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# Effects of the pyrones, maltol and ethyl maltol, on iron absorption from the rat small intestine

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The pyrones, 3-hydroxy-2-methyl-4-pyrone (maltol) and 3-hydroxy-2-ethyl-4-pyrone (ethyl maltol) chelate iron with a high affinity and selectivity. The resulting 1 : 3 (metal–ligand) complexes, being neutral, are able to partition readily across cell membranes and thus may facilitate iron transport across the intestinal wall. Absorption of radioactive iron ($^{59}$Fe) in the presence of these pyrones was investigated in male rats 1, 2, 4 and 6 h after intraduodenal administration of a 7 μg dose and compared with that of $^{59}$Fe given as the sulphate, gluconate, fumarate or complexed to EDTA. Total body absorption and distribution were calculated from the $^{59}$Fe content of various tissue samples. With all the iron preparations used, blood levels of $^{59}$Fe were highest 1 h after injection whilst the $^{59}$Fe content at the major site of deposition, i.e. the bone marrow, increased up to 6 h. No $^{59}$Fe was found in the urine. Total body absorption of $^{59}$Fe was significantly higher from the pyrones than from the other four preparations. Over the dose range 0·7–700 μg, the proportion of $^{59}$Fe absorbed from both iron maltol and iron sulphate decreased with increasing dose. Enhanced $^{59}$Fe uptake from maltol was evident at 0·7–70 μg but not at 700 μg suggesting that use of these pyrones will not result in iron overload. Absorption of $^{59}$Fe given into the stomach was slower in onset but was sustained longer presumably via a steady delivery of iron to the duodenum from the gastric reservoir. The presence of excess maltol to maintain the integrity of the neutral 1 : 3 iron–maltol complex under a variety of physiological conditions did not appear critical in-vivo for effective iron absorption. There was no difference in $^{59}$Fe uptake between maltol, ethyl maltol or a mixture of ethyl maltol and maltol. $^{59}$Fe uptake was significantly enhanced in iron-deficient animals. Within 1 min of i.v. injection of iron complexed to maltol or ethyl maltol, $^{59}$Fe became associated with a plasma protein of molecular ratio similar to that of transferrin. With EDTA this process was much slower, i.e. up to 60 min. The half-life of $^{59}$Fe in the blood was similar whether administered as the maltol, ethyl maltol, sulphate or EDTA; at the 110 μg iron dose it was 133 ± 8 min and at 1 μg it was 74 ± 10 min in iron-replete animals and 44 ± 5 min in iron-deficient animals. The rate of absorption from the duodenum of $^{59}$Fe given as maltol, ethyl maltol or as iron sulphate increased with increasing dose but the shape of the absorption curve depended on the rate of movement of iron along the intestine. It is concluded that the pyrones, maltol and ethyl maltol, are able to enhance the initial stages of iron uptake from the intestinal lumen, possibly by holding the iron in a readily absorbable form, but do not influence subsequent iron distribution and so may provide safe and palatable alternatives to those iron preparations presently available for the treatment of iron deficiency.

The mechanisms by which non-haem iron is absorbed from the small intestine have not been fully elucidated but it has been suggested that uptake of iron into the mucosal cells may be regulated by specific intracellular transporting intermediates (Halliday et al 1976). Both ferritin (Johnson et al 1983) and a transferrin-like protein (Huebers et al 1983), which have been identified in mucosal cells, have been proposed as regulators of uptake (Savin & Cook 1980). However, it is generally recognized that, whatever the nature of the intermediate, the bioavailability of iron within the gut lumen is of critical importance to the uptake process (Bothwell et al 1979; Morris 1983). A variety of intraluminal factors influence inorganic iron availability, not least being the unfavourable pH in the small intestine. In the absence of chelating agents, at neutral to alkaline pH, inorganic iron will hydrolyse to form insoluble polynuclear hydroxide complexes (Aisen 1977). In the presence of chelating agents which can hold the iron in soluble form, absorption will depend on the ease with which the particular chelate can donate its iron.

A group of pyrones has been developed, all of which possess characteristics suitable for chelating iron and may prove useful as alternatives to the

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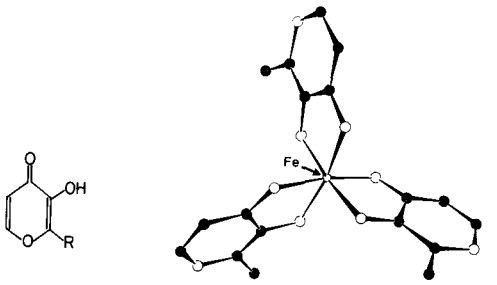


