Effect of ATP depletion and temperature on the transferrin-mediated uptake and release of iron by BeWo choriocarcinoma cells

Arie van der ENDE,*† Atala du MAINE,* Alan L. SCHWARTZ† and Ger J. STROUS*
*Department of Cell Biology, University of Utrecht, Nicolaas Beetsstraat 22, 3511 HG Utrecht, The Netherlands, and †Edward Mallinckrodt Departments of Pediatrics and Pharmacology, Washington University School of Medicine, Division of Pediatric Hematology-Oncology, Children's Hospital, St. Louis, Missouri 63110, U.S.A.

We have recently described the transferrin-mediated uptake and release of iron by BeWo cells [van der Ende, du Maine, Simmons, Schwartz & Strous (1987) J. Biol. Chem. 262, 8910–8916]. We now extend our studies of the mechanisms responsible for uptake and release of iron by these cells. Following preloading, 59Fe release was maximal (about 12%) after about 4 h. Replacement of the extracellular medium with an equal volume of fresh medium either prior to or following the time at which equilibrium was reached further stimulated 59Fe release. Both the rate and maximum amount of iron release decreased if longer loading times were used. Preincubation of BeWo cells for 15 min with 10 mm-sodium cyanide and 50 mm-2-deoxyglucose prior to the determination of 59Fe release did not alter the amount released into medium (which did not contain a high-affinity iron chelator). However, under these conditions, the uptake of 59Fe was dramatically inhibited as a result of prolongation of the transferrin–transferrin-receptor complex recycling time. These results demonstrate that the release of iron from BeWo cells is independent of cellular ATP levels, whereas iron uptake is ATP-dependent. Rates of both 59Fe release and 59Fe uptake were temperature-dependent. Analysis of these data via an Arrhenius plot suggests a single rate-limiting step for the release and uptake processes between 0 and 37 °C. The apparent energies of activation of these processes are very similar (approx. 59.0 kJ/mol for iron release and 50.6 kJ/mol for iron uptake), which raises the possibility that the release and uptake of iron share a common thermodynamically rate-limiting step. Possible mechanisms involved in iron release out of the cell and out of the endosome are discussed.

INTRODUCTION

All living cells, either prokaryotic or eukaryotic, require iron for growth. Iron is an essential component of many enzymes such as cytochromes, succinate dehydrogenase and iron–sulphur proteins, many of which function in the respiration chain. In multicellular organisms, iron is supplied to the cells by the serum protein ferrotransferrin. Transferrin is taken up by the cells via receptor-mediated endocytosis. The iron transport protein can bind either one or two iron atoms, and in its saturated form it has the highest affinity for its receptor at neutral pH 7.4. Receptor-bound ferrotransferrin is taken up by the cells via coated pits and coated vesicles (Aisen & Listowski, 1980; Young & Aisen, 1981), although there are suggestions that iron may also be taken up from transferrin prior to internalization (Thorstensen & Romslo, 1988). The internalized ferrotransferrin–receptor complex is transported intracellularly to a mildly acidic prelysosomal compartment in which the iron moiety dissociates from the polypeptide. Iron moves from this compartment into cellular stores, although the mechanisms are unknown. The apotransferrin–receptor complex remains intact at pH values below 6.5 and recycles to the plasma membrane where it dissociates and the apotransferrin is released into the medium at neutral pH (van Renswoude et al., 1982; Ciechanover et al., 1983; Dautry-Varsat et al., 1983; Klausner et al., 1983). In addition to the predominant intracellular storage forms as ferritin, iron may accumulate into other minor storage pools (Jacobs, 1977; Bakkeren et al., 1985; van der Ende et al., 1987). However, the mechanism of iron transfer to these pools and the relationship between them is still poorly understood.

Cells which function in iron transfer must be able to release accumulated iron, as seen in the macrophage (Saito et al., 1986), hepatocyte (Baker et al., 1981; Mostert et al., 1986) and Sertoli cell (Wauben-Pennis et al., 1988). The mechanism responsible for the release of iron by these cells is still unclear. Recently it was found that the release of iron by BeWo cells is not inhibited by the acidotropic drug primaquine or the ionophore monensin, and is stimulated by exogenous ligands (van der Ende et al., 1987).

