Transferrin Receptor of the Rabbit Reticulocyte†

Adela Leibman and Philip Aisen*

ABSTRACT: A Triton X-100 solubilized macromolecular complex of transferrin and a membrane constituent can be isolated by gel chromatography from rabbit reticulocytes previously incubated with ^{125}I-labeled transferrin. The apparent molecular weight of this complex is close to that of ferritin, or about 445 000. On sodium dodecyl sulfate gel electrophoresis the complex displays two glycoprotein subunits, of molecular weights 176 000 and 95 000, in addition to transferrin. A transferrin-binding fraction with a molecular weight near 400 000, containing these subunits, can also be identified in membranes of nonincubated reticulocytes. The corresponding membrane fraction from mature erythrocytes, which have lost transferrin-binding activity, displays both protein subunits, but the 176 000 molecular weight component fails to give a PAS stain for carbohydrate. Treatment of reticulocytes with Pronase, which destroys the ability of the cells to form specific complexes with transferrin, degrades both components. We believe these results are consistent with the hypothesis that the primary transferrin receptor of the rabbit reticulocyte is a glycoprotein of molecular weight in the range 350 000–400 000, comprised of a combination of two subunits with molecular weights 176 000 and 95 000, respectively. Transferrin-binding activity appears to depend on the carbohydrate moiety of the 176 000 subunit.

Transferrin is the major, and perhaps the only, source of iron for the biosynthesis of hemoglobin by the reticulocyte (Jandl et al., 1959; Hemmaplardh and Morgan, 1974). The initial event in the delivery of iron from protein to cell is the binding of transferrin to specific receptors on the reticulocyte surface (Jandl et al., 1959; Jandl and Katz, 1963). These receptors are vulnerable to proteolytic attack, so that cells treated with Pronase or trypsin no longer take up iron or bind transferrin, although other metabolic functions appear to be intact (Jandl et al., 1959; Hemmaplardh and Morgan, 1975). As the reticulocyte matures into an adult red cell it loses, along with its ability to synthesize hemoglobin, its capacity to interact with transferrin. The fate of the receptor during cell maturation is not known.

Using a variety of detergents to solubilize the stroma of reticulocytes incubated with ^{125}I-labeled transferrin, Garrett et al. (1973) were able to demonstrate a labeled fraction with an apparent molecular size substantially greater than that of transferrin, and presumably representing the transferrin-receptor complex. This intriguing observation prompted study of the transferrin receptor in a number of laboratories (van Bockxmeer et al., 1975; Fielding and Speyer, 1974, 1975; Witt and Woodworth, 1975; Sly et al., 1975), but a generally accepted model of its properties has not yet emerged. In this paper, we present the results of our studies on some properties of the transferrin receptor of the rabbit reticulocyte. Our particular concerns are with its size, subunit structure, and the nature of the receptor-inactivating alterations it undergoes as the cell matures or is treated with proteolytic enzymes.

Materials and Methods

Reagents were the highest quality commercially available. Triton X-100 was obtained from Rohm and Haas, sodium dodecyl sulfate (Sequanal grade) was from Pierce Chemical Co., and acrylamide and *N,N'*-methylenebisacrylamide were from Eastman Kodak Co. Sephadex G-200 was supplied by Pharmacia Fine Chemicals, and Ultragel AcA22 by LKB Instruments, Inc. Other chemicals were analytical grade. Yeast alcohol dehydrogenase, bovine liver catalase, and porcine thyroglobulin were purchased from Schwarz/Mann, horse spleen ferritin was from Miles Laboratories, and rabbit muscle phosphorylase *a* was from Worthington Biochemicals. RNA polymerase from *Escherichia coli* was a gift from Zaharia Hillel.

Transferrin was isolated from rabbit serum prepared from freshly drawn blood or supplied by Pel-Freeze (type 2), by the method of Baker et al. (1968). Labeling of transferrin with ^{59}Fe (to 95% saturation) and ^{125}I was carried out by standard procedures with precautions taken to remove iron not specifically bound to the protein (Harris and Aisen, 1975; Katz, 1961).

