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Significance of non-esterified fatty acids in iron uptake by intestinal brush-border membrane vesicles

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Iron uptake from Fe/ascorbate by mouse brush-border membrane vesicles is not greatly inhibited by prior treatment with a variety of protein-modification reagents or heat. Non-esterified fatty acid levels in mouse proximal small intestine brush-border membrane vesicles show a close positive correlation with initial Fe uptake rates. Loading of rabbit duodenal brush-border membrane vesicles with oleic acid increases Fe uptake. Depletion of mouse brush-border membrane vesicle fatty acids by incubation with bovine serum albumin reduces Fe uptake. Iron uptake by vesicles from Fe/ascorbate is enhanced in an O_2-free atmosphere. Iron uptake from Fe/ascorbate and Fe^{3+}-nitrilotriacetate (Fe^{3+}-NTA) were closely correlated. Incorporation of oleic acid into phosphatidylcholine/cholesterol (4:1) liposomes leads to greatly increased permeability to Yb^{3+}, Tb^{3+}, Fe^{2+}/Fe^{3+} and Co^{2+}. Ca^{2+} and Mg^{2+} are also transported by oleic acid-containing liposomes, but at much lower rates than transition and lanthanide metal ions. Fe^{3+} transport by various non-esterified fatty acids was highest with unsaturated acids. The maximal transport rate by saturated fatty acids was noted with chain length C_{14-16}. It is suggested that Fe transport can be mediated by formation of Fe^{3+}(fatty acid)$_3$ complexes.

Introduction

Iron uptake by isolated intestinal brush-border membrane vesicles has been studied by several groups employing different mammalian species and incubation conditions. Eastham et al. [1] (rat), Cox and O'Donnell [2,3] (rabbit), Marx and Aisen [4] (rabbit) and Muir et al. [5,6] (mouse) employed Fe^{2+}-containing media, while the latter three groups and Simpson and Peters [7,8] (mouse) also studied uptake from stabilized Fe^{3+}-containing media. In most of these studies, at least some of the iron uptake was believed to represent transport across the brush-border membrane. Muir et al. [5] showed that the osmotic experiments used in earlier studies were not sufficient evidence that the uptake was, in fact, transport. Subsequently, Simpson and Peters [9] suggested a model for uptake of iron from Fe/ascorbate solutions which explained apparent contradictions in the earlier studies. This model suggested that uptake consisted of two processes, a rapid, low-capacity, high-affinity binding process and a slower, lower-affinity process sensitive to weak inhibition by NaCl. Evidence from Cox and O'Donnell [3] and Simpson and Peters [10] suggests that the latter

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

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uptake process may be subject to adaptive regulation in circumstances of altered in vivo iron absorption. Simpson and Peters [10] provided further evidence, based on inhibition by NaCl, pH dependence and the effect of membrane-acting agents (cholate, 8-hydroxyquinoline), that the uptake process did indeed represent transport of Fe across isolated brush-border membrane [10]. These workers subsequently showed that this transport process was quantitatively and qualitatively adequate to explain in vivo mucosal uptake of Fe from lumenal Fe/ascorbate solutions in mouse proximal intestine [11]. Further work demonstrated the presence of a cholate-extractable Fe-binding component of both mouse [10] and rabbit [12] proximal intestinal brush-border membrane vesicles. This component had Fe-binding properties (reversibility and pH- and NaCl-sensitive binding) consistent with a potential membrane carrier, and was purified 40-fold from rabbit duodenal brush-border membrane vesicles, and identified as a lipid [12].

Investigation of Fe binding by lipids from rabbit duodenal brush-border membrane vesicles and purified lipids identified non-esterified fatty acids as the Fe-binding lipid [13]. Studies of Fe permeability of model membranes (liposomes) [14] showed that non-esterified fatty acids, at levels reported in isolated intestinal brush-border membrane vesicles [15,16], could induce rapid membrane Fe^{2+} transport. The effects of medium pH and NaCl on this process were similar to those observed on low-affinity Fe uptake by isolated brush-border membrane vesicles.

In this paper non-esterified fatty acid levels in mouse and rabbit brush-border membrane vesicle preparations are reported and the influence of varying their levels on Fe uptake is investigated. The relationship between uptake of Fe from Fe/ascorbate and Fe^{3+}-NTA solutions is also considered. The mechanism of the transport process is investigated.

