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CHARACTERISTICS OF IRON(III) UPTAKE BY ISOLATED FRAGMENTS OF RAT SMALL INTESTINE IN THE PRESENCE OF THE HYDROXYPYRONES, MALTOL AND ETHYL MALTOL

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Abstract—Accumulation of radioactive iron (^{59}Fe) into isolated fragments of rat small intestine in the presence of two hydroxypyrones, maltol and ethyl maltol, was compared with that in the presence of another chelator of iron(III), nitrilotriacetic acid (NTA). The characteristics of uptake were similar with all three ligands. Between 10^{-6} and 10^{-4} M, iron uptake showed saturable kinetics. The uptake was partially inhibited by metabolic inhibitors. Above 10^{-4} M a non-saturable uptake, unaffected by metabolic inhibitors became evident in the presence of the pyrones. The distribution of ^{59}Fe after uptake was determined by gel filtration. At low iron concentrations (10^{-6} M), 35–40% of absorbed iron was associated with proteins of molecular weights similar to those of ferritin and transferrin. At high concentrations (10^{-3} M), the majority of ^{59}Fe was found in a low molecular weight fraction. At each concentration, a small amount of ^{59}Fe was bound to a membrane fraction. 5% Polyethylene glycol, which reduces glycocalyx viscosity enhanced uptake at low iron concentrations (10^{-6} M) but did not affect the non-saturable diffusion seen at higher concentrations (10^{-3} M). The iron(II) chelator, bathophenanthroline sulphonate (10^{-3} M), decreased uptake at low iron concentrations but did not affect the non-saturable uptake. It is suggested that conversion of iron(III) to iron(II) may take place at the mucosal cell surface before uptake via the saturable system. Apparent K_m values for iron uptake via the saturable system were higher in the presence of maltol and ethyl maltol than in the presence of NTA, presumably since the iron binds more avidly to the hydroxypyrones and so is less readily donated. Excess ligand, either pyrone or NTA, reduced the rate at which ^{59}Fe was donated to the uptake system. The V_{max} value for uptake from the pyrones was greater than from NTA. It is concluded that maltol, ethyl maltol and NTA can hold iron(III) in solution and donate it to an endogenous uptake system. But, the hydroxypyrones may be more suitable ligands for the oral administration of iron since, when complexed with iron, they lack the toxic effects associated with iron(III)-NTA and with iron(II) preparations.

The comparison of iron(II) (ferrous) and iron(III) (ferric) uptake by mammalian intestine has recently been the focus of intense research effort. In a study of 14 different iron preparations in man, Dietzfelbinger [1], showed that the iron(III) preparations, without exception, had a lower bioavailability than iron(II) sulphate and were therefore of dubious therapeutic efficacy. Similar conclusions have been reached by others [2, 3]. Unfortunately, orally administered iron(II) sulphate generates hydroxyl radicals in the gastrointestinal tract of mammals [4]. This property together with the associated acidity of iron(II) sulphate may cause irritation and damage to the mucosa. A wide range of side effects have been reported for iron(II) sulphate [5]. Thus, should an efficiently absorbed iron(III) complex be identified, it would be of therapeutic benefit.

Recently, it has been shown that a variety of hydroxypyridones and hydroxypyrones are able to form stable complexes with iron(III) in aqueous media over a wide range of pH [6]. Providing these ligands possess uncharged sidechains, the resultant complexes will be neutral. By selecting suitable substituents, it is possible to adjust the partition coefficient (*n*-octanol/water) of these iron complexes to between 0.1 and 1.0, values which should allow transmembrane diffusion [6]. The hydroxypyrones, maltol (3-hydroxy-2-methyl-4-pyrone) and ethyl maltol (3-hydroxy-2-ethyl-4-pyrone) have been shown to diffuse readily into liposomes and erythrocytes [7]. These particular pyrones have also been investigated *in vivo* in the rat, where they appear to enhance iron absorption from the small intestine [8]. However, it is not clear whether they simply hold the iron in some readily absorbable form or whether they allow diffusion of the intact iron complexes into the mucosal cells. It is important to resolve this question since maltol and ethyl maltol, being relatively non-toxic and rapidly metabolised [9], may be suitable compounds for therapeutic use.

