The regulation of mineral absorption in the gastrointestinal tract

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The absorption of metal ions in the mammalian single-stomached gut is fortunately highly selective, and both luminal and tissue regulation occur. Initially, assimilation of metal ions in an available form is facilitated by the intestinal secretions, chiefly soluble mucus (mucin) that retards hydrolysis of ions such as Cu, Fe and Zn. Metal ions then bind and traverse the mucosally-adherent mucus layer with an efficiency $M^+ > M^{2+} > M^{3+}$. At the mucosa Fe^{3+} is probably uniquely reduced to Fe^{2+}, and all divalent cations (including Fe^{2+}) are transported by a membrane protein (such as divalent cation transporter 1) into the cell. This minimizes absorption of toxic trivalent metals (e.g. Al^{3+}). Intracellular metal-binding molecules (such as mobilferrin) may be present at the intracellular side of the apical membrane, anchored to a transmembrane protein such as an integrin complex. This mobilferrin would receive the metal ion from divalent cation transporter 1 and, with part of the integrin molecule, transport the metal to the cytosol for safe sequestration in a larger complex such as ferritin or 'paraferritin'. β_2-Microglobulin and HFE (previously termed human leucocyte antigen H) may be involved in stabilizing metal mobilferrin–integrin to form this latter complex. Finally, a systemic metal-binding protein such as transferrin may enter the antiluminal (basolateral) side of the cell for binding of the sequestered metal ion and delivery to the circulation. Regulatory proteins, such as HFE, may determine the degree of ion transport from intestinal cells to the circulation. Gradients in pH and perhaps pCa or even pNa could allow the switching of ions between the different transporters throughout this mechanism.

Metal ion absorption: Gastrointestinal tract: Aluminium: Iron: Zinc

The absorption of metal ions in the mammalian single-stomached gut is fortunately highly selective. Thus, essential dietary cations, such as Cu, Zn and Fe are generally absorbed 10–100-fold more efficiently than toxic dietary ions such as Al. In previous reviews (Powell *et al.* 1994; Whitehead *et al.* 1996) we have discussed the relationship between the anatomy, biochemistry and physiology of the gastrointestinal lumen and the regulation of mineral absorption. Here we concentrate on the absorptive role of the most important luminal metal-binding species, i.e. mucus, and explore mechanisms for the subsequent trans-mucosal uptake of metal ions.

Background

Ingested metals may be considered in two categories: those soluble throughout the potential pH range of the gastrointestinal lumen (approximately pH 1–8), such as Na, Mg and Ca, and those susceptible to hydroxy-polymerization, such as Al, Cu, Fe, Mn and Zn. This latter group, termed 'hydrolytic metals', are acid-soluble, but as the pH is raised in the absence of soluble binding ligands, they readily form insoluble hydroxide precipitates. This sometimes led, incorrectly, to the belief that hydrolytic metal ions were absorbed from the 'soluble' acidic gastric environment and not from the 'insoluble' peri-neutral intestinal environment (Stewart, 1989). In fact, the stomach has little absorptive capacity, with a small surface area and a thick protective layer of pH-buffered gelatinous mucus (Fig. 1), and is remarkably impermeable to the absorption of nutrients, including metal ions (Whitehead *et al.* 1997). Nonetheless, the stomach plays an important role in the 'preparation' of ingested metals for later absorption. A marked reduction in gastric acid output (hypochlorhydria) may be ineffective in dissociating a metal ion from its ingested matrix (e.g. Fe in food (Champagne, 1989) or Al in an antacid), leading to a reduced luminal concentration of solubilized and potentially-available metal. Furthermore,

