Leading article

Helping the mucosa make sense of macromolecules

What is the matter with Mary Jane?
She's crying with all her might and main
and she won't eat her dinner – rice pudding again –
What is the matter with Mary Jane?
(A A Milne)

The considerable interest in intestinal absorption of macromolecules stems largely from armchair speculations accompanied by a dearth of well documented experimental data. Essentially it is suggested that enhanced macromolecular absorption of ingested toxins, or protein antigens, or their digestive fragments, or other luminal contents such as microbial products, or digestive enzymes may, if appropriate circumstances prevail, cause or perpetuate various diseases.¹⁻³ The list of disorders is long and varied ranging from coeliac⁴ and inflammatory bowel diseases⁵ and their extra-intestinal manifestations to food allergy,⁶ atopic eczema⁷ and even rheumatoid arthritis,⁸ ankylosing spondylitis⁹ and schizophrenia.¹⁰

That dietary macromolecular absorption occurs in normal subjects has been known for a long time. The oldest and the least sophisticated demonstration is simply observing the immediate clinical effect of an ingested toxin. The most striking example is the effect of botulinus toxin which produces a dramatic symptom complex within 24 hours of ingestion, or the Type 1 immediate hypersensitivity reaction experienced by predisposed individuals after ingestion of certain specific foodstuffs.

The theoretical and proposed consequences of macromolecular absorption in most diseases are neither immediate, nor so clear cut. To obtain circumstantial evidence of absorption of dietary antigen in these disorders two investigational techniques have been used. One can isolate serum immunoglobulins which specifically bind to dietary antigens, or demonstrate serum immune complexes after a test meal which contain the dietary protein. The paper by Husby *et al* in this issue (*Gut* 1987; 28: 1062–72) illustrates an additional technique involving serum immunoassay of ovalbumin and β -lactoglobulin after their oral administration. This study extends previous observations in infants, children with food allergy and adults, with refinements allowing greater sensitivity.

Collectively these immunologically based techniques show that dietary macromolecules are absorbed at least partly intact in health and disease. It seems likely, as intestinal permeability workers have been postulating for years,^{11,12} that in due course many disorders will be shown to be associated with increased macromolecular absorption. It is, however, unlikely that these immunological techniques will find general application as screening

tests because of the considerable technical complexities involved and the existence of highly sensitive non-invasive procedures for assessing permeability.¹¹⁻¹³ How is the technique of Husby *et al* going to give insights into the aetiology and pathogenic mechanisms of disease putatively ascribed to macromolecular absorption? The magnitude of the problem is considerable and hampered by semantic problems as well as by a lack of adequate experimental data. The macromolecules in question are carbohydrates, lipids or proteins, or lipopolysaccharides, glycoproteins and the like. The study of each calls for specialised test substances and techniques, but a sensible start has been made by assuming that proteins or peptides are the most important moieties.¹⁴ The likely site of macromolecular absorption will influence the feasibility and the approach of the study. It will often be difficult to assess whether macromolecular penetration is primary or secondary to the disease process. This is highlighted in Crohn's disease which may reflect both processes. An early hypothesis of the aetiology of Crohn's disease postulated mucosal exposure to a highly specific lipid-soluble macromolecule, originating from luminal microbes or from dietary sources, with increased intestinal permeability as an underlying defect.⁵ The lipid by its interaction with macrophages in turn causes the self-perpetuating chronic inflammatory process. Recent support for this hypothesis was furnished by claims of increased intestinal permeability to polyethylene glycols of mean molecular weight 400 (PEG 400) in asymptomatic close relatives of patients with Crohn's disease.¹⁵ At face value this suggests that an intestinal permeability defect is inherited, as postulated by Shorter *et al*,⁵ or that there is a common environmental factor to which these families are exposed to, such as a dietary irritant or viruses, which increases the absorption of PEG 400. Unfortunately, despite the initial spectacular and widely publicised¹⁶ claims, the use of PEG 400 for assessing intestinal permeability has been fraught with difficulties. Urine recovery of PEG polymers is variable and low after intravenous instillation in man,¹⁷ and detailed analysis of the data in the above study¹⁵ show that apparently normal controls are absorbing five to 15 times less PEG 400 than control subjects studied elsewhere. The data from Crohn's patients and relatives would, however, do well as normal values in previous studies from Sweden, United Kingdom, United States of America and Canada.^{16, 20}

Once the inflammation is established, it disrupts the intestinal barrier as reflected by increased absorption of ⁵¹CrEDTA.²¹ When the disease is particularly active it is associated with extraintestinal symptoms. The extraintestinal symptoms, if indeed at all related to macromolecular absorption, are more likely to have their origin in microbiological products, rather than in a dietary protein, as most patients with Crohn's disease and extra-intestinal symptoms have colonic rather than small intestinal disease. Direct proof for macromolecular involvement in the extraintestinal manifestations of Crohn's disease is lacking. Arthritis and liver damage are a frequent complication of intestinal bacterial overgrowth syndromes. Wands *et al*²² showed convincingly the presence of circulating cryoprotein immune complexes in patients developing arthritis after intestinal bypass for treatment of morbid obesity and the resolution of arthritic symptoms after administration of antibiotics. One of the interesting features here is that the macromolecule need not be specific but must be present in sufficient amounts.