Uptake of iron, complexed to maltol and ethyl maltol, has thus been examined with an *in vitro* method, using isolated fragments of rat small intestine. Similar methods have already been employed to study both iron(III) and iron(II) uptake in human

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and other mammalian intestine [10]. Iron uptake with the hydroxypyrones has been compared with uptake in the presence of nitrilotriacetic acid (NTA), a non-penetrating iron(III) ligand, which is able to hold iron in solution but must donate it to an endogenous uptake system at the cell surface [10].

Preliminary results of this work have already been reported [11].

MATERIALS AND METHODS

Male rats were used, both Sprague–Dawley and Wistar (70–400 g body weight). They were fed a standard laboratory diet (CRM, obtained from Labsure, K. & K. Greef Ltd, Croydon, U.K.) containing 30 mg iron kg^{-1}. Animals to be made iron-deficient were fed from weaning on a special powdered diet (Special Diet Services, Witham, Essex, U.K.) containing less than 10 mg iron kg^{-1}. In these animals, haemoglobin levels assayed by the cyanomethaemoglobin method (Sigma Technical Bulletin No: 525, 1982) were in the range 4–10 g/100 ml blood (normal range 14–20 g/100 ml) and haematocrits were between 16 and 34% (normal range 40–50%). Animals were fasted, but were allowed water *ad lib.*, for 18 hr before use.

Animals were killed by stunning and decapitation. The proximal part of the small intestine (i.e. duodenum and jejunum) was removed, cut lengthwise to expose the mucosal layer and then transversely to produce fragments of between 30 and 50 mg in weight. The fragments were washed in oxygenated buffer (16 mM Hepes, 125 mM NaCl, 3.5 mM KCl, 1 mM $CaCl_2$, 10 mM $MgSO_4$ and 10 mM D-glucose, pH 7.4). In some studies the fragments were pre-incubated for 5 min at 37° in buffer containing the extracellular fluid marker, [^{3}H]-inulin (5 nM) before incubation at 37° in a shaking water bath with buffer containing the appropriate iron ligand solutions (see below). Fragments were removed at intervals, blotted, washed rapidly in ice-cold buffer, blotted again and weighed. For single isotope detection, ^{59}Fe content of the fragments was measured in an LKB-Wallac 1260 Multigamma counter. For dual-isotope detection, tissue fragments were first solubilised in 500 μl Soluene-350 (Canberra Packard, Pangbourne, Berks., U.K.) at 40° for 3–4 hr. Ten millitres PPO/POPOP/toluene scintillant (Toluene Scintillator, Canberra Packard) were added and the mixture left at room temperature for 18 hr to allow chemiluminescence to decay. The ^{59}Fe and tritium contents of the fragments were measured by liquid scintillation β-particle spectrometry with automatic external standardisation to correct for quenching and conversion to d.p.m. for both nucleides.

The amount of ^{59}Fe accumulated by the fragments was calculated, after correction for extracellular fluid volume, as a distribution ratio (tissue/medium) and subsequently converted to pmoles min^{-1} mg^{-1} wet weight of tissue.

Accumulation or uptake of iron into the fragments was assumed to take place at or across the mucosal cell surface since once in buffer, the fragments roll up to expose the mucosal surface to the bathing medium. Indeed, when mucosal and serosal layers were separated by scraping after incubation, the amount of absorbed iron in the serosal layer was found to be very small compared with that in the mucosal layer.

The viability of the isolated fragments was checked by monitoring their oxygen consumption, radioactive 3-*O*-methyl-D-glucose uptake and ultrastructural appearance during incubation. Providing that the length of incubation did not exceed 30 min, no deterioration was evident with any of these parameters.