We have used the choriocarcinoma cell line BeWo of trophoblastic origin as a model system to study the mechanism(s) which underlie transferrin-mediated iron uptake and subsequent iron release. The BeWo cell line, as originally isolated by Patillo & Gey (1968), exhibits

---

Abbreviations used: HCG, Human chorion gonadotropin; DTPA, diethylenetriaminepentaacetic acid; MEM, minimal essential medium; PBS, phosphate-buffered saline.

† To whom correspondence should be addressed.
many of the biochemical and morphological parameters of differen-
tiation associated with the development of syncytiotrophoblast
from cytrophoblast. BeWo cells synthesize and secrete a spectrum of placental hormones
including oestrogenic and progestational steroids, human
placental lactogen and human chorionic gonadotropin
(hCG). Exposure to methotrexate stimulates both alkaline
phosphatase and hCG production; it also causes some
degree of morphological differentiation, resulting in
formation of multinucleated cells which appear to re-
semble the syncytiotrophoblast of the placenta (Speer
et al., 1976; Friedman & Skehan, 1979). The syncyti-
un is the physical barrier between the foetal and maternal
blood circulations. Iron is transported unidirectionally
across this cell layer, which possess an exceptionally high
number of transferrin receptors on the microvilli. Iron is
taken up in a transferrin-dependent manner (Faulk
& Galbraith, 1979; Johnson et al., 1980), but maternal
transferrin does not cross the placenta (Gitlin et al.,
1964; Contractor & Eaton, 1986).

We have recently demonstrated the presence of a large
number of transferrin receptors (3.5 × 10⁶ per cell) and
the rapid accumulation of iron in these cells. The
accumulated iron is released as a low molecular mass
complex via a process which can be stimulated by
exogenously added iron chelators such as apotransferrin,
desferrioxamine and diethylenetriaminepenta-acetic
acid (DTPA) (van der Ende et al., 1987).

In the present study we have further dissected the
mechanism(s) of iron uptake and release by BeWo cells.
Replacement of the extracellular medium with fresh
medium caused an increase in ⁵⁹Fe release from preloaded
cells. Moreover, the release is more efficient when shorter
preloading times are used. The extent and kinetics of this
process suggest that the release occurs from a small,
rapidly releasable intracellular pool. The ⁵⁹Fe release
process is not energy-dependent and occurs over a wide
temperature range. However, while ⁵⁹Fe uptake is
dependent upon cellular ATP, the effect of temperature on
the rate of uptake is similar. The implications of these
processes for transcellular iron movement are discussed.

MATERIALS AND METHODS

Cells

The b24 clone of the human trophoblast-derived cell
line BeWo (Pattillo & Gey, 1968) (obtained from ATCC)
was grown in Eagle's minimal essential medium (MEM)
with 10% foetal calf serum as previously described (van
der Ende et al., 1987).

Materials

Iron-free human transferrin was purchased from
Calbiochem, and Eagle's MEM and foetal calf serum
were from Gibco. Na⁵⁴Cl and ⁵⁹FeCl₃ were obtained from
Amersham. 2-Deoxyglucose was from Sigma. Sodium
cyanide was from BDH.

Labelling of transferrin

Transferrin was fully saturated with iron and radio-
iodinated with ¹²⁵I as described previously (van der
Ende et al., 1987) (specific radioactivity was generally
~ 5 × 10⁶ d.p.m./mg). Loading transferrin with ⁵⁹Fe was
performed according to the procedure of van der Heul
et al. (1978) with specific radioactivity ~ 10⁶ d.p.m./mg.

Uptake of transferrin and iron

Uptake of ¹²⁵I-labelled ferrotransferrin and ⁵⁹Fe-
ferrotransferrin was usually carried out in 60 mm dishes.
Cells were incubated with 20 μg of transferrin/ml in 1 ml
of binding medium (Eagle's MEM buffered with 20 mm-
Na-Hepes, pH 7.4) at the temperature indicated. After
incubation, the cells were washed with ice-cold phos-
phate-buffered saline (PBS; 170 mm-NaCl/3 mm-KCl/
10 mm-Na₂HPO₄/2 mm-KH₂PO₄) and dissolved in 1 ml
NaOH as previously described (van der Ende et al.,
1987). The one-cycle experiments were carried out as
described in detail previously (Ciechanover et al., 1983;
vander Ende et al., 1987).