Materials and Methods

General

Iodoacetic acid, sodium cholate, oleic acid, palmitic acid, sodium ascorbate, bovine serum albumin (fraction V, fatty acid-free), mimosine, arsenazo III, $YbNO_3$ and $TbCl_3$ were from Sigma, 1,5-diphenylcarbazide, trinitrobenzenesulphonic acid and tetranitromethane were from Aldrich Chemical Co. (Gillingham, U.K.). Other chemicals were Analar grade from BDH Chemicals and other lipids were obtained as in Ref. 14. Animals were obtained and treated as in Refs. 7, 9 and 12. $^{59}FeCl_3$ and [1-^{14}C]oleic acid were from Amersham International (Amersham, U.K.).

Protein concentrations and enzyme activities were determined as in Ref. 7. Brush-border membrane vesicles were prepared from mouse small intestine (proximal 6 cm from the pylorus) as described in Ref. 7, and from frozen rabbit duodenum as described in Ref. 12. Fe uptake was determined from an isotonic ^{59}Fe-containing medium (0.1 M mannitol/0.1 M NaCl/20 mM Hepes (pH 7.25)) by the rapid Millipore filtration technique described in Refs. 7 and 9. Fe/ascorbate media were generally prepared, using air-equilibrated buffers, immediately before use in all experiments and preincubated for 5 min at 37 °C before uptake determinations. In some experiments, N_2-bubbled buffers were employed, $FeSO_4$ was substituted for $FeCl_3$, and uptake incubations were performed under N_2.

Mouse brush-border membrane vesicles (approx. 20 mg/ml) were treated with chemical modification reagents (0.9 mM trinitrobenzene sulphonate, tetranitromethane or iodoacetate) in resuspension buffer (0.1 M mannitol/0.1 M NaCl/20 mM Hepes (pH 7.4)/0.1 mM $MgSO_4$) for 30 min at 37 °C before 10-fold dilution with resuspension buffer. The resulting vesicle suspensions were employed for Fe uptake determinations as in Ref. 9 (final concentration of modification reagents was approx. 8.2 μM). Heat-treated vesicles were obtained by heating vesicle suspensions (approx. 2 mg/ml) for 5 min at 95 °C. Medium pH was varied and determined as described in Ref. 10. Experimental results are generally representative of experiments which were reproducible on at least three occasions.

Liposomes were prepared from egg phosphatidylcholine and cholesterol by sonication as in Ref. 14, substituting mimosine or arsenazo III for ferrozine. Egg phosphatidylcholine/cholesterol liposomes were relatively impermeable to Mg^{2+}, Ca^{2+}, Fe^{2+}/Fe^{3+}, Yb^{3+}, Tb^{3+} and Co^{2+}, and hence

were suitable for studies of fatty acid-mediated transport of these metal ions.

Modification of brush-border membrane vesicle non-esterified fatty acid levels

Rabbit duodenal brush-border membrane vesicles were loaded with oleic acid by a method similar to that described by Merrill et al. [17]. Vesicles (approx. 1 mg protein in 1 ml of resuspension buffer) were incubated with 5–250 μM oleic acid (2 mM stock solution sonicated in resuspension buffer) for 30 min at 37 °C. Vesicles were collected by centrifuging for 30 min at 40000 × *g* and washed once with 1 ml of resuspension buffer followed by suspension in 0.1 ml of the same buffer. Use of [^{14}C]oleic acid showed that more than 50% of medium oleic acid was recovered in the vesicle pellet (approx. 20% of vesicle protein was recovered, irrespective of oleic acid concentration).

Mouse brush-border membrane vesicles (approx. 1 mg protein) were depleted of fatty acids by incubation at 37 °C in 5 ml resuspension buffer containing fatty acid-free bovine serum albumin (20 mg/ml), with or without 0.45 mM sodium cholate, for 30 min before collection and washing as above. Control vesicle preparations were obtained by incubation and washing as above in the absence of albumin. Recovery of protein in control and albumin incubations was similar, suggesting negligible contamination of vesicles with albumin.

Determination of brush-border membrane vesicle non-esterified fatty acid levels

Non-esterified fatty acids were determined by the method of Falholt et al. [18]. A series of mouse vesicle preparations were also assayed by the above method, except that non-esterified fatty acids were first isolated by thin-layer chromatography [19]. The values obtained showed a similar range and distribution to those found by direct assay of whole vesicles; thus, the assay was routinely conducted without the thin-layer chromatography step.

