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Absorption of Iron from Iron Succinyl-protein Complexes by Mouse Small Intestine

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Abstract—The absorption of iron from iron succinyl-protein complexes was investigated in mice. ^{59}Fe-labelled succinyl-casein and -albumin complexes, [^{59}Fe]ferritin and $^{59}FeSO_4$, at doses of 20 or 200 μg of iron, were administered orally to normal mice or mice with absorption enhanced by chronic hypoxia. ^{59}Fe from iron succinyl-protein was well absorbed in normal mice (> 10% of dose) and showed enhanced absorption in hypoxic mice (> 40% of dose). Intestinal uptake was predominantly by the duodenum for all compounds studied. In-vivo absorption of ^{59}Fe from an iron succinyl-protein complex was studied using tied-off segments of mouse duodenum, jejunum or ileum of normal or hypoxic mice. Incubation for up to 15 min in duodenum or 60 min in ileum showed very little absorption of ^{59}Fe. No enhancement of absorption was seen in hypoxic mice. It was concluded that absorption of the intact iron succinyl-protein complex cannot explain absorption seen after oral dosing.

Iron deficiency is a widespread nutritional problem, even in advanced countries. Treatment is generally regarded as simple, oral iron sulphate being the prescription of choice. Frequent and severe, but difficult to predict, side effects (Fochi et al 1985) from this treatment suggest that less toxic alternative iron preparations will also play a role in future iron therapy. Iron succinyl-protein complexes represent a new, non-toxic, vehicle for oral administration of therapeutic iron (Cremonesi et al 1984; Pagella et al 1984). Clinical studies have shown that the compounds are well tolerated and the iron is bioavailable (Landucci & Frontespezi 1987; Danisi et al 1987). The mechanism by which iron is absorbed from these compounds is unknown, and we wished to investigate the absorption mechanism by identifying the small intestinal location of absorption and determining whether processing of the iron succinyl-protein complexes by the stomach was required for absorption. In this paper, iron absorption from two iron succinyl-protein complexes (succinyl-albumin, containing 10% iron (ITF241) and succinyl-casein, containing 5% iron (ITF281)), $FeSO_4$ and ferritin were compared in normal mice and in mice with absorption enhanced by chronic hypoxia.

Materials and Methods

Preparation of ^{59}Fe-labelled proteins, ferritin and $FeSO_4$

^{59}Fe-labelled iron succinyl-protein complexes were prepared using $^{59}FeCl_3$ (NEN Research Products, Stevenage, Herts, UK), $FeCl_3$ (BDH Chemicals, Poole, Dorset) and succinylated albumin or casein (Cremonesi et al 1984). [^{59}Fe]ferritin was prepared from rat liver as described by Pippard et al (1982).

Neutral aqueous solutions were used. The concentrations and specific activities of the ^{59}Fe-labelled proteins were; ferritin, 12·8 mM Fe, 7·4 nCi nmol^{-1}; iron succinyl-albumin, 16·8 mM Fe, 37·9 nCi nmol^{-1}; iron succinyl-casein, 13·2 mM Fe, 17·8 nCi nmol^{-1}.

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Animals and hypoxia

Mice were 25 g CD1-strain (Charles Rivers UK Ltd, Margate, Kent UK). Before experiments, the mice were fasted for 16 h and kept in wire-bottom cages. Mice were subjected to hypoxia (0·5 atmospheres) in a specially constructed chamber for 3 days to enhance iron absorption (Raja et al 1987a).

Relative rates and gastrointestinal location of mucosal uptake and absorption of ^{59}Fe-labelled ferritin, iron succinyl-albumin, iron succinyl-casein and $FeSO_4$

Groups of 8 mice were given (p.o.) 100 μL of labelled compound (1 μCi) containing either 20 or 200 μg Fe. After 1 h, mice were killed and the entire gastrointestinal tract removed and divided into stomach, duodenum (first 5 cm of small intestine), jejunum (next 10 cm), ileum (remainder, typically approximately 20 cm) and caecum plus colon and rectum. Contents were washed from duodenum, jejunum and ileum with 5, 10 and 15 mL of 0·15 M NaCl, respectively. Adherent food particles were removed from the mucosal surface with tissue paper. The tissues were counted for radioactivity together with the washings. Liver, kidney, spleen, femur and a blood sample were separately counted for ^{59}Fe as were the separate portions of gastrointestinal tract, washings and remainder of the carcass. The total blood activity was calculated by assuming a blood volume of 1·5 mL per mouse (Paxson & Smith 1968). Bone marrow activity was calculated by assuming that a femur contained 9·5% of the total bone marrow (Keene & Jandl 1965). Carcass activity was derived from the sum of the activities of kidney, liver, spleen, femur, blood and residual carcass activity.

