Biochimica et Biophysica Acta 901 (1987) 52–60
Elsevier

BBA 73628

Comparison of $^{59}Fe^{3+}$ uptake in vitro and in vivo by mouse duodenum

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(Received 16 December 1986)

Key words: Iron absorption; Hypoxia; (Mouse duodenum)

Initial rates of $^{59}Fe^{3+}$ uptake by mouse duodenal fragments (in vitro) and tied-off duodenal segments (in vivo) have been characterised for control and hypoxic animals. $^{59}Fe^{3+}$ uptake by duodenal fragments was rapid, selective and dependent on medium Fe^{3+}-nitrilotriacetate concentration. Most of the $^{59}Fe^{3+}$ uptake (70–75%) occurred via the mucosal route and was dependent on the metabolic state of the tissue. Mucosal uptake showed an adaptive increase following exposure of animals to 3 days hypoxia; the enhancement was due to a 2–3-fold increase in V_{max}^{app}, without any significant changes in the K_m^{app}. Studies of upper small intestine transit times showed a mean residence time of 4–5 min for ^{59}Fe-labelled mouse chow, emphasising the importance of initial uptake measurements. Time courses for in vivo total mucosal uptake exhibited linearity over a wide variety of absorption rates after correction for the permeation by intact metal-chelate complex. The corrected uptake showed a hyperbolic dependence on medium Fe^{3+}-nitrilotriacetate concentration. Kinetic studies revealed a 2–3-fold increase in total mucosal uptake in hypoxia. Mucosa-to-carcass transfer of ^{59}Fe was also markedly increased by chronic hypoxia. The in vitro system exhibits similar qualitative and quantitative kinetics for Fe^{3+} transport via the mucosal membrane to those obtained in vivo. The results observed in vitro are thus valid and provide a convenient method for further studies on Fe^{3+} transport in animals and in man.

Introduction

Iron homeostasis is known to be controlled primarily by the regulation of the rate of intestinal absorption. The absorptive mechanism therefore adapts itself to the increasing iron requirements. Despite intense research, the mechanism of iron absorption and the regulation of this process remains largely obscure. Previous work [1–3] has demonstrated at least two kinetically distinct steps in the movement of iron across the intestinal mucosa: (i) uptake across the luminal brush border membrane of the enterocyte, and (ii) transfer of a portion of this absorbed iron to the plasma. Both steps appear to be subject to adaptive regulation [3].

Cox and Peters [4] have devised an in vitro method for determining the unidirectional uptake of ferric iron by intestinal tissue from humans. At present, this technique is the only suitable method available for determining the kinetic parameters of initial uptake of iron by human intestinal tissue, and is therefore potentially of great importance [5].

The present study was carried out to compare, qualitatively and quantitatively, the initial uptake of Fe^{3+} by intestinal fragments with in vivo measurements of Fe^{3+} influx across the brush border using tied-off segments of mouse intestine. A similar comparison was also performed in animals in

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which iron absorption had been enhanced by hypoxic exposure [6], and provides a validation of the in vitro method for studies in man.

Materials and Methods

Reagents. Tris was purchased from Boehringer Mannheim (Lewes, U.K.). Other biochemical reagents were from either BDH Chemicals (Poole, U.K.; Analar grade) or Sigma Co. Ltd. (Poole, U.K.). Radiochemicals were from Amersham International (Amersham, U.K.).

Animals. Male mice, To strain, 6–8 weeks of age, were fed a standard rodent diet (K and K, Greeff Chemicals Ltd., Croydon, U.K.). The iron content of the diet was 3.6 mmol/kg.

Hypoxia. Mice were subjected to a simulated altitude of 5000 m by placing them in a hypobaric chamber maintained at 53.5 kPa (0.5 atm) for 3 days. The animals received food and water ad libitum during this period, except during the day of the experiment, when the food was removed.

In vitro iron uptake studies. The initial rates of Fe^{3+} uptake by mouse duodenal fragments were determined as reported previously [4,6]. The method satisfies the criteria set by Sallee et al. [7] for the valid measurement of rates of unidirectional uptake. The animals were killed by cervical dislocation and pieces of duodenum (up to 5 cm from the pylorus) removed. The pieces were then sectioned into fragments (2–10 mg), rinsed in oxygenated physiological medium (125 mM NaCl, 3.5 mM KCl, 10 mM D-glucose, 16 mM Na-HEPES (pH 7.4)) and incubated at 37°C in the same buffer containing ^{59}Fe (spec. act. 6.1–41.4 MBq/μmol Fe) as a ferric chelate of nitrilotriacetate (Fe^{3+}/chelate 1:2). [^{57}Co]Cyanocobalamin (spec. act. 0.5–1.0 kBq/pmol), 5 nmol/l, was used as a non-absorbed extracellular fluid marker [4,8].