Determination of thiobarbituric-acid-reactive material

Thiobarbituric-acid-reactive material was assayed after incubation of brush-border membrane vesicles for 30 min at 37 °C (100–1000 μg/ml protein/90 μM Fe/1.8 mM ascorbate/0.1 M NaCl/0.1 M mannitol/20 mM Hepes (pH 7.2)) with or without 50 μM butylated hydroxytoluene (0.01 vol. of 5 mM solution in ethanol). 2 vol. of ice-cold 10% (w/v) trichloroacetic acid were added and the mixture was centrifuged (5 min, 10000 × *g*, Eppendorf 5414 microcentrifuge). The supernatant was boiled for 15 min with an equal volume of 50 mM thiobarbituric acid and centrifuged, and absorbance at 535 nm was recorded. Appropriate blanks lacking Fe, vesicles or both were used to determine thiobarbituric-acid-reactive material production.

Metal ion transport by fatty acid-containing liposomes

Rates of transport using arsenazo-III-containing liposomes with Mg^{2+}, Co^{2+}, Ca^{2+}, Tb^{3+}, Yb^{3+} or Fe^{2+}/Fe^{3+} were obtained from changes in A_{650} using a Perkin Elmer 557 spectrophotometer as described in Ref. 20. Transport of metal ion, as opposed to leakage of arsenazo III, was verified after each determination by addition of excess EDTA as 0.01 vol. of 100 mM Na-EDTA (pH 7.4). Rates of change of A_{650} were converted to uptake of metal ions using calibration factors for each metal ion studied. Fe^{3+} transport from $Fe^{3+}(NTA)_2$ was determined with mimosine-containing liposomes by measuring increases in A_{446}.

Results and Discussion

Involvement of protein in Fe uptake by brush-border membrane vesicles

Marx and Aisen [4] reported that Fe uptake at low Fe/ascorbate concentrations was unaffected by prior boiling of rabbit brush-border membrane vesicles. We observed that Fe^{3+} uptake from Fe^{3+}-NTA by mouse duodenal brush-border membrane vesicles was enhanced by similar heat treatment [7]. Investigation of low-affinity Fe uptake from Fe/ascorbate showed that this is slightly enhanced by 5 min preheating at 95 °C (Table I). This enhancement may result from membrane damage with increased permeability.

A series of covalent protein-modification reagents (iodoacetate modifies thiol and histidyl moieties, trinitrobenzenesulphonate modifies

amino, thiol and histidyl groups and tetranitromethane reacts with tyrosyl side-chains [21]) were found to have little or no inhibitory effect on Fe uptake from Fe/ascorbate by mouse brush-border membrane vesicles (Table I). It should be noted that some of these modifiers may introduce Fe-binding groups or replace one possible Fe binding group with another. The demonstration of the usual long time-course uptake after these modifications was, however, suggestive of a transport process. While the lack of inhibition of uptake, especially by heat treatment, suggests that uptake is not protein-mediated, this cannot be regarded as conclusive evidence.

Correlation of Fe uptake with levels of non-esterified fatty acids

Fig. 1 shows a plot of Fe uptake against non-esterified fatty acid content in mouse brush-border membrane vesicle preparations. A close correlation between the two parameters was obtained by linear regression. It is noteworthy that, as reported previously [9], a wide distribution of Fe uptake values was observed, and it can be seen that it was the higher values which dominate the regression. We suggested previously [11] that these occasional very high vesicle uptake values may not be of physiological significance in vivo. Correlations do not prove causal relations; therefore, experiments in which vesicle fatty acid levels were selectively modified were conducted.

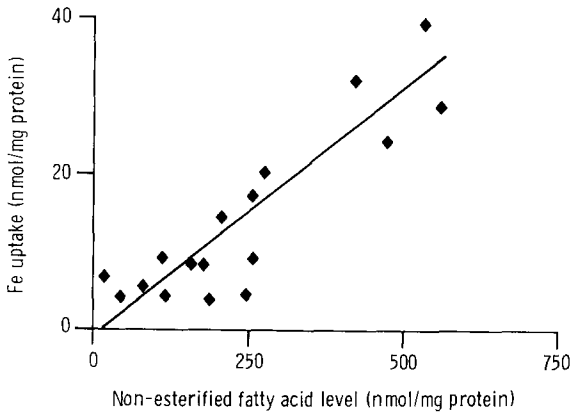

Fig. 1. Relationship between Fe uptake and non-esterified fatty acid content in mouse duodenal brush-border membrane vesicles. Fe uptake was determined by millipore filtration [7,9] after incubation at 37 °C in 91 μM ^{59}Fe/1.82 mM sodium ascorbate/0.1 M mannitol/0.1 M NaCl/20 mM Hepes (final pH 7.25). Non-esterified fatty acids were determined as in Ref. 18. The line was obtained by linear regression ($r = 0.912$, $n = 17$, $P < 0.001$).

