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Fe^{2+} uptake by intestinal brush-border membrane vesicles from normal and hypoxic mice

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Fe^{2+} uptake by mouse intestinal brush-border membrane vesicles consists of two components: a rapid, high affinity ($K_d < 1$ μM), low capacity binding (< 2 nmol/mg protein), presumably to the outside of the vesicles, and a second, large capacity component with an initial rate showing a hyperbolic dependence on medium Fe^{2+} (K_m 35–90 μM). The latter, predominant process is relatively independent of medium ascorbate: Fe^{2+} ratio, is inhibited by Co^{2+} and Mn^{2+} but varies greatly from one membrane preparation to another. This component is strongly inhibited by large extravesicular NaCl and KCl concentrations and may represent transport into the vesicles. No significant change in uptake could be observed in vesicles prepared from hypoxic mice.

Introduction

Four groups of workers have studied Fe^{2+} uptake by intestinal brush-border membrane vesicles. The iron transport properties of brush-border membrane vesicles are of such interest because of the possible role of this membrane in regulating overall iron absorption [1–5]. These studies have been somewhat contradictory leaving some doubt regarding certain crucial points relating to the transport of Fe^{2+} by isolated brush-border membrane vesicles. Eastham et al. [1] showed that Fe^{2+} uptake by rat brush-border membrane vesicles appears to relate to an osmotically active space and hence presumably represents transport across the membrane. The uptake showed saturation kinetics over the range 0–2 μM Fe^{2+} but first-order kinetics over the wider range 0–50 μM. Some doubt has been cast on this study by later workers [3–5] as no ascorbate (to maintain Fe^{2+} in the reduced state) was included. Cox and O'Donnell [2,3] showed, using the same osmotically active solute (cellobiose), that uptake by rabbit brush border membrane vesicles represented binding and showed saturation kinetics over the concentration range 45–450 μM. They further showed that uptake by brush-border membrane vesicles from proximal intestine was enhanced when iron-deficient rabbits and depressed when iron-loaded rabbits, were used as the source of vesicles. Marx and Aisen [4] also used rabbit brush-border membrane vesicles and confirmed, with mannitol, an apparent osmotic dependence of Fe^{2+} uptake. They also showed that boiling the vesicles had little effect on the apparent transport, leading them to conclude [6] that the brush-border membrane played only a passive role in iron absorption.

Recently, Muir et al. [5] showed that the use of potentially iron binding molecules (for example

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Abbreviation: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

sugars, sugar alcohols) as osmotically active solutes could lead to misleading results in the crucial shrinking experiment with brush-border membrane vesicles. They demonstrated that the apparent osmotic dependence of Fe^{2+} uptake by mouse brush-border membrane vesicles with mannitol may be due to Fe^{2+} binding by mannitol, thus raising doubt as to whether Fe^{2+} uptake represents transport across the brush-border membrane. Other osmotically active solutes which do not bind Fe^{2+} show no apparent dependence of brush-border uptake of Fe^{2+} on medium tonicity. They concluded, however, that since the detergent cholate accelerated the exchange of pre-loaded $^{59}Fe^{2+}$ with extravesicular $^{56}Fe^{2+}$, there was a component of Fe^{2+} uptake which did, in fact, represent transport into the vesicle. It seems likely that since Fe^{2+} uptake represents accumulation of Fe^{2+} against a concentration gradient, then binding of Fe^{2+}, either within or to the outside of the vesicles, must occur. The transported component exchanged more rapidly with unlabelled extravesicular Fe^{2+} in vesicles prepared from iron-deficient mice at low medium Fe^{2+} concentrations but less rapidly at higher concentrations. Overall uptake of Fe^{2+} was enhanced in vesicles from proximal intestine of iron-deficient mice.

In this paper we present studies of $^{59}Fe^{2+}$ uptake by normal mouse proximal intestine brush-border membrane vesicles which attempt to show how the above observations regarding osmotic dependence of Fe^{2+} uptake can be reconciled. We also present studies of Fe^{2+} uptake by duodenal brush-border membrane vesicles from mice made hypoxic in order to induce enhanced iron absorption.

