feeding a liquid meal with a marker and sampling intestinal contents in the jejunum or ileum over a 3-hour period. The investigation can be repeated with a low-protein meal to get an idea of endogenous protein secretion. However, since the amount of dietary amino acid fed determines the secretion of pancreozymin and hence of pancreatic enzymes[18], the endogenous protein found with a low-protein meal may not reflect the amount secreted with a high-protein meal. Nevertheless, this technique has

clearly delineated the site at which free amino acids are absorbed in contrast to the site at which peptides are absorbed, and has provided a fresh look at the endogenous protein secreted. It has shown that the equivalent of 25% of the nitrogen fed is passed out in an unassimilable form; however it is not clear if this is exogenous or endogenous. Furthermore it has been useful in providing information about absorptive defects relevant to the *in vivo* state in cystinuria[32].

3.6 Amino-acid uptake in intestinal mucosa obtained by peroral biopsy

It has been shown that the intestinal transport of an amino acid and its uptake by tissue *in vitro* correlate well[33]. This principle has been used to measure intestinal uptake of ^{14}C-amino acids by incubating specimens of human intestinal mucosa *in vitro* with ^{14}C-amino acids. After incubation lasting 30 - 45 minutes the tissue is removed, rinsed with the same amino acid in inactive form and homogenized[34,35]. The results are expressed as percentage uptake by tissue or ratio of concentration of radioactivity in intracellular water (80% of tissue weight) to that in the medium. Various kinetic rates can be measured by incubating with increasing quantities of valine[35]. A Lineweaver-Burk plot will show a typical rectangular hyperbola.

4. APPLICATION OF METHODOLOGY TO CLINICAL SITUATIONS

It is clear from the above considerations that there is room for improvement in the techniques for estimation of absorption of amino acids and protein. In the current status of knowledge the technique used depends upon the information sought from the study. These techniques can be grouped in the following manner.

4.1 Clinical demonstration of protein malabsorption

The magnitude of faecal nitrogen together with the gelatin absorption test would indicate whether there is excessive loss of nitrogen via the gut due to malabsorption of both endogenous and exogenous protein and, if so, it might suggest whether this problem is due to maldigestion and/or poor peptide transport by the intestine. These observations together with a protein-loss study could constitute a crude screening test for the cause of hypoproteinemia in various clinical situations.

The use of tolerance tests for single amino acids, such as histidine or β-alanine, and perhaps including double radioactive markers, followed by a test of absorption of carnosine in the way suggested by Asatoor[14], would be a further refinement, differentiating defects of amino acid transport from those of

peptide transport. Measurement of mucosal uptake of radioactively labelled amino acids and peptides would be another addition to the clinical armamentarium and would delineate the transport defect of amino acids and peptides at the mucosal level[34,35].

4.2 Study of amino-acid and peptide transport

Clearly, marker perfusion using known amino acids and perhaps peptides would be the only reliable method for studying isolated transport mechanisms, for demonstrating the effect of other nutrients on such mechanisms, and for defining specific genetic transport defects[13,15].

4.3 Investigation of pathophysiology in disease

The best way to investigate the pathophysiology of disease would be to emulate the procedure of Nixon and Mawer[3,4]. This could be simplified by collecting, after a standard meal, the intestinal contents at a fixed level, perhaps 140 cm, at which level normals would have absorbed all the amino acids and peptides. The contents could be fractionated to differentiate malabsorption of peptides from that of amino acids. This method theoretically could be refined further by spiking the meal with ^{3}H-labelled albumin and determining the specific radioactivity of amino acid in the initial meal and in the intestinal contents at 140 cm. This difference would clearly indicate the extent and nature of endogenous dilution. Furthermore, if done at different levels, it would show if exogenous and endogenous proteins are being absorbed differently.

In conclusion, the latter method is perhaps the best way of obtaining information although it is tedious and cumbersome. With studies of kinetics of uptake by intestinal mucosal specimens, using media and techniques designed to keep such biopsies functional during the incubation interval, a very good idea can be obtained of the *in vivo* digestion, absorption and mucosal transport kinetics in conditions of disease.

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