Non-radioactive stock solutions of iron–ligand (10–50 mM) were prepared by mixing $FeCl_3$ with the ligands in Hepes buffer, to a metal–ligand ratio of 1:4 or 1:10 with the pyrones or 1:5 with NTA (nitrilotriacetic acid, trisodium salt). Radioactive stock solutions (2 mM) were prepared in a similar way and then mixed with sufficient $^{59}FeCl_3$ so as to achieve a final concentration of ^{59}Fe in the incubation medium of 0.1–0.2 μCi/ml. The radioactive and non-radioactive stock solutions were combined and diluted in Hepes buffer immediately before use so as to obtain a concentration range of iron from 10^{-3} to 10^{-6} M. The extracellular fluid marker and inhibitors were added as required just before use.

$^{59}FeCl_3$, [^{3}H]inulin and 3-*O*-methyl-D-[U-^{14}C]glucose were purchased from Amersham International (Bucks, U.K.), while the pyrones were purchased as Veltol (maltol) and Veltol-plus (ethyl-maltol) from Pfizer Ltd (Sandwich, Kent, U.K.). All other chemicals were either purchased from Sigma Chemical Co. Ltd (Poole, Dorset, U.K.) or were standard laboratory reagents of analytical grade.

To determine the subcellular distribution of ^{59}Fe after absorption, fragments were first incubated for 10 min with iron–ligand solutions containing 2.5 μCi ^{59}Fe ml^{-1}, then sonicated for 20 sec in 1 ml Hepes buffer (pH 7.4) containing 30 mM $NaHCO_3$ and centrifuged for 5 min at 600 *g* to remove unbroken cells and debris. 400 μl of supernatant was then applied to a column of Sephadex G100 (1.7 cm × 50–60 cm) in tandem with a second column of Sepharose 4B (1.7 cm × 50–60 cm). The effective fractionation range of the combined system was 4×10^3 to 2×10^7 Mr (Pharmacia, Uppsala, Sweden). The columns were equilibrated with 25 mM Hepes, 30 mM $NaHCO_3$ and 0.02% sodium azide at pH 7.4 and eluted with the same buffer in 2.5 ml fractions with a flow rate of 6 ml hr^{-1}. The eluate was monitored continuously at 280 nm to detect the protein peaks and all fractions were analysed subsequently for ^{59}Fe content by gamma counting. Recovery of iron was over 70% of that added to the columns. Between each experiment, the columns were flushed through with 20 ml EDTA (1 mM) in Tris buffer (20 mM; pH 7.4), followed by 250–300 ml of the above elution buffer to remove any residual iron bound to the gel. Horse spleen ferritin, human serum transferrin and adenosine-5′-monophosphate were passed through the gel filtration system to identify the elution peaks for the two main iron-binding proteins and for the bed volume.

RESULTS

Characteristics of iron uptake

The uptake of iron from each of the three ligand

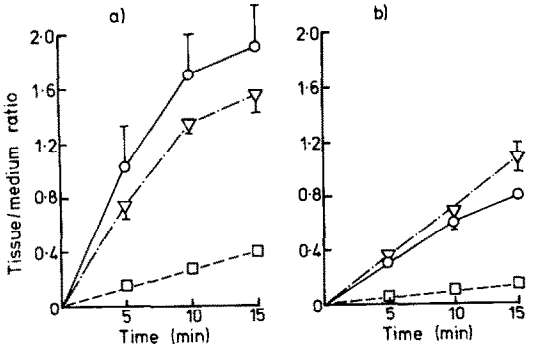

Fig. 1. Effect of time on the uptake of ^{59}Fe into isolated fragments of rat small intestine from iron–NTA (□), iron–maltol (○) and iron–ethyl maltol (▽) with metal:ligand ratios of 1:5, 1:4 and 1:4 respectively. Iron concentrations were (a) 0.02 mM and (b) 0.2 mM. Each point is the mean ± SER of data taken from four separate animals, four values being obtained from each animal.