TABLE I

EFFECT OF HEAT-TREATMENT AND PROTEIN-MODIFYING REAGENTS ON BRUSH-BORDER MEMBRANE VESICLE Fe UPTAKE

Vesicles were pretreated for 5 min at 95 °C or with 0.9 mM of the chemical-modification reagents for 30 min at 37 °C prior to Fe uptake determinations (see Materials and Methods section for details). Fe uptake was determined by Millipore filtration [7,9] after incubation at 37 °C in 91 μM ^{59}Fe/1.82 mM sodium ascorbate/0.1 M mannitol/0.1 M NaCl/20 mM Hepes (final pH 7.25). Data are mean ± S.E. for three separate experiments. *$P < 0.5$ compared to control.

Treatment	Fe uptake (% of untreated control)	
Time (min):	2	10
Tetranitromethane	91 ± 11	101 ± 6
Iodoacetate	95 ± 12	89 ± 6
Trinitrobenzenesulphonate	83 ± 1 *	77 ± 8
Heat treatment	165 ± 16 *	164 ± 21 *

Effect of oleic acid loading on uptake of Fe from Fe / ascorbate by rabbit duodenal brush-border membrane vesicles

Rabbit vesicles were found to have relatively low endogenous non-esterified fatty acid levels (72.9 ± 11.9 nmol/mg protein (S.E.M., $n = 4$)) compared with mouse (see Fig. 1). This value represents approx. 4.5% of total vesicle lipid, assuming 0.5 mg of lipid per mg of protein [15] and a mean molecular weight of 300 for fatty acids. These values suggest that rabbit vesicles would be suitable for loading with oleic acid. Incubation with ^{14}C-labeled oleic acid resulted in label becoming associated with the vesicles, confirming the observations of Merrill et al. [17]. These workers showed that Ca^{2+} binding by rabbit vesicles was enhanced by oleic acid incorporation, and we observed a clear relation between Fe uptake and increasing oleic acid level (Fig. 2) ($P < 0.01$ by linear regression). At higher non-esterified fatty acid levels, (more than 400 nmol/mg protein), Fe uptake was extremely rapid, and thus difficult to measure accurately. In addition, detergent effects of fatty acids may affect retention of

vesicles by millipore filters. These factors may explain the plateauing of uptake at very high (approx. 40% of total lipid) fatty acid levels. The non-linear rise in uptake over the range 90–400 nmol fatty acid/mg protein resembles the high-order dependence of Fe transport on fatty acid levels seen in model membrane systems (liposomes) [14]. That this dependence is not so clearly seen when the endogenous fatty acid level in vesicles is varied (Fig. 1) may relate to changes in fatty acid composition, as well as level. We observed [14] that different fatty acids appear to transport Fe at widely differing rates. This may also account for the higher level of uptake observed with oleic acid compared with endogenous fatty acids for a given fatty acid level (compare Fig. 1 and Fig. 2). We observed that oleic acid gave a higher rate of Fe transport than other fatty acids [14].

Investigation of the pH dependence of uptake revealed the same, characteristic optimum of 6.9 for both control and oleic acid-loaded vesicles. We have shown that Fe binding by non-esterified fatty acids shows a higher optimum (pH 7.1) [12,13] than transport by vesicles [10,12] or fatty acid-mediated transport by liposomes [14] (pH 6.9). The difference, though small, is highly reproducible and characteristic of transport as opposed to binding by fatty acids. These observations suggest