The incubation was terminated by blotting the tissue and rinsing in 1 ml ice-cold buffer on a Vortex mixer. After reblotting and weighing, the radioactivity was measured in the tissue and in an aliquot of the medium with a twin-channel γ-counter (LKB Wallac 1280, Helsinki, Finland). ^{57}Co and ^{59}Fe were determined separately by channel ratio analysis. After correction for the adherent medium (extracellular fluid space), uptakes were expressed as pmol/min per mg wet weight. The apparent kinetic parameters for Fe^{3+} influx, K_m^{app} and V_{max}^{app}, were calculated from concentration-velocity studies by the direct linear plot of Eisenthal and Cornish-Bowden [9]. In some experiments, right-side-out tied-off duodenal segments were prepared in situ in anaesthetised animals, then rapidly removed, rinsed and transferred to the $^{59}Fe^{3+}$-containing medium. Following incubation for 5 min, the segments were removed, rinsed in buffer, and blotted in a similar fashion to the fragments. After removal of the cut ends and any adjoining connective or pancreatic tissue, the duodenal segments were blotted, weighed and counted for $^{59}Fe/^{57}Co$. The uptakes were corrected for the extracellular fluid space and expressed as pmol/min per mg tissue.

In vivo $^{59}Fe^{3+}$ absorption studies. Mice were anaesthetised intraperitoneally with Hypnorm (fentanyl citrate, 0.315 mg/ml; fluanisone 10 mg/ml, Crown Chemical Co. Ltd., Lamberhurst, U.K.)/midazolam. The abdomen was then opened and a 2–3 cm segment of duodenum located immediately distal to the bile duct. The distal end of the duodenum was ligated, care being taken to maintain the integrity of the blood supply. A small incision was made above the ligature and sterile 0.15 M NaCl (prewarmed to 37°C) was pumped through the loop (via the proximal end) at a slow rate. A ligature was tied proximal to the incision following the intraluminal administration of a small volume (50–100 μl) of the $^{59}Fe^{3+}$-containing medium. The medium was similar to that used in the in vitro studies except that cyanocobalamin was omitted. For ^{51}Cr-EDTA studies, the solution contained 100 μM ^{51}Cr (0.05 μCi/nmol) and 400 μM EDTA. In some experiments, the prewashing of duodenal segments with 0.15 M NaCl, before $^{59}Fe^{3+}$ incubation, was omitted.

After incubation for up to 10 min, the animal was killed by cervical dislocation and the duodenal segment was removed, flushed with wash medium, opened, blotted on both surfaces, and any connective, fatty or pancreatic tissue removed. The segment was weighed and counted in a γ-counter (LKB Wallac 1280, Helsinki, Finland). The carcass was similarly counted for 5 min in a high-resolution bulk sample counter [10]. The activity of ^{59}Fe present in either the tissue or carcass was expressed as pmol/mg wet weight intestine. The wet

weight per unit length of duodenum (mean ± S.E. (n)) was similar in controls and mice exposed to 3 days hypoxia (controls, 31.2 ± 1.1 (25) mg tissue cm^{-1}; hypoxic, 36.5 ± 3.6 (10)). The tissue radioactivity is a measure of the duodenal retention (referred to as 'mucosal retention') of $^{59}Fe^{3+}$. The radioactivity in the carcass gives a measure of the 'transfer' of ^{59}Fe from the absorbing cells to the plasma. The sum of the mucosal 'retention' and 'transfer' to the carcass gives a measure of the 'total mucosal uptake' of $^{59}Fe^{3+}$.

The wash (flush) procedure referred to above was optimised by varying the volume of sterile 0.15 M NaCl. Experiments using 250 μM Fe^{3+} and 500 μM nitrilotriacetate showed that the mucosal 'retention' and 'transfer' of $^{59}Fe^{3+}$ was essentially independent of the wash volume in the range 10–30 ml. No additional radioactivity was recovered in the washes after flushing the duodenal segment with 20 ml 0.15 M NaCl containing 100 μM Fe and 200 μM nitrilotriacetate.

Assay of tissue nucleotide levels in mouse duodenum. Mouse duodenal fragments were frozen in liquid nitrogen and ground in a pre-cooled pestle and mortar. The powdered tissue was thawed in approximately 2–3 vol. of 30% ice-cold perchloric acid. The extracts were centrifuged in an Eppendorf 5412 Microfuge and the supernatants were neutralized with potassium carbonate (phenol red). The neutralized extracts were centrifuged as above and the supernatants were stored frozen until nucleotide determination. Nucleotides (i.e., ATP, ADP and AMP) were determined enzymatically essentially as described by Beutler [11]. In some experiments, duodenal tissue was removed from mice under Hypnorm/midazolam anaesthesia, and frozen immediately in liquid nitrogen. Experiments were also performed in which mouse duodenum was freeze-clamped in situ [12] with aluminium tongs precooled in liquid nitrogen.