In-vivo tied-off segments

Mice were anaesthetized with Hypnorm (fentanyl citrate, 0·315 mg mL^{-1}; fluanisone 10 mg mL^{-1} (Crown Chemical Co. Ltd, Lamberhurst, UK))/midazolam mixture (Raja et al 1987b) and the abdomen opened. A segment of intestine (1·5–3 cm) was located and isolated with surgical thread, care being taken to avoid damaging the blood supply. Duodenal segments were located immediately distal to the bile duct,

jejuno-ileal segments were 8 to 20 cm distal to the pylorus and distal ileal segments were within 4 cm of the ileo-caecal valve. Where appropriate, the lumen of the segment was gently washed with 0·5 mL of warm (37°C) 0·15 M NaCl. ^{59}Fe-succinyl-albumin (20 or 100 μg mL^{-1}) was injected so as to avoid damaging the intestinal wall of the segment (see Simpson & Peters (1986) and Raja et al (1987b) for further details). Hypoxia was induced as above.

Statistical analysis of data

Groups of data were tested for normality (Royston 1983) and where they differed significantly from normal, data were characterized by the median and interquartile limits. All data are presented as mean ± s.e.m. for simplicity. Comparison between groups was by *t*-test where valid (Royston 1983) otherwise Wilcoxon's rank sum test (Wetherill 1967) was employed.

Results

Absorption of orally administered ^{59}Fe-labelled compounds

^{59}Fe uptake and distribution of various iron compounds in normal and hypoxic mice at two Fe doses (20 and 200 μg) were studied. In most groups, there was evidence (see Fig. 1 for 20 μg dose data) of a gradient of uptake going down the small intestine (200 μg data were similar). Comparison of duodenum vs ileum uptake reached significance ($P < 0.05$) for iron succinyl-albumin (both hypoxic groups), iron succinyl-casein (both hypoxic groups and 200 μg normal), $FeSO_4$ (both normal groups and 200 μg hypoxic) and ferritin (200 μg normal group). This gradient is even steeper if the data are expressed relative to the length of the segments. The gradient cannot be explained by failure of ^{59}Fe to reach the ileum as at least 60% of the dose had left the stomach and passed the duodenum in all groups of mice in the 1 h incubation period. In the hypoxic animals receiving ferritin, however, there was evidence for greater uptake by ileum than duodenum (hypoxic 20 μg, $P < 0.001$).

Little evidence for any difference in uptake or distribution between iron succinyl-albumin and iron succinyl-casein was observed, with only a single comparison showing a significant difference between the compounds, namely, the ileal uptake in the normal 200 μg group (succinyl-casein > succinyl-albumin, $P = 0.01$). Fig. 2 shows tissue distribution data for mice dosed with each of the two iron succinyl-protein compounds. The tissue distribution of the ^{59}Fe absorbed from iron succinyl-albumin and iron succinyl-casein showed little activity in the kidney with substantial incorporation into the liver, blood and bone marrow, particularly in the hypoxic animals (Fig. 2).

Comparison of normal with hypoxic carcass uptake shows significant increases with hypoxia in the low dose groups for iron succinyl-albumin and iron succinyl-casein ($P < 0.005$) (Fig. 3). The increase at the higher iron dose approaches significance and all tissue uptakes are significantly increased with iron succinyl-albumin ($P \leq 0.002$). There was evidence for decreased carcass uptake (absorption) with increased dose with both $FeSO_4$ and iron succinyl-albumin; however, no comparison reached significance at the 5% level.

Stomach emptying of $FeSO_4$ appeared to be faster than the emptying of other compounds (Fig. 4). Comparing iron succinyl-albumin with $FeSO_4$ showed a significant difference for both hypoxic groups ($P < 0.002$) though the change in normal groups was not significant. Duodenal lumenal concentrations of $FeSO_4$ were significantly reduced by hypoxia at the lower Fe dose ($P < 0.05$), suggesting a more rapid rate of duodenal transit of $FeSO_4$ in hypoxia. Similar data were obtained with the 200 μg dose (not shown).

