Biochimica et Biophysica Acta 856 (1986) 115–122
Elsevier

BBA 72999

Mouse intestinal Fe^{3+} uptake kinetics in vivo. The significance of brush-border membrane vesicle transport in the mechanism of mucosal Fe^{3+} uptake

Robert J. Simpson and Timothy J. Peters

Division of Clinical Cell Biology, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ (U.K.)

(Received September 19th, 1985)

Key words: Iron absorption; Hypoxia; Kinetics; Brush-border membrane; (Mouse intestine)

Initial rates of mucosal uptake of Fe^{3+} from luminal Fe^{3+}-nitrilotriacetate solutions by tied segments of mouse intestine in vivo have been measured. Duodenal uptake showed an approximately hyperbolic dependence of uptake on Fe^{3+} complex concentration ($K_{m(app)}$ 66 μM, V_{max} 6.2 pmol/min per mg intestine) with little dependence on nitrilotriacetate:Fe^{3+} ratio or on added Ca^{2+}. Duodenal uptake was greatly stimulated by hypoxic treatment of mice. Uptake rates by distal ileum were lower than by duodenum and more sensitive to added Ca^{2+}. These results show that isolated duodenal brush-border membrane Fe^{3+} transport characteristics (Simpson, R.J. and Peters, T.J. (1984) Biochim. Biophys. Acta 772, 220–226) are inadequate to explain duodenal Fe^{3+} uptake in vivo. However, ileal uptake can be explained by the properties of isolated ileal brush-border membrane (Simpson, R.J., Raja, K.B. and Peters, T.J. (1985) Biochim. Biophys. Acta 814, 8–12).

Introduction

Studies on Fe^{3+} uptake by mouse duodenal brush-border membrane vesicles have provided evidence that the predominant uptake process for Fe^{3+} represents transport across the isolated brush-border membrane [1,2]. It has been demonstrated that this transport is subject to adaptive regulation [2,3]. Advances in understanding the biochemical mechanism of mucosal Fe^{3+} uptake operating in vivo may be possible by further investigation of isolated brush-border membrane transport of Fe^{3+}. It is essential, however, that the relationship between the transport properties of isolated brush-border membrane and in vivo transport properties of mucosal epithelium be investigated.

In vivo and in vitro studies of Fe^{3+} absorption over many years have delineated two kinetically distinguishable steps in the overall process of Fe^{3+} absorption, termed uptake and transfer [4,5]. The uptake step is usually identified with the brush-border membrane transport [6,7], while transfer presumably includes transport across and from the enterocyte to the plasma. The many reported studies of iron absorption in vivo and in vitro (see references in Ref. 4) suggest that the uptake step is capable of adaptive regulation [5] (and thus is presumably mediated by a controllable transport mechanism). The variety of different luminal conditions which have been employed for in vivo studies, coupled with the known instabilities of Fe^{3+} solutions make it difficult to interpret many previous studies of iron absorption in kinetic terms. In this paper the kinetics of Fe^{3+} uptake by mouse intestine studied in vivo, using conditions comparable to those previously employed for vesicle studies, are presented.

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; NTA, nitrilotriacetate.

Materials and Methods

In vivo $^{59}Fe^{3+}$ absorption. Mice were anaesthetised with Sagatal (May and Baker Ltd., Dagenham, U.K.) and the abdomen opened. Segments of mouse intestine of approx. 2 cm length were located either immediately distal to the bile duct or greater than 20 cm from the pylorus (distal ileum) and the ends tied off with surgical thread. Care was taken to maintain the blood supply of the segment intact. The appropriate radiolabelled incubation solution was injected intraluminally essentially as described in [8]. All incubation solutions contained 0.1 M mannitol, 0.1 M NaCl, 20 mM Hepes (pH 7.4) and were prepared as for vesicle uptake experiments [1]. For iron uptake studies the solution contained $^{59}Fe^{3+}$ (0.1 μCi/nmol) and excess nitrilotriacetate. For ^{51}CrEDTA studies the solution contained 100 μM ^{51}Cr (0.05 μCi/nmol) and 400 μM EDTA. After incubation for up to 10 min the segment was removed, flushed with 20 ml of ice cold wash solution (0.1 mM Fe^{3+}/0.22 mM nitrilotriacetate/0.15 M NaCl), opened, blotted on both surfaces and the connective, fatty or pancreatic tissue trimmed off. The segment was weighed and counted in a gamma counter (Beckman Instruments Inc., Fullerton, CA, U.S.A., Gamma 7000). The mouse was killed and counted for 10 min in a high-resolution bulk sample counter [9]. The animals were placed centrally between two detectors put at optimal separation. Appropriate standards were counted in each gamma counter and the activity of ^{59}Fe in mucosal tissue expressed as pmol/mg (wet weight) of intestine.