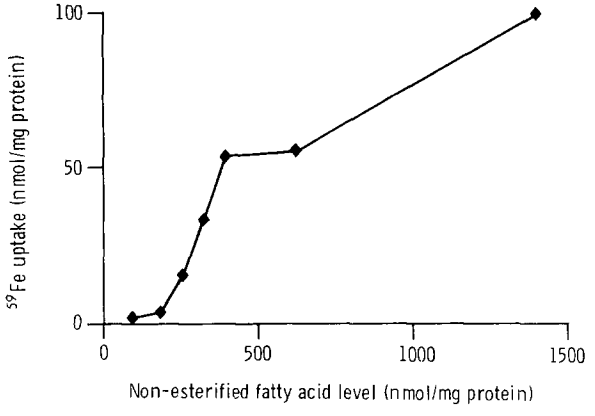

Fig. 2. Effect of oleic acid loading on Fe uptake by rabbit duodenal brush-border membrane vesicles. Vesicles were loaded by incubation with oleic acid followed by washing, and non-esterified fatty acids were determined as described in Materials and Methods. Incubation conditions for Fe uptake were as in Fig. 1.

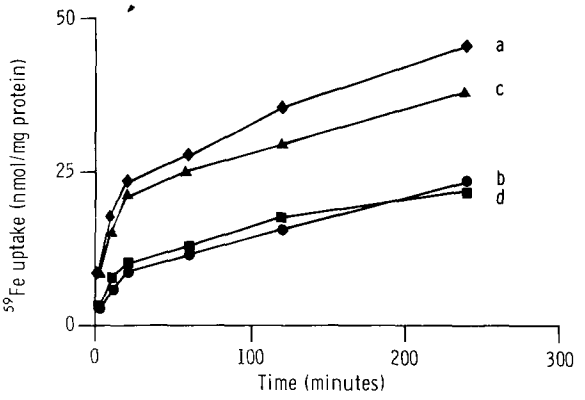

Fig. 3. Effect of prior incubation with bovine serum albumin on Fe uptake by mouse proximal intestine brush-border membrane vesicles. Vesicles were incubated and washed as described in Materials and Methods and Fe uptake and non-esterified fatty acid levels were determined as in Fig. 1. Fatty acid levels (nmol/mg protein) were: (a) 212 (control-incubated); (b) 42.7 (albumin-incubated); (c) 184 (cholate-incubated); (d) 39.9 (albumin- and cholate-incubated).

that the higher Fe uptake values observed in some unmodified vesicle preparations may be explained by the high fatty acid levels observed in these preparations. The role of fatty acids in Fe uptake over the lower, perhaps more physiological, range is best investigated by selectively lowering vesicle fatty acid levels. This was most easily achieved with mouse brush-border membrane vesicles which have higher endogenous non-esterified fatty acid levels than those from the rabbit.

Effect of lowering endogenous non-esterified fatty acid levels on uptake of Fe from Fe/ascorbate by mouse duodenal brush-border membrane vesicles

Incubation of mouse brush-border membrane vesicles with bovine serum albumin (which has high-affinity binding sites for fatty acids [22]) was found to deplete mouse brush-border membrane vesicles of non-esterified fatty acids (Fig. 3), although inclusion of cholate did not enhance this effect. Fe uptake by fatty acid-depleted vesicles was reduced compared with control-incubated vesicles (Fig. 3). This finding suggests that not only the very high Fe uptake values, but also the lower values, can be attributed to the presence of non-esterified fatty acids. Evidence presented in Ref. 11 suggests that vesicle uptake in this range of values is of in vivo significance. Further investi-

gation of the relationship between fatty acid levels observed in isolated brush-border membrane and the levels likely to occur in this membrane in situ in the living animal is required. It is noteworthy that intestinal brush-border membrane is more likely to encounter non-esterified fatty acids at high levels than cell membranes of other tissues.

Relationship between brush-border membrane vesicle uptake of Fe from Fe / ascorbate and Fe^{3+}-NTA solutions

While it is unlikely that Fe^{3+}-NTA solutions contain much Fe^{2+}, air-equilibrated Fe/ascorbate solutions may be expected to contain both Fe^{2+} and Fe^{3+}. We have shown that Fe binding from Fe/ascorbate solutions by fatty acids probably represents Fe^{2+} binding [13], and that Fe transport by fatty acid-containing liposomes relates to Fe^{2+} [14]. Fig. 4 shows that incubation under anaerobic conditions results in increased Fe uptake from Fe/ascorbate solutions by mouse brush-border membrane vesicles. This suggests that Fe uptake from Fe/ascorbate solutions may relate to Fe^{2+} present in the solution. Such experiments are, of course, difficult to perform in vivo and hence, previous studies have concentrated on air-equilibrated Fe/ascorbate solutions. Previously reported observations [7,9] show that uptake of Fe from both Fe^{3+}-NTA and Fe/ascorbate solutions relate to unchelated forms of Fe in the media. Uptake of Fe from both media is also weakly inhibited by NaCl [7,9]; however, the effects of pH and competing metal ions such as Co^{2+} or Ca^{2+} differ for the two media, probably because effects on the stability of the Fe^{3+}-NTA complex, and hence the unchelated Fe^{3+} concentration, predominate in that medium.

