Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 2258–2262, April 1983
Cell Biology

pH and the recycling of transferrin during receptor-mediated endocytosis

(endocytic vesicle/apotransferrin/transferrin receptor)

ALICE DAUTRY-VARSAT*, AARON CIECHANOVER, AND HARVEY F. LODISH

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Communicated by Joseph L. Goldstein, December 27, 1982

ABSTRACT **At pH 5.4, apotransferrin (iron-free transferrin) binds to cell-surface transferrin receptors to the same extent and with the same affinity as does diferric transferrin at pH 7.0. Apotransferrin is quickly dissociated from its receptor when the pH is raised to 7.0. These and other results strongly support a simple model that explains the cycling of transferrin during a single cycle of receptor-mediated endocytosis. Diferric transferrin binds to cell-surface receptors, and the transferrin–receptor complex is endocytosed. The pH of the endocytic vesicle is lowered to 5.5 or below; this causes dissociation of iron from the transferrin–receptor complex, but apotransferrin remains bound to its receptor. The iron remains within the cell, and the apotransferrin–receptor complex is recycled to the cell surface. Upon encountering the neutral pH of the medium, apotransferrin is dissociated from the cell.**

During receptor-mediated endocytosis, ligands bind to specific cell-surface receptors and are internalized in membrane-limited endocytic vesicles (1, 2). Most ligands studied to date are degraded within the cell; examples include asialoglycoproteins, insulin, epidermal growth factor, low density lipoprotein, and human choriogonadotropin (3–8). Although the exact pathway followed by such ligands is not known, many receptor–ligand complexes enter the cell through clathrin-coated pits and vesicles. Dissociation of receptor and ligand then occurs, probably in a prelysosomal vesicle (9–14), and the ligand is transported in a series of vesicles to lysosomes, wherein it is degraded (1, 2, 4, 6–8). Less is known about the pathway taken by the receptor. In several systems the receptor is not degraded but recycles to the cell surface; such is the case for the receptors to asialoglycoprotein (15), insulin (16, 17), α_2 macroglobulin (18), mannose (19), mannose 6-phosphate (20) and low density lipoprotein (21).

The fate of transferrin is different. Transferrin (22), a major mammalian serum glycoprotein, transports iron from sites of absorption and storage to tissue cells. The first step of iron delivery involves binding of ferrotransferrin to specific cell-surface receptors (23) that are found on all growing cells. This iron-loaded transferrin is subsequently internalized by endocytosis, and the iron is delivered to the cell (24–28). However, apotransferrin (iron-free transferrin) is not degraded within the cell, but is exocytosed intact into the medium (ref. 29; unpublished data). Is apotransferrin dissociated from its receptor within the cell? If so, how does it escape degradation by the lysosome and how is it secreted into medium? Or does transferrin remain bound to its receptor in endocytic vesicles? If so, how and when is apotransferrin released from its receptor into the culture medium?

Endocytic vesicles which contain α_2-macroglobulin or transferrin are acidic, with a pH of about 5 (12, 30). In this paper we demonstrate the unique pattern of binding of transferrin and apotransferrin to its receptor as a function of pH. These studies allow us to formulate a model for delivery by transferrin of iron into the cell and for recycling of apotransferrin to the medium.

MATERIALS AND METHODS

Cells. The maintenance of the human hepatoma cell HepG2 (31), on which all experiments were conducted, has been described (32).

Labeling of Proteins. Human transferrin was purchased from Calbiochem. $Na^{125}I$ and $^{59}FeCl_3$ were obtained from the Radiochemical Centre and desferrioxamine (desferal) was obtained from CIBA Pharmaceutical. Transferrin was saturated with iron just prior to iodination as described by Karin and Mintz (27). The degree of iron saturation, as assessed by absorption at 465 nm, was more than 90%. Transferrin, insulin, and asialoorosomucoid were iodinated as described (27, 33). Labeled transferrin and asialoorosomucoid were then filtered through a Sephadex G-25 column equilibrated with 20 mM Na Hepes/0.15 M NaCl, pH 7.3, and were stored at −70°C.

