*Gut*, 1984, **25**, 913–918

*Leading article*

# Coeliac syndrome: biochemical mechanisms and the missing peptidase hypothesis revisted

*'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.[1–3] 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 finger print when coeliac mucosa was compared with control intestine. Staining studies suggested that this peptide was rich in proline.[4 5] Douglas and Booth[6] 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[7–9] examined gluten mucosal digests by column chromatography and claimed to have identified a toxic peptide fraction. They suggest that the toxic fraction caused lysosmal labilisation,[10] an alteration found in coeliac mucosa by cytochemical[11] and biochemical techniques.[12]

The important paper from Woodley and colleagues in this issue of *Gut*[13] 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.[14] 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.[15] Reports of normal activities in untreated coeliac disease[16 17] or persistently reduced activities in treated patients[18] remain unexplained. Further studies of the properties of this enzyme and the associated γ-glutamyl hydrolase activity[19] would clearly be interesting. This enzyme shows the most striking villus to crypt gradient[20] 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.[21] It is possible that a selective defect in one or more of these other 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*[22] and *in vivo*,[23] 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.[24]

It is now clear that protein digestion is a multi-step process with progressive luminal, brush border and cytosolic hydrolysis of peptides. Of paticular 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.[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 $Ca^{2+}$, cross-links adjacent polypeptides by forming a peptide bond between the $\epsilon$-amino groups of lysine residues of one chain with the $\gamma$-carboxyl group of glutamine residues in another.[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[31] and the enzyme has been implicated in cell-cell interaction,[32] keratin formation,[33 34] endocytosis[35 36] and cell proliferation and neoplasia,[37] as well as in fibrin[31] and seminal plug[38] stabilisation. It has also been implicated in lymphocytes,[39] macrophages[40] and erythrocytes[41 42] and in drug[43] reactions. Gliadin would be expected to be an excellent substrate for this enzyme and indeed has been found to be so.[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.[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.[44] Transglutaminase activity has been shown in normal jejunal biopsy specimens in man,[46] which may explain the toxicity of gluten to normal volunteers when given in sufficient quantities.[47] Moreover, the enzyme activity is increased in biopsies from patients with coeliac disease in remission and in relapse[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.[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.

T J PETERS AND I BJARNASON

*Division of Clinical Cell Biology,*
*MRC Clinical Research Centre,*
*Watford Road,*
*Harrow, Middlesex*

**References**

1 Weijers HA, Van de Kamer JH. Coeliac disease. IV A rapid method to test wheat sensitivity. *Acta Paediatr* 1955; **44:** 536–40.
2 Alvey C, Andersom CM, Freeman M. Wheat gluten and coeliac disease. *Arch Dis Child* 1957; **3:** 434–7.
3 Douglas AP, Booth CC. Post-prandial plasma free amino acids in adult coeliac disease after oral gluten and albumin. *Clin Sci* 1969; **36:** 643–53.
4 Pittman FE, Pollitt RJ. Studies of jejunal mucosal digestion of peptic tryptic digests of wheat protein in coeliac disease. *Gut* 1966; **7:** 368–71.
5 Carchon H, Serrus M, Eggermont E. Digestion of gliadin peptides by intestinal mucosa from control or coeliac children. *Digestion* 1979; **19:** 1–5.
6 Douglas AP, Booth CC. Digestion of gluten peptides by normal human jejunal mucosa and by mucosa from patients with adult coeliac disease. *Clin Sci* 1970; **38:** 11–25.
7 Cornell HJ, Townley RRW. Investigation of possible intestinal peptidase deficiency in coeliac disease. *Clin Chim Acta* 1973; **43:** 113–25.
8 Cornell HJ, Rolles CJ. Further evidence of a primary mucosal defect in coeliac disease. *Gut* 1978; **19:** 253–9.
9 Cornell HJ, Maxwell RJ. Amino acid composition of gliadin fractions which may be toxic to individuals with coeliac disease. *Clin Chim Acta* 1982; **123:** 311–9.
10 Cornell HJ, Townley RRW. The effect of gliadin peptides on rat liver lysosomes in relation to the pathogenesis of coeliac disease. *Clin Chim Acta* 1973; **49:** 181–8.
11 Riecken EO, Stewart JS, Booth CC, Pearse AGE. A histochemical study on the role of lysosomal enzymes in idiopathic steatorrhea before and during a gluten-free diet. *Gut* 1966 **7:** 317–32.
12 Peters TJ, Heath JR, Wansbrough-Jones MH, Doe WF. Enzyme activities and properties of lysosomes and brush borders in jejunal biopsies from control subjects and patients with coeliac disease. *Clin Sci* 1975; **48:** 259–67.
13 Bruce G, Woodley JF, Swan CHJ. The breakdown of gliadin peptides by intestinal brush borders from coeliac patients. *Gut* 1984; **25:** 919–24.
14 Peters TJ. The analytical subcellular fractionation of jejunal biopsy specimens. Methodology and characterisation of the organelles in normal tissue. *Clin Sci* 1976; **51:** 557–74.
15 Peters TJ, Jones PE, Wells GP. Analytical subcellular fractionation of jejunal biopsy specimens: enzyme activities, organelle pathology and response to gluten withdrawal in patients with coeliac disease. *Clin Sci* 1978; **55:** 285–92.
16 Fairman MJ, Scott BB, Toothill C, Losowsky MS. Jejunal mucosal gamma glutamyltransferase activity in coeliac disease. *Gut* 1977; **18:** 484–7.
17 Anderson K-J, Schjonsby H, Skagen DW. Jejunal mucosal enzymes in untreated and treated coeliac disease. *Scand J Gastroenterol* 1983; **18:** 251–6.