Measurement of gastrointestinal transit times for ^{59}Fe-labelled diet. Rodent diet (20 g) was labelled by hydrating with 40 g water, adding 10 μCi $^{59}FeCl_3$, mixing thoroughly, then drying overnight at 60 °C. Mice were starved for 6–10 h before feeding 250 mg of ^{59}Fe-labelled diet, which was consumed within 10 min. After 60 min, the mice were anaesthetised with Hypnorm/midazolam, and double ligatures were tied immediately distal, 5 cm distal and 10 cm distal to the pylorus. The digestive tract was removed and divided into stomach, duodenum (first 5 cm), upper jejunum (next 5 cm), ileum (remainder of the small intestine) and caecum. The tied-off duodenal and jejunal segments were opened and flushed with 10 ml 0.15 M NaCl. The collected washings, the duodenum, jejunum and other portions (including contents) of the digestive tract, and the carcass were counted for ^{59}Fe. 1 h post-feeding data were used to calculate transit times as 47.8 ± 5.3% (mean ± S.E., $n = 5$) of the diet passed through the duodenum and upper jejunum within this period. Experiments in which the digestion time was varied showed that a steady-state level of diet was maintained in the upper small intestine over 20–120 min, whilst the total quantity of diet which had passed the proximal intestine increased linearly.

Dietary residence time (min) was calculated from 1/[^{59}Fe passed through the gut segments] × [^{59}Fe in gut contents] × 60. Radioactivity in the carcass was 5.34 ± 1.43% (mean ± S.E., $n = 5$) of the total counts administered. Uptake of ^{59}Fe by gut was also a relatively small proportion of the total counts.

Results

Tissue viability

Electron microscopy revealed no morphological changes following incubation of the duodenal fragments for up to 15 min in an iron-containing medium (Fig. 1).

Assay of tissue nucleotide levels showed that the removal of the duodenum from the intact animal leads to a rapid fall in the ATP/AMP ratio and the energy charge (Table I). A further significant drop in both the ratio and the energy charge occurred during the preparation of duodenal fragments. However, the metabolic viability of the tissue was apparent by the recovery in the energy charge after incubation of the tissue in a physiological medium for 5 min. No further changes were observed when the fragments were incubated for a longer period (15 min). Fragments that were freeze-clamped after 5 min incubation showed no significant difference in the energy charge as compared to those plunged into liquid