The wash media used were the same as those employed in vesicle transport experiments [1,2]. The procedure was optimized by varying the wash volume. The uptake after 10 min incubation in experiments with 100 μM $^{59}Fe^{3+}$ and 200 μM NTA was found to be essentially independent of the volume of wash medium in the range 10–30 ml and 20 ml was therefore selected. ^{59}Fe content of intestine was presumed to represent activity in intestinal mucosa as comparison of counts in whole tissue with counts in mucosal scraping revealed that 86 $\pm$ 3.3% (S.E., $n = 10$) of the radioactivity was in the mucosa (normal tissue, 10 min incubation, 100 μM Fe^{3+}, 200 μM nitrilotriacetate). The radioactivity in the carcass was used to calculate transfer of ^{59}Fe, in pmol/mg of absorbing intestine, using the measured length of the weighed portion and the length of the tied-off segment to calculate the weight of absorbing gut.

In some experiments the gut segment was prewashed with 0.15 M NaCl, immediately before the absorption study. The tied off segment was cut open at the distal end and 1 ml of 0.15 M NaCl (warmed to 37°C) pumped through at a rate slow enough to avoid excessive distention of the gut. The segment was tied-off immediately proximal to the cut and the incubation medium injected.

General methods and materials. Iron determinations were performed by atomic absorption spectrophotometry as in Ref. 2 and with ferrozine as in Ref. 1. Protein and enzyme determinations were performed as in Ref. 1. Gut fragments were homogenized in 1 ml of 50 mM mannitol/2 mM Hepes (pH 7.1) with a Duall homogeniser (Kontes Glass Co., Vineland, NJ, U.S.A.). Materials were obtained as described in Refs. 1 and 2. ^{51}CrEDTA was from Amersham International, Bucks., U.K.

Preparation of brush-border membrane vesicles and determination of Fe^{3+} uptake were performed as described in Refs. 1 and 2.

Animals. 6–8-week-old male To-strain mice were used throughout. Hypoxia was induced by placing animals in a hypobaric chamber at 0.5 atmospheres for 3 days. Food and water were given freely except on the day of an experiment.

Results and Discussion

In vivo studies of the kinetics Fe^{3+} uptake by mouse intestine

In order to provide kinetically useful information from in vivo studies, it is necessary to study the early time course for uptake of $^{53}Fe^{3+}$ and establish linearity. The separate time courses for radioactivity in intestine and carcass in some experimental conditions are shown in Fig. 1. The time course of radioactivity in intestine is curved after an initial rapid phase and shows a tendency to reach a steady-state value. The time course for radioactivity in the carcass shows a noticeable lag. These characteristics, combined with the good linearity of the total mucosal uptake data (see Fig. 3 below) are consistent with the idea that uptake and transfer are sequential steps in the Fe^{3+} absorption pathway.

