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BIRES CHANDRA GUHA MEMORIAL LECTURE 1981
Protein Digestion and Absorption — An Overview

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I feel deeply grateful to INSA for considering me worthy of this honour to deliver the B C Guha Memorial Lecture. Since this is a mixed audience I shall endeavour to make my talk understandable by those who are not biochemists. First, a few words about Dr B C Guha in whose honour the lecture is being given. Dr Guha was an outstanding biochemist of our country. In addition to his service to the country in various ways, the most notable was his contribution to our understanding on the mode of biosynthesis of vitamin C. He placed our country on the international scientific map by this original contribution. He built up and nurtured an active school of research on vitamin C. While delivering this lecture I pay my humble tribute and homage to a great scientist.

The gastrointestinal tract (GI tract for short) is the first port of entry for various compounds found in the diet, directly or processed in the tract biochemically. The digestion of food materials is somewhat limited in the mouth (salivary amylase hydrolysis of carbohydrates like starch and glycogen) and in the stomach (gastric pepsin hydrolysing the proteins). The bulk of the food materials are digested and absorbed in the small intestine which is ideally engineered by nature for this purpose:

(a) It is a large organ, perhaps the largest in the body.

(b) It is a very long tube so that food materials are well exposed to hydrolytic enzymes in the lumen of this tube and in a sense outside the body.

(c) The absorptive surface is very specially designed to give a large surface area almost equal to a tennis court(single, that is).

(d) In collaboration with the pancreas which produces trypsin, chymotrypsin and carboxy-peptidase and liver which produces bile acids and bile salt, the small intestine carries out the digestion of proteins, lipids (fats) and carbohydrates to smaller units which are efficiently absorbed invariably by an 'active' process. In this process, a metabolite is transported or pumped 'uphill' or 'against a concentration gradient' and it requires an input of energy. The molecular basis for this energy transduction is still not fully understood. After this initial entry into the enterocyte (a name given for the epithelial cell of the intestine), metabolites enter through two routes, the lymphatic

route (fats, fat-soluble vitamins, etc.) and the portal route (water-soluble compounds, vitamin B_{12} and other water-soluble vitamins, etc.).

(e) The small intestine is anatomically distinguished by three segments—duodenum (just below the stomach), jejunum (mid gut, most important quantitatively) and ileum (distal end before it joins the large intestine).

(f) Since it is a large organ there is a surplus capacity to handle all the food materials.

Surgical resection of portions of the gut in duodenal ulcers etc., does not, therefore, seriously hamper its function. Also, there is no qualitative anatomical distinction in the functions between the different regions of the gut, except for the absorption of vitamin B_{12} and reabsorption of bile salts for which a specific region in the terminal ileum is necessary.

Disorders of the Intestine

(i) Nontropical sprue (celiac disease): the etiology of this disorder is well known. It is a gluten-induced enteropathy and affects a certain proportion of the population who are 'gluten sensitive'. They exhibit flat mucosa, mal-absorption and the morphology and function reverts to normal if gluten-containing foods like wheat, rye, etc., are withdrawn from the diet. It is extremely rare in India.

(ii) Tropical sprue (mal-absorption syndrome): so-called because of the reports of diarrhoea among British soldiers in the tropics. It is now known in other parts of the world. Charaka, the eminent physician of ancient India gives a reasonably accurate clinical description of this disorder in Charaka Samhita. The etiology is not clear but it may be of viral origin.

(iii) Lactase deficiency and *lactose intolerance:* this is one of the most common disorders since it is prevalent among the coloured populations of the world. The white populations generally have high levels of the enzyme, the only exception being the Greek Cypriots. The enzyme deficiency in India was shown in our laboratory in 1967, by analysis of the biopsy specimens of intestinal mucosa.

(iv) Cystinuria and *Hartnup disease:* These are genetic disorders affecting transport of basic and neutral amino acids respectively (for a further discussion on these disorders refer to a later section).

Protein Absorption

Development of the classical concept

Reaumur in 1753 and Spallanzani in 1763 carried out some remarkable experiments on the fate of food administered in perforated metal tubes to birds, animals and human beings. Sixty years later in 1824 Prout discovered HCl in stomach juice and in 1836 pepsin was discovered. These were land-marks in the understanding of GI tract physiology and established the 'solvent power' of stomach juice. The work of the American surgeon Beaumont with his famous patient Alexis St. Martin, who had a hole (fistula) in his intestine, needs a special mention here. Trypsin was discovered in the middle of the 19th century and in 1897 Buchner's discovery of the catalytic power of yeast extracts heralded the golden era of enzymology. There have been significant advances in transport mechanisms from about 1950 onwards. Wiseman, Wilson (USA), Fisher, Smyth, Parsons (UK) and their groups established the general features of amino acid transport. This is an active mechanism and exhibits group specificity. Genetic disorders like cystinuria (defect in basic amino acid transport) and Hartnup

disease (defect in neutral amino acid transport), confirmed the validity of these mechanisms. Thus, the classical concept of protein-absorption emerged suggesting that proteins are hydrolysed via peptides completely to free amino acids which are then actively absorbed. This dogma of the classical concept also implied that protein absorption takes place *only* in the form of amino acids, since no peptides are found in the portal blood after a protein meal and since proteases and peptidases are present in the GI tract to convert proteins to amino acids.