For loading transferrin with ^{59}Fe, 0.8 μmol of $^{59}FeCl_3$ was neutralized with sodium hydroxide; 16 μmol of nitrilotriacetic acid was added, and the solution was neutralized again. After the solution was boiled for 5 min under nitrogen and cooled on ice, the solution was added to 0.36 μmol of transferrin dissolved in 100 μl of 0.1 M $NaClO_4$/20 mM $NaHCO_3$/10 mM Tris·HCl, pH 7.6. After a 1-hr incubation at room temperature, the mixture was chromatographed on a Sephadex G-25 column (1 × 50 cm) equilibrated in 20 mM Tris·HCl (pH 7.6). [^{59}Fe]Transferrin (diferric) was recovered and dialyzed overnight in 20 mM Hepes (pH 7.5).

Iodinated unloaded transferrin (^{125}I-apotransferrin) was prepared prior to each experiment from iodinated iron-loaded transferrin (^{125}I-transferrin). To a solution of ^{125}I-transferrin, NaOAc was added to a final concentration of 0.1 M and a final pH of 4.9, and desferrioxamine was added to a final concentration of 100 μM; after 2 hr of incubation at room temperature, 1 M NaOH was added to a final concentration of 65 mM to neutralize the apotransferrin solution.

Protein Determination. Quantitations of cellular protein were carried out as described by Lowry *et al.* (34). The total cell protein of 4×10^6 HepG2 cells is 1 mg.

Binding Assays. Binding assays were performed in 35-mm tissue culture dishes containing $3–5 \times 10^6$ cells (70–90% confluence). Binding assays with [^{59}Fe]transferrin were performed in 60-mm tissue culture dishes grown to the same ex-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: CURL vesicle, compartment of uncoupling of receptor and ligand.

* On leave from the Institut Pasteur, Paris, France.

tent of confluence. Prior to binding assays, cells were washed twice at 4°C in binding medium [protein-free minimal essential medium (GIBCO) containing 20 mM Hepes (pH 7.3) gassed with 95% CO_2/5% O_2]; they were then incubated in the same medium for 30 min at 37°C to ensure that any receptor-bound transferrin would be dissociated. Unless otherwise stated, binding reactions were carried out in duplicate 35-mm dishes at 4°C for 2 hr; each dish contained 1 ml of binding medium with 30 nM, 30 nM, or 40 nM, of ^{125}I-transferrin, ^{125}I-labeled insulin, or ^{125}I-labeled asialoorosomucoid, respectively. After being washed with unbound ligand, the cells were solubilized in 1 M sodium hydroxide, and radioactivity was assayed in a Packard gamma counter. Nonspecific binding, determined by performing the binding assay in the presence of a 200-fold excess of the appropriate unlabeled ligand, was less than 10% of the specific binding and was subtracted from all data.

Binding at pH 5.4 was performed in protein-free minimal essential medium (GIBCO) containing 25 mM NaOAc (pH 5.4).

RESULTS

Transferrin Bound to Its Receptor Unloads Its Iron at Acid pH but Remains Bound to Its Receptor. The two bound iron atoms are dissociated from diferric transferrin at a pH of less than 5 (22, 35, 36). Because the pH of endocytic vesicles is also less than 5, we wondered whether iron from receptor-bound transferrin is also dissociated at acid pH and, more importantly, whether the resultant apotransferrin dissociates from its receptor under these conditions. To this end, either ^{125}I-labeled ferrotransferrin or [^{59}Fe]transferrin was bound at 4°C to HepG2 cells at neutral pH. Unbound ligand was removed, and the cells were incubated at 4°C for 10 min in the presence of 50 μM desferrioxamine, a potent iron chelator, at different pH values ranging from 4.5 to 7.5. The amount of radioactivity associated with the cells and present in the incubation buffer was determined. Treatment at acid pH in the presence of iron chelator released the iron associated with transferrin, but the transferrin protein itself remained tightly bound to its receptor (Fig. 1). Upon prolonged incubation at pH 5.0, all of the iron could be removed, (first-order rate constant of ≈ 0.12 min^{-1}).