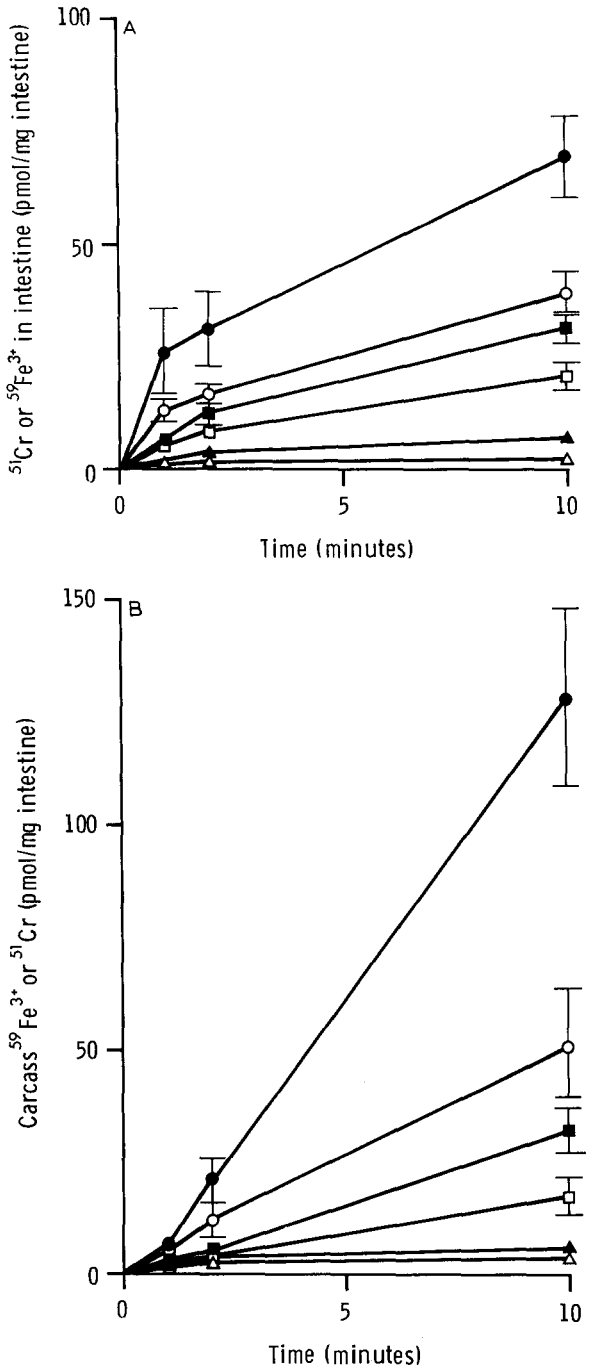

Fig. 1. Uptake into intestinal mucosa (A) and transfer to carcass (B) of $^{59}Fe^{3+}$ and ^{51}Cr-EDTA by mouse duodenal tied-off segments in vivo. Experiments were performed as described in Materials and Methods. Data points are mean ± S.E. for 3–8 animals. Incubation media were varied as follows: (□), 100 μM $^{59}Fe^{3+}$, 200 μM nitrilotriacetate; (○), 400 μM $^{59}Fe^{3+}$, 800 μM nitrilotriacetate; (■), 400 μM $^{59}Fe^{3+}$, 800 μM nitrilotriacetate, animals subjected to 3 days of hypoxia (0.5 atmosphere) and absorption studies performed within 4 h of removal from hypoxic chamber; (△) 100 μM ^{51}CrEDTA instead of Fe^{3+}-NTA; (■) 100 μM $^{59}Fe^{3+}$, 160 μM nitrilotriacetate; (▲) 100 μM $^{59}Fe^{3+}$, 10 mM nitrilotriacetate.

Total mucosal uptake can be obtained by summation of intestine and carcass radioactivity (Fig. 2) and at the highest measured rates shows reasonable linearity. At the lower rates, however, it is not possible to ignore the permeation of whole metal-chelate complex which occurs in intestine by an unknown pathway [10]. The stable metal complex ^{51}CrEDTA is a useful marker for this pathway. Fig. 1 demonstrates that this gives similar values for radioactivity in duodenal mucosa and in the whole animal to those obtained by stabilising the Fe^{3+}-NTA complex with a large excess of nitrilotriacetate (molar ratio of NTA : Fe^{3+} of 100 : 1). Similar data can be obtained with the more stable iron complex $^{59}Fe^{3+}$-EDTA. The total mucosal uptake of ^{51}CrEDTA is low compared with that of $^{59}Fe^{3+}$-NTA at low NTA : Fe^{3+} ratio (Fig. 2) and the utpake of ^{51}CrEDTA can be used to correct the total iron uptake. The resulting total mucosal uptake time courses are plotted in Fig. 3 to emphasise their general linearity, extrapolating to zero at $t = 0$. This allows the determination of an initial rate of $^{59}Fe^{3+}$ total mucosal uptake.

Data presented for absorption of Fe^{3+} in this paper assume that the uptake of radiolabel (^{59}Fe) represents net flux rather than exchange of label between the lumen and other pools of $^{56}Fe^{3+}$. In order to test this assumption, movements of total iron ($^{59}Fe + ^{56}Fe$) must be determined. However, the changes of iron levels observed in most of these experiments were too small for a detectable increase in tissue iron to be observed with iron assays. The alternative to this would be observations of decreased luminal total Fe^{3+} but these may also be complicated by iron release from the intestine into the lumen (see Ref. 4) during the incubation. Using hypoxic mice, with prewashed duodenal segments and a luminal Fe^{3+} concentration of 100 μM, a net decrease in luminal total iron could be detected by chemical determination of the luminal contents collected after incubation in tied segments. After 10 min incubation, the luminal total iron had declined to 41 ± 10 μM (mean ± S.E., $n = 4$). The mean drop in luminal