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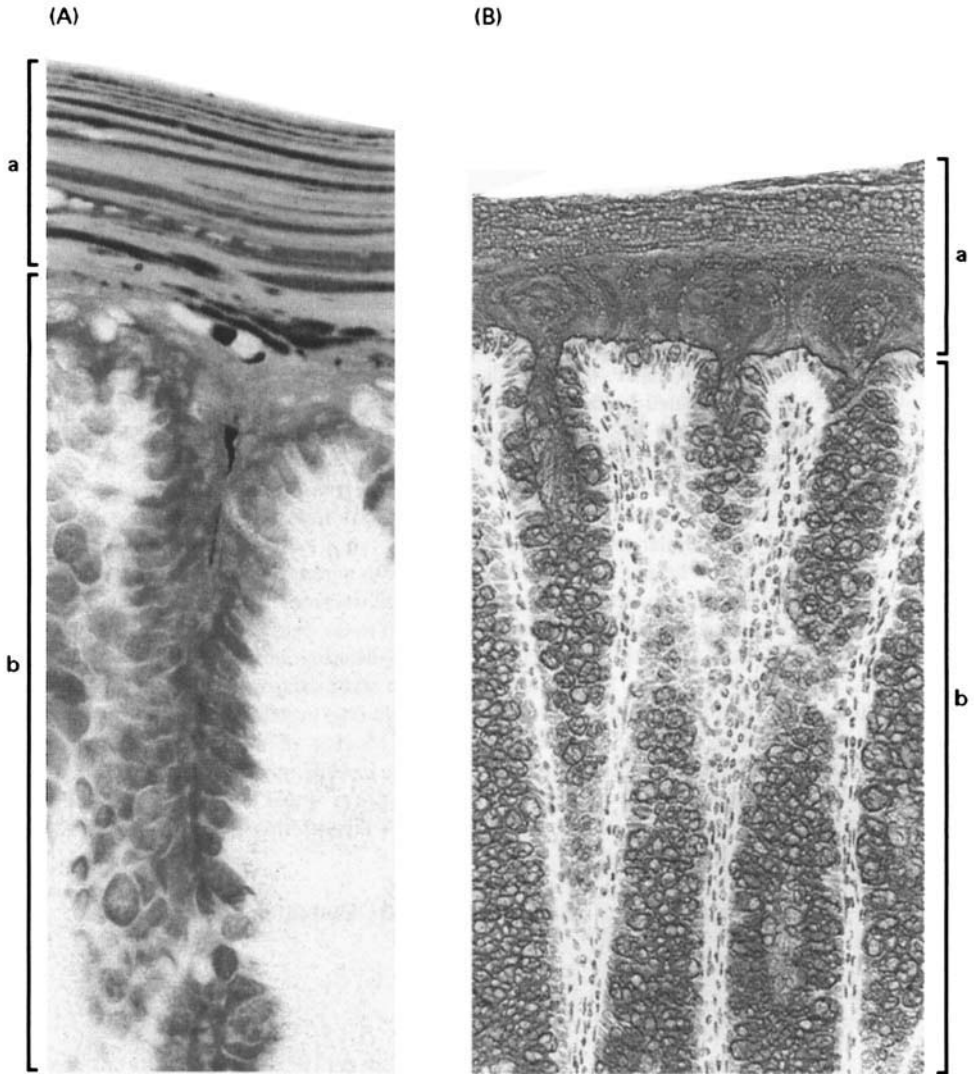


Fig. 1. (A) A distinctly-structured, laminated, mucus layer (a) overlies the mammalian gastrointestinal tract (b; showing surface pyloric mucosal cells of the human stomach). Glycoprotein from pyloric gland cells was stained with paradoxical concanavalin A (dark bands in (a)), while glycoprotein of surface mucus cells was stained with the galactose oxidase-cold thionine reaction (light bands in (a) and dark areas in (b)). (B) The ubiquitous nature of sialic acid in the mucus layer of the human intestine (colon) is shown (dark areas). This section was stained with a periodic acid–sodium borohydride–potassium hydroxide–periodic acid Schiffs reagent sequence. Both the mucus layer (a) and the goblet cells of the mucosa (b) stain red (dark areas), indicating the presence of 8-O-acetyl-neuraminic acid, a histochemical marker of the large intestine. Regular whorl formation in the mucus layer above crypt openings suggests that mucus is expelled from goblet cells in a controlled fashion into the mucus layer. (Micrographs provided by Professor Katsuyama's group, Shinshu University, Japan.)

secreted gastric juice, which mixes with the luminal contents, contains solubilized mucin (once termed 'gastroferrin', see p. 148) that helps hydrolytic metals to remain soluble at near-neutral pH such as that occurring with transient gastric acid buffering by food or following gastric emptying into the small bowel (Powell *et al.* 1994). Indeed, it is the small intestine, which also secretes numerous endogenous ligands and again most notably mucin, that is the predominant site of mineral absorption (Whitehead *et al.* 1996).

Luminal mucin: gastroferrin

The large glycoprotein mucin is secreted throughout the gastrointestinal tract which provides both the mucosally-adherent gelatinous layer (mucus, see p. 150) and a soluble luminal form (Hunter *et al.* 1989). Much of the soluble form occurs as a degradation product of the mucus layer, and its role in metal binding has been well demonstrated (Crowther, 1982; Crowther & Marriott, 1984; Conrad *et al.* 1991). Between 1963 and 1973 a number of authors demonstrated an Fe-binding glycoprotein in human gastric juice that was

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termed 'gastroferrin' (Davis *et al.* 1969; Rudzki *et al.* 1973; Rudzki & Deller, 1973). This isolated glycoprotein maintained Fe^{3+} in solution at neutral pH, suggesting that it played a role in facilitating intestinal Fe absorption, although it was present in gastric juice at similar levels in healthy individuals and in those with Fe-related disorders (Rudzki *et al.* 1973). Significant characterization of gastroferrin was carried out by Rudzki *et al.* (1973) and Rudzki & Deller (1973), who demonstrated its carbohydrate and amino acid content, homology with blood-group antigens, and interactions with metal ions. These findings, in particular the blood-group antigen activity, identify gastroferrin as gastric mucin, and indeed, information on the role of gastroferrin in promoting intestinal Fe absorption is identical to that from recent work with intestinal mucin and Fe (Conrad *et al.* 1991; Fig. 2). Interestingly, much of the Fe is bound non-stoichiometrically by mucin (Rudzki *et al.* 1973), with the glycoprotein stabilizing colloidal poly-hydroxy ion cores and preventing their further polymerization (Rudzki *et al.* 1973; Fig. 2). *In vitro*,