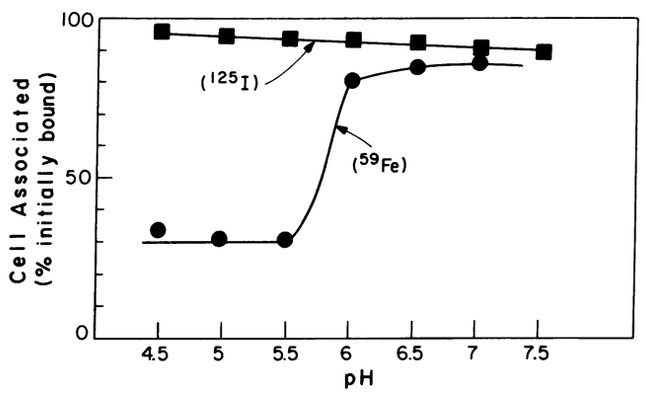

FIG. 1. Treatment of diferric transferrin bound to HepG2 cells at 4°C with buffers of various pH. ^{125}I-Transferrin (■) or [^{59}Fe]transferrin (●) was bound to HepG2 cells at 4°C in binding medium. After unbound ligand was removed, the cells were incubated at 4°C for 10 min in buffers at different pH values; the buffer was collected, the cells were dissolved in 1 M NaOH, and radioactivities in both were assayed. Buffers at pHs 4, 4.5, 5, and 5.5 were composed of 25 mM NaOAc/0.15 M NaCl/2 mM $CaCl_2$/50 μM desferrioxamine; buffers at pH 6, 6.5, and 7 were 25 mM Na Pipes/0.15 M NaCl/2 mM $CaCl_2$/50 μM desferrioxamine; and the buffer at pH 7.5 was 25 mM Na Hepes/0.15 M NaCl/2 mM $CaCl_2$/50 μM desferrioxamine. Initial binding of ^{125}I-transferrin was 275 fmol/mg of protein and of [^{59}Fe]transferrin was 285 fmol/mg of protein.

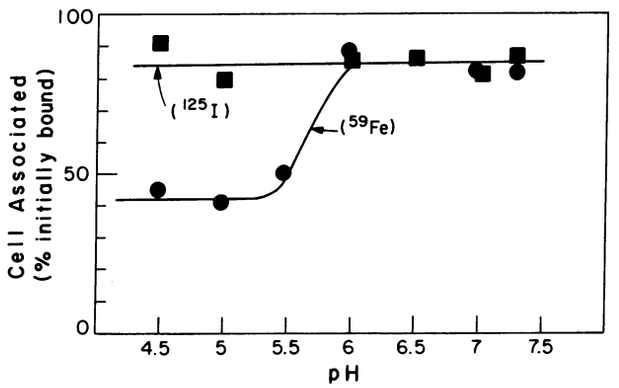

FIG. 2. Treatment at various pHs of diferric transferrin bound to HepG2 cells at 37°C in the presence of energy inhibitors. ^{125}I-transferrin (■) and [^{59}Fe]transferrin (●) were bound at 4°C to HepG2 cells in binding medium containing 10 mM NaCN and 20 mM 2-deoxyglucose. Excess ligand was removed. The cells were then incubated for 15 min at 4°C in phosphate-buffered saline containing 10 mM NaCN and 20 mM 2-deoxyglucose; this buffer was removed, and to each 35-mm dish, 2 ml of incubation buffer prewarmed to 37°C was rapidly added while the dishes were transferred to a 37°C water bath. After 2 min at 37°C, the buffer was collected; radioactivity in the cells was counted as well as that in the buffer. The incubation buffers were the same as in Fig. 1 and contained in addition 10 mM NaCN and 20 mM 2-deoxyglucose. Initial binding of ^{125}I-transferrin was 275 fmol/mg of protein and of [^{59}Fe]transferrin was 285 fmol/mg of protein.

A similar result was obtained at 37°C (Fig. 2). It was necessary to add 10 mM NaCN and 20 mM 2-deoxyglucose to the incubation medium to completely block the internalization of transferrin bound to its receptor: the presence of these energy inhibitors did not affect the binding of transferrin in any way (K_d, maximum binding) (data not shown). A 2-min incubation at 37°C was chosen because, at pH 7 and at 37°C, diferric transferrin dissociated from its cell-surface receptor with a first-order constant of 0.092 (half-time of 7.5 min; data not shown). Upon prolonged incubation at pH 5, all of the iron was released from membrane-bound transferrin with a first-order rate constant of about 0.46 or a half-time of 1.5 min (data not shown). We conclude that treatment at acid pH in the presence of an iron chelator will remove iron from transferrin bound to its receptor but will not remove transferrin from that receptor.