Cellular release of iron

The assay was essentially the same as described pre-
viously (van der Ende et al., 1987). Cells were pre-
loaded with ⁵⁹Fe by receptor-mediated endocytosis of
⁵⁹FeFerrotransferrin for 15 h. Thereafter, cells were
rapidly washed three times with PBS at 0 °C followed by
a 10 min incubation in sodium acetate buffer, (25 mm-
sodium acetate/150 mm-NaCl/2 mm-CaCl₂) pH 4.5, at
0 °C and subsequently three incubations in binding
medium for 30 min at 0 °C. After washing, the cells
were incubated at 37 °C with prewarmed and pregassed
(O₂/CO₂; 19:1) Eagle's MEM (with or without
additions) for the times and under the conditions
indicated in the text. At the appropriate times, samples
of the media and/or cells were evaluated for ⁵⁹Fe.

During all experiments, cell viability was monitored by
Trypan Blue exclusion.

RESULTS

Effect of extracellular media replacement on the release of iron

In an earlier study, we observed that the release of
preloaded iron by BeWo cells was stimulated by the
presence of exogenous iron chelators (van der Ende
et al., 1987). In the absence of added chelator, the release
of iron into the extracellular medium (MEM) reached
saturation after 2–4 h. Therefore, we examined the effect
of replacement and composition of the medium on
the release of iron. Cells were preloaded with ⁵⁹FeFerrotransferrin by overnight incubation. After ex-
tensive washing, the release medium (i.e. fresh MEM)
was added to the cells and the kinetics of ⁵⁹Fe release
were determined. As seen in Fig. 1, the release of ⁵⁹Fe is
a process which can be described as pseudo-first order.
At equilibrium (roughly 4 h), approx. 12% of the
preloaded ⁵⁹Fe is released. Replacement of the
extracellular medium with an equal volume of fresh
medium either prior to or following the time at which
equilibrium is reached (e.g. 180 or 300 min), further
stimulated ⁵⁹Fe release as seen in Fig. 1. In addition, the
total amount is proportional to the extracellular medium
volume, with increased release seen with larger volumes
(results not shown).

The presence of an iron chelator in the medium could
easily explain these observations. Since various amino
cids can serve as weak iron chelators (Schneider & Erni
1982) we examined the effect of alterations in the
composition of the medium on ⁵⁹Fe release. However,
there was no increase in ⁵⁹Fe release in MEM
supplemented with 2-fold essential amino acids when
Effect of ATP depletion and temperature on the iron metabolism of BeWo cells

Fig. 1. Effect of medium refreshment on the release of iron by BeWo b24 cells

Cells were preloaded with $^{59}$Fe by transferrin-mediated endocytosis for 16 h. After an extensive wash procedure, cells were reincubated in MEM for the times indicated. At time points 180 min and 300 min, the medium was changed for fresh medium (A), while in the control plates the medium was not changed (B). After incubation, the cells were washed with PBS and subsequently dissolved in 1 M-NaOH.

Compared to MEM alone (results not shown). Similarly, supplementation with 2-fold vitamins or 2-fold glucose failed to stimulate $^{59}$Fe release (results not shown), whereas $^{59}$Fe release is stimulated approx. 2-fold by the presence of exogenous chelator apotransferrin (2.5 μM) (van der Ende et al., 1987). Other potential iron chelators include both phosphate and citrate (Baker et al., 1981; Mostert et al., 1986; Andersson & Porath, 1986). Neither the absence or the presence of 1 mM-PO$_4$, or of 1 mM-citrate (not normally present in MEM) altered $^{59}$Fe release. All of these results taken together are consistent with the notion that in the absence of high-affinity iron chelator (e.g. 2.5 μM-apotransferrin), $^{59}$Fe release from BeWo cells is diffusion-limited.

Additional characteristics of the pool available for rapid release were examined by evaluating $^{59}$Fe release following different times of preloading. As seen in Fig. 2, the fraction of $^{59}$Fe release decreases with the loading time, as well as the rate of release (Fig. 2b). These data support the presence of an intracellular $^{59}$Fe pool which is rapidly loaded from external sources and from which iron is available for rapid release.