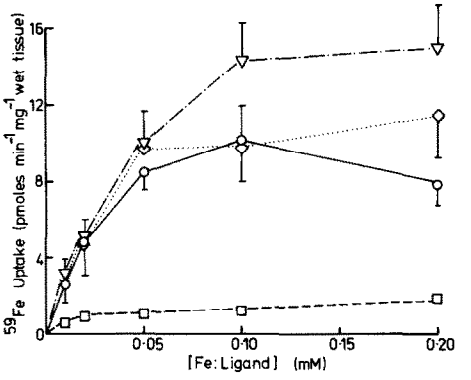

Fig. 2. Effect of iron concentration on the uptake of ^{59}Fe into isolated intestinal fragments from iron–NTA (□), iron–maltol (○), iron–ethyl maltol (▽) and iron–ethyl maltol/maltol (4/1) (◇) with metal:ligand ratios of 1:5, 1:4, 1:4 and 1:4 respectively. Each point is the mean ± SEM of data taken from five to twelve separate animals, four values being obtained from each animal.

mixtures was found to be linear up to 10 min (Fig. 1) with no detectable efflux of iron.

With a fixed incubation time of 10 min, it was possible to calculate initial rates of iron uptake at different iron–ligand concentrations. With each of the three iron–ligand preparations, the process or processes involved in iron uptake appeared to be saturable over the range 10^{-6}–10^{-4} M (Fig. 2). The apparent K_m and V_{max} values for iron uptake, obtained from Lineweaver–Burk plots of the data, are shown in Table 1. Although the affinity of iron for the uptake system from the iron pyrones is lower than from iron–NTA, the capacity is higher. No significant differences in iron uptake were detectable between normal and iron-deficient animals with any of the iron pyrone preparations, although the K_m for uptake with iron–NTA did appear to be lower in iron deficiency. At higher iron concentrations, above 10^{-4} M, non-saturable uptake of iron became apparent.

Uptake was slowed considerably by reduction in temperature. By studying uptake over a range of different temperatures, it was possible to calculate Q_{10} values for each of the iron–ligand preparations. These were found to lie in the range 1.5–2.5.

Over the concentration range 10^{-6}–10^{-4} M the rate of iron uptake was not altered when nitrogen, instead of oxygen, was bubbled through the incu-

Table 1. Kinetic constants for uptake of ^{59}Fe into isolated fragments of rat small intestine when presented as iron:NTA (1:5), iron:maltol (1:4), iron:ethyl maltol (1:4) or iron:ethyl maltol/maltol 4/1 (1:4)

	NTA	Maltol	Ethyl maltol	Ethyl maltol/maltol
Control animals				
K_m (μM)	19 ± 3 (N = 4)	41 ± 8 (N = 7)	165 ± 37 (N = 6)	101 (69–157) (N = 3)
V_{max} (pmol min^{-1} mg^{-1} wet tissue)	1.4 ± 0.2 (N = 4)	15 ± 3 (N = 7)	45 ± 9 (N = 5)	24 (16–34) (N = 3)
Iron deficient animals				
K_m (μM)	12 ± 2 (N = 5)	56 ± 12 (N = 5)	173 ± 73 (N = 4)	n.d.
V_{max} (pmol min^{-1} mg^{-1} wet tissue)	1.5 ± 0.1 (N = 5)	16 ± 3 (N = 5)	42 ± 12 (N = 4)	n.d.

Statistical analysis by Student's *t*-test shows:

K_m in controls	Fe:maltol vs Fe:NTA	$P < 0.05$
V_{max} in controls	Fe:maltol vs Fe:NTA	$P < 0.002$
K_m in controls	Fe:maltol vs Fe:ethyl maltol	$P < 0.005$
V_{max} in controls	Fe:maltol vs Fe:ethyl maltol	$P < 0.005$
K_m for Fe:NTA	Fe deficient vs controls	$P < 0.05$
K_m for Fe:maltol	Fe deficient vs controls	N.S.
V_{max} for Fe:NTA	Fe deficient vs controls	N.S.
V_{max} for Fe:maltol	Fe deficient vs controls	N.S.