Insulin and Asialoglycoprotein Are Dissociated from Their Receptor at Acid pH. In addition to transferrin receptor, asialoglycoprotein (32) and insulin receptors (33) are present on the

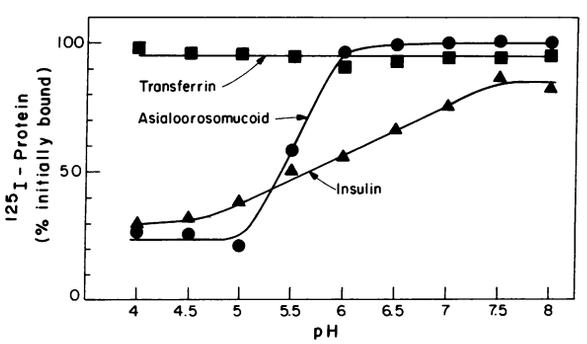

FIG. 3. Treatment at 4°C at various pHs of transferrin, insulin, and orosomucoid bound to HepG2 cells. ^{125}I-Labeled insulin (▲), ^{125}I-transferrin (■), and ^{125}I-labeled asialoorosomucoid (●) were bound to HepG2 cells at 4°C. After washing off unbound ligand, the cells were incubated at 4°C for 5 min at different pHs. The buffers were the same as in Fig. 1 but contained no desferrioxamine. The buffer at pH 8 was 25 mM Na Hepes/0.15 M NaCl/2 mM $CaCl_2$. ^{125}I-Protein, ^{125}I-labeled protein.

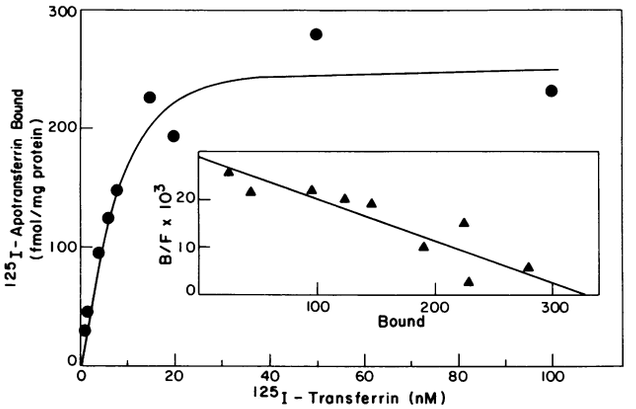

FIG. 4. Binding of apotransferrin at 4°C and pH 5.4 to HepG2 cells. ^{125}I-Apotransferrin was prepared, and binding was measured at the indicated concentrations in minimal essential medium buffered at pH 5.4 in the presence of 50 μM desferrioxamine. (*Inset*) Scatchard analysis of the same data. B/F, bound/free.

surface of HepG2 cells. The effect of acid pH on these receptor–ligand complexes is shown in Fig. 3. Insulin, asialoorosomucoid, and transferrin, each labeled with ^{125}I, were bound to cells at 4°C. Excess unbound ligand was removed, and the cells were treated for 10 min at 4°C in buffers with pH values ranging from 4 to 8. The stability of the receptor–transferrin complex was not affected by pH, whereas both insulin and asialoorosomucoid are dissociated from their respective receptors at pHs of 5.0 or less. This result underscores the marked differences between the transferrin–receptor complex and other ligand–receptor complexes.

Apotransferrin Binds Tightly to Its Receptor at Acid pH but Does Not Bind at Neutral pH. The above results suggest that apotransferrin has a high affinity for its receptor at pH 5–5.5. The experiment in Fig. 4 shows this directly. Scatchard (37) analysis of the data gave 58,000 binding sites for iron-free transferrin per cell and an apparent dissociation constant $K_d = 13 \times 10^{-9}$ M. The binding assay was performed in the presence of 50 μM desferrioxamine to prevent loading of apotransferrin with iron present in buffers. Fig. 5 shows the binding of iron-loaded transferrin at pH 5.4 to HepG2 cells. Scatchard analysis of these data showed 35,000 transferrin binding sites and an apparent dissociation constant $K_d = 13 \times 10^{-9}$ M. These numbers are similar to those obtained for binding of iron-loaded transferrin to HepG2 cells at neutral pH (60,000 receptors with $K_d = 7 \times 10^{-9}$ M; ref. 33).