18 Cohen MI, McNamara H, Blumenfeld O, Arias IM. The relationship between glutamyl transpeptidase and the syndrome of coeliac sprue. In: Booth CC, Dowling RH. eds. *Coeliac disease,* London: Churchill Livingstone, 1970: 91–102.
19 Smith GD, Peters TJ. Analytical subcellular frationation of rat liver with special reference to the localisation of putative plasma membrane marker enzymes. *Eur J Biochem* 1980; **104:** 305–11.
20 Sepulveda FV, Burton KA, Clarkson GM, Syme G. Cell differentiation and L ornithine decarboxylase activity in the small intestine of rats fed low and high protein diets. *Biochem Biophys Acta* 1982; **716:** 439–42.
21 Nicholson JA, Peters TJ. The subcellular localisation of peptide hydrolase activity in the human jejurnum. *Eur J Clin Invest* 1979; **9:** 349–54.
22 Bjarnason I, Peters TJ. *In vitro* determination of small intestinal permeability: demonstration of a persistant defect in patients with coeliac disease. *Gut* 1984; **25:** 145–50.
23 Bjarnason I, Peters TJ, Veall N. A persistant defect in intestinal permeability in coeliac disease demonstrated by a $^{51}$Cr-labelled EDTA absorption test. *Lancet* 1983; **1:** 323–5.
24 Gardner MLG, Lindblad BS, Barston D, Matthews DM. Transmucosal passage of intact peptides in the guinea pig small intestine *in vivo*: a reappraisal. *Clin Sci* 1983; **64:** 433–9.
25 Douglas AP. The binding of a glycopeptide component of wheat to intestinal mucosa of normal and coeliac human subjects. *Clin Chim Acta* 1976; **73:** 357–61.
26 Weiser MM, Douglas AP. An alternative mechanism for gluten toxicity in coeliac disease. *Lancet* 1976; **1:** 567–9.
27 Koltgen E, Volk B, Kluge F, Gerok W. Gluten, a lectin with oligo mannosyl specificity and the causative agent of gluten-sensitive enteropathy. *Biochem Biophys Res Commun* 1982; **109:** 168–73.
28 Colyer J, Farthing MJC, Kumar PJ, Clark ML, Ohannesian AD, Waldron NM. Re appraisal of the 'lectin hypothesis' in the pathogenesis of coeliac disease. *Clin Sci* 1984; **66:** 59P.
29 Folk JE. Transglutaminases *Ann Rev Biochem* 1980; **49:** 517–31.
30 Folk JE. Mechanism and basis for specificity of transglutaminase-catalyzed-episolon-(gamma-glutamyl)lysine bond formation. *Adv Enzymol* 1983; **54:** 1–56.
31 Chung SI. Comparative studies on tissue transglutaminase and factor XIII *Ann N Y Acad Sci* 1972; **202:** 240–55.
32 Birckgichler PJ, Orr GR, Conway E, Patterson MK. Transglutaminase activity in normal and transformed cells. *Cancer Res* 1977; **37:** 1340–4.
33 Buxman NM, Wuepper KD. Isolation, purification and characterisation of bovine epidermal transglutaminase. *Biochem Biophys Acta* 1976; **452:** 356–9.
34 Peterson LL, Buxman MM. Rat hair follicle and epidermal transglutaminases. Biochemical and immunochemical isoenzymes. *Biochem Biophys Acta* 1981; **657:** 268–76.
35 Davies PJA, Davies DR, Levitzki A, *et al.* Transglutaminase is essential in receptor mediated endocytosis of $\alpha_2$-macroglobulin and polypeptide hormones. *Nature* 1980; **283:** 162–7.
36 Haigler HT, Maxfield FR, Willingham MC, Pastan I. Dansyl cadaverine inhibits internalisation of $^{125}$I-epidermal growth factor in BALB 3T3 cells. *J Biol Chem* 1980; **255:** 1239–41.
37 Maddox MK, Russell DH. Increased nuclear conjugated polyamines and transglutaminase during liver regeneration. *Proc Natl Acad Sci* 1981 **78:** 1712–6.
38 Williams-Ashman HG, Beil RE, Wilson J, *et al* Transglutaminases in mammalian reproductive tissues and fluids: relation to polyamine metabolism and semen coagulation. *Advan Enzyme Regul* 1980; **18:** 239–52.
39 Novgrodsky A, Quittner S, Rubin AL, Stenzel KH. Transglutaminase activity in human lymphocytes: early activation by phytomitogens. *Proc Natl Acad Sci* 1978; **75:** 1157–61.
40 Murtaugh MP, Mehta K, Johnson J, Myers M, Juliana RL, Davies PJA. Induction of tissue transglutaminase in mouse peritoneal macrophages. *J Biol Chem* 1983; **258:** 11074–81.
41 Lorand L, Weissmann LB, Epel DL, Bruner-Lorand J. Role of the intrinsic transglutaminase in the $Ca^{2+}$ medicated cross-linking of erythrocyte proteins. *Proc Natl Acad Sci* 1976; **73:** 4479–81.
42 Siefreng GE, Apostol AB, Velasio PT, Lorand L. Enzymatic basis for the $Ca^{2+}$ induced cross linking of membrane proteins in intact human erythrocytes. *Biochemistry* 1978; **17:** 2598–604.
43 Russell DH, Womble JR. Transglutaminase may mediate certain physiological effects of edogeneous amines of amine-containing therapeutic agents. *Life Sci* 1982; **30:** 1499–508.