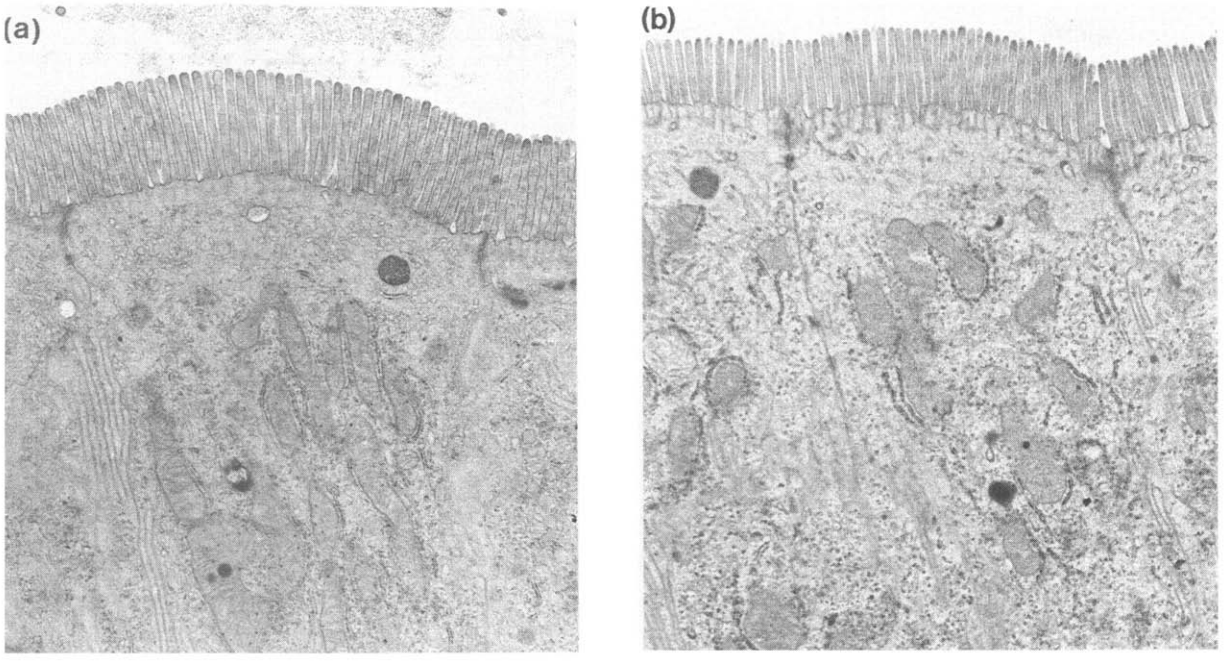

Fig. 1. Electron micrographs showing the apical region of mouse duodenal mucosa. (a) Control tissue. (b) Tissue incubated for 15 min in iron-containing physiological medium. Total magnification: (a) 13200, (b) 9895.

nitrogen. The inclusion of inhibitors of glycolysis (NaF) or oxidative phosphorylation (dinitrophenol) resulted in a small decrease in energy charge. The decrease was much greater when both inhibitors were present. These results suggest that the tissue can compensate for the loss of one or other energy-generating pathway.

In vitro Fe^{3+} uptake studies

Fig. 2 shows the effect of varying the rinse time for tissue samples, on the amount of radioactivity associated with the duodenal fragments. An appreciable amount of both ^{59}Fe and ^{57}Co was associated with the fragments following incubation in the physiological medium. After a short rinse (1–3 s), there is a decline in tissue radioactivity. Further washings (3–7 s) had little additional effect. Prolonged washing (over 7 s) causes a further loss in the tissue-associated ^{59}Fe. This may be due to cellular breakdown. The amount of ^{57}Co associated with the duodenal fragments was identical in the control and hypoxic groups. A rinse period of 3 s has thus been used routinely in all experiments. The amount of ^{59}Fe in the mucosal scrapings was $73.7 \pm 3.2\%$ (mean $\pm$ S.E., $n = 5$) of the total activity in the duodenal fragments. There was a close correlation between uptake and tissue wet weight for control and hypoxic tissue ($r =$

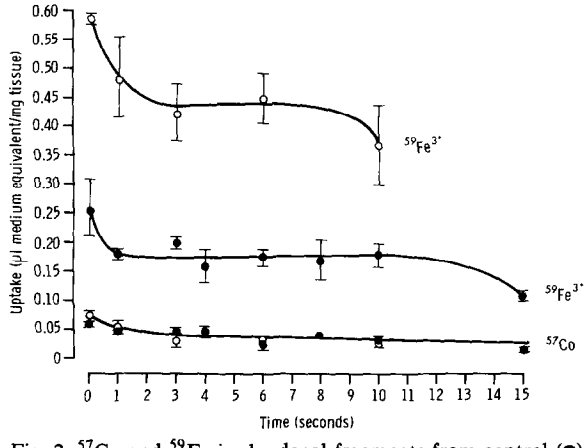

Fig. 2. ^{57}Co and ^{59}Fe in duodenal fragments from control (●) and hypoxic (○) animals. Uptakes were performed as in the methods section and the rinse time varied. Incubation time = 5 min. Medium concentrations were: $Fe^{3+} = 250$ μmol/l, nitrilotriacetate = 500 μmol/l, and cyanocobalamin = 5 nmol/l. Results show means ± S.E. for three experiments with several fragments used for each experiment.

TABLE I

NUCLEOTIDE LEVELS AND ENERGY CHARGE FOR DUODENAL SAMPLES

Preparation	*n*	ATP/AMP ratio	Energy charge **
Freeze-clamped in situ	4	14.0±0.8	0.90±0.01
Segments frozen immediately after removal	5	6.0±0.8 [b]	0.80±0.02 [a]
Fragments prepared from tissue removed from animal	7 *	1.9±0.4 [d]	0.59±0.05 [c]
Fragments incubated for 5 min	5 *	5.8±0.4	0.82±0.01
Fragments incubated for 15 min	5 *	6.2±0.7	0.83±0.02
Fragments incubated for 5 min in NaF-containing medium	3 *	5.2±1.5	0.79±0.05
Fragments incubated for 5 min in DNP-containing medium	3 *	5.1±1.0	0.79±0.04
Fragments incubated for 5 min in medium containing NaF plus DNP	3 *	3.0±0.6 [f]	0.73±0.04 [e]

* Mice sacrificed via cervical dislocation. Perchloric extracts were prepared and assayed for nucleotide levels essentially as in Ref. 11. Except where indicated, tissue samples were plunged into liquid nitrogen. The physiological media used were iron-free, and aerated continuously with 95% O_2/5% CO_2 at 37°C. Concentrations of NaF and dinitrophenol (DNP) in assay medium were 10 and 0.1 mM, respectively.