Reticulocytes were obtained from rabbits bled four or five times (12–15 mL/kg of body weight each time) during the week prior to an experiment. Reticulocyte counts were generally in the range of 20–25%. With heparin as an anticoagulant, plasma and buffy coat of freshly drawn blood were removed by aspiration after centrifugation for 6 min at 2500*g*. Red cells were then washed with isotonic phosphate buffer (pH 7.4) as specified by Dodge et al. (1963), prior to incubation with transferrin or preincubination with Pronase.

Incubation of reticulocytes with labeled transferrin (1.5 mg/mL of total volume) was carried out at a hematocrit of 50% in Hank's solution enriched with 0.1% glucose. Incubation was stopped after 30 min by addition of 4 vol of ice-cold isotonic phosphate buffer, following which cells were washed three times in the cold with the same buffer.

Preincubation of reticulocytes with Pronase was carried out for 10 min at 37 °C in isotonic phosphate buffer, using 0.4 mg of the enzyme preparation per mL of cell suspension.

Reticulocyte ghosts were prepared by the methods of Dodge et al. (1963). Solubilization of the membranes was accomplished by taking 0.4 vol of washed ghosts to 1 vol with 5

† From the Departments of Biophysics and Medicine, Albert Einstein College of Medicine, New York, New York 10461. *Received August 12, 1976.* This work was supported, in part, by Grant No. AM-15056 from the National Institutes of Health.

imosm[1] phosphate buffer and sufficient Triton X-100 to achieve a final concentration of 1% (v/v). This was incubated at 4 °C for 1 h and then centrifuged for 1 h at 0 °C and 48 000*g*. Over 90% of the radioactivity in the ghosts was recovered in the supernatant, 1 mL of which was taken for column chromatography. Although other detergents were of equal or greater effectiveness in solubilizing reticulocyte stroma, Triton X-100 was chosen for our studies because, in the concentrations used, it appeared to have minimal effect on the iron-binding properties of transferrin.

Chromatography of the solubilized reticulocyte membranes was attempted on columns of Sepharose 2B and Sepharose 6B, following the methods of Speyer and Fielding (1974). Because we could not achieve satisfactory resolution of the putative transferrin-receptor complex from free transferrin with Sepharose as the support medium, we turned to the use of Ultragel AcA22. Columns (1.6 × 100 cm) were equilibrated with 1% Triton X-100 in 5 imosm phosphate buffer (pH 7.4) containing 0.02% sodium azide, and the same buffer was used as the eluting solvent. The temperature of the column was maintained at 18 °C. Fractions approximately 1 mL in volume were collected at a flow rate of 3 mL/h. Blue dextran was used to measure void volume (49 mL), and Dnp-alanine to estimate the included volume (145 mL) of the column. K_d was calculated from the usual formula (Fish, 1975). To achieve maximum accuracy in characterizing elution behavior, fractions were weighed (Fish et al., 1969; Fish, 1975). This overcame difficulties due to variations in volume of individual fractions collected during runs. Reference standards for calibrating the column were radioiodinated by the method of Katz (1961).

Transferrin-binding activity of reticulocyte membrane fractions was assayed as follows. Membranes from 5 mL of packed, washed, nonincubated reticulocytes were isolated and solubilized as described and then chromatographed on the column of AcA22 gel. Fractions were collected and pooled in 5-mL quantities. Each pooled volume was incubated with 160 μg of ^{125}I-labeled transferrin for 10 min at 37 °C and then concentrated to a volume near 1 mL by ultrafiltration using the Schleicher and Schuell collodion membrane apparatus. This concentrated sample was then applied to a 1.6 × 35 cm column of Sephadex G-200, equilibrated with 1% Triton X-100 in 5 imosm phosphate buffer (pH 7.4), and eluted with the same buffer. Free and "bound" transferrin are well separated in such a column (van Bockxmeer et al., 1975), so that the amount of radioactivity recovered just after the void volume provides a measure of the relative transferrin-binding activity of the fraction.