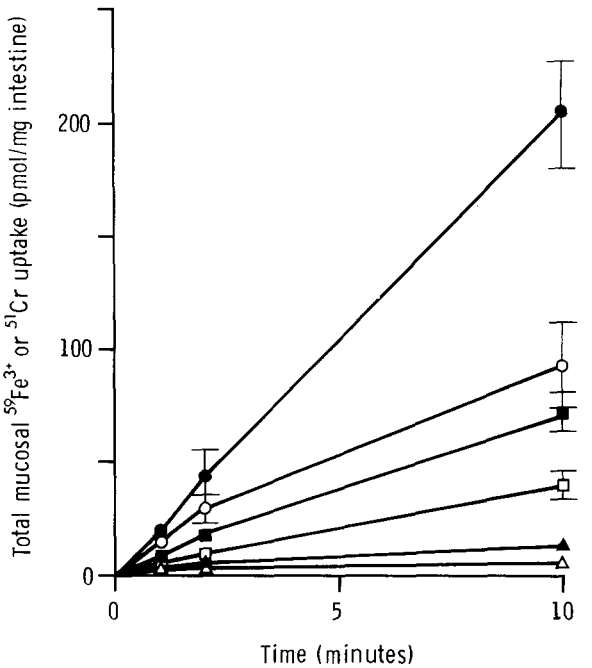

Fig. 2. Total mucosal uptake of $^{59}Fe^{3+}$ obtained by combining mucosal and carcass uptake in Fig. 1. Symbols and experimental details are as in Fig. 1.

$^{59}Fe^{3+}$ in these experiments was 77 ± 7% ($n = 4$). Control incubations lacking iron revealed that 23 ± 5 μM ($n = 4$) endogenous iron is present in the luminal contents after these incubations. It can thus be seen that the decrease in ^{59}Fe is matched by the loss in total iron from the lumen of the tied of segments (77 ± 7% and 82 ± 11%, respectively). The chemical form of the tissue iron released into the lumen in the control incubations is unknown but if it is ferritin (as is most cellular iron) it should not intrude on the uptake studies discussed in this paper.

As discussed by Forth and Rummel [4] tracer techniques of the type used here cannot quantitatively determine the rate of the transfer step in iron absorption due to the unknown degree to which isotope is diluted by mucosal ^{56}Fe. Thus the rates quoted here for transfer simply represent the minimum rate of iron movement to the animal's body from the gut. For the study of the initial unidirectional mucosal uptake rate from the lumen, this uncertainty is not relevant since the fate of the ^{59}Fe after the step of interest should not affect that step when initial rates are studied.

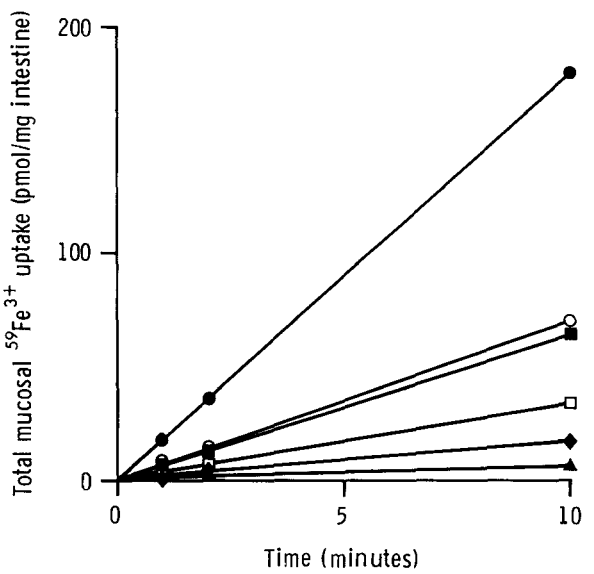

Fig. 3. Total mucosal uptake data corrected for permeation of whole metal complex. Symbols are as in Fig. 1, (◆) represents data for an experiment in which the incubation medium contained 25 μM $^{59}Fe^{3+}$ and 50 μM nitrilotriacetate.

Characteristics of in vivo $^{59}Fe^{3+}$ uptake by mouse duodenum and uptake by isolated brush-border membrane vesicles

Our previous studies of $^{59}Fe^{3+}$ uptake by brush-border membrane vesicles revaled that this uptake rate depended on the medium concentration of unchelated Fe^{3+} rather than $Fe^{3+}(NTA)_2$ complex. This observation was based on studies of the effect of varying medium Fe^{3+} and nitrilotriacetate concentrations at constant NTA : Fe^{3+} ratio (Fig. 4), varying the NTA : Fe^{3+} ratio, or on the effect of divalent cations, such as Ca^{2+}, on the uptake rate.