** Energy charge [18] = 1/2(([ADP] + 2[ATP])/([ATP] + [ADP] + [AMP])).

Significant differences by Student's '*t*' test: [a] $P < 0.01$; [b] $P < 0.001$ compared to freeze-clamped duodenal segments. [c] $P < 0.01$; [d] $P < 0.001$ compared to segments frozen immediately after removal. [e] $P < 0.05$; [f] $P < 0.001$ compared to fragments incubated for 5 min in physiological medium.

+0.91, +0.93, respectively). The intercept did not differ significantly from zero.

Our previous studies [6] have shown that the initial uptake process is rapid and involves the transfer of iron from the chelate at the cell surface whilst the intact chelate is excluded from cell entry. This observation was based on studies of the uptake of $^{59}Fe^{3+}$, [^{14}C]nitrilotriacetate, [^{57}Co]cyanocobalamin and ^{51}Cr-EDTA by mucosal fragments. The uptake of ^{51}Cr-EDTA was very

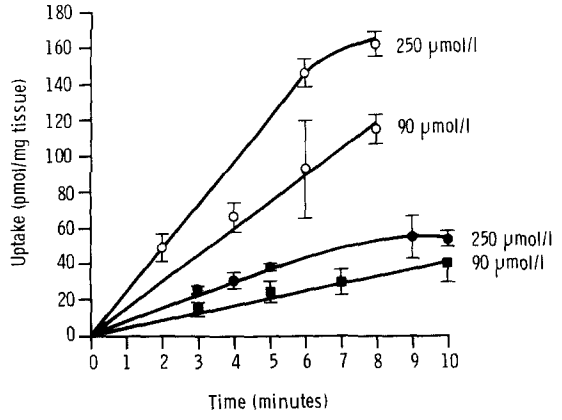

Fig. 3. Time course for $^{59}Fe^{3+}$ uptake by duodenal fragments from control (closed symbols) and hypoxic (open circles) animals at two different medium iron concentrations. The iron in the medium ws presented as a ferric chelate of nitrilotriacetic acid (Fe^{3+}/chelate = 1:2). Results are means ± S.E. for up to three experiments with several fragments used for each experiment.

similar to that of [^{57}Co]cyanocobalamin. Furthermore, the uptake by fragments from control and hypoxic animals is linear over short incubations (up to 5 min, Fig. 3) at all iron concentrations used, and demonstrates saturation kinetics with varying medium Fe^{3+}-nitrilotriacetate concentrations [6]. Uptake by the serosal surface of tied-off duodenal segments have shown that about 25–30% of the in vitro uptake by fragments could be accounted for by non-mucosal tissue [6].

The uptake of $^{59}Fe^{3+}$ by duodenal fragments shows an adaptive increase following exposure of animals to 3 days hypoxia (Figs. 2 and 3) and is accounted for by a 2–3-fold change in the V_{max}^{app}, with no significant alterations in the K_m^{app} [6]. The serosal uptake does not contribute to the enhanced rate of uptake of $^{59}Fe^{3+}$ by fragments from hypoxic animals (K_m^{app} and V_{max}^{app} values for serosal uptake: controls, 124 μmol/l and 3.2 pmol/mg per min; hypoxic, 97.6 μmol/l and 4.1 pmol/mg per min, respectively).

In vivo studies

In vivo experiments with tied-off duodenal segments were performed to confirm that the rate of Fe^{3+} uptake by tissue fragments gives a measure of Fe^{3+} influx across the brush border of the

duodenal mucosa. In experiments in which the effect of varying the volume of 0.15 M NaCl used to pre-wash the loop (before $^{59}Fe^{3+}$ incubation) on the rate of $^{59}Fe^{3+}$ absorption was investigated, no significant differences were observed between the various volumes used; therefore, 0.6 ml was used subsequently in all experiments. Light microscopy demonstrated no morphological changes in the pre-washed duodenum as compared to unwashed tissue.

The mean residence times for the ^{59}Fe-labelled diet in the duodenum and proximal jejunum of normal mice were 0.14 ± 0.06 and 0.58 ± 0.18 min/cm (mean $\pm$ S.E., $n = 5$), respectively. The mean total residence time for the proximal third of the small intestine was 4.37 ± 1.33 min ($n = 5$). As the diet resides only for a short period in the proximal small intestine, the early time courses for $^{59}Fe^{3+}$ 'uptake' and 'transfer' must be investigated in order to obtain kinetically useful information.