Electrophoretic analysis of membrane fractions, including staining procedures, was carried out according to the procedures of Fairbanks et al. (1971). Removal of Triton X-100 prior to electrophoresis resulted in sharper electrophoretic patterns. This was accomplished by adding sodium dodecyl sulfate to a concentration of 0.1% to the fraction to be studied and passing it through a 0.9 × 11 cm column of BioBeads SM-2 equilibrated with 0.01 M Tris–0.001 M EDTA buffer (pH 8.0), containing 0.1% sodium dodecyl sulfate (Holloway, 1973). Samples were then concentrated 10 times by vacuum dialysis against Tris–EDTA–sodium dodecyl sulfate buffer (Fairbanks et al., 1971) and taken for electrophoresis after addition of dithiothreitol. Gels were calibrated for molecular weight estimations with thyroglobulin (subunit mol wt 335 000), RNA polymerase (β,β′ subunits, average mol wt 160 000), phosphorylase *a* (92 500), transferrin (77 000), catalase (57 500), ovalbumin (43 000), and RNA polymerase (α subunit, 40 000).

[1] Abbreviations used are: imosm, ideal milliosmolar; Dnp, dinitrophenyl; EDTA, ethylenediaminetetraacetic acid; PAS, periodic acid–Schiff's reagent.

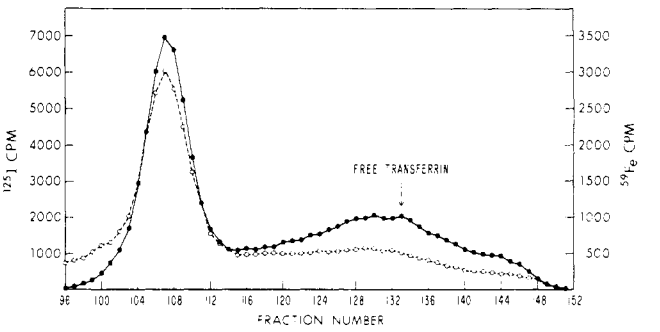

FIGURE 1: Gel filtration chromatogram of Triton X-100 solubilized reticulocyte membranes prepared from cells incubated with doubly labeled transferrin. The eluting buffer was 5 imosm phosphate (pH 7.4) containing 1% Triton X-100. The column, measuring 1.6 × 100 cm, was maintained at 18 °C: (●-●) ^{125}I; (O- - -O) ^{59}Fe.

Results

Identification of a High Molecular Weight Transferrin-Bearing Component in the Membranes of Reticulocytes Incubated with Transferrin. Attempts to reproduce with rabbit reticulocytes the results reported by Speyer and Fielding (1974) for human reticulocytes were not successful. Using Sepharose 2B and Sepharose 6B as support media we could not obtain adequate resolution of the putative transferrin–receptor complex from free transferrin. The former generally appeared as a shoulder or broadening on the high molecular weight side of the free transferrin peak. The use of Sephadex G-200, which resulted in satisfactory separation of free transferrin and the transferrin–receptor complex from reticulocytes, was also unsatisfactory because the complex eluted close to the void volume and outside the linear operating range of the gel. Accordingly, we turned to LKB AcA 22 gel as a support medium, with results presented in Figure 1. About 55% of the total ^{125}I activity applied to the column eluted as a single, symmetrical peak very near the elution position of ferritin (mol wt 445 000), well resolved from the peak corresponding to free transferrin, and well within the operating range of the gel. This corresponds to 22% of the total receptor activity of intact reticulocytes from which membranes were isolated for this experiment. Recovery of ^{59}Fe in the eluted fractions paralleled the appearance of ^{125}I, although the ratio of the isotopes differed in the two peaks. When the ratio of ^{59}Fe to ^{125}I in the transferrin used for incubation is normalized to unity, a ratio of 0.77 is obtained for the isotopes in the peak of the heavy transferrin-containing fraction, and a ratio of 0.54 for the isotopes in the lighter fraction.