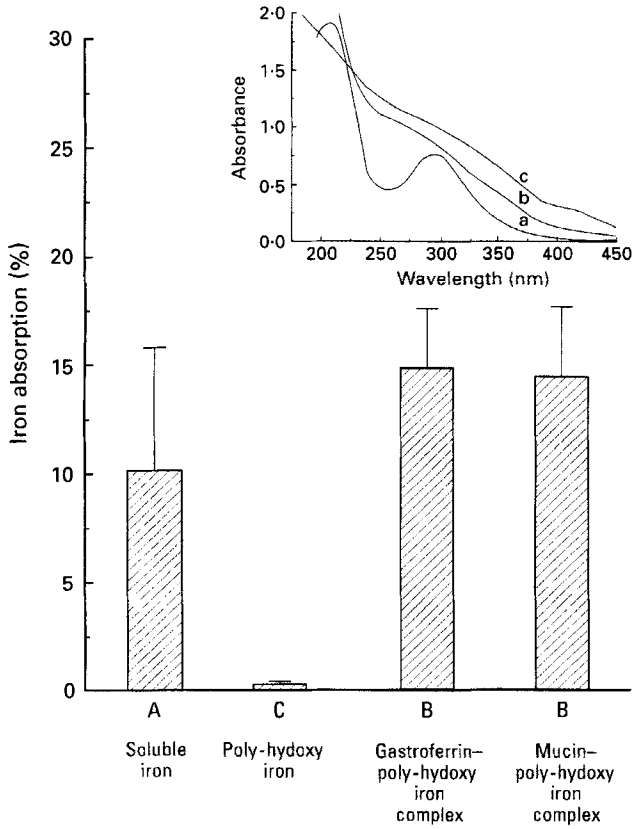


Fig. 2. A solution of ferric chloride at pH 2 has a characteristic u.v. spectrum (a in inset) and, although unphysiologically acidic for the small intestine, it is fully soluble, allowing some absorption of iron when directly introduced to the bowel (A). In contrast, at neutral pH, the solution forms poly-hydoxy iron species that yield a light-scattering spectrum (c in inset) and, although now at physiological pH, are unavailable for absorption in the bowel (C). However, iron bound to gastroferrin (or mucin) maintains its characteristic poly-hydroxy spectrum at neutral pH (b in inset) but is also available for absorption in the bowel (B). Values are means and standard deviations represented by vertical bars. (Data combined from Rudzki *et al.* 1973 and Conrad *et al.* 1991, with permission.)

these aggregates varied in size from 2 to 20 nm diameters (Rudzki *et al.* 1973) and, by comparison with the similar poly-hydroxyferric fructose system (Bates *et al.* 1972; Bates, 1973), are expected to be easily dissociable, thus maintaining Fe in a readily-available form. Indeed, instillation of the $Fe(OH)_3$–mucin complex into the rat gastrointestinal tract yields effective absorption that is clearly not seen with $Fe(OH)_3$ alone or even with 'uncomplexed' $Fe(OH)_3$ + mucin (Rudzki *et al.* 1973; Conrad *et al.* 1991; Fig. 2). Soluble mucins from the stomach and intestinal tract behave similarly, not only in their Fe-binding capacity, but also in their binding to other metals. Combining data from a number of papers (Rudzki *et al.* 1973; Crowther, 1982; Crowther & Marriott, 1984; Conrad *et al.* 1991) suggests that the affinity of gastrointestinal mucin for metals follows the pattern: $Fe^{3+} > Al^{3+}$, $Cr^{3+} > Pb^{2+} > Zn^{2+} > Co^{2+} > Ca^{2+} > Na^{+}$, Cs^{+} (i.e. $M^{3+} > M^{2+} > M^{+}$). Although initially this appears to hold few surprises, given the nature of metal binding to polyelectrolytes, metal–mucin interactions are not so simple. First, although poly-hydroxy Fe clearly interacts with mucin, it also appears that monomeric ionic Fe presented to mucin by low-molecular-mass ligands also binds to mucin (Conrad *et al.* 1991). Indeed, we have shown that potential ligands of low molecular mass are secreted in the gastrointestinal fluids (such as lactate, pyruvate, histidine and bicarbonate; Powell *et al.* 1990; Whitehead *et al.* 1996); these may transiently stabilize metal hydroxy-polymerization, and could allow stoichiometric donation to mucin.

Second, there may be more than one binding site on mucin, since the molecule contains both sulfated groups (sulfated mucins) and carboxylate groups (sialomucins; Rhodes, 1989). Metal ions are anticipated to bind preferentially to one site or another, with covalent-type binding by 'soft' metals (e.g. Pb^{2+}, Cd^{2+}) to sulfated groups and ionic-type binding by 'hard' metals (e.g. Al^{3+}, Fe^{3+}) to carboxylate groups. However, some previously unpublished findings (J Quarterman, unpublished results; Fig. 3) suggest that Zn has two pH-dependent binding sites on mucin. Such