The vesicle uptake rate is unaffected by varying Fe^{3+} concentrations from 5 to 100 μM at constant NTA : Fe^{3+} ratio (Fig. 4). It should be noted that, based on the dissociation constants K_1 and β_2 of Sillen and Martell [11] for Fe^{3+}(-NTA)$_2$, the species Fe^{3+}-NTA_2 and Fe^{3+}-NTA will all vary markedly in an experiment such as in Fig. 4, however, the total unchelated Fe^{3+} species (presumably oligo- or polynuclear Fe species) will not vary greatly. In contrast, over the concentration range 0–400 μM, in vivo uptake shows a hyperbolic dependence on Fe^{3+} concentration (Fig. 5). Only Fe^{3+}-NTA_2, the predominant Fe^{3+} species

in these experiments, would be expected to increase approximately linearly over the range 10–100 μM.

In vivo mucosal Fe^{3+} uptake from $Fe^{3+}(\text{-NTA})_2$ is only slightly stimulated by the addition of 1 mM Ca^{2+} (Table I). This effect may be contrasted with the 7-fold increase in vesicle uptake reported previously with the same concentration of Ca^{2+}, Fe^{3+} and NTA [1]. Table I also shows that in vivo uptake is stimulated less than 2-fold on reducing the NTA : Fe^{3+} ratio from 2 : 1 to 1.6 : 1. In contrast vesicle uptake increases by 4-fold in similar circumstances (taken from Ref. 1).

Uptake is markedly stimulated by exposure of mice to 3 days of hypoia (Fig. 3, Table II); however, there is no change in uptake of ^{51}CrEDTA (Table II). Prewashing the duodenum led to a further marked increased in Fe^{3+} uptake in hypoxic animals. This finding suggests that a factor may be present in hypoxic-mouse gut which inhibits Fe^{3+} uptake. This factor does not appear to have a significant effect in normal mice (Tables I and II).

The increase in initial uptake of Fe^{3+} by brush-border membrane vesicles elucidated by hypoxic treatment of mice has been previously reported to be less than 2-fold [2]. The vesicle studies were performed using the same medium Fe^{3+} and nitrilotriacetate concentrations as in Table II.

The characteristics of in vivo $^{59}Fe^{3+}$ absorption by mouse distal ileum

The absorption of Fe^{3+} by mouse distal ileum is different from that observed in duodenum. Fig. 6 shows that the permeation of the marker ^{51}CrEDTA is similar in the ileum and duodenum (Fig. 2). There is, however, no detectable transfer of $^{59}Fe^{3+}$ into the animal, after correction for the entry of intract complex.

The observation that distal regions of the small intestine exhibit decreased total mucosal uptake with little or no capacity to transfer iron to the plasma has been made before in studies with Fe^{2+} [4,13] and Fe^{3+} [4]. The latter study, however, used $FeCl_3$ at pH 2 which may have a deliterious effect on the mucosa of distal ileum.

The ileal uptake of $^{59}Fe^{3+}$ shows a greater stimulation by Ca^{2+} (Fig. 6) than in duodenum, though the penetration of ^{51}CrEDTA was unaffected by this ion (after 10 min incubation control ^{51}CrEDTA uptake was 7.2 ± 0.8 (S.E., $n = 8$) pmol/mg of gut and with 1 mM $CaCl_2$ it was 8.3 ± 2.6 ($n = 3$) pmol/mg of gut). After correcting for the penetration of ^{51}CrEDTA, the in-