Peptides as intermediates in protein absorption

Even around the time 1950 onwards there was considerable evidence against this classical concept, but all this was totally ignored until recently. For example, Messerli (1920) observed better growth of rats fed on enzymic digest of protein than those fed on acid hydrolysates. In the 50s London showed that the rate of release of amino acids by proteolysis is very much slower than their appearance in blood after a protein meal. In 1959 Newey and Smyth showed absorption of small peptides like Gly-Gly, Gly-Gly-Gly and Gly-Gly-Gly-Gly. Absorption of small peptides has been amply demonstrated in the last decade, in addition to our work in India, by the pioneering work of Prof. Matthews and co-workers in UK and Prof. Adibi and co-workers in USA (Matthews 1975). There were many technical difficulties in establishing peptide transport. Most peptides are hydrolysed rapidly either in the membrane or inside the cell and so the peptides are not detectable. Also, there would be confusion between intact peptide entry and transport of constituent amino acids after enzymic hydrolysis. Some of these difficulties have been overcome by adopting short incubation periods to minimise hydrolysis, use of slowly hydrolysable peptides like glycyl-sarcosine or of glycyl-proline which is not hydrolysed by the membrane.

Table 1 *Specificity of the monkey intestinal Glycyl-L-[^{14}C] leucine-uptake system*

Peptide	Inhibition (%)	Peptide	Inhibition (%)
Gly-Gly	20	Gly-Asp	50
Gly-Ala	50	Gly-Glu	42
Gly-Val	65	Gly-Asn	63
Gly-Ile	78	Ala-Asp	62
Gly-Leu	86	Met-Glu	64
Gly-Ser	70	Asp-Gly	40
Gly-Phe	73	Glu-Ala	54
Gly-Trp	67	Glu-Val	58
Gly-His	63	Glu-Glu	54
Gly-Met	86	Gln-Gln	76
Ala-Gly	70	Tyr-Lys	77
Ala-Ala	73	Lys-Phe	67
Ala-Leu	92	Lys-Lys	62
Ala-Ser	74	Gly-D-Leu	10
Ala-Phe	86	Z-Gly-Leu	30
Ala-Met	86	Gly-β-Ala	20
Leu-Gly	87	Gly-γ-Abu	32
Ser-Leu	85	Gly-Gly-Leu	58
Met-Leu	91	Gly-Leu-Gly	58
Gly-Pro	69	Gly-Gly-Gly	20
Pro-Gly	51		

In these experiments, the concentration of ^{14}C-labelled glycyl-L-leucine was 0.5 mM and the concentration of the unlabelled peptides was 10 mM in all cases. All the peptides, except glycyl-D-leucine, contained amino acids of the L-configuration

Z, N-benzyl-oxycarbonyl; Gly-γ-Abu, Glycyl-γ-aminobutyric acid

An elegant and perhaps the most decisive proof for the existence of peptide transport came from studies on two human genetic disorders—Hartnup disease and cystinuria. In both these cases the 'affected' amino acids were well absorbed when presented as peptides.

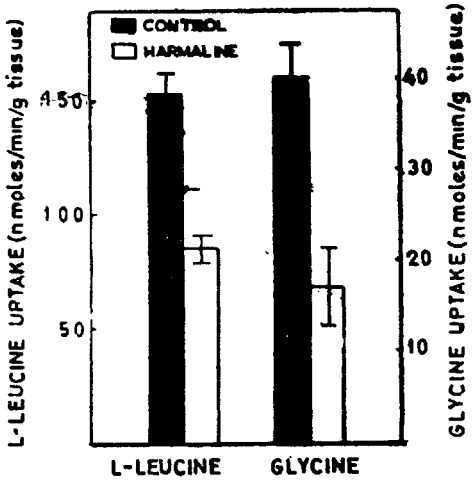

Figure 1 Uptake of L-leucine (1 mmole/l) and glycine (1mmole/l) by monkey intestinal strips in the presence and absence of harmaline (4 mmole/l). The mean and SE are shown (N=6)