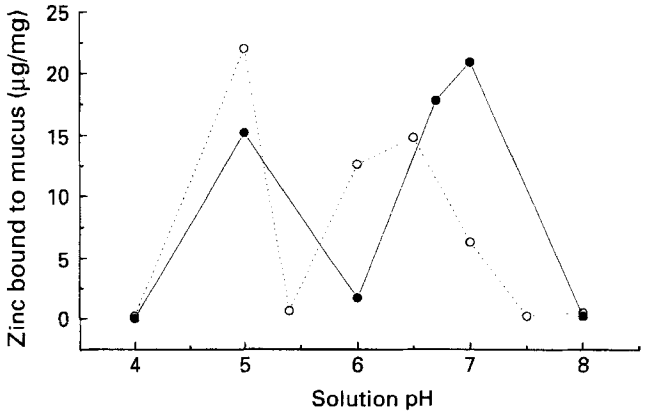


Fig. 3. Dialysis results showing zinc binding to isolated porcine mucus with increasing pH (●) and decreasing pH (○). The data suggest that two pH-sensitive binding sites exist on mucus for zinc (and possibly other metal ions), and that a pH of 5·5–6 may facilitate dissociation of the metal–mucus complex. (Data provided by Dr J. Quarterman, Aberdeen, UK; formerly of The Rowett Institute, Aberdeen.)

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observations on the complex interaction between metal ions and mucins make it difficult to interpret competition studies between different metals for mucin, or between mucin and different ligands for metals. For example, Rudzki *et al.* (1973) showed that in the presence of a tenfold molar excess of ascorbate or citrate Fe would not bind to mucin. However, with identical concentrations mucin-bound Fe could not be released by citrate or ascorbate (Rudzki *et al.* 1973). Thermodynamic measurements require the reverse reaction to equilibrate similarly to the forward reaction, but kinetic effects, particularly marked in colloidal and/or polyelectrolyte-containing systems, often preclude the simple generation of thermodynamic data. Hence, the proposed Fe–mucin dissociation constant of 10^{-4} M (Conrad *et al.* 1991) may not be reliable or even relevant *in vivo* during transit of the (non-equilibrium) gastrointestinal tract. Nevertheless, we have demonstrated *in situ* the marked luminal interaction of ingested metal (Al) and intestinal mucin (Powell, 1994; Whitehead *et al.* 1995; Fig. 4), which is favoured by the large concentration of mucin in the intestinal lumen and its overall high capacity for metal ions and/or their polyhydroxy species. Further work will be required to delineate the exact *in vivo* nature of the soluble mucin–metal interactions, the influence of endogenous or dietary ligands, and how mucin promotes the availability of dietary metals to the mucosally-adherent mucus layer for the next phase of absorption.

Mucosally-adherent mucus layer

Some remarkable work from Katsuyama's group (Ota & Katsuyama, 1992; Shimizu *et al.* 1996) has recently demonstrated the *in situ* structure of the gastrointestinal mucosally-adherent mucus layer. This leaves little doubt that not only is this a continuous thick layer in constant contact with the mucosa, but it is an alternating laminated array of distinct glycoproteins, rather than an amorphous gel (Fig. 1). Within this layer is a pH gradient influenced only at its surface by the luminal pH and otherwise controlled by the mucosal microclimate pH at the tissue–mucus interface (Powell *et al.* 1994). This again allows order (acid–base control) in the mucus layer, compared with the variable pH of the gastrointestinal lumen. Although the contribution to metal binding and transport by the individual components in mucus has not yet been demonstrated, we have shown using snap-frozen sections that Al is avidly bound *in situ* by the intestinal mucus layer (Powell, 1994; Whitehead *et al.* 1995, 1996). There is clear delineation of the mucus, which stains well for Al, and the mucosa, which lacks any detectable staining (Fig. 4), in keeping with the poor absorption (approximately 0·1 %) of the metal (Powell & Thompson, 1993). In contrast, Quarterman (1987), using an elegant agar cast technique, was able to demonstrate both mucus uptake and subsequent mucosal transfer of the much better-absorbed element Zn (Fig. 5). These findings provide strong evidence for the assimilation of metal ions from the luminal milieu by the intestinal mucus layer, and suggest that those ions easily absorbed traverse the layer, whereas those poorly absorbed remain bound and are shed with the mucus back into the lumen, and excreted. Indeed, binding to mucus appears to follow the pattern $M^{3+} > M^{2+} > M^{+}$ (see p. 149), and for metal absorption the pattern is $M^{+} > M^{2+} > M^{3+}$ (Whitehead *et al.* 1996). Thus, the mucus barrier may act initially as a 'coarse filter' in regulating metal uptake. As we have discussed elsewhere (Whitehead *et al.* 1996), the extent of luminal hydroxy-polymerization, rates of metal–ligand exchange, and mucosal transport systems (see p. 150) will all additionally act to control the degree of metal absorption. In addition, pH (and perhaps pCa or pNa) gradients may play an important role in regulating uptake, and it is interesting to note that the pH at which there is least interaction of mucus and Zn (approximately pH 6·0) appears to be the mammalian mucosal microclimate pH of the proximal small bowel (Lucas & Blair, 1978). This may thereby facilitate cellular uptake of ions by dissociation of the metal–mucus complex and promotion of the final step, i.e. the metal–epithelial cell interaction.