FIG. 1. Structure of pyrone and diagrammatic representation of the neutral 1:3 iron–pyrone complex: R = $CH_3$, 3-hydroxy-2-methyl-4-pyrone (maltol), R = $CH_2CH_3$, 3-hydroxy-2-ethyl-4-pyrone (ethyl maltol).

preparations currently available for the treatment of iron deficiency (Fig. 1) (Hider et al 1984). The 3-hydroxy-2-methyl-4-pyrone (maltol) has an affinity for iron much higher than that of many established chelates, e.g. relative stability constants of ferric iron for maltol (Hider, unpublished observations), citric acid and EDTA (Aisen 1977) at pH 7·4 being 25, 17 and 22, respectively. Furthermore, maltol is highly selective for iron, having a much lower affinity for other metal ions, e.g. relative stability constants of maltol at pH 7·4 for copper, lead and zinc being 10, 9 and 7, respectively (Bakaj et al, unpublished observations). It has been shown in-vitro that despite the high affinity, maltol is able to donate its iron readily to transferrin, the rate of transfer comparing most favourably with other iron chelates, e.g. the half-lives for transfer of iron to apotransferrin from maltol, citric acid and EDTA being 2 min, 8 h and 4 days, respectively (Bakaj et al unpublished observations). At neutral pH and above, it is able to form an uncharged 1:3 metal–ligand complex which shows partition characteristics favouring diffusion across cell membranes (Bakaj 1984) in contrast to other iron chelates (partition coefficients in octanol/Tris HCl at 20 mM, pH 7·4 and 20 °C for iron–maltol, iron–EDTA and iron–ascorbate of 0·5, 0·001 and 0·0015, respectively).

The present study was undertaken to investigate the effect of the pyrones, maltol and ethyl maltol, on intestinal iron absorption in experimental animals, to compare uptake with that from currently available iron preparations and to determine whether iron in the presence of these pyrones, owing to the lipophilic nature of the complexes formed, would by-pass the normal uptake and regulatory mechanisms. Preliminary results of this work have already been reported (Callingham et al 1984). The rat was used since it absorbs non-haem iron extremely well (Cook et al 1973) and so has already been the subject of extensive investigation (Forth & Rummel 1975). It will be necessary to confirm by clinical studies that the results obtained in the rat are relevant to man who absorbs non-haem iron less readily (Bothwell et al 1979).

## MATERIALS AND METHODS

Male Wistar rats, 200–400 g were used. Most of the following experiments were performed on normal, iron-replete animals, fed a standard laboratory diet (CRM obtainable from Labsure, K. & K. Greef Ltd, Croydon, UK) containing 30 mg iron $kg^{-1}$. In these animals, haemoglobin levels assayed by the cyanmethaemoglobin method (Sigma Tech. Bulletin No. 525, 1982), were in the range 14–20 g/100 mL and haematocrits between 40 and 50%. Some experiments were on animals made iron-deficient by feeding them from weaning on a special powdered diet (Special Diet Services, Witham, Essex, UK) containing less than 10 mg iron $kg^{-1}$. In these animals, haemoglobin levels ranged between 4 and 10 g/100 mL and haematocrits between 16 and 34%. Growth was greatly retarded in these animals so that by the time their weight was ca 200 g, their age-matched, normally fed litter mates were twice their size. Thus both age-matched and size-matched normal animals were used as controls in these studies. Animals were starved 24 h before use.

*Absorption and distribution of iron in whole animals*

Animals were anaesthetized with ether. Aliquots, 400 μL, of the appropriate iron-containing solutions were injected via a small abdominal incision into the duodenum 2–3 cm beyond the pylorus. The body wall was sutured and the animals allowed to recover. In later experiments, the iron solutions were administered via an oral cannula into the stomach and no anaesthetic was necessary. 1, 2, 4, and 6 h after injection, the animals were killed by ether overdose and samples taken of blood, liver and skeletal muscle together with urine, kidneys, femurs, spleen and the unabsorbed contents and the washed segments of the small intestine divided into 5 portions from pylorus to caecum. Their content of radioactive iron ($^{59}Fe$) was measured in a Packard Auto Gamma 500 counter. The amount of iron entering the body was calculated by adding together the various tissue levels of $^{59}Fe$ excluding the intestine and its contents. The total skeletal muscle mass was taken to be 30% of the total body weight and total blood volume to be

5·9 mL/100 g body weight (Wang 1959). In a preliminary experiment, 8 rats, previously given either $^{59}$Fe–maltol (n = 4) or $^{59}$Fe sulphate (n = 4) into the duodenum were dissected and all parts of each animal taken for radioactive counting in order to recover the entire $^{59}$Fe dose injected and thereby identify the major sites of accumulation of $^{59}$Fe. The greatest amount of radioactivity was found in the marrow-containing areas of the bone, particularly the upper and lower spine, limb bones and scapulae. It was apparent from the data that the ratio of the amount of $^{59}$Fe in these areas to the amount of $^{59}$Fe in the two femurs was constant (10·7 ± 0·3%). $^{59}$Fe content of the two femurs could thus be taken as an index (i.e. 1/12) of $^{59}$Fe present in the total bone marrow.