44 Bruce SE, Patel E, Peters TJ. Transglutaminase activity of rat gastro-intestinal tract. *Clin Sci* 1984; **66:** 64P.
45 Van de Kamer JH, Weijers HA. Coeliac disease. V Some experiments on the cause of the harmful effect of wheat gliadin. *Acta Paediatr* 1955; **44:** 465.
46 Bruce SE, Bjarnason I, Peters TJ. Jejunal transglutaminase: demonstration of activity, enzyme kinetics, substrate specificity and levels in patients with coeliac disease. *Clin Sci* 1984; **66:** 64P.
47 Doherty M, Barry RE. Mucosal changes in subjects with overt small bowel disease. *Lancet* 1981; **1:** 517–20.
48 Watford M, Lund P, Knebs HA. Isolation and metabolic characteristics of rat and chicken enterocytes. *Biochem J* 1979; **178:** 589–96.
49 Porteous JW. Glutamate, glutamine, aspartate, asparagine, glucose and ketone body metabolism in chick intestinal brush border cells. *Biochem J* 1980; **188:** 619–32.
50 Windmueller HG, Spaeth AE. Respiratory fuels and nitrogen metabolism *in vivo* in small intestine of fed rats. Quantitive importance of glutamine, glutamate and aspartate. *J Biol Chem* 1980; **255:** 107–12.