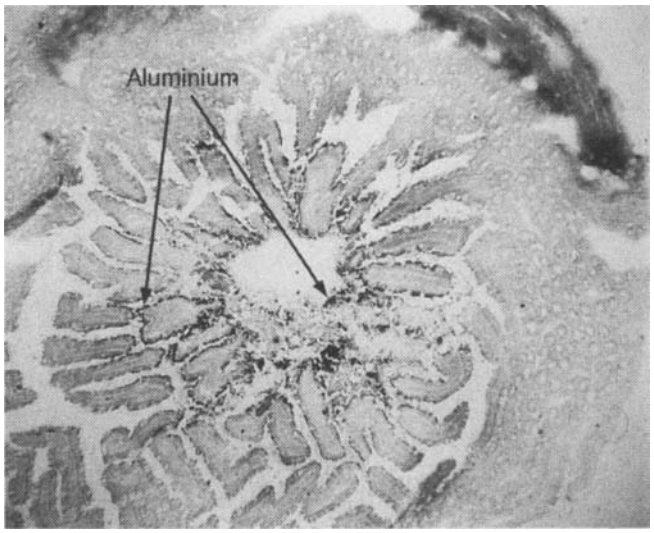


Fig. 4. Solochrome azurine stain showing association of aluminium with rat intestinal tissue following oral administration of aluminium sulfate in deionized water. Aluminium (shown as dark areas) is localized with the mucus layer at the villi surfaces and with soluble mucin in the lumen.

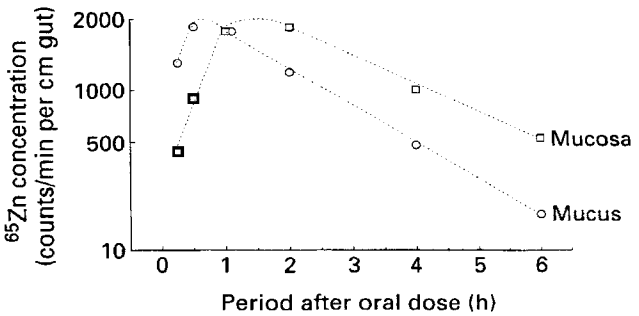


Fig. 5. Uptake of ^{65}Zn (counts/min per cm gut) into the mucus layer (○) and mucosa (□) of rats following an oral dose of zinc sulfate. (Data modified from Quarterman, 1987, with permission.)

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Mucosal regulation

Absorption from the mucus layer and into the intestinal mucosa may occur either through the enterocyte (transcellular) or between enterocyte junctions (paracellular; Powell *et al.* 1994). The latter route is inefficient (usually $< 1\%$) and likely, therefore, only to be important in the absorption of non-essential polyvalent metals ($M^{>2+}$, e.g. Bi and Al) that lack a transport system. Efficiency of this route ('paracellular leakage') may be increased by the ingestion, or mucosal application of penetration enhancers. Such substances either chelate intercellular Ca^{2+} (e.g. citrate), since Ca^{2+} is important in normal maintenance of the tight junctional integrity, or undergo rapid enterocyte metabolism (e.g. glucose) causing cytoskeletal contraction and an increase in junctional space (Powell *et al.* 1994). However, for the absorption of most essential metals a transcellular route facilitated by a mucosal transport system is likely to operate. Until recently, direct evidence was lacking on the molecular mechanisms involved in metal ion uptake at the intestinal mucosa. Advances in molecular techniques have generated much new information, chiefly on the acquisition of Fe and Zn, underlying their physiological importance, but the broad concepts are likely to be applicable to other essential elements such as Cu and Mn. The following section outlines some of this newer information and speculates how it may be pieced together, rather than providing a comprehensive review.

It is well recognized that in the absence of genetic haemochromatosis body Fe stores influence inversely the efficiency of Fe absorption. However, there is a complex sequence of factors controlling this Fe homeostasis at the intestinal level that is quite different from the normal transferrin-associated Fe uptake seen in cells of other tissues. Three steps are considered in the uptake of metal ions by enterocytes. The first is transport through the luminal (apical) cell membrane, the second is handling within the enterocyte, and the third is transport through the antiluminal (basolateral) membrane into the circulation.

