A Review of Current Knowledge of Gastrointestinal Absorption of Intact Proteins Including Medicinal Preparations of Proteolytic Enzymes

M. L. G. GARDNER

Introduction

The scientific rationale for the use of oral preparations of enzymes, such as Phlogenzyme (containing trypsin and bromelain), Mulsal-N (containing trypsin, bromelain, and papain), and Wobenzyme (containing trypsin, chymotrypsin, papain, and bromelain), for treatment of extra-gastrointestinal diseases in Man hinges critically upon the question of whether the enzymes (or a therapeutically significant amount of them) can be absorbed across the gastrointestinal tract in intact form. This is an area, formerly regarded as distinctly controversial, where opinion among gastrointestinal physiologists has changed markedly in the past two decades, and which is currently attracting renewed research activity on mechanisms of absorption. It is now accepted beyond reasonable doubt that significant (albeit small) amounts of many macromolecules can be absorbed in intact and biologically active form.

The possibility that absorption of intact proteins may be relevant in the pathogenesis of several diseases and some food idiosyncrasies and the substantial commercial interest in devising preparations of therapeutic peptides (notably insulin) and proteins for oral administration have stimulated new work in this area. Likewise, recent interest in developing macromolecular probes of intestinal permeability for clinical, diagnostic, and research purposes has led to several recent investigations of the extent of intact protein absorption of protein probes (notably ovalbumin and α-lactoglobulin) in humans in health and disease.

There are identifiable reasons why, historically, views as to the form(s) in which dietary proteins and their digestion products enter the circulation have changed several times, and why current opinion is strongly swinging towards the view that intestinal absorption of small amounts of proteins and other macromolecules is a normal physiological event, and also that a greater amount of some small peptides (incompletely digested) can be absorbed than had hitherto been realised. These reasons have been reviewed by Gardner [5, 7], and they can be summarised briefly as follows:

1. Discovery of the pancreatic and intestinal proteases and peptidases producing free amino acids within the intestinal lumen, followed by observation of an increase in free amino acids in blood following ingestion of protein, together with the subsequent characterisation of amino acid carrier systems in the brush-border membrane of the small intestine, detracted attention from the possibility that forms (such as intact peptides and proteins) other than free amino acids might enter the body during assimilation of a protein meal.
2. Substantial methodological difficulties in reliably quantifying the amounts of intact peptides and proteins crossing the gastrointestinal tract under unequivocally physiological conditions. These difficulties include ensuring adequate specificity of analytical methods, overcoming the lack of methods for estimation of peptides as a general class of compound, and the problem of peptides and proteins being cleared (e.g. by tissue uptake or by hydrolysis) before or during sample collection and handling.

3. The fairly recent elucidation of the specialised Peyer’s patch route for antigen sampling and absorption (e.g. [20, 21, 30, 5–7]), and awareness that this route permits absorption of particles as large as intact viruses (e.g. [34]).

Further, until recently a general lack of realisation of the potential clinical and therapeutic significance of absorption of small quantities of intact proteins and peptides has undoubtedly contributed to the relatively slow advance of knowledge on this topic. There are now new, urgent incentives for more attention to be devoted to gastrointestinal absorption of intact proteins and peptides.

Authoritative statements, clearly affirming the view that significant amounts of intact protein molecules can (and do) cross the gastrointestinal tract of healthy humans, include the following quotations from monographs and reviews:

- Immunological evidence of the absorption from the gastro-intestinal tract of protein fragments large enough to exhibit the specificity of the parent protein has been obtained many times. (Fisher [4], p. 10)

- The gastrointestinal tract is assumed to be an impenetrable barrier to the uptake of intraluminal antigens. Despite this popular notion, increasing experimental and clinical evidence suggests that the mucosal barrier to antigenic material may be incomplete, allowing for absorption of macromolecules, not in quantities to be of nutritional importance, but in quantities that may be antigenic or biologically active. This observation could mean that the intestinal tract represents a potential site for the absorption of toxic quantities of bacterial breakdown products, endotoxins, proteolytic and hydrolytic enzymes or of ingested antigens . . . (Walker [29], p. 202, emphasis added)

- There is now no reasonable doubt that small quantities of intact proteins do cross the gastrointestinal tract in animals and adult humans, and that this is a physiologically normal process required for antigen sampling by subepithelial immune tissue in the gut. (Gardner [6], p. 345)

- In conclusion, numerous electron microscopic and biochemical studies have shown that proteins in the 0.005 μm range can cross the intestinal barrier of adults by an endocytotic mechanism . . . an analysis of the intestinal transport of a variety of macromolecular substances has shown that the adult mammalian intestinal epithelium retains the ability to transport macromolecules to a limited degree. (Weiner [32], pp. 870, 878)

- In spite of overwhelming evidence to the contrary, familiarity with the much discussed concept of closure . . . may have led around 1970 to the impression that the mature gut was unable to absorb any protein at all. At least that was the situation complained of by Warshaw, Walker & Isselbacher (1974). However not many years later it became accepted that small amounts of intact proteins did normally reach the bloodstream of adult animals including man. (Matthews [16], p. 113)

- Throughout the years there have been many reports on the oral activity of peptide and protein drugs. But the doses required are often excessive in comparison with parenteral doses. (Lee et al. [13], p. 691)

- It has been found that the small intestine is permeable to several digestive enzymes . . . In the adult mammal . . . small amounts [of intact dietary protein] may escape complete digestion [and] reach subepithelial sites . . . such protein movement may occur in one of several ways . . . (Sanford [24], pp. 80, 149)