*Pharmacokinetic studies*

Rats were anaesthetized with sodium pentobarbitone (60 mg kg$^{-1}$ i.p.) and cannulae inserted into trachea, jugular vein and carotid artery. In early studies, a cannula was also inserted into the other carotid artery to monitor blood pressure but this was discontinued since the disruption to the circulation combined with the blood sampling led to poor survival. Heparin, further anaesthetic as required, and the iron solutions were introduced via the jugular cannula. Blood samples, usually around 100–200 μL, were taken at suitable time intervals, in some cases up to 4 h, by allowing blood to drip from the carotid cannula. Care was taken to ensure that total blood loss was kept to a minimum, i.e. generally no more than about 10% of the total blood volume. In a series of studies where the iron was administered as above into the duodenum, the jugular vein was not cannulated and heparin and further anaesthetic were given into the peritoneum. Rats remained under anaesthesia throughout the experiments.

*Preparation of solutions*

Iron solutions for absorption studies were prepared by mixing $^{59}FeCl_3$ (Amersham International, Bucks., UK) with $FeCl_3$, the appropriate ligand and with buffer so that the final 200 μL aliquots for injection contained 20 mM Tris-Cl, pH 7·0, 1 μCi $^{59}$Fe and 0·0625, 0·625, 6·25 or 62·5 mM Fe (i.e. doses per animal of 0·7, 7, 70 and 700 μg). Except where stated, the 1:3 iron–pyrone complexes were prepared by mixing together metal and ligand in a ratio of 1:4. For preparation of $^{59}FeSO_4$ solutions, $^{59}FeCl_3$ was mixed with $FeSO_4$ before addition of buffer to allow adequate time for distribution of the isotope between the two oxidation states of iron. Even at the lowest concentration, $^{59}$Fe represented no more than 10% of the total iron present. For the pharmacokinetic studies, iron solutions given in 400 μL aliquots contained (mM) Hepes 10, pH 7·4, NaCl 140, KCl 4, $CaCl_2$ 1 and $MgSO_4$ 10 with 1 μCi $^{59}$Fe and 5 mM $FeCl_3$ mixed with pyrones in a metal–ligand ratio of 1:3 or 1:4 with NTA (nitriloacetic acid, trisodium salt) 1:5 and with EDTA (ethylenediaminetetraacetic acid, disodium salt) at 1:1. The pyrones were purchased as Veltol (maltol) and Veltol-plus (ethyl maltol) from Pfizer Ltd, Sandwich, Kent. All other chemicals were either purchased from Sigma Chemical Co. Ltd, Poole, Dorset or were standard laboratory reagents of analytical grade.

*Gel filtration of plasma*

For crude separation of high and low molecular weight species, 200 μL aliquots of plasma, divided from the cells by centrifugation at 6000*g* for 2 min at room temperature (20 °C), were passed down 10 mL columns (PD-10) of Sephadex G-25 (Pharmacia, Uppsala, Sweden) previously equilibrated with 20 mM Hepes, pH 7·4 in 154 mM NaCl and eluted in 1 mL fractions. For gel filtration with greater resolving power, 400 to 500 μL aliquots of plasma were applied to a column (1·6 × 43 cm) of Sephacryl S-300 (effective fractionation range molecular ratio (Mr) 10 000–1 500 000; Pharmacia) equilibrated with buffer as above and eluted in 1 mL fractions with a flow rate of 1 mL min$^{-1}$. All fractions were analysed by gamma counting. The Sephacryl column was calibrated with standard proteins; thyroglobulin, ferritin, both human and bovine transferrin, bovine serum albumin and ovalbumin, the Mr of these being taken as 669 000, 440 000, 78 000, 67 000 and 43 000, respectively.

## RESULTS AND DISCUSSION

*Absorption and distribution of $^{59}$Fe after intraduodenal administration of a 7 μg iron dose of iron–maltol. A comparison with iron fumarate, sulphate, gluconate and EDTA*

With all iron preparations studied, the amount of $^{59}$Fe absorbed into the body appeared to be maximal by 1 h and no further increases up to 6 h were seen (Fig. 2). However the distribution of $^{59}$Fe between blood and bone marrow continued to change such that blood levels of $^{59}$Fe were highest at 1 h and decreased thereafter whilst the $^{59}$Fe content of the bone marrow and spleen rose steadily so that by 6 h most of the $^{59}$Fe absorbed was in the bone marrow