Importantly, apotransferrin shows very little detectable specific binding to cell-surface receptors at pH 7.3. At the

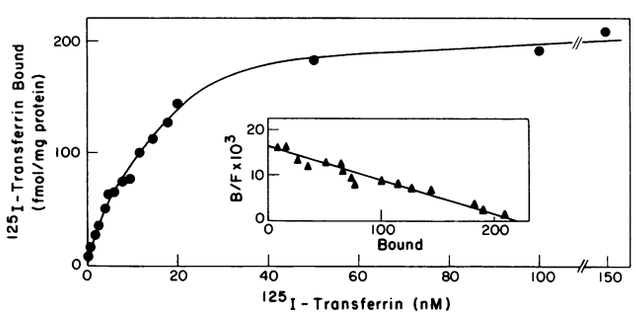

FIG. 5. Binding of transferrin at 4°C and pH 5.4 to HepG2 cells. Binding of ^{125}I-transferrin was measured at the indicated concentrations in minimal essential medium buffered at pH 5.4. (*Inset*) Scatchard analysis of the same data. B/F, bound/free.

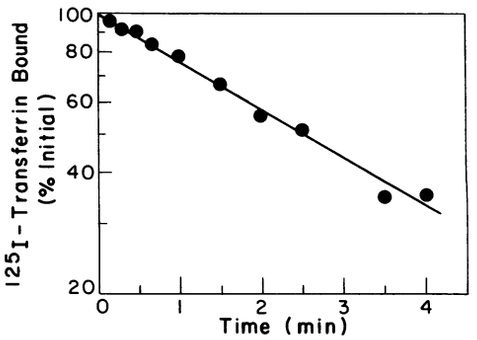

FIG. 6. Dissociation of apotransferrin from HepG2 cells at 4°C. ^{125}I-Transferrin was bound to HepG2 cells at 4°C and excess ligand was removed. The cells were then treated at 4°C for 10 min in 25 mM NaOAc, pH 5/0.15 M NaCl/2 mM $CaCl_2$/50 μM desferrioxamine to remove iron from transferrin. This acidic solution was removed, and binding medium containing 50 μM desferrioxamine and unlabeled diferric transferrin (10 μg/ml) at 4°C was added for different times. This medium was collected, and the radioactivity in the medium and the cells was assayed.

saturating concentration for holotransferrin (30 nM), the binding of apotransferrin is at most 5% of that of holotransferrin. If apotransferrin is added to cells in the presence of 50 μM desferrioxamine and 100 μM ferrous ammonium citrate to overcome the desferrioxamine, it becomes loaded with iron and binds to cells as well as does holotransferrin (data not shown). As an additional control, we showed that the presence of 50 μM desferrioxamine does not affect the binding of iron-loaded transferrin or of asialoorosomucoid to HepG2 cells (data not shown).

A final experiment (Fig. 6) showed that apotransferrin, bound to its receptor at pH 5.4, is rapidly dissociated when the pH is raised to 7.3. Diferric transferrin was bound to HepG2 cells at 4°C and pH 7.3, and excess ligand was removed. Transferrin bound to its surface receptor was stripped of iron by incubation of the cells for 10 min at 4°C at pH 5 in the presence of 50 μM desferrioxamine. This treatment caused loss of iron, but the apotransferrin remained bound to its receptor. The acidic buffer was removed, and the cells were incubated at neutral pH and at 4°C for different times (Fig. 6). Apotransferrin, bound to its receptor at acid pH, dissociated from that receptor at pH 7.3 with a half time of 2.5 min at 4°C. (The rate of dissociation was the same whether or not unlabeled transferrin was included in the dissociation reaction.) In contrast, diferric transferrin dissociated from its surface receptor under these conditions with a half-time of 90 min. At 37°C, the respective half-lives were 16 sec and 7.5 min (data not shown).