Effect of ATP depletion on iron release

The data presented above suggest that the iron release does not depend on cellular energy requirements. To investigate this, cells were incubated with sodium cyanide and 2-deoxyglucose in order to inhibit glycolysis and oxidative phosphorylation respectively and to deplete ATP levels. Following preloading of $^{59}$Fe for 16 h, BeWo cells were incubated with 10 mM-sodium cyanide and 50 mM-2-deoxyglucose for 15 min prior to the de-

Fig. 2. Effect of the preloading time on the release of iron by BeWo cells

Cells were washed with PBS and preincubated for 45 min in MEM at 37 °C. After preincubation, the cells were preloaded with $^{59}$Fe by incubation with 20 μg of $[^{59}$Fe]ferrotransferrin/ml for the times indicated. After the extensive wash procedure, the release was followed by incubation in MEM supplemented with 10% foetal calf serum (a). (b) log [(1 - $B_i$)/$B_{max}$] plotted against the time, where $B_i$ is the amount of $^{59}$Fe released at time point $t$, and $B_{max}$ is the maximal $^{59}$Fe released (30 min preloading time). Correlation coefficients vary between 0.980 and 0.998. Preloading times: ● 30 min; ○ 60 min; ■ 120 min; □ 300 min.

termination of $^{59}$Fe release. As seen in Fig. 3(a), there was only a small (less than 10%) effect on $^{59}$Fe release, when in MEM only. Additional experiments at concentrations up to 250 mM-sodium cyanide in the presence of 50 mM-2-deoxyglucose (results not shown)
Cells were preincubated for 15 min with 50 mM-2-deoxyglucose and 10 mM-sodium cyanide prior to the determination of the release or uptake of iron by BeWo cells. (a) Release. After preincubation with the drugs, iron release by cells was measured in MEM containing the drugs. (b) Uptake. After preincubation with the drugs, $[^{59}\text{Fe}]$ferrotransferrin (20 $\mu$g/ml) was added to the medium and incubated for the indicated times. Non-specific $^{59}\text{Fe}$ uptake was determined by adding a 50-fold excess of unlabelled transferrin.

and 250 mM-2-deoxyglucose in the presence of 10 mM-sodium cyanide (Fig. 4a) confirmed the lack of effect (i.e. less than 12\% inhibition) of these agents.

These results are in agreement with the results displayed in Fig. 1 and Fig. 2, and are consistent with the concept that in the absence of a high-affinity chelator, iron release from BeWo cells is diffusion-limited.
Fig. 5. Effect of ATP depletion on one cycle of endocytosis of transferrin by BeWo b24 cells

Cells were preincubated in 50 mM-2-deoxyglucose and 10 mM-sodium cyanide in MEM for 15 min. After preincubation cells were incubated with 10 µg of 125I-ferrotransferrin/ml in binding medium at 0 °C for 2 h, whereafter they were washed three times with ice-cold PBS. The cells were then warmed to 37 °C by incubation in binding medium supplemented with 100 µg of ferrotransferrin/ml. At the indicated times, medium was removed and the cells were washed with a sodium acetate buffer, pH 4.5, for 10 min and subsequently with binding medium for another 10 min, whereafter the cells were dissolved in 1 M-NaOH. The medium (∙), the acid/neutral wash (indicating the surface bound transferrin, ○) and the cells in 1 M-NaOH (□) were counted separately in a gamma-counter.