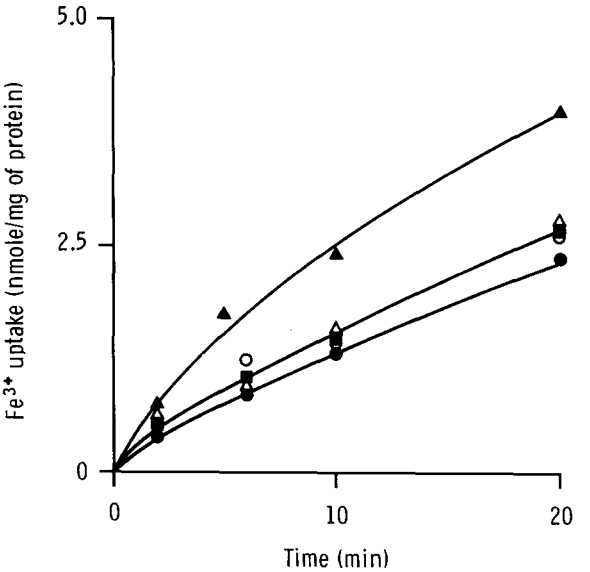

Fig. 4. Initial $^{59}Fe^{3+}$ uptake by duodenal brush-border membrane vesicles at various medium Fe^{3+} and constant NTA : Fe ratio. Uptake was determined by millipore filtration as described in Ref. [1]. Medium Fe^{3+} : NTA was 1 : 2 with Fe^{3+} concetration of: (▲), 361 μM; (△), 91 μM; (■), 30 μM; (○), 13 μM and (●), 5 μM.

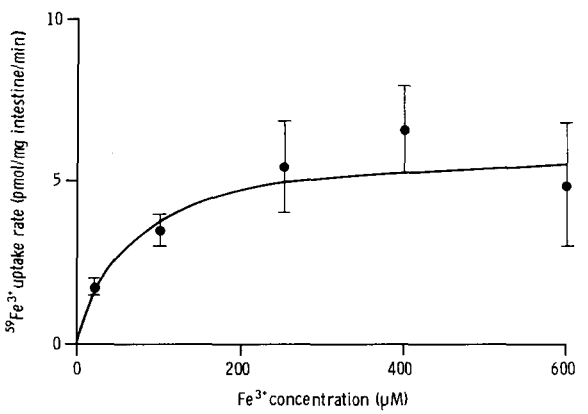

Fig. 5. Initial mucosal uptake rate for $^{59}Fe^{3+}$ by duodenum in vivo at various medium Fe^{3+} and constant NTA : Fe ratio. Data were obtained from tied-off segments incubated for 10 min in vivo as described in the Methods section. Each point is ±S.E. for 4–8 determinations. The curve was obtained by fitting the data to a hyperbola and is characterised by a 'K_m' of 66 μM and a V_{max} of 6.2 pmol/min per mg intestine.

TABLE I

TOTAL MUCOSAL UPTAKE OF Fe^{3+} FROM DUODENAL TIED SEGMENTS

In all experiments in part (a), the medium contained 0.1 M mannitol, 0.1 M NaCl, 20 mM Hepes (pH 7.4 ± 0.1), 100 μM Fe^{3+} and 200 μM nitrilotriacetate. Further additions were made as detailed below. In part (b) only the nitrilotriacetate concentration was varied. Uptake studies were performed as described in Materials and Methods. Incubation time was 10 min. Values have been corrected for absorption of ^{51}CrEDTA as described in the text and show mean ± S.E. values for (*n*) animals. In part (a) all values are not significantly different from control ($P > 0.05$).

Experiment	$^{59}Fe^{3+}$ uptake (pmol/mg of gut)	Uptake relative to control
(a) Control	35 ± 6 (8)	1
+1 mM $MgCl_2$	51 ± 4 (4)	1.4
+1 mM $CaCl_2$	56 ± 5 (3)	1.6
Prewashed loop	45 ± 8 (7)	1.3
(b) NTA : Fe^{3+} ratio		
1.6	66 ± 8 (4) *	1.9
2.0	35 ± 6 (8)	1
100	7 ± 3 (6) **	0.2

* $P < 0.05$ compared with control.
** $P < 0.01$ compared with control.

crease in total $^{59}Fe^{3+}$ mucosal uptake in the presence of Ca^{2+} was about 4-fold. The uptake of ^{51}CrEDTA represents a major correction to the total $^{59}Fe^{3+}$ uptake so accurate measurements of specific $^{59}Fe^{3+}$ uptake in ileum are difficult. Brush-border membrane vesicles from distal ileum have been reported to show a 4-fold stimulation in initial uptake in response to the addition of 1 mM $CaCl_2$ to the medium [2].

Comparison of Fe^{3+} transport by isolated brush-border membrane vesicles and by whole tissue

The comparison of initial transport rates by isolated brush-border membrane vesicles with total uptake by mucosal epithelium in vivo is possible if a brush-border membrane marker enzyme is used to assess the brush-border membrane surface in each preparation and if both represent initial influx rates. This assumes that the marker enzyme and Fe^{3+} transporters share the same topological distribution on the brush-border membrane. The rates involved in studies of in vivo Fe^{3+} uptake are sufficiently slow to make limitation by unstirred layers small [14] hence a comparison is reasonable. It has been shown previously [1,15] that studies of $^{59}Fe^{3+}$ uptake by brush-border membrane vesicles do indeed measure the net initial influx rate for Fe^{3+}. As discussed above, the in vivo measurements here also appear to represent a net initial influx rate. It could be argued that $^{59}Fe^{3+}$ uptake by brush-border membrane vesicles is rate determined by a step subsequent to the membrane, the overall uptake masking a rapid initial phase. It has been demonstrated, however, that the uptake rates measured with brush-border membrane vesicles are indeed determined by the membrane transport rate [1].