Data shown as means ± SEM of N different experiments (number as shown in parentheses). Each experiment involved 5 different iron concentrations with 4 fragments sampled per concentration.

bation mixture. Nor was there any decrease in iron uptake in the presence of either 1 mM KCN, 0.1 mM 2,4-dinitrophenol or 5 mM NaF in agreement with observations made by others using brush border vesicles [12]. However, with a combination of 10 mM iodoacetate, 0.1 mM dinitrophenol, nitrogen bubbled through the mixture and the omission of glucose, a reduction in iron uptake from iron–NTA was seen (tissue/medium ratio after 10 min incubation in presence of inhibitors: 0.29 ± 0.07 compared with a control value of 1.67 ± 0.37, $N = 8$, $P < 0.001$). No reduction with this combination of inhibitors was observed with the iron pyrones. Uptake of 3-*O*-methyl-D-glucose (1 mM), a process known to require energy, was decreased by this combination of metabolic inhibitors (tissue/medium ratio after 10 min incubation in presence of inhibitors: 0.79 ± 0.21 compared with a control value of 2.74 ± 0.14, $N = 5$, $P < 0.001$).

Distribution of absorbed ^{59}Fe within the mucosal cells

The uptake of ^{59}Fe as described above was measured as that ^{59}Fe associated with the tissue fragments in excess of that amount accountable as extracellular. From this, one could not say whether the excess iron represented uptake into the cells or binding to the outside of the cells. To determine with what cellular components the ^{59}Fe became associated, intestinal fragments were incubated for 10 min with iron–NTA or maltol (10^{-6}, 10^{-4} and 10^{-3} M). The mucosal cells were scraped off, homogenised and samples of the homogenates separated by gel filtration as described in Materials and Methods. The elution profile of ^{59}Fe could be resolved into four peaks (Fig. 3), one appearing at the void volume, presumably containing membranous material; the second and third peaks eluting at positions corresponding to ferritin and transferrin; and the fourth peak associated with low molecular weight material eluting at the bed volume.

The percentage distribution of ^{59}Fe within these peaks depended upon the nature of the ligand used (Fig. 4). At 10^{-6} M iron no significant differences between iron pyrones and iron–NTA were evident. At 10^{-4} M a higher percentage of iron from the iron pyrones was found in the ferritin and transferrin peaks, whilst more iron from iron–NTA appeared with the low molecular weight material. At 10^{-3} M only a small percentage of ^{59}Fe could be found in the peaks corresponding to ferritin and transferrin, the majority of ^{59}Fe eluting with the low molecular weight material. Thus at low iron concentrations, at least 35–40% of iron in the intestinal homogenates appeared to be associated with soluble iron-binding proteins suggesting that some uptake of iron must have occurred. But at saturating concentrations of iron, the actual proportion of iron associated with protein within the tissues was very small.

Effect of polyethylene glycol on iron uptake

Polyethylene glycol (PEG) has been shown to enhance intestinal absorption of drugs by modifying the viscosity of the overlying glycocalyx [13]. It was of interest, therefore, to determine whether this agent could modify the uptake of iron by isolated intestinal fragments.

Preliminary studies with PEG 400 and PEG 600 showed that both compounds were capable of enhancing iron uptake. With a range of concentrations of PEG 400 at a fixed concentration of iron–NTA (10^{-5} M), maximum enhancement of uptake was seen in the presence of 5% w/v of PEG 400. The effect of 5% w/v PEG 400 was examined on iron uptake after 10 min incubation at 5×10^{-6}, 10^{-4} and 10^{-3} M of iron ligand. PEG 400 caused a significant increase in iron uptake, at 5×10^{-6} M iron, both with iron–NTA (tissue/medium ratio in the presence of PEG: 2.7 ± 0.6 compared with 1.0 ± 0.1 in control tissues, $N = 5$, $P < 0.05$) and with iron maltol (tissue/medium ratio