In order to obtain an estimate of the size of the heavy transferrin fraction, the AcA 22 column was calibrated with the following reference standards (molecular weight given in parentheses): porcine thyroglobulin (669 000), horse spleen ferritin (445 000), bovine liver catalase (232 000), and yeast alcohol dehydrogenase (150 000). The Triton X-100–phosphate buffer was used for development of the chromatogram in each case. A straight line was obtained in a semilogarithmic plot of the molecular weight vs. K_d of these standards, lending some credence to the reliability of the gel filtration procedure for estimating molecular size. The putative complex of transferrin and its receptor consistently eluted with a K_d value identical or close to that of ferritin. Free transferrin did not fall

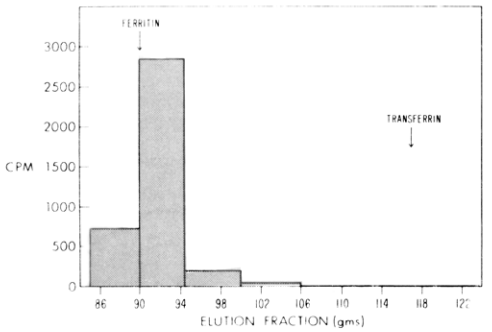

FIGURE 2: Relative transferrin-binding activity of reticulocyte membrane fractions. Membranes were solubilized with Triton X-100 and then fractionated on the column described in Figure 1.

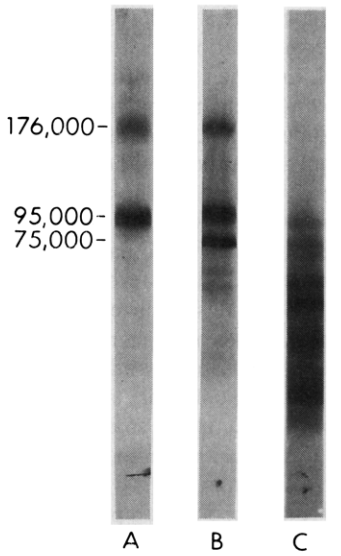

FIGURE 3: Sodium dodecyl sulfate gel electrophoresis patterns stained with Coomassie blue of Triton X-100 solubilized membrane fractions. (A) Mol wt 400 000 fraction of Triton X-100 solubilized membranes of mature erythrocytes. Strongly positive PAS reaction is present only in the band corresponding to a mol wt of 95 000. (B) Mol wt 445 000 peak of Figure 1. Over 95% of the ^{125}I activity added to the gel is present in the 75 000 mol wt band. Strongly positive PAS reactions were given by the three major bands. (C) Mol wt 400 000 fraction of Triton X-100 solubilized membranes from Pronase-treated reticulocytes.

on the straight line given by the standards, possibly because its marked deviation from spherical symmetry (Bezkorovainy and Rafelson, 1964) results in a Stokes radius greater than that indicated by its molecular weight. Additionally, the molecular weight of transferrin, 77 000, is close to the lower operating limit of the gel, where deviation from ideal behavior may be encountered.

Because of the possibility that the high molecular weight transferrin-bearing peak might represent an aggregate of transferrin, or other artifact, rather than a specific complex of transferrin and a membrane component, a control experiment was performed. Transferrin labeled with ^{125}I was incubated for 10 min at 37 °C with a preparation of Triton X-100 solubilized membranes from mature rabbit erythrocytes. To assure binding, the concentration of transferrin used was approximately 10 times that observed in peak fractions from membrane digests of reticulocytes incubated with transferrin, or 150 μg/mL. When chromatographed on the AcA 22 gel column, a single symmetrical peak of radioactivity, with a K_d identical with that of free transferrin in buffer alone, was consistently observed, so that aggregation of transferrin did not appear to be a problem.

Transferrin-Binding Activity of Reticulocyte Membrane Fractions. As shown in Figure 2, the transferrin-binding activity of solubilized reticulocyte membranes was present in fractions with apparent molecular weights centered near 400 000. If the molecular weight of the transferrin–receptor complex is taken as 445 000, the expected molecular weight of the receptor itself would be 445 000 − 77 000 or 368 000. The difference between this figure and 400 000 is, we feel, within the experimental error and reliability of the chromatographic procedures used for estimating molecular weight. We feel, therefore, that our results indicate that the primary transferrin receptor of the rabbit reticulocyte has a molecular weight in the range 350 000–400 000.

Because of the possibility that the receptor might persist in a "masked" state in mature cells, the transferrin-binding activity of solubilized membranes from mature cells was assayed. No transferrin-binding component could be demonstrated in these membranes.