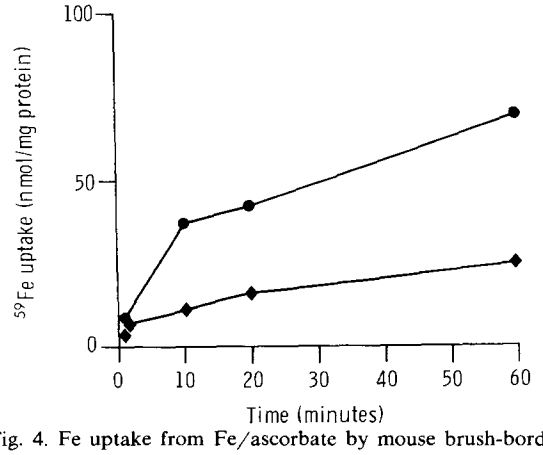

Fig. 4. Fe uptake from Fe/ascorbate by mouse brush-border membrane vesicles in the presence and absence of O_2. Media were as in Fig. 1 (◆) or prepared with $FeSO_4$, and all media were gassed thoroughly with N_2 and incubated in capped tubes flushed with N_2 (●).

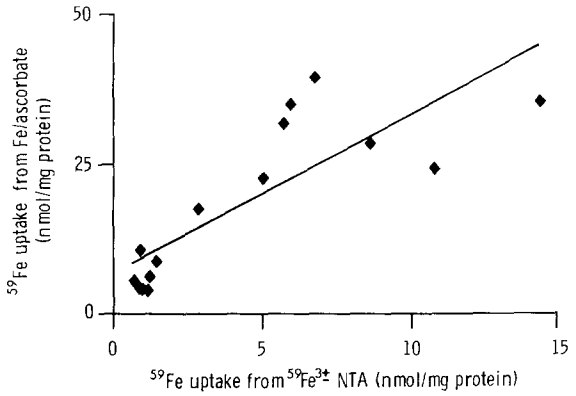

Fig. 5. Relationship between mouse duodenal brush-border membrane vesicle initial Fe uptake rate from Fe^{3+}-NTA and Fe/ascorbate solutions. Uptake from 400 μM $^{59}Fe^{3+}$/800 μM NTA/0.1 M mannitol/0.1 M NaCl/20 mM Hepes (pH 7.4) (incubation time 5 min) and from 91 μM ^{59}Fe/1.82 mM sodium ascorbate (incubation time 1 min) in the same medium (in place of Fe^{3+}/NTA) was determined as described in Refs. 7 and 9, respectively. The line was obtained by linear regression ($r = 0.825$, $n = 16$, $P < 0.001$).

Fig. 5 shows that vesicle Fe uptake rates from the two media, although quantitatively different, show a reasonable correlation. We have also found that the cholate-extractable Fe binder in rabbit duodenal brush-border membrane vesicles (subsequently identified as non-esterified fatty acids [13]) binds Fe from both Fe^{3+}-NTA and Fe/ascorbate media (unpublished observation). These results suggest that the same, non-esterified fatty acid-mediated mechanism can account for Fe uptake from both Fe^{3+}-NTA and Fe/ascorbate media. Further evidence that fatty acids can mediate Fe^{3+} transport was found in studies using liposomes (Fig. 6).

In order to observe transport, a potent, water-soluble Fe^{3+} chelator, giving a highly coloured Fe^{3+} complex, must be incorporated inside the liposomes. Mimosine was found to be a suitable chelator. Fig. 6 shows initial transport rates for