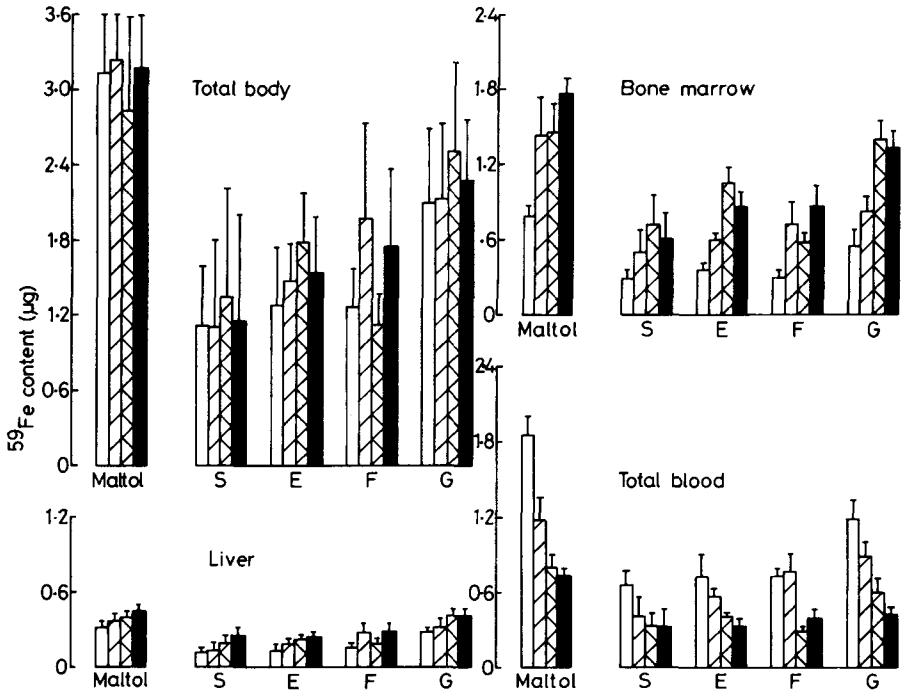


FIG. 2. Amount of $^{59}$Fe (in μg) in total body, blood, bone marrow and liver 1 (□), 2 (▨), 4 (▩) and 6 (■) h after intraduodenal administration of 7 μg iron given either as the sulphate (S), fumarate (F), gluconate (G), with EDTA (E) or complexed to maltol. Each value represents the mean ± s.e.m. of 6 animals. Statistical analysis by Student's *t*-test of data on total body $^{59}$Fe combined from all four times (i.e. n = 24) shows: maltol (3·08 ± 0·9 μg) vs sulphate (1·2 ± 0·9 μg) $P < 0·001$, vs EDTA (1·51 ± 0·5 μg) $P < 0·001$, vs fumarate (1·22 ± 0·8 μg) $P < 0·001$, vs gluconate (2·25 ± 0·7 μg) $P < 0·001$.

(Fig. 2). A small percentage of the $^{59}$Fe administered was detectable in the liver and the amount increased up to 6 h (Fig. 2). No radioactivity was found in the urine. This was to be expected since it is well known that elimination of iron from the blood does not occur via renal excretion or, under normal circumstances or to any great extent, via hepatic extraction, but mainly by uptake into the bone marrow (Bothwell et al 1979). Iron loss from the body occurs normally via the gastrointestinal tract primarily by desquamation of epithelia into the lumen and also via extravasation of red cells (Bothwell et al 1979), but the time scale of these processes is such that losses during the 6 h experiments described above would be negligible.

Although it has generally been observed that iron is absorbed better in the ferrous state than when given as solutions of ferric salts (Bothwell et al 1979), there is controversy over this, based on the relative bioavailability of the di- and trivalent forms of the iron (Forth & Rummel 1975; Schäfer & Forth 1984). Despite any such differences, it was apparent that with 7 μg iron per rat (equivalent to about 2 mg per 70 kg man), significantly higher absorption of $^{59}$Fe occurred when the iron was given in the ferric form as the maltol complex than when presented as iron–EDTA or in the ferrous state as sulphate, fumarate or gluconate (Fig. 2).