Comparison of the vesicle and in vivo uptake rates (Table III) indicate that the rates observed with purified brush-border membranes are inadequate, by a factor of nearly 10, to account for those observed in vivo in normal tissue. Many observations noted above also point to qualitative discrepancies between the in vivo and the isolated brush-border membrane transport characteristics.

Preparation of brush-border membrane vesicles by methods not involving treatment with 10 mM $MgCl_2$, namely disruption of brush border with

TABLE II

IN VIVO $^{59}Fe^{3+}$ MUCOSAL UPTAKE BY HYPOXIC MICE

Uptake data (mean ± S.E. for (*n*) determinations), after 10 min incubation, for normal and hypoxic mouse duodenum from media containing either 400 μM $^{59}Fe^{3+}$ and 800 μM nitrilotriacetate or 100 μM ^{51}CrEDTA. The data for Fe^{3+} uptake have been corrected for uptake of whole metal complex with the ^{51}CrEDTA uptake values. Statistical analysis: comparison of normal and hypoxic mice (a) $P < 0.01$; comparison of uptake by hypoxic mice (prewashed) with hypoxic (non washed) gut segment (b) $P < 0.01$.

Experiment	Total mucosal uptake (pmol/mg intestine)	
	^{59}Fe	^{51}CrEDTA
Normal mouse	66.5 ± 14.0 (6)	5.7 ± 1.5 (4)
Normal mouse, prewashed segment	60.0 ± 2.0 (3)	– –
Hypoxic mouse	182 ± 24 (5) [a]	5.4 ± 1.5 (3)
Hypoxic mouse, prewashed segment	377 ± 36 (8) [b]	5.9 ± 2.7 (3)

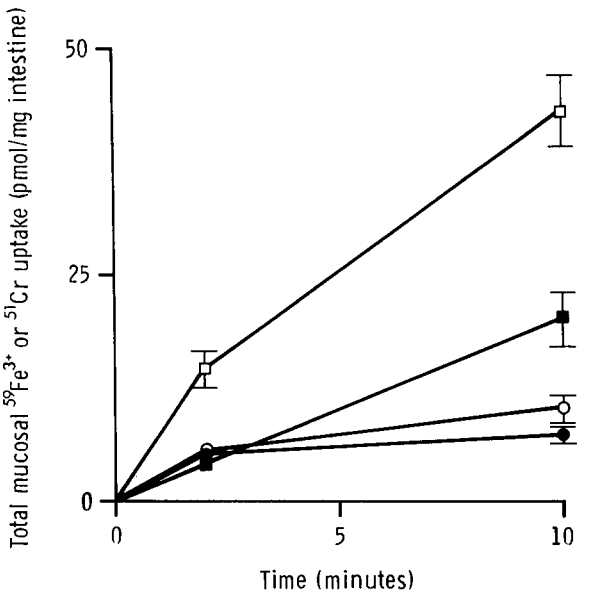

Fig. 6. Total mucosal uptake of $^{59}Fe^{3+}$ and ^{51}CrEDTA by tied-off segments of distal ileum. Incubation media were as in Fig. 1 only (○) 100 μM $^{59}Fe^{3+}$, 200 μM nitrilotriacetate; (□) 400 μM $^{59}Fe^{3+}$, 800 μM nitrilotriacetate; (●) 100 μM ^{51}CrEDTA, No Fe^{3+}-NTA; (■) 100 μM $^{50}Fe^{3+}$, 200 μM nitrilotriacetate and 1 mM $CaCl_2$.

TABLE III

COMPARISON OF BRUSH-BORDER MEMBRANE AND IN VIVO Fe^{3+} UPTAKE RATES

$^{59}Fe^{3+}$ uptake rates by brush-border membrane vesicles in vitro and total mucosal uptake of $^{59}Fe^{3+}$ in vivo expressed relative to the activity of Zn^{2+}-resistant α-glucosidase, a brush-border membrane marker enzyme [16]. All experiments were performed with 0.1 M mannitol, 0.1 M NaCl, 20 mM Hepes (pH 7.4±0.1), 400 μM Fe^{3+} and 790–800 μM nitrilotriacetate and tissue from normal mice. Data were calculated from (a) Fig. 3, (b) and (d) Ref. [2], (c) Fig. 6, and the value for α-glucosidase in duodenum of 0.055±0.005 (S.E., $n=16$) munits/mg of gut in distal ileum.