DISCUSSION

Ligands that enter a cell by receptor-mediated endocytosis are rapidly found in small intracellular vesicles. Tycko and Maxfield (12) showed that α_2 macroglobulin is found in an intracellular nonlysosomal compartment with an internal pH of about 5. More recently, Van Renswoude *et al.* (30) showed that, after endocytosis, transferrin is found within an acidic intracellular compartment that can be separated from lysosomes by density gradient fractionation.

The present experiments have been carried on HepG2 cells, which bear on their surface 60,000 high-affinity receptor sites for transferrin ($K_d = 7 \times 10^{-9}$ M). At 4°C, transferrin binds to these receptors, but is not internalized. After warming to 37°C, about 40% of surface-bound diferric transferrin is eluted intact into the binding medium (with a half-time of 7.5 min).

The balance is internalized, presumably into endocytic vesicles, with a half-time of about 3 min. All of the internalized iron remains within the cells, but all of the internalized iron-free transferrin protein is secreted into the medium, with a half-time of about 8 min (unpublished data).

The present studies have clarified the fate of receptor-bound diferric transferrin in such an acidic compartment. At pH values from 4.5 to 7.5, transferrin remains bound to its receptor (Figs. 1–3). The binding of iron to transferrin is destabilized at acid pH (22, 35, 36). At pH 5–5.5, in the presence of desferrioxamine, iron is rapidly removed from transferrin bound to its receptor. Importantly, apotransferrin remains tightly bound to its receptor at these acidic pHs. When the apotransferrin–receptor complex is subjected to neutral pH, apotransferrin rapidly dissociates from the receptor, with a half-time (2.5 min at 4°C, or 16 sec at 37°C) that is 30–40 times faster than that for the diferric transferrin–receptor complex (90 min at 4°C and 7.5 min at 37°C) (unpublished data).

These results suggest a novel model for the delivery of iron to cells and for the cycling of transferrin (Fig. 7). Iron-loaded transferrin binds to its receptor on the cell surface at neutral pH; under these conditions, binding of apotransferrin is negligible. After binding, diferric transferrin is internalized by receptor-mediated endocytosis. Within 2–6 min, the transferrin–receptor complex moves to an acidic nonlysosomal compartment. There, perhaps in the presence of an iron-chelating component, iron is released from transferrin; we do not know how the iron is transported across the membrane of the vesicle into the cytoplasm. Apotransferrin remains bound to its receptor, and together they are recycled to the cell surface. Upon reaching neutral pH, (either at the cell surface or just prior to it in an intracellular vesicle), apotransferrin rapidly dissociates from its receptor. The free receptor on the cell surface is available for another cycle of receptor-mediated endocytosis. The released apotransferrin will be transported in the blood to a loading site where two ferric ions will be re-added.

Other ligands subjected to receptor-mediated endocytosis, such as insulin, asialoglycoproteins, low density lipoproteins, and lysosomal enzymes, are transported in intracellular vesicles to lysosomes while their receptors recycle to the cell surface. The compartment in which dissociation of asialoglycoproteins from their receptor occurs has been identified (13, 14). These vesicles, called "CURL" (compartment of uncoupling of receptor and ligand) contain ligand within their lumen. Little receptor is found in the vesicle membrane; most of the receptor is concentrated in detached tubular membranous extensions that are free of ligand. CURL vesicles appear to transform into secondary lysosomes wherein the ligand is degraded. The tubular vesicles appear to be an intermediate in recycling of the asialoglycoprotein receptor to the surface. We assume that CURL vesicles have a pH less than 5.5. All of the above-mentioned ligands are released from their respective receptors at pH values less than 5–5.5 (Fig. 3; 3, 20, 38), and low density lipoprotein does not bind to its specific receptor below pH 6 (39). Transferrin is unique in that it does not dissociate from its receptor at pH values below 5.5. We speculate that the diferric transferrin–receptor complex enters CURL vesicles, wherein the iron is lost; the apotransferrin–receptor complex would enter the tubular vesicles associated with the CURL and, thus, would be recycled to the cell surface. To test this model, it will be necessary to conduct immuno-electron-microscopic studies (14), in which the fates of transferrin and its receptor are followed in the same cell.