*Absorption of $^{59}$Fe after intraduodenal administration of 0·7, 7, 70 and 700 μg iron given as iron–maltol or iron sulphate*

It was found with both iron compounds that the proportion of iron absorbed decreased with increasing dose (Fig. 3). Such limitation on iron uptake capacity is already well documented (Forth & Rummel 1975; Jacobs & Worwood 1981). But whereas uptake of iron from maltol was significantly higher than that from sulphate over the dose range 0·7–70 μg iron per rat (equivalent to 0·2–20 mg per 70 kg man), no differences were seen at the highest dose studied, i.e. 700 μg iron per rat or 200 mg per 70 kg man. It has already been suggested by others that transfer of iron occurs in two ways, at high dose levels by simple diffusion and at low doses via a

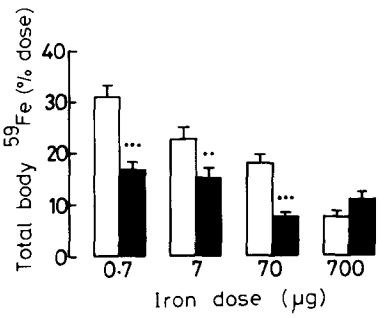


FIG. 3. Amount of $^{59}$Fe (% of dose given) in total body 1, 2, 4 and 6 h after intraduodenal administration of 0·7, 7, 70 and 700 μg iron given either as sulphate (■) or complexed to maltol (□). Data at all four times has been combined so that each value represents the mean ± s.e.r. of 16 animals. Statistical analysis by Student's *t*-test shows: ***$P < 0{\cdot}001$, **$P < 0{\cdot}02$, *$P < 0{\cdot}05$.

Table 1. Total body uptake of $^{59}$Fe shown as percentage of dose administered. Comparisons between 0·7 and 70 μg iron doses given either as 1 : 4 or 1 : 10 iron–maltol into the stomach or into the duodenum.

| Dose of iron (μg) | 1 : 4 iron–maltol | | 1 : 10 iron–maltol | |
|---|---|---|---|---|
| | Stomach | Duodenum | Stomach | Duodenum |
| 0·7 | 45·0 ± 3·8 (n = 4) | 40·3 ± 7·4 (n = 4) | 35·7 ± 8·8 (n = 4) | 49·8 ± 7·0 (n = 4) |
| | *42·7 ± 5·6 (n = 8) | | **42·8 ± 8·3 (n = 8) | |
| 70 | 21·1 ± 4·7 (n = 4) | 23·8 ± 6·5 (n = 4) | 27·3 ± 10·8 (n = 4) | 18·5 ± 2·4 (n = 4) |
| | *22·4 ± 5·3 (n = 8) | | **22·9 ± 7·6 (n = 8) | |

Values shown are means ± standard error of ratio.
* 1 : 4 iron–maltol 0·7 vs 70 μg iron dose $P < 0{\cdot}002$.
** 1 : 10 iron–maltol 0·7 vs 70 μg iron dose $P < 0{\cdot}02$.
Statistical analysis by Student's *t*-test.

carrier-mediated process (Bothwell et al 1979). It appears, therefore, that maltol in some way is able to enhance the high affinity, lower capacity transfer seen at the lower doses without increasing the uptake at the higher doses. The implications of this interpretation may be important to the way in which maltol has its effect. It means that maltol will not cause iron overload at high doses to any greater extent than other commercially available iron preparations but yet can be used more effectively at lower doses.

*Absorption of $^{59}$Fe from the stomach*

It has been shown in-vitro that at neutral pH the uncharged 1 : 3 iron–maltol complex is the predominant species, but that under acidic conditions 1 : 1 and 1 : 2 charged species may also be formed (Stefanovic et al 1968; Bakaj et al, unpublished observations). It was possible therefore that in the stomach, dissociation of ligand from metal could occur so releasing free maltol to diffuse away and leaving only the charged 1 : 1 and 1 : 2 species to pass into the duodenum. To avoid this possibility, in the initial experiments outlined above, the iron was given directly into the duodenum. However, when iron–maltol was given by tube directly into the stomach, considerable absorption of iron was detected and by 4 h there appeared to be no significant difference in total body uptake of $^{59}$Fe between stomach and duodenal administration (Table 1). Similar comparisons were made in pharmacokinetic experiments in which iron uptake was monitored by sampling blood at intervals from anaesthetized animals. Although the rise in radioactive iron levels in the blood seen after duodenal administration was somewhat delayed in the animals given iron into the stomach, nevertheless plateau levels of iron were no different and indeed were maintained longer, presumably due to a slow but steady delivery of iron–maltol from the reservoir in the stomach to the absorptive surfaces of the duodenum (Fig. 4). Only a small amount of $^{59}$Fe was absorbed into the blood