Effect of ATP depletion on iron uptake

In order to determine which step in transferrin-mediated iron uptake is inhibited by ATP depletion and to confirm the depletion of ATP stores in BeWo cells under these conditions, we examined the effect of these agents on receptor-mediated endocytosis and uptake of 59Fe from [59Fe]ferrotransferrin during receptor recycling, as this process is energy-dependent (Ciechanover et al., 1983; Clarke & Weigel, 1985; McBabee & Weigel, 1987). As expected, the uptake of 59Fe was dramatically inhibited in cells following incubation with 10 mM-sodium cyanide and 50 mM-2-deoxyglucose as seen in Fig. 3(b). Again, higher concentrations of up to 250 mM-sodium cyanide (+50 mM-2-deoxyglucose) (results not shown) and 250 mM-2-deoxyglucose (+10 mM-sodium cyanide) (Fig. 4a) did not further decrease the uptake of 59Fe. The uptake in the presence of the inhibitors is about 25% of the control level, most probably caused by a prolonged recycle time as observed with the asialoglycoprotein receptor (McBabee & Weigel, 1987). Independent evidence in support of this is seen in Fig. 5, in which the effect of ATP depletion on a single cycle of 125I-labelled ferrotransferrin uptake was examined. As demonstrated earlier, a single cohort of transferrin molecules loaded with iron enters cells, discharges iron to intracellular stores and recycles back to the extracellular medium (Ciechanover et al., 1983; van der Ende et al., 1987). However, as seen in Fig. 5, preincubation with sodium cyanide plus 2-deoxyglucose dramatically alters the normal ferrotransferrin cycle; 125I-labelled ferrotransferrin leaves the cell surface with the expected kinetics (half-time approx. 3–4 min, see Ciechanover et al., 1983; van der Ende et al., 1987). Extracellular transferrin accumulates from the cell-surface transferrin receptor with approx. 30% remaining in the media, consistent with the well-described kinetics of transferrin–transferrin receptor dissociation at 37 °C (Ciechanover et al., 1983). In addition, as expected, approx. 50% of the cell surface transferrin moves into the BeWo cell (Fig. 5). However, this pool of transferrin molecules remains within the cell and is not able to (or at a far lower rate) recycle back to the extracellular medium. This observation is consistent with confirmatory data in Fig. 4(b). Here we see that a 2 h preincubation with increasing amounts of 2-deoxyglucose (0–100 mM) plus 10 mM-sodium cyanide causes a depletion of the surface transferrin receptors (measured by 125I-ferrotransferrin binding at 0 °C) to approx. 30% of the control amount. The total number of transferrin receptors was measured by 125I-ferrotransferrin binding at 0 °C but in the presence of 0.1% saponin (Fallon & Schwartz, 1986). This detergent permeabilizes but does not solubilize the cell membrane, enabling macromolecules as large as 2 × 10^6 kDa to enter the cell (Shepherd et al., 1984). Saponin does not cause the loss of membrane-bound molecules (Simmons & Schwartz, 1984). As seen in Fig. 4(b), the total number of transferrin-binding sites remains virtually the same with increasing concentrations of 2-deoxyglucose. This means that in the presence of 2-deoxyglucose and sodium cyanide, only 10% of the transferrin receptors are at the cell surface instead of the normal 40%. Fig. 5 shows that the endocytosis rate is normal compared to control cells. To maintain the distribution of the transferrin receptors in drug-treated cells, the externalization rate has to be significantly reduced, and hence the recycle time of the receptor increased (Ciechanover et al., 1983). This is in fact observed: Fig. 5 shows that during the course of the experiment, no visible reduction of the intracellular transferrin is observed, while Fig. 3(b) shows a more than 4-fold reduction in iron uptake. In conclusion, iron uptake via the transferrin–transferrin-receptor cycle in BeWo cells is dependent upon cellular ATP levels. In this cycle the rate of endocytosis is normal, whereas the externalization is inhibited.

Effect of temperature on iron uptake and release

In order to further characterize the processes involved in the release of 59Fe from BeWo cells, we examined the effect of temperature on both 59Fe release and 59Fe uptake. As seen in Fig. 6, the rate of 59Fe release is temperature-dependent, with increases in the rate of release with elevated temperature. Analysis of the data using the Arrhenius equation (see Fig. 6b) demonstrates a linear relationship between the logarithm of the rate of 59Fe release and 1/temperature in degrees Kelvin. This suggests a single rate-limiting step for the release process between 0 and 37 °C. From these data, the apparent energy of activation of this process is approx. 59.0 kJ/mol. Similarly, from an analysis of the effect of temperature on the rate of 59Fe uptake in these same cells, the apparent energy of activation of the uptake process is approx. 50.6 kJ/mol (Fig. 6). The value of 59.0 kJ/mol for the apparent energy of activation for the uptake of 59Fe via transferrin-mediated endocytosis is in close agreement with the value found for the endocytosis of asialo-orosomucoid (Weigel & Oka, 1985) but sub-
stantially lower than the values reported by others for the endocytosis of several other proteins (Steinman et al., 1974; Kaplan & Nielsen, 1979; Dunn et al., 1980).