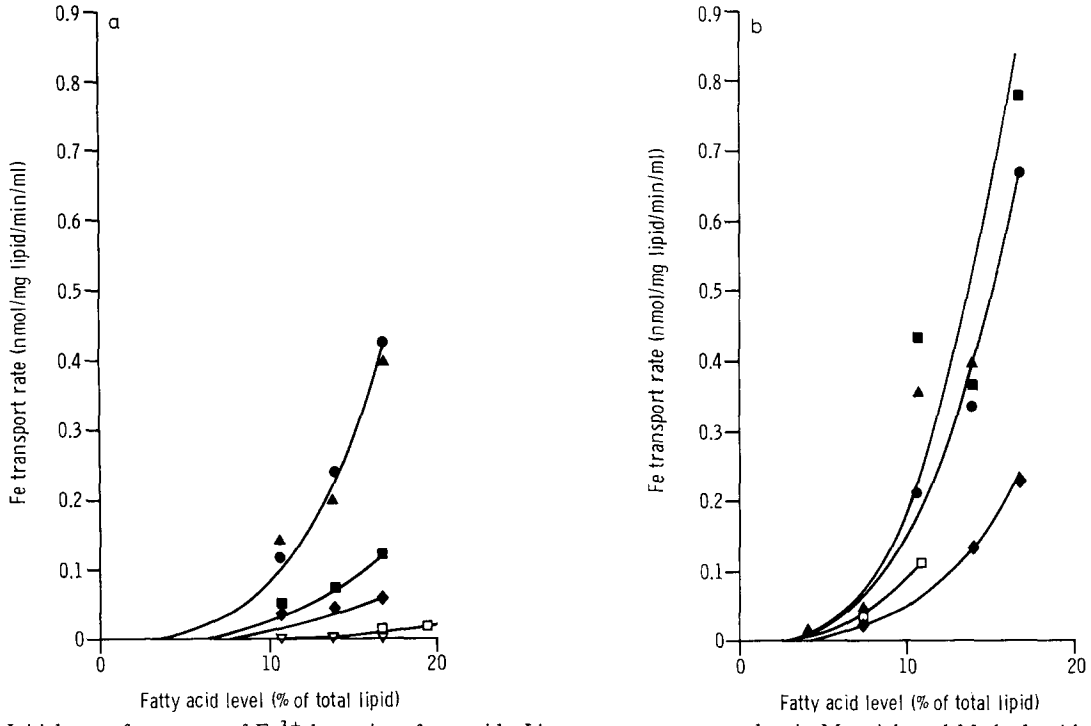

Fig. 6. Initial rate of transport of Fe^{3+} by various fatty acids. Liposomes were prepared as in Materials and Methods with various concentrations of different fatty acids and 50 mM mimosine in 0.1 M mannitol/60 mM NaCl/20 mM Hepes (pH 7.4) inside. Transport was measured from A_{446} after adding 0.1 vol. of 1 mM Fe^{3+}/2 mM NTA/0.1 M mannitol/0.1 M NaCl/20 mM Hepes (pH 7.4). Data points are means of duplicate determinations. (a) shows data for saturated fatty acids: (▽) capric; (□) lauric; (◆) arachidic; (■) stearic; (▲) palmitic; (●) myristic acid. (b) shows data for unsaturated fatty acids: (◆) arachidonic; (□) linoleic; (●) γ-linolenic; (▲) linolenic; (■) oleic acid.

various fatty acids from Fe^{3+}-NTA-containing media. The observed rates are lower than those noted previously with the Fe/ascorbate media [14], probably due to competition between NTA and fatty acids for Fe^{3+}. As shown previously [14], the transport rate shows a curvilinear dependence on liposome fatty acid level (Fig. 6) which can be fitted by a third-order (i.e., three fatty acids per Fe transported) kinetic model (see Ref. 14). Unsaturation of fatty acids generally leads to increased Fe transport (Fig. 6b cf. a). Transport by saturated fatty acids is maximal for chain length 14–16 carbon atoms.

Transport of other metal ions by non-esterified fatty acids

The above observations suggest that Fe^{3+} can be transported by non-esterified fatty acids. Fig. 4 and Ref. 14 may also suggest that Fe^{2+} can be transported by fatty acids, although the presence of Fe^{3+} in Fe/ascorbate media at pH 7.2 is difficult to prevent. In order to investigate this question further, transport of several metal ions was investigated using liposomes incorporating oleic acid (Table II).

The transport appears to be relatively selective for lanthanide and transition metal ions. The virtual absence of transport of Mg^{2+} and the effect of the addition of EDTA further confirmed that the observed transport of transition metal ions and Ca^{2+} is not due to leakage of arsenazo III. The rate of Ca^{2+} transport is in agreement with observations made by others [23]. It is clear that both divalent and trivalent metal ions can be transported by non-esterified fatty acids.