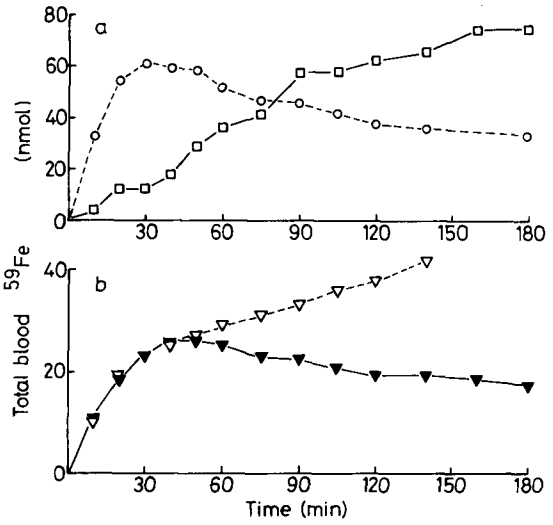


FIG. 4. Change in blood content of $^{59}$Fe in four anaesthetized rats after administration of 110 μg iron dose (a) or 11 μg iron dose (b) either into the stomach (□) or into the duodenum (○, ▼, ▽) over the course of 3 h. Values shown are in nmoles exogenous iron in total blood and are estimated from single blood samples.

from the stomach itself, i.e. when the pylorus was clipped off. Presumably some diffusion of the iron–ligand complex can take place across the stomach wall but this seems to be limited.

*Effect of excess ligand on $^{59}Fe$ absorption from iron–maltol given either into the stomach or into the duodenum*

To test whether in-vivo excess ligand, which should push the equilibrium in favour of the 1 : 3 complex, was necessary at low iron concentrations, uptake of $^{59}Fe$ given either into the duodenum or into the stomach was compared at metal–ligand ratios of 1 : 4 and 1 : 10. No differences were apparent in $^{59}Fe$ uptake either at the low dose of 7 μg iron or at the higher dose of 70 μg iron (Table 1). It is probable that in-vivo excess ligand can diffuse rapidly from the gut lumen so that, irrespective of the original metal–ligand ratio given, the same mixture of iron–maltol complexes remains behind for absorption. Indeed, results of in-vitro studies (Barrand et al 1986) to investigate the uptake of both metal and ligand into the intestinal wall suggest that maltol, when not bound to iron, quickly enters the tissues in a non-saturable manner.

*Absorption of $^{59}Fe$ complexed either to maltol, ethyl maltol or a 4 : 1 ethyl maltol/maltol mixture*

The effect of maltol on $^{59}Fe$ uptake from the stomach was compared with that of ethyl maltol to see whether $^{59}Fe$ might be taken in more readily with the more lipophilic pyrone. The partition coefficients (octanol/water) for the two iron complexes differ considerably i.e. 0·5 for iron(maltol)$_3$ and 4·2 for iron(ethyl maltol)$_3$ (Bakaj 1984). Solutions for injection into the stomach were prepared just before use since iron–ethyl maltol tended to aggregate, particularly at high concentration. Addition of a small amount of maltol to the ethyl maltol at a ratio of 4 : 1 (ethyl maltol–maltol) was able to reduce this aggregation substantially. No significant differences in $^{59}Fe$ uptake between the three iron–pyrone preparations were seen (Table 2), despite the greater lipophilicity of the iron–ethyl maltol complex (Bakaj 1984). The action of these pyrones on iron uptake may thus be somewhat more complicated than simple diffusion of the metal–ligand complexes across the intestinal brush border into the mucosal cells.

*Absorption of $^{59}Fe$ from iron–maltol in iron-deficient animals*

Uptake of $^{59}Fe$ from iron–maltol given into the stomach at a dose level of 7 μg was investigated in iron-deficient and in both age- and size-matched normal animals. Iron uptake from iron sulphate was comparable with that from iron–maltol in the iron-deficient animals. In the normal iron-replete animals, no differences in iron uptake between the size-matched and the age-matched groups were evident so data from these two groups were pooled. As expected, total body uptake of iron from iron–maltol 4 h after administration was considerably increased in the iron-deficient animals (21·8 ± 3·2% in normal animals, n = 9, compared with 64·2 ± 5·4% in iron-deficient animals, n = 8: $P < 0.001$). Significant differences were evident also at iron doses of 70 μg and 0·7 μg. Recent studies have shown that mucosal uptake is increased in iron-deficient animals whilst entry of iron into the mucosal storage pool is decreased (Nathanson et al 1985). It has been suggested that transferrin-mediated iron uptake in the mucosa may be responsible for this increase (Savin & Cook 1980; Huebers et al 1983; Osterloh et al 1985). Clearly maltol is able to donate its iron to whatever system is enhanced in the iron-deficient condition.

Table 2. Total body uptake of $^{59}Fe$ shown as percentage of dose administered given into the stomach in the presence of the following ligands.

| Dose of iron (μg) | Maltol | Ethyl maltol | Ethyl maltol/maltol 4 : 1 mixture |
|---|---|---|---|
| 0·7 | 35·0 ± 6·4 (n = 10) | 29·6 ± 4·9 (n = 10) | 39·7 ± 7·6 (n = 8) |
| 7·0 | 35·3 ± 5·9 (n = 10) | 34·3 ± 6·6 (n = 10) | 26·2 ± 5·2 (n = 11) |
| 70 | 20·3 ± 4·7 (n = 14) | 31·2 ± 5·1 (n = 10) | 26·5 ± 3·2 (n = 10) |

Values shown are means ± standard error of ratio.