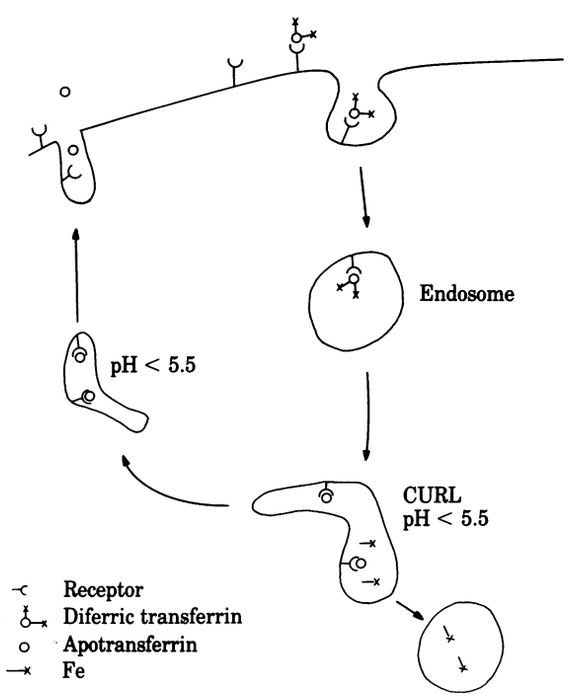

FIG. 7. The transferrin cycle. See text for details.

At pH 5.4, the binding constant of apotransferrin to the transferrin receptor ($K_d = 13 \times 10^{-9}$ M) is similar to that for binding of diferric transferrin to the same receptor at neutral pH ($K_d = 7 \times 10^{-9}$ M; ref. 33). We can detect little specific binding of apotransferrin to the transferrin receptor at pH 7.3, and we estimate that the dissociation constant is greater than 7×10^{-7} M. Bridges *et al.* (29) also found no binding of apotransferrin to surface receptors at neutral pH. Workers who claim binding of apotransferrin to cell-surface receptors at neutral pH (27, 40) do not utilize iron chelators in the binding reaction; under these conditions, we have found that holotransferrin is generated by the trace levels of iron present in all salt solutions. Our results suggest that the conformation of apotransferrin at pH 5.4 is similar to that of diferric transferrin at neutral pH, especially that of the receptor binding site. In contrast, the conformation of apotransferrin or of its receptor, or both, at pH 5.4 must be different from the conformations at neutral pH. Further comparison of the conformation and physiochemical properties of apo- and diferric transferrin, and of the transferrin receptor at neutral and acidic pH are needed to understand these pH-dependent changes of affinity between transferrin and its receptor.

We are grateful to Dr. Richard Klausner for making his data available to us prior to publication. We thank Miriam Boucher for preparation of the manuscript. This work was supported by Grants AI-08814 and GM27981 from the National Institutes of Health. A.D.-V. is the recipient of a fellowship from the International Union Against Cancer. A.C. is supported by the Melvin Brown Memorial Fellowship through the Israel Cancer Research Fund and by a postdoctoral fellowship from the Leukemia Society of America.

1. Goldstein, J. L., Anderson, R. G. W. & Brown, M. S. (1979) *Nature (London)* **279**, 679–685.
2. Pastan, I. H. & Willingham, M. C. (1981) *Annu. Rev. Physiol.* **43**, 239–250.
3. Ashwell, G. & Morell, A. G. (1974) *Adv. Enzymol.* **41**, 99–128.
4. Bridges, K., Harford, J., Ashwell, G. & Klausner, R. D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 350–354.
5. Tsai, J. R. & Seeman, M. (1981) *Biochim. Biophys. Acta* **673**, 259–269.
6. Carpenter, G. & Cohen, S. (1976) *J. Cell Biol.* **71**, 159–171.