The third interesting and relevant aspect of macromolecular absorption in Crohn's disease comes from the observation that total parenteral nutrition and elemental diets are an effective treatment for exacerbations.^{23,24} The reason(s) for the success of this treatment is uncertain, but unlikely to have anything to do with the aetiology of Crohn's disease. Certainly exclusion of dietary proteins is a possibility, but as elemental diets have several other potentially beneficial effects,²⁵ such as alteration of the intestinal bacterial flora, diminished biliary and pancreatic output and supply of nutrition and calories, it is unlikely that any single factor is responsible. Of equal importance, if confirmed, are the claims that certain specific foodstuffs cause symptomatic relapse after treatment with elemental diets,²⁶ but they are diverse and one would therefore not expect a single protein to be solely responsible.

It may be that the faulty regulating mechanism(s) governing macromolecular absorption in these circumstances are so interrelated that they cannot be separated. It is therefore of particular interest that we may now have a reliable model of Crohn's disease in man that lends itself much more readily to study.^{27,28} It is now widely accepted that longterm non-steroidal anti-inflammatory drug (NSAID) treatment causes small intestinal inflammation in 70% of patients receiving these drugs.^{27,30} Furthermore, NSAID induced small intestinal inflammation in man has not only similar, albeit less severe, complications as small bowel Crohn's disease namely blood and protein loss,³¹ ileal dysfunction²⁸ and strictures,³² but may respond to the same treatments.³³ The pathogenesis of the NSAID lesion³⁴ initially involves increased intestinal permeability, mediated by decreased mucosal prostaglandins with bile acids playing a passive permissive role. The increased intestinal permeability may then allow dietary macromolecular access to the mucosa where, in continued cooperation with NSAID effect on chemotaxis and neutrophil function, paves the way for bacterial invasion. This provokes an inflammatory reaction. It seems likely that study of the mechanisms by which NSAID's damage the small intestine will provide insight into the pathogenesis of inflammatory bowel disease, but it is already clear that the pathogenesis of NSAID induced inflammation involves several interacting factors.

How does Husby and his colleagues' technique help in the understanding of these questions and what potential role has it in conditions which are more clearly linked to dietary proteins? It is not going to help us in circumstances where we have identified the causative agent, such as in coeliac disease. There is no good test substitute for gluten and the way forward has been detailed elsewhere.³⁵ There is, however, an outstanding and important uncertainty regarding the physiological regulatory mechanisms for macromolecular absorption. Dietary proteins are subjected to progressive proteolytic degradation by stomach and pancreas proteases and at the brush border. Immunoglobulins are postulated to limit protein absorption. In particular it is suggested that IgA deficiency leads to enhanced macromolecular absorption, explaining its association with autoimmune diseases.^{2,36} Intestinal permeability, in particular the intercellular pathway that ⁵¹CrEDTA and oligosaccharides take, is important for the uptake of hydrophylic macromolecules, but the role of M cells, macrophages and classical cell mediated and humoral immunological factors local mucosal and systemic, are somewhat blurred. The potential application of the

technique for Husby *et al* is to use their marker in conjunction with highly sensitive and specific permeability probes in various conditions where proteolysis is limited, such as pernicious anaemia or pancreatic insufficiency, alone or in combination with IgA deficiency. Such studies will give valuable information on their relative importance in regulating macromolecular absorption. Before embarking on such analysis we must call for further validation of the technique along the lines that have been set down for inert permeability probes. In particular, we need to know whether serum concentrations of ovalbumin and lactoglobulin after oral administration or eventually the area under the absorption curve, accurately reflect the amount absorbed and whether the elimination of these markers by the reticulo endothelial system or by conventional immunological mechanisms differ in health and disease. While this may be investigated by intravenous administration of the test substances, the technique offers the possibility of carrying out some more elegant and sophisticated flux studies integrating the disappearance of one marker after iv administration with the appearance of another which has been taken orally as is used in, for example, calcium absorption studies. Integration of such results with appropriate animal models should considerably increase our understanding of the regulatory mechanisms of macromolecular absorption and its possible role(s) in the pathogenesis of inflammatory bowel diseases.

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