There was a broadly similar level of uptake by the ileum in all groups of mice, irrespective of dose, compound or

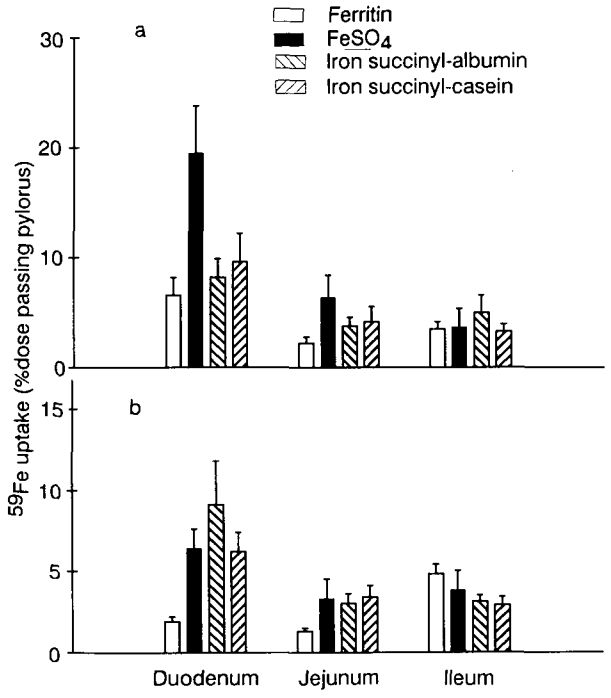

FIG. 1. ^{59}Fe distribution after oral dosing with 20 μg ^{59}Fe-labelled-ferritin, -$FeSO_4$, -succinyl-albumin or -succinyl-casein. Uptake of ^{59}Fe into duodenal, jejunal and ileal walls 1 h after oral dosing to normal (a) or hypoxic (b) mice with ferritin, $FeSO_4$, iron succinyl-albumin and iron succinyl-casein. Data are means ± s.e.m. for n = 8.

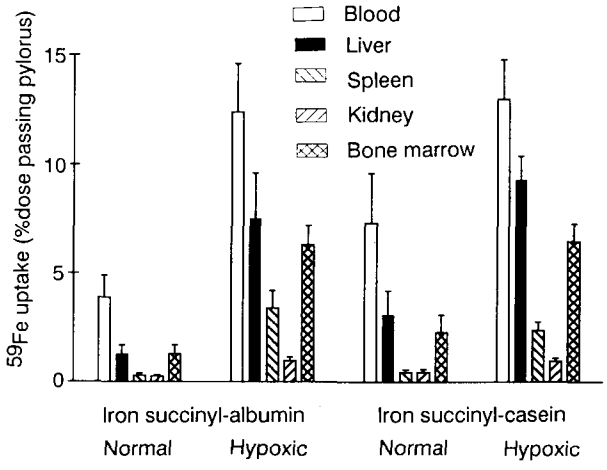

FIG. 2. ^{59}Fe distribution after oral dosing with 20 μg ^{59}Fe-labelled-ferritin, -$FeSO_4$, -succinyl-albumin or -succinyl-casein. Uptake of ^{59}Fe into blood, liver, spleen, kidney and bone marrow after oral dosing to normal or 3-day hypoxic mice. Data are means ± s.e.m. for n = 8.

Table 1. Uptake and absorption of iron succinyl-albumin complex by tied-off intestinal segments.