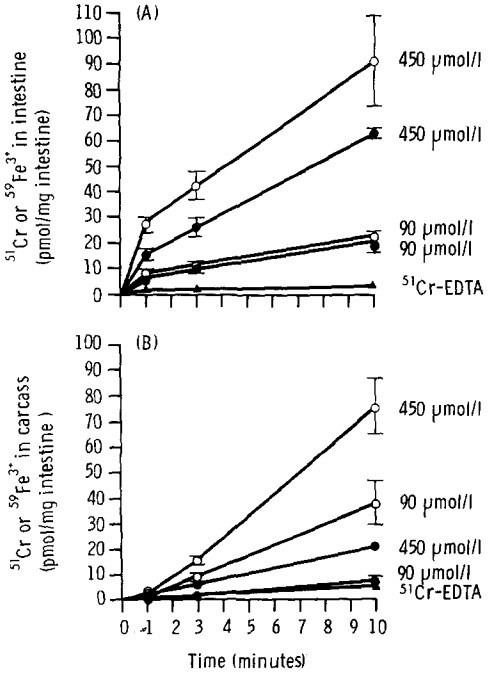

Fig. 4. Uptake into the intestinal mucosa (A) and transfer to the carcass (B) of $^{59}Fe^{3+}$ and ^{51}Cr-EDTA by tied-off mouse duodenal segments. Results are means $\pm$ S.E. for two to seven experiments. Fe^{3+} uptakes were performed at two medium iron concentrations with segments from control (●) and hypoxic (○) mice. Uptake of ^{51}Cr-EDTA (100 µmol/l) by control animals (▲).

Fig. 4 shows the time courses, at two different concentrations of iron, for the amount of radioactivity found in the intestine (duodenum) and carcass. Mucosal $^{59}Fe^{3+}$ levels (Fig. 4A) approach steady-state levels following an initial rapid uptake phase. Segments prepared from animals exposed to 3 days hypoxia showed increased amounts of radioactivity in the intestine; this increase was more marked at the higher medium iron concentration. Conversely, the time courses for the 'transfer' of radioactivity to the carcass showed a distinct lag (Fig. 4B). Hypoxic exposure resulted in a marked increase in the amount of $^{59}Fe^{3+}$ transferred to the body following a 10 min intraluminal-incubation with $^{59}Fe^{3+}$-containing medium. When the amount of radioactivity in the intestine and carcass were summed, good linearity was apparent for the total mucosal uptake of $^{59}Fe^{3+}$. These observations suggest that 'uptake' and 'transfer' are sequential steps in the $^{59}Fe^{3+}$ absorption pathway.

It is noteworthy, however, that there is considerable permeation (up to 25% at the lower rates) of the whole-metal complex which occurs by an unknown pathway [13] and is suitably demonstrated by the use of ^{51}Cr-EDTA, a stable-metal complex of low molecular weight. ^{51}Cr-EDTA uptake showed first-order dependence on medium Cr-EDTA for concentrations up to 1 mM, consistent with non-facilitated uptake. Hypoxic ex-

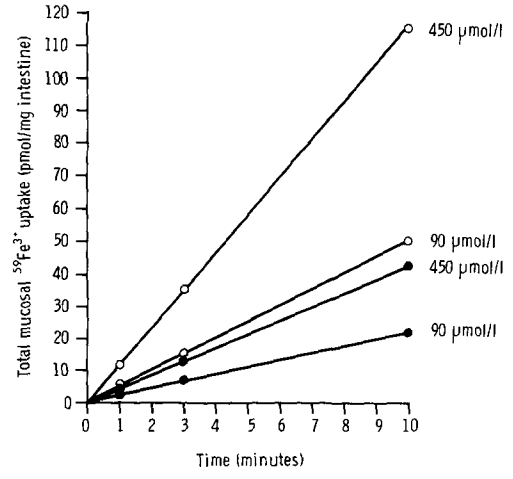

Fig. 5. Total mucosal uptake of $^{59}Fe^{3+}$ corrected for the permeation of whole metal complex using ^{51}Cr-EDTA. Symbols as in Fig. 4.

posure of animals did not affect the rate of uptake of ^{51}Cr-EDTA by tied-off duodenal segments (10 min incubation, 100 μM ^{51}Cr-EDTA; controls, 9.1 ± 1.7 (3) pmol/mg; hypoxic, 11.6 ± 1.3 (3)). Correcting for the permeation of the metal complex, the total mucosal $^{59}Fe^{3+}$ uptake time course shows excellent linearity for at least 10 min and extrapolates through zero at $t = 0$ (Fig. 5). This therefore allows the determination of initial rates of total mucosal uptake of $^{59}Fe^{3+}$. These experiments assume that the uptake of $^{59}Fe^{3+}$ is represented by net flux rather than radiolabel exchange between the lumen and other intracellular pools of $^{56}Fe^{3+}$. Chemical analysis of the amount of iron ($^{56}Fe^{3+}$ and $^{59}Fe^{3+}$) in the lumen before and after a 10 min intraluminal incubation has revealed that the loss in radiolabel is paralleled by a similar decrease in the total iron [14].