Mechanism of non-esterified fatty acid-mediated transport of Fe ions

The data presented here and previously [14] allow some insight into a possible mechanism for

TABLE II

UPTAKE RATES OF METAL IONS BY OLEIC ACID-CONTAINING LIPOSOMES

Liposomes (10 mg egg phosphatidylcholine/2.5 mg cholesterol/1 mg oleic acid) were prepared by sonication as in Ref. 14 in 0.5 ml 5 mM arsenazo III/0.1 M NaCl/20 mM Hepes (pH 7.25) followed by gel filtration [14] to remove extraliposomal arsenazo III. Metal-ion transport was determined from changes in A_{650} (compared to at 700 nm) after addition of 100 μM (0.01 vol. of a freshly prepared 10 mM solution of metal ion (as chloride, nitrate or sulphate salt) at $t = 0$ (see Materials and Methods). Incubation temperature was 37 °C. Data are mean ± S.E. for three separate experiments. Fe ions were introduced by adding freshly dissolved $FeSO_4$.

Metal	Initial uptake rate (nmol/mg lipid per ml per min)
Fe^{2+}/Fe^{3+}	17.3 ±2.9
Co^{2+}	2.03 ±0.47
Tb^{3+}	1.23 ±0.09
Yb^{3+}	1.03 ±0.07
Ca^{2+}	0.029 ± 0.006
Mg^{2+}	0.011 ± 0.004

this fatty acid-mediated metal ion transport. Oxidation of polyunsaturated fatty acids has been shown to generate ionophores [24], while the possibility of membrane damage due to lipid peroxidation could be important when Fe^{2+}/Fe^{3+} or Co^{2+} are present. The latter possibility is unlikely to explain the rapid transport of the other metals, especially Tb^{3+}. On the other hand, the fact that Fe is not transported by control egg phosphatidylcholine liposomes in spite of the presence of polyunsaturated fatty acid side-chains, yet is rapidly transported when saturated fatty acids are introduced into the membrane, also makes the possibility of a peroxidation/damage mechanism seem unlikely. Furthermore, the proposal that oxidized fatty acids can act as ionophores [24] specifically excluded oleic acid from being active. We demonstrated previously that the antioxidant butylated hydroxytoluene does not inhibit Fe uptake by brush-border membrane vesicles [9]. Assays of product of thiobarbituric acid-reactive material by vesicles on incubation with Fe/ascorbate showed that this antioxidant inhibited peroxidation by 88 ± 3% (S.E.M., $n = 4$). This suggests that vesicle Fe uptake is not a result of vesicle lipid peroxidation.

Other work suggests that it is the side-chain of the fatty acid, not the carboxyl group, which produces the structure responsible for metal ion transport by oxidized fatty acids [25]. Positive evidence that the fatty acid carboxyl group is important in the transport of Fe ions was presented in Ref. 14. The transport was inhibited by NaCl and H^+. Both effects could be attributed to competition between Fe ions and Na^+ or H^+ for fatty acids. In particular, the pH dependence of transport was consistent with the pK_a of non-esterified fatty acids in membranes. The apparent stoicheometry of three fatty acids per Fe^{3+} transported (Fig. 6) is consistent with formation of a neutral lipophilic Fe^{3+}(fatty acid)$_3$ complex as an intermediate in the transport. This mechanism is the simplest one which can explain the observations made here and in Ref. 14. The differing transport rates observed with various fatty acids (Fig. 6) suggests that transport rate relates to partition of the Fe^{3+}(fatty acid)$_3$ complexes in the lipid phase of the membrane. It is possible that transport of Fe from Fe/ascorbate media is mediated either via Fe^{3+}(fatty acid)$_3$ if traces of O_2 are present, or via Fe^{2+}(fatty acid)$_2$. The above results (Fig. 4, Table II) suggest both mechanisms are possible.

Conclusions

Studies of modulating non-esterified fatty acid content and correlation with non-esterified fatty acid levels in brush-border membrane vesicles shows that uptake of Fe from both Fe/ascorbate and Fe^{3+}-NTA media by rabbit and mouse proximal intestine vesicles can be explained by non-esterified fatty acid-mediated transport. Studies with liposomes show that non-esterified fatty acids can transport several different metal ions. The kinetics of Fe^{3+} transport are consistent with a mechanisms involving formation of neutral Fe^{n+}(fatty acid)$_n$ complexes.

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