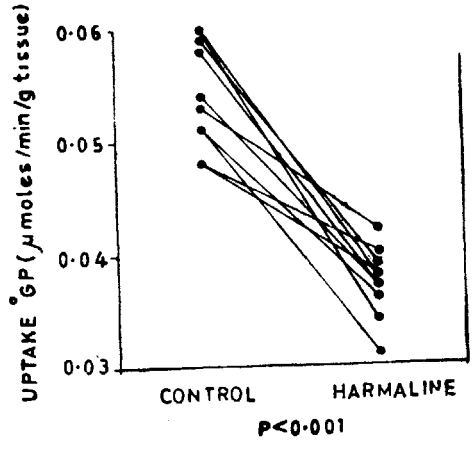

Figure 2 Uptake of glycyl [^{14}C]-proline by monkey intestinal strips in presence and absence of harmaline (4 mM)

Wide-spectrum dipeptide transport system

A systematic study of dipeptide transport in our laboratory during 1970–1975 (Das & Radhakrishnan 1975) led to the discovery of a transport system of wide specificity, reminiscent of the bacterial transport system. The main features are: broad specificity, stereospecificity, dependence on energy and a clear distinction from amino acid transport.

Harmaline, a plant glycoside is a well-known inhibitor of amino acid transport. It also inhibits sodium-dependent dipeptide transport. For example, it inhibits transport of glycyl-proline (not hydrolysed by the membrane, but intracellularly) very significantly. Glycyl-proline shares the same general transport system as for many other dipeptides. Thus, harmaline is considered as a general inhibitor of dipeptide transport (Ganapathy & Radhakrishnan 1980).

In the GI tract at any given time after a protein meal and at different regions of the gut, there will be a mixture of unpredictable composition of peptides, amino acids and undigested protein. It is a very dynamic system and it is not easy to quantitate the relative role of peptides vs. amino acids in absorption. However, as a first step, we have approached this problem as follows.

A crystalline protein of known primary structure is subjected to hydrolysis in vitro by all the proteolytic enzymes known to be present in the GI tract, until no further hydrolysis takes place ('limit hydrolysis'). In a typical experiment the mixture after limit hydrolysis contained 60–70% of peptides and the remaining as free amino acids. This work would strongly suggest that peptide absorption is quantitatively perhaps more important than amino acid absorption. The enzymes

Table 2 *Substrate specificity of the 'master' dipeptidase (from Das & Radhakrishnan 1973)*

C-Terminal N-Terminal	Gly	Ala	Ile, Leu Val	Ser, Thr,	Phe, Tyr Trp, His	Met	Asp, Glu	Lys, Arg
Gly	Gly-Gly	Gly-Ala	Gly-Ile Gly-Leu Gly-Val	Gly-Thr	Gly-Trp Gly-Phe Gly-His	Gly-Met	Glu-Gly	Gly-Lys
Ala	Ala-Gly	Ala-Ala	Ala-Leu Ala-Val	Ala-Ser	Ala-Phe	Ala-Met	Ala-Asp	Ala-Arg Ala-Lys
Ile, Leu, Val	Leu-Gly	Leu-Ala	Leu-Leu Val-Leu	Leu-Ser	Leu-Phe	Leu-Met		
Ser, Thr	Ser-Gly	Ser-Ala	Ser-Leu His-Leu	Ser-Ser	Ser-Phe	Thr-Met		
Phe, Tyr Trp, His	Phe-Gly	Phe-Ala	Phe-Leu Tyr-Leu Trp-Leu		Phe-Phe	Phe-Met	Tyr-Glu	Tyr-Lys
Met	Met-Gly	Met-Ala	Met-Leu Met-Val	Met-Ser Met-Thr	Met-Phe Met-Tyr	Met-Met	Met-Glu	
Asp, Glu	Glu-Glu Asp-Gly	Glu-Ala	Glu-Val		Glu-Tyr		Glu-Glu Glu-Asp	Glu-Lys Asp-Lys Lys-Lys
Lys, Arg	Lys-Gly Arg-Gly	Lys-Ala Arg-Ala	Lys-Leu		Lys-Phe		Lys-Asp Arg-Asu	Arg-Arg Arg-Lys

used in this study are: pepsin, trypsin, carboxypeptidase, chymotrypsin and leucine-amino-peptidase (Hellier et al. 1976b).

After the peptides enter the cell, they are very rapidly hydrolysed by powerful peptidases in the cytosol, thus accounting for the absence of peptides in circulation. The most thoroughly characterized dipeptidase designated 'master dipeptidase' was discovered in our laboratory in 1973. It is so called because of its extremely wide substrate specificity. It is a remarkable enzyme with a molecular activity of 10^6 moles substrate hydrolysed per mole of enzyme per minute. It is one of the most active enzymes in the body and perhaps hydrolyses about 80% of the dipeptides derived from proteins. The remaining dipeptides are hydrolysed by dipeptidases like prolidase, prolinase, glycyl-glycine hydrolase and glycyl-histidine hydrolase which have somewhat limited specificity.