Experiment	Time (min)	Gut	Transfer	Mucosal uptake (pmol (mg gut)$^{-1}$)
(a) Fe concentration = 20 μg mL^{-1}				
Normal, duodenum	5	2·2±4·3	1·8±0·9	4·0±1·0
Normal, duodenum	10	3·0±0·6	2·5±0·7	5·5±0·9
Normal, duodenum	15	2·9±0·5	2·9±0·7	5·8±1·2
Normal, duodenum, prewashed	5	2·5±0·7	2·0±0·4	4·5±1·1
Normal, duodenum, prewashed	10	1·9±0·2	2·5±0·6	4·4±0·8
Normal, duodenum, prewashed	15	4·8±0·9	2·7±0·8	7·5±1·3
Hypoxic, duodenum	10	1·4±0·3	3·0±0·2	4·4±0·2
Hypoxic, duodenum, prewashed	10	3·7±1·4	1·7±0·4	5·4±1·6
Normal, jejuno-ileum prewashed	15	3·3±1·3	1·2±0·3	4·5±1·4
Normal, distal ileum prewashed	15	9·0±2·9	2·1±0·8	11·0±3·6
(b) Fe concentration = 100 μg mL^{-1}				
Normal, duodenum	10	10·8±2·9	11·7±2·5	22·5±5·3
Normal, duodenum, prewashed	10	16·8±3·8	5·4±1·5	22·3±3·3
Normal jejuno-ileum	60	24·1±7·9	2·7±0·5	26·8±8·3
Normal, distal ileum	60	23·0±10	6·1±2·4	29·1±8·9
Hypoxic, jejuno-ileum	60	32·8±10	1·9±0·6	34·7±10·4

Tied-off segments were prepared as described in the methods section and uptake of ^{59}Fe into the gut wall (Gut) and carcass (Transfer) determined. Mucosal uptake is the sum of gut plus carcass uptakes. Data are means±s.e.m. for n=6.

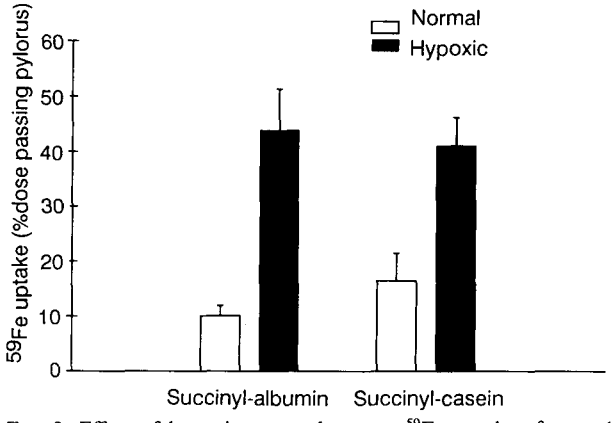

FIG. 3. Effect of hypoxia on total carcass ^{59}Fe uptake after oral dosing with 20 μg iron succinyl-protein complexes. Open bars, normal mice; closed bars, 3 day hypoxic mice. Data are means ±s.e.m. for n=8.

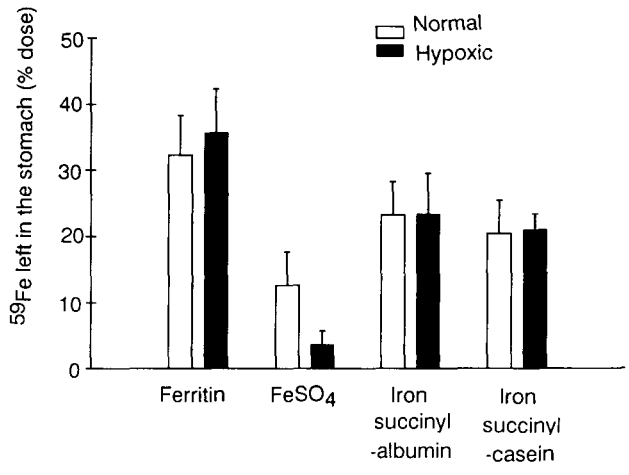

FIG. 4. Emptying of iron compounds from mouse stomach. ^{59}Fe left in stomachs of normal or 3 day hypoxic mice 1 h after dosing with 20 μg of various iron compounds. Data are means±s.e.m. for n=8.

hypoxia. This uptake could be significant in the absorption of the compounds, but only if the ileum can transfer this ^{59}Fe to the circulation at a significant rate. The fact that iron from succinyl-casein was taken up by ileum to a greater extent than from succinyl-albumin, yet actual absorption (carcass uptake) of the compounds was similar, suggests that ileum may not be the site of absorption of the compounds. The data from these experiments support a primarily duodenal location for uptake of iron from succinyl-albumin and succinyl-casein.

Absorption of ^{59}Fe-succinyl-protein complexes by tied-off intestinal segments

Table 1 shows iron absorption by tied-off segments of mouse intestine. The effects of incubation time, prewashing, location in the small intestine, concentration of Fe and hypoxia on absorption are shown.