Preparation	Approximate initial uptake rate (nmol/min per munit α-glucosidase)
(a) In vivo duodenal tied-off loops	0.14
(b) Duodenal brush-border membrane vesicles	0.015–0.02
(c) In vivo ileal tied-off loops	0.06 –0.08
(d) Ileal brush-border membrane vesicles	0.06 –0.20

Tris or NaSCN, does not lead to large enhancement of the Fe^{3+} uptake rate [2]. Similarly, preloading of vesicles with unlabelled Fe^{3+} [2] suggests that the discrepancy cannot be explained by in vivo uptake representing an isotope exchange phenomenon while the uptake by vesicles is a slower net flux of Fe^{3+}. With distal ileum, the quantitative and qualitative discrepancies between isolated brush border membrane and in vivo uptake do not occur and Table III demonstrates the quantitative agreement.

It is clear that further attempts to characterise biochemically the Fe^{3+} transport displayed by isolated duodenal brush-border membrane vesicles must take into account the discrepancies between vesicle uptake and in vivo duodenal mucosal uptake.

It is possible that a cellular mechanism for Fe^{3+} uptake, operating in duodenum but not ileum and greatly increased in response to hypoxia is not expressed in brush-border membrane vesicles, perhaps because of the loss of the cellular reducing power or ATP. It is noteworthy that the characteristics of the uptake displayed by vesicles would be important in conditions of low pH, where unchelated Fe^{3+} may persist for longer periods than at neutral pH. We have not extended our study of Fe^{3+} uptake by brush-border membrane vesicles to low pH but data in Ref. 17 imply that the uptake of Fe^{3+} by vesicles also occurs at lower pH. The question of whether an Fe^{3+} uptake mechanism which interacts with weak Fe^{3+} complexes (such as are present in dilute Fe^{3+}-$(NTA)_2$ solutions at neutral pH) is more important than one which interacts with unchelated Fe^{3+} cannot be unequivocally answered without a better knowledge of the chemistry of iron during in vivo digestion of food.

Acknowledgements

R.J.S. is an MRC training fellow. We thank Ingvar Bjarnason for useful discussions and Sandra Ember for typing this manuscript.

References

1 Simpson, R.J. and Peters, T.J. (1984) Biochim. Biophys. Acta 772, 220–226

2 Simpson, R.J., Raja, K.B. and Peters, T.J. (1985) Biochim. Biophys. Acta 814, 8–12
3 Simpson, R.J., Raja, K.B. and Peters, T.J. (1983) Clin. Sci. 65, 68P
4 Forth, W. and Rummel, W. (1973) Physiol. Rev. 53, 724–792
5 Marx, J.J.M. (1979) Haematologica (Pavia) 64, 479–493
6 Aisen, P. (1982) Clin. Haematol. 11, 241–257
7 Bedard, Y.C., Pinkerton, P.H. and Simon, G.T. (1971) Blood 38, 232–245
8 Charley, P.J., Stitt, C., Shore, E. and Saltman, P. (1963) J. Lab. Clin. Med. 61, 397–410
9 Cronquist, G., Mackenzie, J. and Smith, T. (1975) Int. J. Appl. Radiat. Isot. 26, 89–91
10 Bjarnason, I., Smethurst, P., Levi, A.J. and Peters, T.J. (1985) Gut 26, 579–586
11 Sillen, L.G. and Martell, A.E. (1971) Special Publication No. 25, The Chemical Society, Burlington House, London
12 Johnson, G., Jacobs, P. and Purves, L.R. (1983) J. Clin. Invest. 71, 1467–1476
13 Wheby, M.S., Jones, L.G. and Crosby, W.H. (1964) J. Clin. Invest. 43, 1433–1442
14 Dainty, J. and House, C.R. (1966) J. Physiol. (London) 182, 66–78
15 Simpson, R.J., Weir, M., Gibson, J.F. and Peters, T.J. (1984) Biochem. Soc. Trans. 12, 859
16 Peters, T.J. (1976) Clin. Sci. Mol. Med. 51, 557–574
17 Marx, J.J.M. and Aisen, P. (1981) Biochim. Biophys. Acta 649, 297–304