Nutritional importance of peptide transport

1. Simultaneous entry of several amino acids (as units of peptides) leads to maintenance of uniform levels of all amino acids in circulation.
2. There may be considerable saving in the energy economy of the cell.
3. Many peptides of dietary origin may act as vehicles of transport, in a 'piggy-back' type of mechanism, of other molecules, drugs and metal ions.

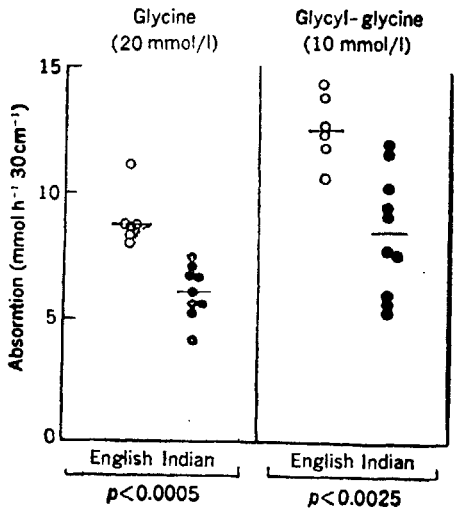

Figure 3 Absorption of glycine (20 mM) and glycyl-glycine (10 mM) in normal English and Indian people. Horizontal lines represent means

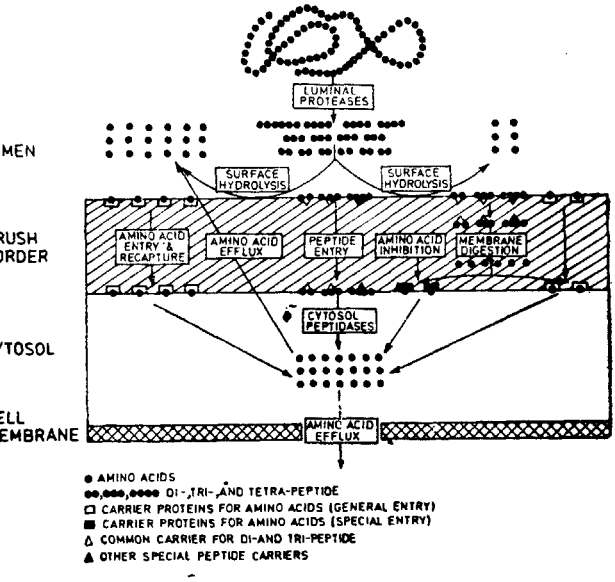

Figure 4 A general scheme of protein digestion and absorption

4. It is possible that the primary structure of the protein may be of greater importance than has been hitherto recognised. Some of the peptides may have a direct effect on the absorption process itself and some others may exert a systemic effect.
5. In Indian subjects we have demonstrated impaired absorption of amino acids and peptides. (Matthews 1975, Premalatha & Radhakishnan, unpublished data). The effect of this deficit on the overall nutritional status of our population in India requires further study.

General scheme of protein-absorption

Based on available information, from our work and of others, a general scheme is proposed (Radhakrishnan 1977). It provides a working model for further experimentation and perhaps an approach for the treatment of certain transport disorders in the human.

The main features are as follows:

(a) Proteins of dietary origin are broken down in the GI tract to a mixture of amino acids and peptides (2–6 residues), in which peptides form the predominant fraction.

(b) The free amino acids are absorbed by the well-established group specific systems.

(c) The amino acid transport systems may also operate as a 'recapture' mechanism for amino acids effluxed into the lumen from inside the cell.

(d) Peptides are presumed to be handled by one of the following three mechanisms:

(i) Peptides cross the membrane intact but are rapidly hydrolysed inside the cell before entering the blood stream.

(ii) Peptides are hydrolysed at the surface by 'membrane digestion' and the resulting amino acids behave like luminal amino acids being transported by group specific transport systems.

(iii) Peptides are hydrolysed in the membrane but the resulting amino acids do not equilibrate with luminal amino acids.They are transported by a special mechanism not yet rigorously established. Kinetically it would be difficult to distinguish this type of transport from that of intact peptides into the cell.

(e) Certain unique peptides which are not hydrolysed either in the membrane or in the cytosol, may be transported by peptide transport systems and enter into circulation intact exerting biological effects.

I like to thank the Indian National Science Academy for giving me this opportunity to review our work on protein absorption and I thank you all for your presence and for the patience with which you have listened to me.

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