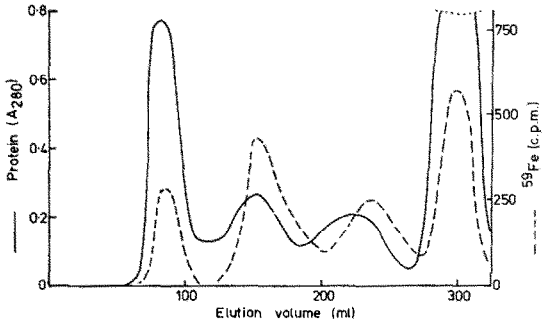

Fig. 3. Gel filtration profile showing subcellular distribution of ^{59}Fe in homogenates of intestinal mucosa taken 10 min after incubation of intestinal fragments with 0.02 mM iron–maltol (1:4, metal:ligand). 400 μl of homogenate were applied to a column of Sephadex G100 in tandem with a column of Sepharose 4B and eluted in 2 ml fractions with 20 mM Hepes buffer, pH 7.4, containing 30 mM $NaHCO_3$ and 0.02% sodium azide. Protein (——) was monitored continuously at 280 nM. ^{59}Fe content (– – –) of each fraction was measured in gamma counter.

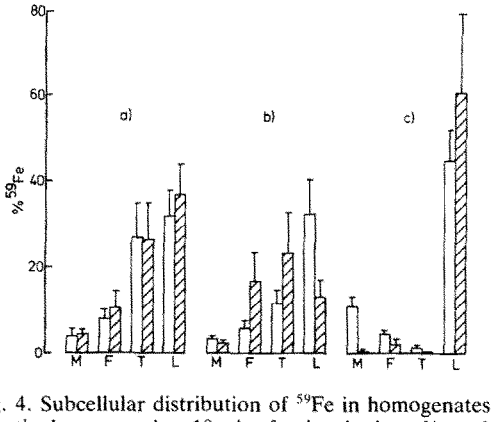

Fig. 4. Subcellular distribution of ^{59}Fe in homogenates of intestinal mucosa taken 10 min after incubation of intestinal fragments with iron–NTA (1:5, metal:ligand) (□) and iron–maltol (1:4, metal: ligand) (▨) at iron concentrations of (a) 10^{-6} M, (b) 10^{-4} M and (c) 10^{-3} M and separated by gel filtration as described in Fig. 3. Values shown are percentages ± SER of ^{59}Fe applied to the columns recovered in membrane fraction (M), ferritin–weight protein fraction (F), transferrin–weight protein fraction (T) and low molecular weight fraction (L) from homogenates obtained from four separate animals.

in the presence of PEG: 3.4 ± 0.3 compared with 1.0 ± 0.2 in control tissues, N = 5; P < 0.001). However, no significant increases in iron uptake, due to PEG 400, were seen with either ligand at 10^{-4} M iron, at which concentration the rate of uptake is maximal or at 10^{-3} M iron where simple diffusion may make an appreciable contribution to iron accumulation.

Effect of excess ligand on iron uptake

At pH 7.4 the neutral (1:3, iron:pyrone) complex is the predominant species, but at low concentrations and low pH values, 1:1 and 1:2 charged species are also formed [14], which may exhibit different uptake characteristics. Further, the addition of excess ligand may not only influence the formation and amounts of the charged complexes but may also affect the uptake of iron directly. In order to test this possibility, the uptake of iron was examined in the presence of increasing concentrations of free maltol. As can be seen in Fig. 5, excess maltol inhibited the uptake of ^{59}Fe. A similar effect was seen with NTA. Kinetic analysis revealed that the inhibition by both ligands was probably competitive in nature (Fig. 6).

Effect of an iron(II) chelator on iron uptake

In the presence of the selective iron(II) chelator, bathophenanthroline sulphonate (BPS), a similar inhibition of ^{59}Fe uptake from iron–NTA and iron maltol was seen. The tissue/medium ratio after 10 min incubation of 10^{-6} M iron–NTA in the presence of 10^{-3} M BPS was 0.48 ± 0.06 compared with control of 1.1 ± 0.15 (P < 0.01, N = 11). With 10^{-6} M iron maltol as the complex, tissue/medium ratios of 0.2 ± 0.06 in the presence of BPS and 0.8 ± 0.19 (P < 0.02, N = 9) were found. The integrity of the fragments in 10^{-3} M BPS was tested by monitoring the uptake of 3-*O*-methyl-D-glucose. No effect on the tissue accumulation of glucose was seen (tissue/medium ratios of ^{14}C after 10 min incubation with 1 mM 3-*O*-[^{14}C]methyl-D-glucose of 0.52 ± 0.09 and 0.53 ± 0.04, N = 4, in BPS and control respectively).

