Leading article

Coeliac syndrome: biochemical mechanisms and the missing peptidase hypothesis revisited

'A powerful idea communicates some of its strength to him who challenges it' [Marcel Proust]

Shortly after the recognition by Dicke that wheat proteins, in particular gluten, are the specific precipitant of a relapse in children with coeliac disease, it was suggested that this disorder was because of a failure of the small intestinal mucosa of coeliac patients to detoxify gluten. Frazer showed by careful feeding studies that, whereas a peptic tryptic-pancreatic digest of gluten induced a relapse in these patients, after incubation with normal intestine, the resultant mixture was no longer toxic. Since that period the missing peptidase hypothesis has waxed and waned in popularity, waging a love-hate relationship with the so-called immunological hypothesis.

Various ingenious methods have been applied to test the missing peptidase hypothesis. Early whole body studies were performed by administering large quantities of gluten to normal volunteers and to patients with coeliac disease with subsequent measurements of amino acids, in particular glutamine concentrations in the serum, in the hope that they would reflect altered handling of the peptides by the brush border. Serum glutamate concentrations were found to be significantly higher in the coeliac patients and it was concluded that gluten digestion was not specifically impaired. It was, however, clear that this approach lacked sensitivity because of the various factors affecting postprandial amino acid concentrations in the peripheral blood and biochemical techniques were therefore applied directly to the jejunal mucosa.

Examination of mucosal digests of gluten peptic-tryptic fractions showed a distinct peptide fingerprint when coeliac mucosa was compared with control intestine. Staining studies suggested that this peptide was rich in proline. Douglas and Booth repeated these studies and failed to find any difference between normal and coeliac mucosal digests. This should have laid the peptidase hypothesis to rest, but there have been subsequent conflicting reports in this area. Cornell and colleagues examined gluten mucosal digests by column chromatography and claimed to have identified a toxic peptide fraction. They suggest that the toxic fraction caused lysosomal labilisation, an alteration found in coeliac mucosa by cytochemical and biochemical techniques.

The important paper from Woodley and colleagues in this issue of Gut also examines the possibility of impaired gluten proteolysis by coeliac mucosa. Using isolated brush border membranes from normal and coeliac...
mucosa in conjunction with a novel highly sensitive assay for glutamate release from gluten peptides, the authors did not detect differences between membranes from normal subjects and patients with coeliac disease in remission. Although this is further evidence against the peptidase hypothesis, certain reservations remain. It is, for instance, of some concern that 80% of the brush border was lost during the purification procedure and enzyme rates were determined over a 60 minute incubation period. Furthermore, there is always a nagging concern whether the putative peptidase remains stable in the incubation medium. It is also possible that examination of the products of gliadin proteolysis may fail to detect a subtle, highly specific defect in gliadin hydrolysis.

Gluten is an unusual protein in that it contains approximately 45% of its residue in the form of glutamine. This is responsible for its unique properties in bread making and also, presumably, for its pathogenic role in coeliac disease. This high glutamine content has focused interest on peptidases implicated in the cleavage of this residue. It is clear from the elegant studies of Woodley that overall glutamate release from gluten peptides is normal in coeliac mucosa. Other enzymes are, however, implicated in glutamine metabolism. The principal activity which has been investigated in this respect is γ-glutamyl transferase. Subcellular localisation studies have shown that most of this activity has a brush border localisation but some activity is found in the cytosol and basal lateral membrane. Careful studies using subcellular fractionation techniques have shown reduced brush border γ-glutamyl transferase activity in coeliac mucosa returning to normal values with successful gluten withdrawal. Reports of normal activities in untreated coeliac disease or persistently reduced activities in treated patients remain unexplained. Further studies of the properties of this enzyme and the associated γ-glutamyl hydrolase activity would clearly be interesting. This enzyme shows the most striking villus to crypt gradient and studies of its development during intestinal morphogenesis could be of considerable interest.

Peptide hydrolysis does not occur solely at the brush border and activities are found in the lysosomal, cytosolic and basal-lateral membrane locations. It is possible that a selective defect in one or more of these organelles might be implicated. Similarly, attention has been focused mainly on gluten degradation by the enterocytes but defects in the metabolism of the protein, or more likely of certain peptide fragments, may involve intramucosal elements including crypt, lymphoid, vascular, or other interstitial cells. This point is particularly relevant following the demonstration, both in vitro and in vivo, of a persistently increased permeability of the intestinal mucosa of patients with coeliac disease in complete remission, to low molecular probes (<1000 daltons), similar in size to that of the smallest toxic gluten fragment. In a recent reappraisal it is considered that up to 10% of lumen peptides may be absorbed intact.