Arguably, transmembrane shuttles are not required for the passage of divalent ions across the intestinal mucosa, as facilitated transfer could be achieved through intracellular chelating agents providing the necessary gradient (Whitehead *et al.* 1996). However, recent findings strongly support the presence of transmembrane transporters. A transferrin-like protein (melanotransferrin) linked to a cell-surface glycosyl-phosphatidylinositol has been described in fetal porcine intestinal cells, but its role in adult mammals was not demonstrated, and further information is required to assess its role in neonatal Fe absorption (Danielsen & Deurs, 1995). The transmembrane dimeric integrin complex with an α chain of 150 kDa and β chain of 90 kDa has been strongly supported by Conrad and colleagues (Conrad *et al.* 1993*b*; Umbreit *et al.* 1998) for a role in apical Fe transportation. Typically, the α chain has several cation-binding sites often occupied by Ca, but as discussed by Umbreit *et al.* (1998) integrins lack normal transport-protein properties. Integrins are transmembrane molecules and their extracellular domains function as adhesion sites for cell–cell or cell–matrix interactions, while their intracellular cytoplasmic tail provides linkage for internal cytoskeletal proteins. Thus, it is proposed that these integrins are involved in the 'docking' of intracellular Fe carriers such as mobilferrin (see p. 151), facilitating the uptake of ions by enterocytes without themselves undertaking transport (Umbreit *et al.* 1998). Indeed, the actual transmembrane transport of Fe may involve a ferrireductase enzyme that has been demonstrated at the mucosal surface and uniquely reduces Fe^{3+} to Fe^{2+} (Riedal *et al.* 1995; Pountney *et al.* 1996). This would allow subsequent Fe absorption through a common divalent cation transporter, and protect against inadvertent uptake of the highly toxic Al^{3+} ion and other trivalent metal ions that do not have redox potential. A divalent cation transporter (divalent cation transporter 1) has recently been cloned that is expressed at high levels in enterocytes of the proximal small bowel and appears to have many transmembrane domains, making its expression at the cell surface likely (Gunshin *et al.* 1997). This protein shows significant homology with the natural resistance-associated macrophage protein family, and while it avidly binds Fe^{2+}, it also has a broad substrate range of other divalent cations, including Cu^{2+}, Mn^{2+} and Zn^{2+} (Gunshin *et al.* 1997). This binding of metal ions, which is a proton-coupled process, is favoured at the mildly acidic pH typically found in the micro-environment of the gastrointestinal mucus layer. As discussed previously, the pH gradient of the mucus layer may serve to de-couple metal–mucus interactions (Fig. 4) at the mucosal surface and promote the coupling of apical transporter and metal ion. Internalization of the metal ion (with or without its binding protein) has not been directly demonstrated, although findings with fetal melanotransferrin suggest this mechanism (Danielsen & Deurs, 1995).

In some tissues such as the liver, Fe-responsive proteins appear integral to the regulation of cellular Fe concentrations acting as Fe sensors in the cell cytosol, but there is little evidence for an action of Fe-responsive proteins in intestinal epithelial cells (Chrichton & Ward, 1998). Instead, the recently-identified *HFE* gene (previously termed *HLA-H* due to the similarity with the human leucocyte antigen family), that generally carries a point mutation in genetic haemochromatosis, may be involved in controlling Fe levels in the absorptive intestinal cells (Feder *et al.* 1996). This gene encodes for a protein (HFE; previously termed human leucocyte antigen H) that is related to the major histocompatibility complex class I and, similarly, associates with β_2-microglobulin and is expressed at the cell surface (Feder *et al.* 1998). Thus, HFE has only a short cytoplasmic tail and a single membrane-spanning region that is inconsistent with a role as an ion transporter (Gunshin *et al.* 1997). More recent findings suggest that the role of HFE is in regulation of Fe transfer from epithelial cells to the circulation by regulated interaction with the basolateral transferrin receptors (Feder *et al.* 1998; Lebron *et al.* 1998; see p. 152).

Intracellular metal-binding proteins have been reported, most notably mobilferrin (Conrad *et al.* 1990; Conrad *et al.* 1990, 1994, 1996; Umbreit *et al.* 1998). Mobilferrin is a 56 kDa intracellular protein that has been isolated from both rat and human small intestine and is homologous with the Ca-binding protein calreticulin (Conrad *et al.* 1993*a*). Interestingly, calreticulin has chaperone activity in the formation of major histocompatibility complex class I

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complexes (Umbreit *et al.* 1998), so perhaps it has similar activity for the putative Fe-regulating protein HFE (which is major histocompatibility complex class I-like). Mobilferrin binds metal ions such as Ca, Cu and Zn with relatively high affinity, and is a particularly strong binder of Fe (Conrad *et al.* 1990). These interactions are acid-sensitive, again suggesting pH gradients as a means to associate and dissociate metal–mobilferrin complexes. As we have previously noted (Whitehead *et al.* 1996), it remains to be established if trace-metal–mobilferrin complexes are sufficiently stable in the presence of the relatively high levels of intracellular Ca. Nonetheless, a number of papers from Conrad's group (Conrad *et al.* 1990, 1993*a*,*b*, 1994; Umbreit *et al.* 1996, 1998) point to a key role for this molecule, at least in intracellular Fe regulation. In fact this work goes further, suggesting that following acquisition of the Fe, mobilferrin binds into a large protein complex termed 'paraferritin' containing solubilized integrin, mobilferrin, metal ion, β_2-microglobulin and 'unidentified polypeptides' (Umbreit *et al.* 1996, 1998). Since β_2-microglobulin normally stabilizes major histocompatibility complex class I–peptide complexes (Song & Harding, 1996), it may be that the major histocompatibility complex class I-like putative Fe-regulating protein HFE is also involved in this complex. Similarly, for Fe, ferritin sequesters excessive levels of the metal, possibly providing a short-term depot of Fe before the cell is sloughed into the lumen, while preventing Fe-related free-radical damage to the cell, but a significant role in Fe transport has not been shown. Indeed, a number of molecules, such as metallothioneins, exist in the cell cytosol that are likely to be involved in the transient sequestering of absorbed metal ions, particularly when the influx of metal ions is high (Davis *et al.* 1998).