The in vivo total mucosal uptake of $^{59}Fe^{3+}$, corrected for the permeation of whole metal complex, shows a hyperbolic dependence on the Fe^{3+} concentration (Fig. 6). A similar dependence was observed in animals exposed to 3 days hypoxia, and is consistent with observations in vitro. When these data were analysed, K_m^{app} values of 129 and 119 μmol/l and V_{max}^{app} values of 6.00 and 15.1 pmol/mg per min were obtained for the control and hypoxic animals, respectively. A 2–3-fold increase in the V_{max}^{app} is still apparent in hypoxic animals, although the values are lower than those obtained in vitro [6]. Similar kinetic parameters have been reported for control animals using a cation-free incubation medium [14]. In experiments in which unwashed duodenal segments were used for $^{59}Fe^{3+}$ incubation studies, a marked decrease in the adaptive response to hypoxia was found ($P < 0.01$; Table II). This is attributable mainly to a significant change in the mucosal uptake. Normal animals also showed a small but significant decrease in the mucosal 'retention' when compared to controls with pre-washed segments. These observations may suggest the existence of an inhibitory luminal factor in hypoxic animals and to a small extent in control animals.

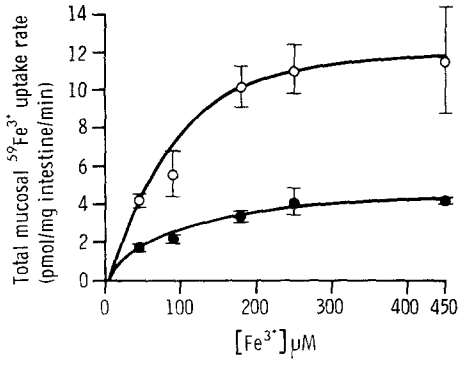

Fig. 6. Total mucosal uptake of $^{59}Fe^{3+}$ by tied-off duodenal segments in control (●) and hypoxic (○) animals. Fe^{3+}/nitrilotriacetate = 1:2. The uptakes have been corrected for ^{51}Cr-EDTA uptake. Medium iron concentration range: 45–450 μmol/l. Each data point is the mean ± S.E. for three to eight determinations.

Discussion

Integrity of incubated duodenal mucosa

Microscopy, at both light and electron level, revealed no morphological changes in incubated fragments as compared to control tissue. Previous studies with NaF and dinitrophenol [15] have

TABLE II

EFFECT OF PRE-WASHING THE DUODENAL SEGMENT ON $^{59}Fe^{3+}$ ABSORPTION

For pre-washing, 0.6 ml prewarmed 0.15 M NaCl was used. Incubation = 10 min. Fe^{3+}, 250 μmol/l; nitrilotriacetate, 500 μmol/l. Results are means ± S.E. for (n) animals. Total mucosal uptake = $^{59}Fe^{3+}$ in intestine plus $^{59}Fe^{3+}$ in carcass.

Group	Duodenal segment	n	$^{59}Fe^{3+}$ in intestine (pmol/mg)	$^{59}Fe^{3+}$ in carcass (pmol/mg)	Total mucosal uptake of $^{59}Fe^{3+}$ (pmol/mg)
Controls	prewashed	8	32.1 ± 2.7	21 ± 2.6	53.1 ± 4.8
	unwashed	7	23.6 ± 2.1 [a]	21 ± 2.4	44.6 ± 2.4
Hypoxic	prewashed	7	52 ± 3.2	88.3 ± 12.4	140.3 ± 13.5
(3 days)	unwashed	10	30.9 ± 4.3 [b]	62.4 ± 7.8	93.2 ± 8.8 [b]

Significant difference from values obtained with pre-washed segments, by Student's 't' test: [a] $P < 0.05$; [b] $P < 0.01$.

suggested that the mucosal Fe^{3+} uptake mechanism is highly dependent on the metabolic state of tissue. These studies demonstrated that Fe^{3+} uptake decreases by 14–24% with either inhibitor alone, and by 37–50% when both are present. Our studies show that these effects can be related to changes in the energy charge. The findings demonstrate that the decrease in energy charge which results on tissue removal is insufficient to inhibit the Fe^{3+} uptake rate, and it is only when the energy charge drops below 0.8 that inhibition occurs.