Methods

General methods and materials. Enzyme and protein determinations and materials were as described in Ref. 7. Brush-border membrane vesicles were prepared from mouse duodenal or duodenal plus jejunal mucosal scrapings by the method of Kessler et al. [8] as described in Ref. 7 and suspended in 0.1 M mannitol/0.1 M NaCl/0.1 mM $MgSO_4$/20 mM Hepes (pH 7.4). Male, To strain mice (6–8 weeks old) were used throughout. Hypoxia was induced by placing mice in a hypobaric chamber at 0.5 atmospheres for up to 3 days. Food and water were given freely at all times.

$^{59}Fe^{2+}$ uptake by brush-border vesicles. $^{59}Fe^{2+}$ uptake by freshly prepared brush-border membrane vesicles was determined using a method based on that used previously for $^{59}Fe^{3+}$ uptake [7] and is similar to the techniques used by other workers [1–5]. 10 mM $FeCl_3$ and $^{59}FeCl_3$, both in 10 mM HCl, were mixed to give approx. 20000 cpm/nmol Fe. Na ascorbate (as a 0.1 M solution) was added, followed by the appropriate H_2O and 0.2 M mannitol/0.2 M NaCl/40 mM Hepes (pH 7.4) required to give the necessary final Fe^{2+} concentration in 0.1 M mannitol/0.1 M NaCl/20 mM Hepes. Other additions were made in place of H_2O. After preincubation at 37°C, 50 μl of this medium was mixed with 5 μl of vesicle suspension (10–30 μg of vesicle protein) and incubated for the appropriate time at 37°C before 50 μl was removed, spotted onto a moist 0.22 μm Millipore filter in a Millipore filtration manifold (Cat. No. XX2702550). Filters were immediately washed with 10 ml of ice cold 0.1 mM $^{56}Fe^{2+}$/2 mM Na ascorbate/154 mM NaCl, blotted and counted at constant geometry in a γ counter (Beckman Gamma 7000).

In preliminary experiments where various washing procedures were investigated, the above method was selected as it yielded low retention (about 0.01%) of radioactivity in blank incubations lacking vesicles and when vesicles were present represented a relative plateau in the washout curve (uptake after 3 min incubation varied by less than 10% on increasing the wash to 15 ml or reducing it to 5 ml). Omitting Fe^{2+} and ascorbate or substituting 0.1 mM Fe^{3+} and 0.22 mM nitrilotriacetate, respectively, in the wash caused apparent uptake to increase by about 10%. Only in experiments where high medium Fe^{2+} concentrations (greater than 91 μM) were present did the blank uptake differ significantly from background radioactivity, in which case the necessary corrections to uptake were made.

Data shown are from representative experiments since qualitative observations show good reproducibility, although absolute uptake rates varied greatly from one membrane preparation to another. Kinetic data were analysed using the method of Eisenthal and Cornish Bowden [9].

Fresh vesicles were used for all experiments as storage at −70°C occasionally led to low uptake. This was found to be associated with large quantities of brush-border marker enzyme passing through the millipore filters after storage. 0.22 μm Millipore filters give very high, reproducible retention of freshly prepared mouse brush-border membrane after washing with 10 ml of wash medium similar to that described above [7], even in experiments where vesicles were osmotically shrunk.

Results and Discussion

Time-course of $^{59}Fe^{2+}$ uptake

Fig. 1 shows the uptake of Fe^{2+} by mouse brush-border membrane vesicles as a function of time in isotonic medium and in medium made hypertonic by the addition of NaCl. It may be seen that, as was found with Fe^{3+} [7], the majority of Fe^{2+} taken up by the vesicles at this Fe^{2+} concentration (91 μM) is associated with an apparent osmotically active space and presumably is transported into the vesicles. This is true even for incubation times up to 120 min, when uptake has largely ceased.