Finally, a basolateral transporter, such as transferrin for Fe, appears to shuttle ions from the cell through the basolateral membrane to the circulation. A lack of mRNA for transferrin in the enterocyte suggests that there is no constitutive expression of the protein in intestinal cells (Chrichton & Ward, 1998). However, basolateral transferrin receptors (there is normally no apical expression) may allow influx of transferrin and efflux of Fe–transferrin (Chrichton & Ward, 1998). This flux of transferrin appears to be one major regulatory mechanism in Fe homeostasis, because in genetic haemochromatosis there is inefficient interaction of the transferrin receptor with the (abnormal) Fe-regulatory HFE protein (Feder *et al.* 1998) and hence upregulation of the receptor (Chrichton & Ward, 1998). Similarly, for Zn, a basolateral Zn transporter (Zn transporter-1) partly regulates flux from intestinal cells (McMahon & Cousins, 1998).

Hence, in summary, assimilation of metal ions in an available form is facilitated by the intestinal secretions, chiefly soluble mucus (mucin). Metal ions then bind and traverse the mucosally-adherent mucus layer with an efficiency $M^+ > M^{2+} > M^{3+}$. At the mucosa, Fe^{3+} is uniquely reduced to Fe^{2+}. Divalent cations (including Fe^{2+}) are transported by a membrane protein (such as divalent cation transporter 1) into the cell. Intracellular metal-binding molecules (such as mobilferrin) may be present at the intracellular side of the apical membrane, anchored to a transmembrane protein such as an integrin complex. This mobilferrin would receive the metal ion from divalent cation transporter 1 and, with part of the integrin molecule, transport the metal to the cytosol for safe sequestration in a larger complex such as ferritin or 'paraferritin'. Finally, a systemic ion-binding protein such as transferrin may enter the antiluminal (basolateral) side of the cell for binding of the sequestered metal ion and delivery to the circulation. This would allow some external regulation, by the liver, of the release of Fe from the enterocytes to the circulation, perhaps determined by the extent of liver Fe stores. Regulatory proteins, such as HFE, may determine degree of ion transport from intestinal cells to the circulation. Gradients in pH and perhaps pCa or even pNa could allow the switching of ions between the different transporters throughout this mechanism.

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References

Bates GW (1973) Complex formation, polymerization, and autoreduction in the ferric fructose system. *Bioinorganic Chemistry* **2**, 311–327.

Bates GW, Boyer J, Hegenauer JC & Saltman P (1972) Facilitation of iron absorption by ferric fructose. *American Journal of Clinical Nutrition* **25**, 983–986.

Champagne ET (1989) Low gastric hydrochloric acid secretion and mineral bioavailability. In *Advances in Experimental Medicine and Biology*, vol. 249, *Mineral Absorption in the Monogastric GI Tract*, pp. 173–184 [F Dintzis and J Laszlo, editors]. New York: Plenum Press.

Chrichton RR & Ward RJ (1998) *Iron Homeostasis*. Basel, Switzerland: Marcel Dekker.

Conrad ME, Umbreit JN & Moore EG (1991) A role for mucin in the absorption of inorganic iron and other metal cations: a study in rats. *Gastroenterology* **100**, 129–136.

Conrad ME, Umbreit JN & Moore EG (1993*a*) Rat duodenal iron-binding protein mobilferrin is a homologue of calreticulin. *Gastroenterology* **104**, 1700–1704.

Conrad ME, Umbreit JN, Moore EG & Heiman D (1996) Mobilferrin is an intermediate in iron transport between transferrin and haemoglobin in K562 cells. *Journal of Clinical Investigation* **98**, 1449–1454.

Conrad ME, Umbreit JN, Moore EG, Peterson RDA & Jones MB (1990) A newly identified iron binding protein in duodenal mucosa of rats: purification and characterization of mobilferrin. *Journal of Biological Chemistry* **265**, 5273–5279.

Conrad ME, Umbreit JN, Moore EG, Uzel C & Berry MR (1994) Alternate iron transport pathway: Mobilferrin and integrin in K562 cells. *Journal of Biological Chemistry* **269**, 7169–7173.

Conrad ME, Umbreit JN, Peterson RDA, Moore EG & Harper KP (1993*b*) Function of integrin in duodenal mucosal uptake of iron. *Blood* **81**, 517–521.

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Crowther RS (1982) Cation induced changes in the biophysical properties of mucus glycoproteins. PhD Thesis, University of London.

Crowther RS & Marriott C (1984) Counter-ion binding to mucus glycoproteins. *Journal of Pharmacy and Pharmacology* **36**, 21–26.

Danielsen EM & Deurs BV (1995) A transferrin-like GPI-linked iron-binding protein in detergent-insoluble noncaveolar microdomains at the apical surface of fetal intestinal epithelial cells. *Journal of Cell Biology* **131**, 939–950.

Davis PS, Multani JS, Cepeeneek CP & Saltman P (1969) Isolation of gastroferrin from human gastric juice. *Biochemical and Biophysical Research Communications* **37**, 532–537.

Davis SR, McMahon RJ & Cousins RJ (1998) Metallothionein knockout and transgenic mice exhibit altered intestinal processing of zinc with uniform zinc-dependent zinc transporter-1 expression. *Journal of Nutrition* **128**, 825–831.

Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, Dormishian F, Domingo R Jr, Ellis MC, Fullan A, Hinton LM, Jones NL, Kimmel BE, Kronmal GS, Lauer P, Lee VK, Loeb DB, Mapa FA, McClelland E, Meyer NC, Mintier GA, Moeller N, Moore T, Morikang E, Prass CE, Quintana L, Starnes SM, Schatzman RC, Brunke KJ, Drayna DT, Risch NJ, Bacon BR & Wolff RK (1996) A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nature Genetics* **13**, 399–408.