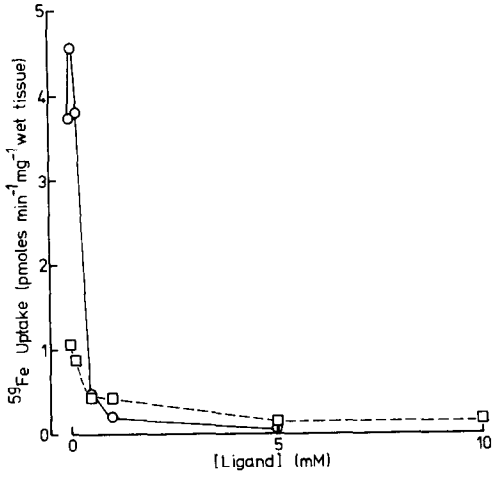

Fig. 5. Effect of excess ligand on uptake of ^{59}Fe into isolated intestinal fragments from iron–NTA (□) and iron–maltol (○) with initial metal:ligand ratios of 1:5 and 1:3 respectively and at a single iron concentration of 0.02 mM. Each point is the mean of data taken from two separate animals, four values being obtained from each animal.

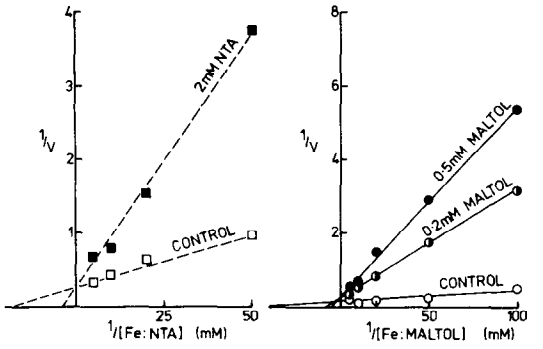

Fig. 6. Effect of excess ligand on the kinetics of ^{59}Fe uptake into isolated intestinal fragments from iron–NTA (□) and iron–maltol (○) with control metal:ligand ratios of 1:5 and 1:4 respectively. Data is presented as a Lineweaver–Burk plot with V in units of pmoles min^{-1} mg^{-1} wet tissue. Each point is the mean of data taken from two (for NTA) or six (for maltol) separate animals, four values being obtained from each animal.

DISCUSSION

The technique described in this paper provides a useful *in vitro* method for investigating the uptake of iron across the intestinal mucosal cell surface and for comparing iron uptake in the presence of various chelating agents. Although it is iron accumulation that is measured, this probably represents uptake rather than superficial binding. The gel filtration studies show that there is little membrane bound iron but a large proportion of iron associated with intracellular proteins and low molecular weight material.

Iron uptake into intestinal cells has been studied extensively *in vitro* both in intestinal fragments [10] and in brush border vesicles [15] with iron(III) and iron(II) complexes [see 15, 16]. In this study, an attempt has been made to establish whether or not the characteristics of iron(III) uptake from the iron–pyrone complexes, maltol and ethyl maltol, resemble those previously described by comparing uptake of iron from the pyrones with that from NTA, a ligand, which does not enter the mucosal cells [10] and must donate its iron to the endogenous system at or near the cell surface.