It is now clear that protein digestion is a multi-step process with progressive luminal, brush border and cytosolic hydrolysis of peptides. Of particular interest is the demonstration by Matthews and colleagues of peptide carriers at the brush border membrane. Defects in these peptide transporters could interfere with glutaminyl residue metabolism by affecting their cellular compartmentalisation. Simple measurements of peptide hydrolysis in tissue homogenates, or with solubilised cell
membrane components would not show such defects, if they existed, in coeliac mucosa.

An alternative biochemical approach to explain the toxicity of gluten was formulated with the lectin hypothesis which postulates the existence of abnormal brush border glycoproteins to which gluten, or a fraction thereof, binds because of its lectin properties.\textsuperscript{25–28} Cell damage is initiated with a compensating increase in cell turnover, the immature cells being even more susceptible to gluten toxicity because of less complete glycoproteins on the cell surface. According to the hypothesis, the binding is a passive process and only possible because of faulty glycosylation of membrane proteins. An alternative, related possibility, has more recently been raised: whether an enzyme exists which is capable of facilitating gluten binding to membrane components: transglutaminase is an obvious candidate. This enzyme, which shows as absolute requirement for Ca\textsuperscript{2+}, cross-links adjacent polypeptides by forming a peptide bond between the ε-amino groups of lysine residues of one chain with the γ-carboxyl group of glutamine residues in another.\textsuperscript{29, 30} Transglutaminase activity has long been recognised in other tissue sites. Plasma fibrin stabilising activity (factor XIII) has been shown to be due to transglutaminase activity\textsuperscript{31} and the enzyme has been implicated in cell-cell interaction,\textsuperscript{32} keratin formation,\textsuperscript{33, 34} endocytosis\textsuperscript{35, 36} and cell proliferation and neoplasia,\textsuperscript{37} as well as in fibrin\textsuperscript{31} and seminal plug\textsuperscript{38} stabilisation. It has also been implicated in lymphocytes,\textsuperscript{39} macrophages\textsuperscript{40} and erythrocytes\textsuperscript{41, 42} and in drug\textsuperscript{43} reactions. Gliadin would be expected to be an excellent substrate for this enzyme and indeed has been found to be so.\textsuperscript{44}

An early observation, little studied at present, is that gluten which has been selectively deamidated – that is, in which the amides of the glutamine residues are cleaved without effecting the polypeptide backbone, is no longer toxic to patients with coeliac disease.\textsuperscript{45} This observation strongly implicates defects in the metabolism, or binding of the glutamine residues in the pathogenesis of coeliac disease. Transglutaminase is an enzyme with such a role and deamidation of gluten renders it no longer a substrate for this enzyme.\textsuperscript{44} Transglutaminase activity has been shown in normal jejunal biopsy specimens in man,\textsuperscript{46} which may explain the toxicity of gluten to normal volunteers when given in sufficient quantities.\textsuperscript{47} Moreover, the enzyme activity is increased in biopsies from patients with coeliac disease in remission and in relapse\textsuperscript{44} and thus it might have an important role in gluten cell membrane interactions, a key step in most hypotheses of coeliac disease. These studies also raise the question of whether we have been searching for an enzyme defect, when increased activity may more adequately explain the pathological picture. Further studies, including the cellular and subcellular localisation of the activity, are necessary in normal and in coeliac mucosa. These observations do, however, indicate new biochemical approaches to the study of coeliac disease.

Recent interest in glutamate and glutamine metabolism by rat small intestine have stressed the importance of intracellular (cytosolic and mitochondrial) glutaminase, glutamate dehydrogenase and aspartate and alanine amino-transferases in enterocyte intermediate metabolism.\textsuperscript{48, 49, 50} There is a clear need for similar studies in man of normal and gluten-sensitive subjects.

There is therefore a need for a new detailed examination of gluten
degradation, transfer, binding and metabolism by intestinal mucosa from control subjects and particularly from patients with coeliac disease in full remission. *In vitro* techniques with organ culture procedures should be coupled with *in vivo* perfusion, or metabolic balance studies. This approach should have preceded the large number of *in vitro* gliadin cytotoxicity studies, which so far have yielded only conflicting and confusing results. It is surely to the benefit of immunological and biochemical protagonists, that the basic biochemistry of gliadin handling by the small gut be elucidated in detail.

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**References**

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