*Pharmacokinetic studies*

Attempts were made to compare the kinetics of absorption of iron given as a pyrone complex, as iron sulphate or as iron–EDTA. For this purpose, elimination rate constants for the different iron preparations were first estimated.

*(i) After intravenous administration.* The rates of disappearance of $^{59}Fe$ from the blood after i.v. injection of a 110 μg iron dose complexed to maltol, ethyl maltol, NTA, EDTA or given as iron sulphate were estimated by monitoring blood iron levels over 80 min in anaesthetized animals. Although whole blood was taken, the rates of disappearance pre-

sumably relate to plasma iron since within the time scale studied, incorporation of $^{59}$Fe into red cells via erythropoiesis is not appreciable (Bothwell et al 1979). Little difference was found between the iron preparations in the rates at which $^{59}$Fe was cleared from the blood (half-life for iron after injection with ethyl maltol, NTA and sulphate being 133 ± 8 min, n = 14 and for iron after injection with maltol being 133 ± 10 min, n = 5). Values of a similar magnitude have been determined by others from data obtained over a 4 h period (Geisser & Müller 1984). No significant difference was evident in the half-life of iron in the blood of iron-deficient animals at the 110 μg iron dose level (138 ± 16 min, n = 4). Yet very much shorter than normal half-lives for iron in the plasma have been observed in iron deficiency both in dogs (Nathanson et al 1985) and in man (Bothwell et al 1979). The possibility was considered that with a 110 μg dose of iron, the amount of added iron may be large compared with the endogenous plasma iron levels and may approach the upper limits of total iron binding capacity in rat plasma, thus modifying the half-lives calculated. Indeed in subsequent experiments in which a 100-fold smaller concentration of added iron was used, even in the normal animals the half-life of $^{59}$Fe appeared to be shorter (74 ± 10 min, n = 4 at 1 μg iron compared with 133 ± 10 min, n = 5 at 110 μg iron dose level, $P < 0.01$). It was difficult to estimate the half-life in the iron-deficient animals at the lower 1 μg iron dose since blood concentration of $^{59}$Fe (expressed as the natural logarithm) was not linear with time over the course of 80 min but showed two distinct phases. If data from the first 40 min only were used, then a half-life of 44 ± 5 min, n = 4, could be calculated, this being significantly shorter ($P < 0.02$) than that estimated above in normal iron-replete animals.

*(ii) Tissue distribution after intravenous administration.* The distribution of $^{59}$Fe between tissues at the end of these pharmacokinetic experiments, i.e. 80 min after intravenous injection, was similar to that seen within the same time after intraduodenal administration (Fig. 2), except that a small amount could also be detected in the urine, the percentage appearing after 110 μg iron dose of iron–maltol being significantly more than that after a 1 μg iron dose (2·6 ± 1%, n = 4 after 110 μg iron compared with 0·3 ± 0·2%, n = 4 after 1 μg iron, $P < 0.005$). The highest urine levels were found after $^{59}$Fe–EDTA injection (13·5 ± 2%, n = 4 after 110 μg iron dose of iron–EDTA compared with 2·6 ± 1%, n = 4 with iron–maltol, $P < 0.002$). In contrast, the proportion of $^{59}$Fe found in the bone marrow after injection of $^{59}$Fe–maltol (11 ± 2%, n = 4) was significantly higher ($P < 0.02$) than that after $^{59}$Fe–EDTA (4·7 ± 0·5%, n = 4), indicating that EDTA probably delays the donation of iron to transferrin receptors in the marrow. A higher proportion of $^{59}$Fe was found in the bone marrow of iron-deficient animals after injection of iron–maltol at iron doses both of 1 μg (41·8 ± 3%, n = 5, in anaemic rats compared with 25·5 ± 3%, n = 4, in normal animals, $P < 0.005$) and of 110 μg (17 ± 0·5%, n = 4, in iron deficiency compared with 11 ± 2%, n = 4, in normals, $P < 0.01$).

*(iii) After intraduodenal administration.* The shapes of the curves obtained by monitoring blood levels of $^{59}$Fe after intraduodenal injection were variable and, irrespective of the nature of the iron preparation given, but seemed to depend on how quickly the iron was propelled along the small intestine, i.e. the slower the movement, the more prolonged the rise in blood level. Various studies both in-vivo (Huebers et al 1983) and in-vitro (Muir et al 1984) have shown that iron absorption occurs primarily in the upper part of the small intestine. Thus the longer the iron is retained in this portion of the gut, presumably the greater the absorption possible. Movement of iron from stomach to caecum was slowed in the anaesthetized animals compared with the conscious animals so that uptake of iron was maintained longer. Although other studies have used the areas under the curve for comparing absorption of iron from different iron preparations (Christensen et al 1984), it was not possible in this work to obtain kinetic constants of absorption owing to the variable shapes of the curves.