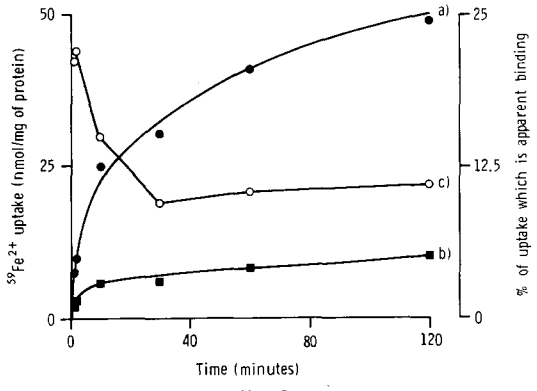

Fig. 1. Time-course of $^{59}Fe^{2+}$ uptake by mouse duodenal brush-border vesicles. Curve (a) vesicles were prepared in 0.1 M mannitol/0.1 M NaCl/0.1 mM $MgSO_4$/20 mM Hepes (pH 7.4) and added to 10 vol. of 0.1 mM $^{59}Fe^{2+}$/2.0 mM sodium ascorbate/0.1 M mannitol/0.1 M NaCl/20 mM Hepes (pH 7.4). 50 μl was removed at the indicated times of incubation at 37°C and uptake determined by Millipore filtration. Curve (b) as (a) except that the incubation medium contained additional NaCl to generate a total osmolarity (calculated assuming ideal solute behaviour) of 2.78 osM or 8.68-times that of (a). Curve (c) % of uptake which is not osmotically active calculated from the data points of (a) and (b) according to data point $c = (b - (a - b) \cdot 0.115/0.885) \cdot 100/a$ which is the numerical equivalent of extrapolating a line drawn through the uptake values determined, for a given medium Fe^{2+} concentration at two medium osmolarities (0.32 osM and 2.78 osM) to infinite medium osmolarity.

The uptake in hypertonic medium may be used to calculate the quantity of Fe^{2+} uptake which is apparently not related to an osmotically active space and thus may be binding to the outside of the vesicles (Fig. 1c). This proportion declines rapidly from an initially higher value, consistent with a small component of rapid binding superimposed on a longer, dominant time-course of a second component of uptake.

This demonstrates that NaCl may be used to dissect the overall uptake into two components, one dependent on NaCl and one independent of NaCl. Uptake is, however, found to be independent of NaCl if large NaCl concentrations (0.5 M) are added to the medium after incubation for 60 min with Fe^{2+}. This is true even if incubation with the large NaCl concentration is allowed to proceed for a further 30 min. It was also found that washing the vesicles after uptake experiments, with 10 ml of 1.5 M NaCl had no effect on apparent uptake compared with vesicles washed with isotonic NaCl.

The uptake time-course in isotonic medium is qualitatively similar to that shown by Cox and O'Donnell [2] with rabbit brush-border vesicles at a similar medium Fe^{2+} concentration. This time-course differs from that observed at very low Fe^{2+} concentrations by Muir et al. [5], Marx and Aisen [4] and Eastham et al. [1] with, respectively, mouse, rabbit and rat vesicles. These workers observed a rapid uptake which terminates after 2–3 min. Muir et al. [5] have shown that this time-course is predominantly that of a binding process with a small component which may be intravesicular.

Cox and O'Donnell [2] have shown with rabbit vesicles that uptake after 80 min shows no apparent osmotically active component [2]. This observation appears to conflict with Fig. 1, hence further studies of uptake after short (1 min) and long (60 min) incubations with Fe^{2+} in the presence of different osmotically active solutes were instigated. KCl demonstrated a large apparent osmotically active component in initial Fe^{2+} uptake ($t = 1$ min). At longer incubation times ($t = 60$ min), NaCl and KCl showed similar low apparent

TABLE I

RELATIVE INITIAL AND END-POINT UPTAKE OF Fe^{2+} IN MANNITOL COMPARED WITH NaCl INCUBATION BUFFERS AT CONSTANT TONICITY

Vesicles were prepared in 0.15 M NaCl (or 0.3 M mannitol)/20 mM Hepes (pH 7.4)/0.1 mM $MgSO_4$ and uptake time-courses were determined in 0.15 M NaCl (or 0.3 M mannitol)/20 mM Hepes (pH 7.4). Results are mean ± S.E. uptake for three vesicle preparations. Other conditions as in Fig. 1.