Over the concentration range 10^{-6}–10^{-4} M, the uptake of iron, both from iron–NTA and from the iron–pyrones, showed saturable kinetics. Above these concentrations non-saturable uptake became apparent in the presence of the pyrones. With iron–NTA, the saturable component appeared partially sensitive to metabolic inhibition. With the iron–pyrones, this inhibition was not evident. It has been found in human mucosal samples that a combination of inhibitors is required to reduce iron uptake [10]. Iron uptake from both iron–NTA and from the iron–pyrones exhibited temperature sensitivity with Q_{10} values of approximately 2. In these respects, the

uptake of iron from the pyrones resembles that seen by others for iron uptake *in vitro* [15, 16]. The presence of two components to uptake is consistent with that observed *in vivo* [8].

The existence of two components of uptake may explain the differential effects of PEG. At low concentrations of iron where the saturable system predominates, supply of iron from the iron–ligand complex is likely to be the rate limiting step. Thus, by reducing the viscosity of the overlying glycocalyx, PEG is able to enhance uptake [13]. At saturating concentrations, the supply of substrate may no longer be rate-limiting and so PEG may have little effect.

The distribution of iron within the tissues appears to depend on the concentration of iron used. At low concentrations, a large proportion of the absorbed iron is associated with proteins in the tissue. At high iron concentrations, where diffusion may be occurring, the majority of the iron is found in a low molecular weight pool. Inevitably, there will be some redistribution of the iron during homogenisation of the tissue, particularly when the iron then becomes exposed to the very high affinity transferrin. This emphasises the need to interpret experimental results of this nature with care. Nevertheless it is clear that iron taken up from the iron pyrones ultimately binds to proteins in the intestinal mucosa of similar weight to ferritin and transferrin.

The characteristics of iron uptake from both iron–NTA and the iron–pyrones are such that it could be concluded that the ligands donate their iron to the same endogenous system. However, the apparent affinity of iron for this system is greater from iron–NTA than from the iron–pyrones. This may be explained in terms of the relative affinities of iron for the pyrones and NTA (K_{aff} values of iron of $\log\beta_2 = 24$ and $\log\beta_3 = 28$ for NTA and maltol, respectively). Donation of iron to an endogenous system would clearly be easier from the lower affinity iron–NTA complex. Likewise, excess ligand, either pyrone or NTA, would be predicted to compete for iron with the endogenous system and so, as shown here, reduce iron uptake in a competitive manner. Such inhibition with excess ligand has already been documented for iron–NTA [17]. Interpretation of the exact meaning of the K_m values is complicated by the fact that they may represent composites of the kinetic parameters of several competing interactions as well as representing the overall affinity of the uptake system itself.

It is more difficult to explain why the V_{max} values for iron uptake are greater from the iron–pyrones than from iron–NTA. Possibly the pyrones which themselves enter the cells [11] may modify transfer of iron across the membrane or its dissociation from the carrier inside the cell. Such an effect is clearly not possible for a non-penetrating ligand like NTA. Addition of excess pyrone, however, fails to augment the V_{max} any further and in fact appears to compete with the initial donation of iron to the carrier.

The actual mechanism of iron uptake at the mucosal cell surface is still not clearly elucidated. One suggestion that has been made is that conversion of iron(III) to iron(II) takes place at the intestinal cell surface before uptake can occur [18]. Such conversion has been demonstrated in microorganisms [19] and in plants [20]. If reduction of this kind is in fact involved in the saturable uptake of iron into isolated intestinal fragments, then this would explain why the iron(II) chelator, bathophenanthroline sulphonate, inhibits iron uptake at low iron concentrations. Experiments to investigate this further are now in progress. It is interesting to note that not only iron–NTA but also the iron–pyrones are affected by this agent.

In conclusion, the characteristics of iron(III) uptake in the presence of the hydroxypyrones are similar to those for uptake in the presence of the non-penetrant iron(III) ligand, NTA. Thus it appears that despite their high affinity for iron, the pyrones at low iron concentrations are able to donate iron to the endogenous uptake system. Iron–NTA is known to be toxic, generating hydroxyl radicals [21] and including the formation of lipid peroxides in membranes [22]. In contrast iron complexes of the hydroxypyrones, maltol and ethyl maltol, lack these undesirable properties [21]. The hydroxypyrones may thus prove to be suitable and effective ligands for oral administration of iron in the less toxic iron(III) form.

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