*Disposition of $^{59}$Fe in the plasma*

The mechanism of action of maltol is not yet clearly defined but the available evidence would suggest that maltol enhances only the initial step in iron uptake into the intestinal mucosal cells. Subsequent distribution of iron to the tissues was the same for iron–maltol as for the other iron preparations studied and also rates of elimination were identical. It seems that once through the intestinal wall, iron complexed to maltol must become transferred to the iron-carrying plasma protein, transferrin. To confirm this, gel filtration studies were carried out on plasma samples taken 10 to 30 min after duodenal administration of $^{59}$Fe complexed to maltol, ethyl maltol or EDTA and it could be shown that $^{59}$Fe eluted from the gel column at a peak corresponding

to that of transferrin. Even after intravenous injection, $^{59}Fe$ in plasma samples taken within 1 min eluted from a PD-10 column in the high molecular weight protein fraction and subsequent gel filtration on Sephacryl showed this $^{59}Fe$-bound protein to correspond in size to transferrin. It is known from in-vitro studies that iron may be transferred readily from maltol to transferrin with a half-life of around 2 min (Bakaj et al, unpublished observations). With iron–EDTA somewhat slower transfer was seen in-vivo, a proportion of $^{59}Fe$ remaining in a small molecular weight fraction of the plasma up to 1 h after injection (Fig. 5). This slow transfer, though

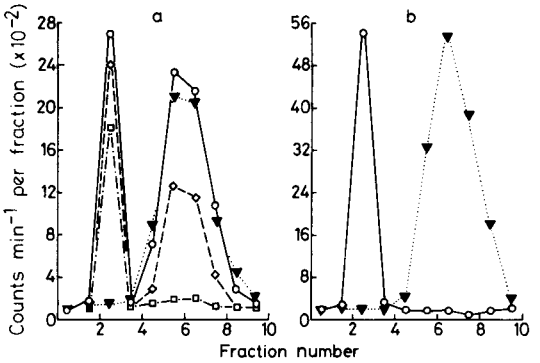


FIG. 5. Gel filtration profiles of plasma samples taken 1 (○), 10 (◇) and 60 (□) min after intravenous injection of 110 μg dose of $^{59}Fe$ given either as iron–EDTA (a) or as iron–maltol (b). 200 μL aliquots of plasma were applied to PD-10 columns containing Sephadex G-25M and 1 mL fractions eluted. Values shown are total counts $min^{-1}$ per fraction. Profiles of standard solutions (▼) both of $^{59}Fe$–EDTA and $^{59}Fe$–maltol are also given for reference.

considerably faster than that seen in-vitro (Bates et al 1967), probably accounts for the higher urinary excretion and lower bone marrow uptake of iron after intravenous administration of iron–EDTA described above.

In conclusion it seems that iron in the presence of the pyrones, maltol and ethyl maltol, is absorbed at low doses more readily from the small intestine than iron given in other currently available iron preparations. At high doses there is no greater uptake of iron than that seen with iron sulphate. These pyrones should therefore not lead to any greater danger of iron overload which might be expected if the normal mechanisms regulating absorption were to be bypassed. It is well recognized that iron balance is regulated primarily through modulation of uptake (Nathanson et al 1985). Indeed, the iron–pyrone complexes in-vivo appear to donate their iron rapidly to transferrin. The mechanisms by which these pyrones exert their enhancing action are not entirely clear but may relate to their ability to hold the iron within the gut lumen in a form available for absorption and possibly also to carry the iron into the intestinal epithelium. Simple diffusion of the metal–ligand complexes into the mucosal cells would not explain the saturation seen at the higher doses. Experiments on isolated intestinal fragments to probe the initial steps of uptake of these iron–pyrone complexes show that these ligands modify both the affinity and capacity of the mucosal uptake system for iron (Barrand et al 1986). Maltol and ethyl maltol have been used for some time as flavour enhancers in various foods and previous studies have shown them to be non-toxic (Gralla et al 1969; Rennhard 1971). Unlike most currently available iron preparations, iron co-ordinated to maltol is present in the ferric form. This may prove to be a valuable feature of the iron–pyrones since it is recognized that ferrous iron is able to enter a Fenton type reaction (Aisen 1977) which generates toxic free hydroxyl radicals leading to tissue damage (Slivka et al 1986). It seems that these pyrones may prove to be safe, palatable and non-irritant alternatives to those preparations currently available for the treatment of iron deficiency.

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