Experiment	Incubation time (min)	Ratio (uptake in mannitol incubation buffer)/(uptake in NaCl incubation buffer)
Vesicles prepared in mannitol resuspension buffer	1	1.15 ± 0.32
	60	0.92 ± 0.17
Vesicles prepared in NaCl resuspension buffer	1	0.88 ± 0.20
	60	0.80 ± 0.07

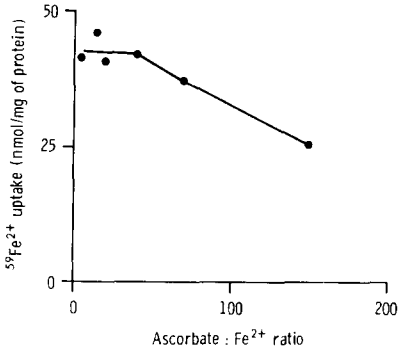

Fig. 2. Dependence of $^{59}Fe^{2+}$ uptake on medium ascorbate: Fe^{2+} ratio. Medium and vesicles were prepared as in Fig. 1a containing various sodium ascorbate concentrations to generate the indicated ascorbate: Fe^{2+} ratio. The final Fe^{2+} concentration was 91 μM. Incubation was for 5 min at 37°C.

binding (less than 25% of uptake) while Hepes-Na and mannitol demonstrated a higher apparent binding (57 ± 14% ($n = 4$) and 95 ± 2.5% ($n = 5$), respectively (means ± S.E.)). In other experiments, the effect of varying NaCl and mannitol concentrations at constant osmolarity was investigated. It was found that relative uptake with mannitol compared with NaCl as major solutes in the incubation buffer was generally similar when mannitol resuspension buffer is used (Table I). This effect was slightly different if vesicles were prepared in NaCl resuspension buffer (Table I).

Experiments in isotonic media containing 0.1% cholate demonstrated an accelerated uptake rate with no effect on the endpoint uptake. Higher cholate concentrations or solubilization of the vesicles with 1% deoxycholate resulted in a marked reduction in apparent uptake, presumably due to Fe^{2+} binding components of the vesicles no longer being retained by the filters.

The dependence of uptake on ascorbate: Fe^{2+} ratio

Fig. 2 shows that the initial uptake of Fe^{2+} is not greatly affected by a wide variation in ascorbate: Fe^{2+} ratio at a constant Fe^{2+} concentration of 91 μM. The slight decline in uptake observed with very high ascorbate: Fe^{2+} ratios may be explained by the presence of Fe^{2+}-ascorbate complexes in the medium. Fig. 2 is consistent with a K_a for Fe^{2+}-ascorbate complex of approx. 10^2 M^{-1} if uptake is related to free Fe^{2+} rather than to the Fe^{2+}-ascorbate complex. Reported K_a values for Fe^{2+} ascorbate complexes at 25°C are $10^{0.82}$ M^{-1} for neutral Fe-ascorbate (at pH 7.4) and $10^{0.21}$ M^{-1} for (Fe-ascorbate)$^+$ [10] at 25°C in 3 M $NaClO_4$ [10]. Based on our K_a value, 17% of Fe^{2+} would be bound to ascorbate at 100 μM Fe^{2+} and 2 mM ascorbate but 50% would be bound at 10 mM ascorbate. In order to further examine a possible involvement of ascorbate itself in the uptake process, we examined Fe^{2+} uptake in the presence of isoascorbate (D-*erythro*-hex-2-enoic acid, sodium salt). In three experiments, uptake by duodenal brush border vesicles after 1 min was examined in medium containing 90 μM Fe^{2+} and 1.8 mM ascorbate or 1.8 mM isoascorbate. The result was a ratio of uptake in the presence of ascorbate to uptake in the presence of isoascorbate of 0.86 ± 0.42 nmol/mg protein per min (mean ± S.E.), suggesting that the nature of the Fe^{2+} reducing agent does not greatly influence the uptake rate.

The above experiments suggest that Fe^{2+} uptake rates probably relate to free Fe^{2+} and are relatively independent of ascorbate: Fe ratio. Thus experiments where Fe^{2+} concentration is varied at constant ascorbate concentration (as in Ref. 2) or at constant ascorbate: Fe^{2+} ratio (see below) should give similar results.