Duodenal segments

It was found that no significant increase in ^{59}Fe retained in the gut wall, ^{59}Fe transferred to the carcass or total mucosal uptake occurred on increasing the incubation time from 5 to 15 min in non-washed duodenum ($P>0·25$). Experiments were also performed in which contents of the small intestine were removed by prewashing before injection of ^{59}Fe. These experiments were intended to show the influence (if any) of endogenous luminal ligands on the absorption of iron succinyl-albumin. In prewashed intestine, the increase in gut and total mucosal uptake on increasing incubation time from 5 to 15 min was not significant ($P>0·05$); no change in ^{59}Fe transfer occurred ($P>0·4$).

No significant increases in absorption occurred in hypoxic animals (prewashed or not) ($P>0·3$). There was a significant decrease in gut uptake by non-washed intestine ($P<0·05$). Prewashing had no significant effect on any parameter of absorption in normal mice, when compared with non-washed segments. In hypoxic mice, prewashing had no significant effect on gut or total mucosal uptake ($P>0·1$); however, transfer was significantly decreased ($P<0·02$). This

decrease was offset by the tendency of gut uptake to increase so that total mucosal uptake showed a tendency to increase.

Increasing the concentration of iron succinyl-albumin by five-fold resulted in approximately five-fold increases in most parameters. At this higher concentration, prewashing tended to reduce ^{59}Fe transfer to the carcass ($P \simeq 0{\cdot}05$), however, once again this was balanced by an increase in gut retention so that no change in total uptake occurred.

Jejunal/ileal segments

Absorption parameters showed no significant change in more distal regions of the small intestine (prewashed) ($P > 0{\cdot}1$) at 20 $\mu g\ mL^{-1}$ Fe for 15 min incubation. Longer incubations (60 min) with higher concentrations of iron succinyl-albumin (100 $\mu g\ mL^{-1}$) may reflect more closely the conditions of the oral dosing experiments described above. In these conditions, transfer to the carcass is very low (Table 1b), lower than is observed in the duodenum at only 15 min incubation. The difference is significant when comparing jejuno-ileal segments with duodenal segments ($P < 0{\cdot}01$). No increase in uptake or transfer was observed in hypoxic animals ($P > 0{\cdot}2$). No difference between jejuno-ileal and distal ileal segments was observed ($P > 0{\cdot}5$).

Discussion

The data show that iron is well absorbed from both ferritin and the iron succinyl-protein complexes after oral administration to mice. The two iron succinyl-protein compounds demonstrate similar absorption behaviour despite structural differences (i.e. succinyl-albumin compared with succinyl-casein). This suggests that a similar absorption mechanism may operate for both.

The iron succinyl-albumin is poorly taken up when injected intact into tied-off segments of duodenum. In order to make a rough comparison, an uptake of 5 pmol mg^{-1} of intestine represents approximately 3% of the dose; thus after 10 min a duodenal segment has transferred approx. 2% of the luminal dose to the carcass in hypoxic mice while more than 40% of the oral dose reaches the carcass in such mice after oral dosing.

The comparatively slow rate of uptake of iron from the iron succinyl-protein compounds resembles that seen in mice when iron is given bound to lactoferrin or transferrin (Simpson et al 1986) and in rats when iron is given as ferritin (Huebers et al 1983) or iron hydroxide (Bernier et al 1986). This slow uptake may represent uptake by endocytic processes, involving apical pits, as has been observed with polymerized iron (Deutschlander et al 1975) and haemoglobin (Parmley et al 1981). Another possibility is non-specific adsorption to the mucosal surface. The absence of an uptake time course in tied-off segments and the presence of this uptake in all regions of the intestine, support this latter possibility. The absorption of iron from the complexes following oral administration, seems to be primarily duodenal. Several studies suggest that only proximal intestine has a significant capacity to transfer iron to the circulation (Johnson et al 1983; Simpson & Peters 1986).

This finding, that iron from the compounds is available for absorption after oral but not intraluminal administration, resembles observations made on the absorption of iron from polymeric iron species in rats (Terato et al 1973; Bernier et al 1985, 1986). The suggestion can be made that either iron is dissociated from the iron succinyl-protein compounds by the stomach, or structural changes to the compounds occur during digestion in the stomach which render them suitable for absorption by the intestine.

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