In vitro $^{59}Fe^{3+}$ uptake

In this paper, a similar incubation medium was employed for comparing the uptake of $^{59}Fe^{3+}$ by mouse duodenal fragments in vitro with the in vivo total mucosal $^{59}Fe^{3+}$ uptake using tied-off duodenal segments. A weak, low molecular weight chelator of Fe^{3+} (namely nitrilotriacetate) has been used to maintain the iron in the medium in a soluble and oxidized state. Glover and Jacobs [16] have shown that a substantial proportion of iron in a test meal is bound to low molecular weight compounds during digestion in the human jejunum. Kane and Miller [17] have found that dialysability of iron after simulated digestion in vitro correlates with in vivo bioavailability of different forms of food iron. These findings suggest that low molecular weight complexes of iron are important donors of iron to the intestinal iron transport systems in vivo and justifies the use of nitrilotriacetate chelates in the present study.

Previous studies of iron absorption have paid little attention to the residence time of diet in the proximal small intestine during absorption. Our experiments show this time to be very short, and therefore, in vitro and in vivo studies of initial rates of Fe^{3+} transport must be performed. In addition, suitable markers (i.e., [^{57}Co]cyanocobalamin, ^{51}Cr-EDTA) must be used to correct for both medium adhering to the tissue and for the mucosal permeation of whole-metal complex. The initial uptake of $^{59}Fe^{3+}$ by duodenal fragments determined by the in vitro technique was noted to be rapid, and studies with [^{14}C]nitrilotriacetate-metal complex have revealed that there is specific uptake of Fe^{3+} from the complex [6].

The major proportion of the specific uptake by duodenal fragments is expected to occur via the large exposed surface area of the villi and microvilli. It is, however, possible that some entry occurs via the serosal route or via the cut surfaces. Studies on the relationship between the corrected uptake of $^{59}Fe^{3+}$ and wet weight of tissue indicated that uptake via the cut surfaces is negligible, since a non-zero intercept would occur if such a process was significant. The serosa, on the other hand, does account for an appreciable proportion of the overall uptake by duodenal fragments; it does not, however, contribute to the enhancement in the rate of uptake by fragments from hypoxic animals. As iron uptake by duodenal fragments shows a hyperbolic dependence on the medium concentration of Fe^{3+}-nitrilotriacetate, and is inhibited by metabolic inhibitors [15], a carrier mechanism, whose activity increases in hypoxia, is envisaged.

In vivo $^{59}Fe^{3+}$ uptake studies

Pre-washing of the duodenal loop, prior to $^{59}Fe^{3+}$ incubation, was performed in order to make the comparison between the in vivo and in vitro systems more compatible. Time courses for mucosal $^{59}Fe^{3+}$ 'retention' and 'transfer' were studied for incubation times of 1–10 min. The total mucosal uptake of $^{59}Fe^{3+}$ exhibited linearity (especially after correction for the permeation of ^{51}Cr-EDTA) for a variety of experimental conditions and absorption rates. Hypoxia resulted in a 2–3-fold increase in the total mucosal uptake. Although both the 'uptake' and 'transfer' steps were enhanced, the latter showed a more marked response. However, the precise rate of movement of iron to the body remains unknown owing to the uncertainty of the degree to which the radioisotope is diluted by the mucosal $^{56}Fe^{3+}$ [2].

The corrected total mucosal uptake of $^{59}Fe^{3+}$ shows a hyperbolic dependence on Fe^{3+}-nitrilotriacetate in both control and hypoxic animals. Such a dependence is consistent with the in vitro observations. The V_{max}^{app} values obtained in vivo were however about 30–40% lower than the values obtained in vitro. The K_m^{app} values, on the other hand, were fairly similar. It should be noted that up to 30% of the total uptake by fragments is due to uptake via a non-mucosal route. Thus, on correcting for this, the uptake rate obtained in vitro

(corrected V_{max}^{app}, 7.0 pmol/mg per min) is similar to that for the in vivo Fe^{3+} influx across the brush border membrane (V_{max}^{app}, = 6.0 pmol/mg per min). These observations suggest that the Fe^{3+} uptake rates observed in the fresh pieces of intestine in vitro are, indeed, valid reflections of the in vivo conditions.

Conclusions

The in vivo total mucosal uptake of Fe^{3+} across mouse duodenal brush border membrane exhibits similar kinetics to those observed in vitro, and indicates that the Fe^{3+} uptake rates observed with duodenal fragments are valid measurements. This system thus provides a suitable in vitro method for determining the kinetics of Fe^{3+} uptake into mucosal epithelium in both normal and adapted animal models, and supports its use in man. In addition to the initial uptake step, the rate of movement of iron from mucosa to the body also plays an important role in the regulation of iron absorption.

Acknowledgements

We are most grateful to Professor T. Iancu and Mrs J. Roberts for their expert assistance with the electron microscopy, and to Ms Rosamund Greensted for secretarial assistance.

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