The effect of Fe^{2+} concentration at constant Fe^{2+} : ascorbate ratio on initial $^{59}Fe^{2+}$ uptake

Fig. 3 shows the dependence of initial $^{59}Fe^{2+}$ uptake on Fe^{2+} concentration at a constant ascorbate : Fe^{2+} ratio of 20 : 1. Also shown is the uptake in hypertonic media with the same ascorbate and Fe^{2+} concentrations. It can be seen that uptake in isotonic media shows a saturation effect over the range 50–500 μM Fe^{2+}. Studies of the uptake time-course show no change in the general form of the time-course over this range. In very hypertonic media, uptake is nearly independent of Fe^{2+} concentration. Thus at low concentrations of Fe^{2+} (< 15 μM) we found a large proportion of uptake is not osmotically dependent, confirming the observation of Muir et al. [5]. The time-courses of Fe^{2+} uptake presented by Muir et al. [5], Eastham et al. [1] and Marx and Aisen [4] at very low Fe^{2+} are consistent with a rapid process, presumably binding to the outside of the vesicles (see above). If such a binding process has a K_d of less than approx. 1 μM then this bound component will be independent of medium Fe^{2+} concentration over the range shown in Fig. 3 and correction to the total uptake can be made to reveal the dependence of the second component on medium Fe^{2+} concentration.

The results of Cox and O'Donnell show [2] a dependence of total uptake on medium Fe^{2+} which is clearly non-hyperbolic, since their 1/(velocity of uptake) versus 1/(Fe^{2+} concentration) plots are obviously curved. This curvature, though slight, was found by us to be a reproducible feature with mouse brush-border membrane vesicles (see Fig. 4a). This curvature is removed by correcting for the 'binding' component, revealing a better hyperbolic relationship between uptake rate and medium Fe^{2+} (Fig. 4b). Such a curve, which may indicate a carrier mediated transport process, may be characterised by a maximal initial uptake rate and a K_t, analogous to the K_m of the Michaelis-Menten relationship. We have found K_t values in the range 35–90 μM for normal mouse proximal intestine brush-border membrane vesicles. These values are lower than those obtained by Cox and O'Donnell with rabbit vesicles (120–130 μM [2]). This difference can probably be attributed to their failure to make the correction described above. This would bias the uptake at lower substrate concentrations to give an apparently high K_t.

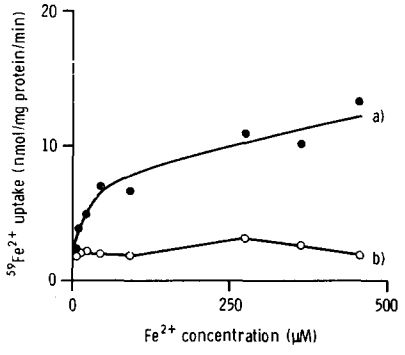

Fig. 3. Initial $^{59}Fe^{2+}$ uptake at different medium Fe^{2+} concentrations. (a) $^{59}Fe^{2+}$ uptake was determined as in Fig. 1a after 1 min incubation with various medium Fe^{2+} concentrations at a constant ascorbate : Fe^{2+} ratio of 20 : 1. Curve (b) as (a) only the media contained sufficient NaCl to generate an osmolarity of 2.0 osM or 6.25-times that in (a).

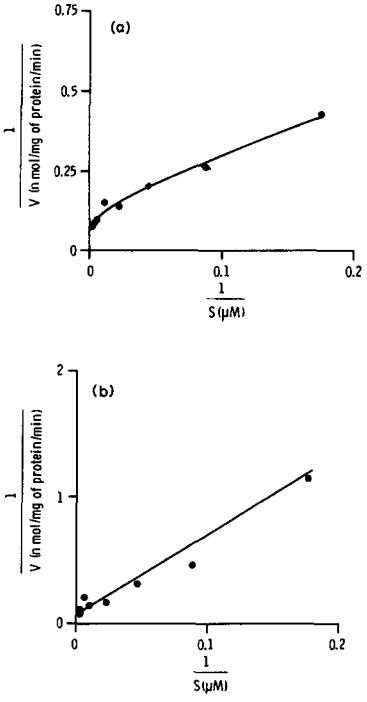

Fig. 4. Double-reciprocal plots of total uptake (a) and 'transport' components of uptake (b) of $^{59}Fe^{2+}$ by brush-border membranes at various medium Fe^{2+} concentrations. (a) Data from Fig. 3a. (b) Data calculated from Fig. 3 a and b using: 'transport' component = $(a - b)\cdot(1-(0.16/0.84))$. $V = ^{59}Fe^{2+}$ uptake rate, $S = Fe^{2+}$ concentration.