Feder JN, Penny DM, Irrinki A, Lee VK, Lebron JA, Watson N, Tsuchihashi Z, Sigal E, Bjorkman PJ & Schatzman RC (1998) The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. *Proceedings of the National Academy of Sciences USA* **95**, 1472–1477.

Gunshin H, Mackenzie B, Berger UV, Gunshin Y, Romero MF, Boron WF, Nussberger S, Gollan JL & Hediger MA (1997) Cloning and characterisation of a mammalian proton-coupled metal-ion transporter. *Nature* **388**, 482–488.

Hunter AC, Allen A & Garner A (1989) Studies on mucus biosynthesis in the gastrointestinal tract. In *Symposia of the Society for Experimental Biology,* no. 43, *Mucus and Related Topics,* pp. 27–36 [E Chantler and N Ratcliffe, editors]. Cambridge: Cambridge Society for Experimental Biology.

Lebron JA, Bennett MJ, Vaughn DE, Chirino AJ, Snow PM, Mintier GA, Feder JN & Bjorkman PJ (1998) Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. *Cell* **93**, 111–123.

Lucas ML & Blair JA (1978) The magnitude and distribution of the acid microclimate in proximal jejenum and its relation to luminal acidification. *Proceedings of the Royal Society London* **A200**, 27–41.

McMahon RJ & Cousins R (1998) Mammalian zinc transporters. *Journal of Nutrition* **128**, 667–670.

Ota H & Katsuyama T (1992) Alternating laminated array of two types of mucin in the human gastric surface mucous layer. *Histochemical Journal* **24**, 86–92.

Pountney DJ, Raja KB, Bottwood MJ, Wrigglesworth JM & Simpson RJ (1996) Mucosal surface ferricyanide reductase activity in mouse duodenum. *Biometals* **9**, 15–20.

Powell JJ (1994) Aluminium in the gastrointestinal tract. PhD Thesis, United Medical and Dental Schools, University of London.

Powell JJ, Gartland KPR, Nicholson JK, Ainley CC & Thompson RPH (1990) Bile, pancreatic juice and small bowel secretions contain endogenous metal binding ligands. *Gut* **31**, A1197.

Powell JJ & Thompson RPH (1993) The chemistry of aluminium in the gastrointestinal lumen and its uptake and absorption. *Proceedings of the Nutrition Society* **52**, 241–253.

Powell JJ, Whitehead MW, Lee S & Thompson RPH (1994) Mechanisms of gastrointestinal absorption: dietary minerals and the influence of beverage ingestion. *Food Chemistry* **51**, 381–388.

Quarterman J (1987) Metal absorption and the intestinal mucus layer. *Digestion* **37**, 1–9.

Rhodes JM (1989) Colonic mucus and mucosal glycoproteins: The key to colitis and cancer. *Gut* **30**, 1660–1666.

Riedal HD, Remus AJ, Fitscher BA & Stremmel W (1995) Characterization and partial purification of a ferrireductase from human duodenal microvillus membranes. *Biochemical Journal* **309**, 745–748.

Rudzki Z, Baker RJ & Deller DJ (1973) The iron-binding glycoprotein of human gastric juice: II nature of the interaction of the glycoprotein with iron. *Digestion* **8**, 53–67.

Rudzki Z & Deller DJ (1973) The iron binding glycoprotein of human gastric juice: I isolation and characterization. *Digestion* **8**, 35–52.

Shimizu T, Akamatsu T, Sugiyama A, Ota H & Katsuyama T (1996) *Helicobacter pylori* and the surface mucous gel layer of the human stomach. *Helicobacter* **1**, 207–217.

Song R & Harding CV (1996) Roles of proteasomes, transporter for antigen presentation (TAP), and β2-microglobulin in the processing of bacterial or particulate antigens via an alternative class I MHC processing pathway. *Journal of Immunology* **156**, 4182–4190.

Stewart WK (1989) Aluminium toxicity in individuals with chronic renal disease. In *Aluminium in Food and the Environment,* pp. 7–19 [R Massey and D Taylor, editors]. London: Royal Society of Chemistry.

Umbreit JN, Conrad ME, Moore EG, Desai MP & Turrens J (1996) Paraferritin: A protein complex with ferrireductase activity is associated with iron absorption in rats. *Biochemistry* **35**, 6460–6469.

Umbreit JN, Conrad ME, Moore EG & Latour LF (1998) Iron absorption and cellular transport: the mobilferrin/paraferritin paradigm. *Seminars in Haematology* **35**, 13–26.

Whitehead MW, Farrar G, Christie G, Blair JA, Thompson RPH & Powell JJ (1997) Mechanisms of aluminium absorption in the rat. *American Journal of Clinical Nutrition* **65**, 1446–1452.

Whitehead MW, Powell JJ & Thompson RPH (1995) The gut mucus layer regulates metal absorption? *Gut* **36**, A48.

Whitehead MW, Thompson RPH & Powell JJ (1996) Regulation of metal absorption in the gastrointestinal tract. *Gut* **39**, 625–628.

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