**DISCUSSION**

In an earlier study, we described that BeWo cells can release previously accumulated iron in significant quantities, whereas the cervical epithelial HeLa cells do not show significant release of their accumulated iron (van der Ende et al., 1987). The release of iron by BeWo cells is stimulated by exogenously added chelators such as apotransferrin, lactoferrin, DTPA and desferrioxamine. Lactoferrin, DTPA (unable to enter the cell) and desferrioxamine (able to enter the cell, Bottomley et al., 1985) are more or less competitive with apotransferrin in chelating the released iron. Moreover, when no chelators are present in the medium, the released iron has a low molecular mass form. The nature of this complex is unknown. It elutes in the included volume of a Sephadex G-100 gel-filtration column and is able to pass through a filter with a 30 kDa molecular mass cutoff (van der Ende et al., 1987). Here we show that the release of iron by BeWo cells is dependent on replacement of the release medium (a limiting component in the medium was excluded by varying the composition of the release medium). Also, the extent of iron release appeared to depend on the time of iron uptake, i.e. the shorter the time of iron uptake the more efficiently intracellular iron is released. These results, together with the earlier data, are interpreted as follows. Iron is taken up by the cell and intracellularly dissociated from transferrin in an acidic compartment (van Renswoude et al., 1982; Dautry-Varsat et al., 1983). The iron atoms are then transferred in an unknown way to a cytosolic pool which contains the iron in a low molecular mass form. From this pool, iron can be transferred to cytosolic ferritin for long-term storage or trafficked to the external medium. From the low molecular mass pool, the iron is rapidly released to the external medium until an equilibrium is reached across the plasma membrane. A rapidly releasable pool of iron has also been observed using rat Sertoli cells (Wauben-Pennis et al., 1988), rat hepatocytes (Octave et al., 1983) and macrophages (Saito et al., 1986). Release from the ferritin (high molecular mass) pool is much slower and occurs via the intracellular pool of rapidly releasable iron. This pool is in equilibrium with cytoplasmic ferritin (van der Ende et al., 1987), but can be stimulated by adding external chelators, which remove the low molecular weight iron from the medium. Release of iron, uncoupled from endocytosed transferrin, has also been observed recently in experiments *in vivo* using the perfused human placenta. It was shown that maternal transferrin is not transferred, but that iron is transferred to the foetal circulation where it becomes associated with foetal transferrin (Contractor & Eaton, 1986).

The uptake of iron by BeWo cells was severely inhibited following ATP depletion. Single-cycle experiments showed that the internalization was not inhibited. However, the externalization rate for apotransferrin appeared to be dramatically decreased and might even be completely inhibited. The decrease in the number of transferrin-binding sites from the cell surface after cellular ATP depletion confirmed this notion. This appears to be a general property of receptor-mediated endocytosis, as similar results have been observed for the asialoglycoprotein receptor (Clarke & Weigel, 1985; McAbbee & Weigel, 1987). These studies demonstrated that a single cycle of endocytosis of the asialoglycoprotein receptor was not influenced by ATP depletion, whereas the continuous recycling of this receptor was inhibited. In addition, the asialoglycoprotein receptor does not return to the cell surface, with loss of cellular ATP. Evidently the first steps in endocytosis (pinching off coated vesicles) need far less energy than later steps.

---

**Fig. 6.** Effect of temperature on the uptake and release of transferrin and iron by BeWo b24 cells

For uptake measurements (○), cells were preincubated in MEM at 37 °C for 60 min and were then incubated for 4 h at the indicated temperature with [59Fe]ferritransferrin in binding medium. After incubation, the cells were washed with binding medium three times and subsequently dissolved in 1 M-NaOH. Release (●) of iron at different temperatures was determined as follows. Cells were preloaded with 59Fe as indicated in the legend of Fig. 1. After washing, the cells were reincubated in binding medium at the indicated temperatures for 4 h. (a) Rate of uptake of release versus temperature. (b) Arrhenius plot, in which log rate is plotted against 1/temperature in K. The correlation coefficients are \( r = 0.979 \) (uptake) and \( r = 0.995 \) (release).
such as uncoating of the vesicles and acidification of endosomes. The results of studies in vitro are in agreement with these observations: energy required for pinching off the coated vesicles is driven by the free-energy change associated with the self-assembly of clathrin, whereas ATP is needed to uncoat coated vesicles (Schmid et al., 1984; Moore et al., 1987). Moreover, acidification of endosomes requires ATP-driven proton pumps (Forgac et al., 1983; Stone et al., 1983; Yamashiro et al., 1983).