The nature of the Fe^{2+} uptake process

Muir et al. [5] provided a partial explanation of overall Fe^{2+} uptake by interpreting data at low medium Fe^{2+} as being binding with a transport component. We have shown uptake at low medium Fe^{2+} to be nearly independent of medium NaCl concentration. Uptake at higher medium Fe^{3+} is characterized by a striking inhibition by medium NaCl (or KCl). This inhibition is not observed if NaCl is added after uptake has occurred, thus showing that the NaCl dependent uptake cannot be simple binding to the outside of vesicles which is competitive with NaCl. This uptake is thus a two-step process, as would be transport across the vesicle membrane followed by binding inside the vesicle. Muir et al. [5] tried to use equilibrium isotope exchange to demonstrate such a two step process in Fe^{2+} uptake at low medium Fe^{2+} concentration, arguing that such a demonstration would imply that the uptake was a transport process. Unfortunately neither equilibrium isotope exchange nor the NaCl experiments can distinguish between a two-step binding to the outside of the vesicles or transport followed by binding. In fact, the best evidence that uptake reflects transport, is the demonstration that removal of the membrane barrier with detergents accelerates uptake without affecting the end point. Such an acceleration can be observed in Fe^{2+} uptake.

Alternative explanations for the data presented here and in Ref. 2 include: (a) Fe^{2+} uptake is predominantly transport, dependent on an osmotically active space which can only be demonstrated by NaCl or KCl either because mannitol and cellobiose permeate the vesicles very rapidly or because they stimulate uptake by some other mechanism to compensate for the inhibitory effect of vesicle shrinking; (b) Fe^{2+} uptake is a two step process which is not affected by osmotic gradients but which is weakly inhibited by NaCl (and to a similar degree by KCl). Note that the latter possibility could also be consistent with uptake being a transport process. Neither of these explanations is entirely consistent with the available data, especially with the small effect of varying mannitol and NaCl concentrations at constant osmolarity on Fe^{2+} uptake. The main aim of these experiments was to establish whether or not Fe^{2+} uptake by brush-border membrane vesicles represents transport across the brush-border membrane and this explanation is the most consistent with the available data.

Mixing Fe^{2+} with membranes in air-saturated medium may be expected to lead to peroxidation and possible damage to the vesicles. It was found that inclusion of the lipid soluble antioxidant butylated hydroxytoluene (50 μM added as 0.01 vol. of a 5 mM solution in ethanol) had little effect on initial uptake of Fe^{2+} (uptake after 1–10 min was $89 \pm 7\%$ (mean $\pm$ S.E.; $n = 4$) of controls). It should also be noted that rapid Fe^{2+} transport is not observed with erythrocyte plasma membranes or artificial membranes (e.g. phosphatidylcholine/cholesterol liposomes) (Ref. 11; Simpson, R., unpublished data), suggesting that brush-border membrane vesicles possess specific Fe^{2+} transport properties.

The effect of divalent cations on Fe^{2+} uptake

Divalent transition metal ions show some tendency to compete with Fe^{2+} for absorption and it has been suggested that some (especially Co^{2+}) may be transported by the Fe^{2+} absorption system [15]. Cox and O'Donnell [3] found only slight inhibition of Fe^{2+} uptake by Co^{2+} with rabbit brush-border membrane vesicles. However, we find Co^{2+} gives an approx. 50% inhibition at a $Co^{2+}:Fe^{2+}$ ratio of 10 whereas Ca^{2+} and Mg^{2+} have little effect (Table II). Mn^{2+} shows similar inhibition to Co^{2+}. Investigation of the effect of Co^{2+} and Mn^{2+} on the apparent binding component of uptake revealed a lower effect than is observed on total uptake. When correction was

TABLE II

EFFECT OF DIVALENT CATIONS ON Fe^{2+} UPTAKE BY MOUSE BRUSH-BORDER MEMBRANE VESICLES

Uptake was determined as in Fig. 1a only the appropriate metal chloride was added immediately prior to the experiment to a final concentration of 0.91 mM (10 times the final Fe^{2+} concentration). Uptake (mean $\pm$ S.E. for (n) vesicle preparations) was expressed relative to controls without metal chloride.