7. Goldstein, J. L. & Brown, M. S. (1977) *Annu. Rev. Biochem.* **46**, 897–930.
8. Ascoli, M. & Puett, D. (1978) *J. Biol. Chem.* **253**, 4892–4899.
9. Willingham, M. C. & Pastan, I. (1980) *Cell* **21**, 67–77.
10. Wall, D. A., Wilson, G. & Hubbard, A. L. (1980) *Cell* **21**, 79–93.
11. Pastan, I. H. & Willingham, M. (1981) *Science* **214**, 504–509.
12. Tycko, B. & Maxfield, F. R. (1982) *Cell* **28**, 643–651.
13. Schwartz, A. L., Geuze, H. J. & Lodish, H. F. (1982) *Proc. R. Soc. London Ser. B*, in press.
14. Geuze, H. J., Slot, J. W., Strous, G. J. A. M., Lodish, H. F. & Schwartz, A. L. (1982) *Cell* **32**, 277–287.
15. Harford, J. & Ashwell, G. (1982) in *The Glycoconjugates*, ed. Horowitz, M. (Academic, New York), Vol. 4, pp. 27–55.
16. Krupp, M. & Lane, M. D. (1981) *J. Biol. Chem.* **256**, 1689–1694.
17. Fehlmann, M., Carpenter, J.-L., Van Obberghen, E., Freychet, P., Thamm, P., Saunders, D., Brandenburg, D. & Orci, L. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 5921–5925.
18. Van-Leuven, F., Cassiman, J.-J., & Van-den-Bergh, H. (1980) *Cell* **20**, 37–43.
19. Stahl, P., Schlesinger, P. H., Sigardson, E., Rodman, J. S. & Lee, Y. C. (1980) *Cell* **19**, 207–215.
20. Gonzalez-Noriega, A., Grubb, J. H., Talkad, V. & Sly, W. S. (1980) *J. Cell Biol.* **85**, 839–852.
21. Brown, M. S., Anderson, R. G. W., Basu, S. K. & Goldstein, J. L. (1982) *Cold Spring Harbor Symp. Quant. Biol.* **46**, 713–721.
22. Aisen, P. & Listowski, I. (1980) *Annu. Rev. Biochem.* **49**, 357–393.
23. Jandl, J. H. & Katz, J. H. (1963) *J. Clin. Invest.* **42**, 314–326.
24. Sullivan, A. L., Grasso, J. A. & Weintraub, L. R. (1976) *Blood* **47**, 133–143.
25. Morgan, E. H., Huebers, H. & Finch, C. A. (1978) *Blood* **52**, 1219–1228.
26. Octave, J.-N., Schneider, Y.-J., Crichton, R. R. & Trouet, A. (1981) *Eur. J. Biochem.* **115**, 611–618.
27. Karin, M. & Mintz, B. (1981) *J. Biol. Chem.* **256**, 3245–3252.
28. Octave, J.-N., Schneider, Y.-J., Hoffmann, P., Trouet, A. & Crichton, R. R. (1982) *Eur. J. Biochem.* **123**, 235–240.
29. Bridges, K., van Renswoude, J., Kempf, C., Ashwell, G. & Klausner, R. D. (1983) *J. Biol. Chem.*, in press.
30. Van Renswoude, J., Bridges, K. R., Harford, J. B. & Klausner, R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 6186–6190.
31. Knowles, B. B., Howe, C. C. & Aden, D. P. (1980) *Science* **209**, 497–499.
32. Schwartz, A. L., Fridovich, S. E., Knowles, B. B. & Lodish, H. F. (1981) *J. Biol. Chem.* **256**, 8878–8881.
33. Ciechanover, A., Schwartz, A. L. & Lodish, H. F. (1983) *Cell* **32**, 267–275.
34. Lowry, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
35. Princiotto, J. V. & Zapolski, E. J. (1975) *Nature (London)* **255**, 87–88.
36. Lestas, A. N. (1976) *Br. J. Haematol.* **32**, 341–350.
37. Scatchard, G. (1948) *Ann. N.Y. Acad. Sci.* **51**, 660–672.
38. Posner, B. I., Josefsberg, Z. & Bergeron, J. J. M. (1978) *J. Biol. Chem.* **253**, 4067–4073.
39. Basu, S. K., Goldstein, J. L. & Brown, M. D. (1978) *J. Biol. Chem.* **253**, 3852–3856.
40. Ward, J. H., Kushner, J. P. & Kaplan, J. (1982) *J. Biol. Chem.* **257**, 10317–10323.