The last step in iron release from BeWo cells is the transfer of a low molecular mass iron component across the plasma membrane. This process may be comparable to the release of iron atoms out of the acidic endosome during transferrin-mediated iron uptake, as the iron uptake and release show nearly identical dependence on temperature. The energies of activation obtained from the respective Arrhenius plots are in close approximation. Given the many assumptions which must be taken into account in any determination of apparent energies of activation of rate-limiting steps in overall processes (e.g. that the substrate for the overall process is saturating at all temperatures, etc.), it is surprising that the effects of temperature on these two processes are so similar.

This raises the issue as to whether the two processes share a common thermodynamically rate-limiting step. Specifically, the steps in which iron has to pass the membrane are most likely similar, i.e. when it moves out of the endosome into the cytosol and out of the cytosol into the medium respectively. How the iron atoms are transferred out of the acidic compartment after dissociation from transferrin during iron uptake and out of the cytosol during iron release is still unclear. Iron is bound to transferrin in the Fe(III) form. Release of iron [in the Fe(III) form] from transferrin can be established by lowering the pH to about 5.5, as occurs in the mildly acidic endosome within the cell (van Renswoude et al., 1982; Dautry-Varsat et al., 1983). Thereafter, the iron atoms have to be transferred to the cytosol. This may occur via the Fe(II) form. This is also the form of iron which enters cytosolic ferritin, after which the iron is oxidized to the Fe(III) form [for a recent overview see Crichton & Charlotte-Wauters (1987)]. Indeed, a transferrin reductase which is apparently associated with the transferrin receptor has been implicated in transferrin-mediated iron uptake, and might convert ferric iron released in the acidic compartment to ferrous iron (Sun et al., 1987). As seen above, iron which has entered the cytosol may have two fates (i.e. to ferritin for long term storage, or to the medium upon release). The form in which iron is released is unknown, but is chelatable by apotransferrin, DTPA and desferrioxamine. Apotransferrin binds only ferric iron [but can accept both ferric iron and ferrous iron: the latter is oxidized by the protein; Koijoma & Bates (1981)]. However, DTPA and desferrioxamine are specific Fe⁺ chelators. Thus, either the iron in the cytosol is in the Fe(III) form, or is converted to the Fe(II) form in the extracellular medium, e.g. when transferred across the plasma membrane.

Remarkably, the release of iron out of the cytosol into the external milieu is not inhibited by ATP depletion. The results obtained with replacement of the release medium support this. Moreover, we have previously shown that the release of iron by BeWo cells is not inhibited by primaquine or monensin, while iron uptake was inhibited under the same conditions, suggesting that vesicular transport is not involved in the release of iron (van der Ende et al., 1987). One attractive possibility is that the redox reaction of Fe(II)/Fe(III), which is linked to membrane-located electron transport, is the driving force in the release of iron from cells. Another possibility is that a carrier-mediated mechanism is operative in iron transport into the cytoplasm, as well as in iron release out of the cytoplasm and into the extracellular medium. Further studies will be necessary to compare both processes and resolve their mechanisms.

The authors wish to thank Drs. Paulette Wauben-Penris and Willem Stoovogel for their stimulating discussions and critical comments. This research was supported in part by Grant GM 38284 from the National Institutes of Health, Grant 0316/87 from the North Atlantic Treaty Organization, and by grants from The National Foundation, The Konigin Wilhelmina Fonds, the American Cancer Society and the American Heart Association. A.L.S. is an established investigator of the American Heart Association.

REFERENCES


Vol. 259
Pattillo, R. A. & Gey, G. O. (1968) Cancer Res. 28, 1231–1236

Received 10 August 1988/17 November 1988; accepted 3 January 1989


A. van der Ende and others