Addition	Relative Fe^{2+} uptake (%)
$MgCl_2$	92 ± 7 (3)
$CaCl_2$	82 ± 9 (3)
$CoCl_2$	45 ± 3 (6)
$MnCl_2$	57 ± 4 (8)

made to yield the inhibitory effect of Co^{2+} and Mn^{2+} on the transport comport of uptake, this was found to be not significantly different from the inhibitions shown in Table II. These results suggest the Co^{2+} may be a useful tool in further investigations of Fe^{2+} absorption. It is also noteworthy that Mg^{2+} has no effect at concentrations far greater than those present in our experiments from the vesicle preparation medium.

$^{59}Fe^{2+}$ uptake by duodenal brush-border membrane vesicles from normal and hypoxic mice

Studies of Fe^{2+} uptake by several different preparations of membranes revealed a great variability in absolute uptake, even though repeated determinations with the same preparation usually differed by less than 20%. This variability (Fig. 5) was observed even when several fresh preparations from individual mice were assayed in parallel on the same day or when duodenal plus jejunal scrapings of four to eight mice were pooled. This variability does not, however, affect qualitative results such as as are shown in Figs. 1–3. The origin of this variability is not known, however, the possibility that contaminating mitochondria (which can take up Fe^{2+} [13]) are responsible, is unlikely as Fe^{2+} uptake experiments with crude mitochondrial fractions isolated from homogenates prepared in the same manner as for the brush-border membrane preparation revealed that less than 10% of the uptake observed here could be accounted for by such mitochondrial contamination.

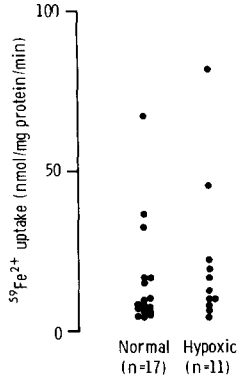

Fig. 5. Initial uptake of $^{59}Fe^{2+}$ by duodenal brush-border membrane vesicles from normal and hypoxic mice. Vesicles were incubated for 1 min at 37°C (as in Fig. 1a) with 91 μM $^{59}Fe^{2+}$ and 1.8 mM sodium ascorbate. Each data point is the mean of duplicate determinations. Vesicles were prepared from normal mice or mice subjected to 3 days of hypoxia.

Examination of several experiments revealed that initial uptake rate values from both normal and hypoxic-mouse brush-border membrane vesicles were not normally distributed [14] (see Fig. 5) and did not differ significantly following hypoxic treatment of the animals ($p > 0.2$) with non-parametric statistical tests [15]. Previous workers have found significantly enhanced Fe^{2+} and Fe^{3+} uptake by mouse brush-border membrane vesicles in iron deficiency [5], enhanced Fe^{2+} uptake by rabbit brush-border membrane vesicles in iron deficiency [2] and enhanced Fe^{3+} uptake by mouse brush-border membrane vesicles after 3 days of hypoxia [15]. Clearly the striking enhancements noted in iron deficiency [2,5] are not present in the hypoxic animal. However, the change observed in Fe^{3+} uptake by brush-border membrane vesicles in hypoxia [15] was small and a similar change could be obscured by the scatter of the data presented here. These results suggest that the adaptive mechanism leading to the enhancement of iron absorption in hypoxia may differ from that operating in iron deficiency.

Conclusions

Fe^{2+} uptake by mouse brush-border membrane vesicles demonstrates two components; a rapid, low capacity, high affinity binding component and a slower, high capacity transport component. The overall initial uptake rate does not show a significant change in brush-border membrane vesicles from hypoxic mice and the mechanism of enhanced iron absorption in hypoxia may differ from that in iron deficiency.

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