Sodium Dodecyl Sulfate Gel Electrophoresis of Red Cell Membrane Components. The fraction with maximum ^{125}I activity from the chromatogram shown in Figure 1 was subjected to sodium dodecyl sulfate gel electrophoresis, with results displayed in Figure 3B. Three distinct protein fractions were observed. One of these, with a mol wt near 75 000, contained over 95% of the radioactivity applied to the gel, and therefore represents transferrin itself. The other two fractions had weights of 95 000 and 176 000, respectively. Each gave a strong PAS stain for carbohydrate, as did the transferrin band.

Membranes of mature erythrocytes were also solubilized and chromatographed, and fractions with an apparent molecular weight near 400 000 were pooled and subjected to sodium dodecyl sulfate gel electrophoresis. Results, shown in Figure 3A, indicate the presence of the two heavier protein bands observed in the putative transferrin–receptor complex. With the PAS stain, however, an important difference became evident: virtually no carbohydrate was demonstrable in the 176 000 mol wt band.

Membrane fractions with an apparent mol wt near 400 000, obtained from Pronase-treated reticulocytes, failed to display distinctively either the 176 000 or the 95 000 mol wt band (Figure 3C). Instead, there were numerous lower molecular weight components not found in untreated cells. Pronase-treated reticulocytes in our laboratory displayed less than 5% of the iron-uptake activity of untreated cells and have been reported to show less than 5% of the transferrin-binding ability of normal reticulocytes (Hemmaplardh and Morgan, 1975).

Discussion

The first evidence for the existence of specific transferrin receptors on the reticulocyte membrane was adduced by Jandl and coworkers (1959), who observed that trypsinization of reticulocytes abolished their ability to take up iron from transferrin, apparently without impairing other metabolic functions of the cells. Subsequently, Jandl and Katz (1963) were able to show that transferrin would bind to reticulocytes, but not to mature erythrocytes, in a time-, temperature-, and energy-dependent fashion, and that this binding was destroyed by treatment with trypsin in parallel with the destruction of the iron-sequestering ability of reticulocytes. By incubating human reticulocytes with an excess of radioiodinated human transferrin until a steady state in cell-bound radioactivity was achieved, an estimate of 50 000 available transferrin receptors

per reticulocyte was obtained. In rabbit reticulocytes, the number of transferrin binding sites per cell ranges from 200 000 to 560 000 (Baker and Morgan, 1969), the variation perhaps reflecting the relative maturity of the cells (Harris and Aisen, 1975).

With the development of methods for solubilizing, isolating, and characterizing membrane constituents came a surge of interest in the primary transferrin receptor of the reticulocyte. Garrett et al. (1973) were the first to report the identification of a complex of transferrin and a macromolecular component of stroma from rabbit reticulocytes incubated with transferrin doubly labeled with iodine and iron. The complex, solubilized with deoxycholate, sodium dodecyl sulfate, or Triton X-100, eluted very close to the void volume of a Sephadex G-200 column, consistent with a molecular weight greater than 200 000. With Bio-Gel A-15m as the support medium, the elution profile of the complex indicated a molecular weight in the range 350 000–700 000, although it was poorly resolved from free transferrin. Because the iodine:iron ratio in this complex was only 10–15% of that in the doubly labeled transferrin with which the reticulocytes were incubated, it was felt that the stromal component could bind both iron and transferrin.

For the most part our studies corroborate as well as extend these findings. Preliminary experiments showed that somewhat more than half the radioactivity associated with the stroma of reticulocytes incubated with ^{125}I-labeled transferrin was associated with a fraction migrating near the void volume and outside the linear operating range (Andrews, 1965) of a Sephadex G-200 column, but well separated from free transferrin. Using Ultragel AcA 22 as support medium, and the dilute phosphate–Triton X-100 buffer as the developing solvent, this heavy fraction consistently eluted similarly to a ferritin standard (Figures 1 and 2). On this basis, the complex of transferrin and a stromal constituent would appear to have a mol wt near 445 000. The characterization of membrane components by gel chromatography in detergent solutions is still somewhat uncertain (Tanford et al., 1974), particularly since different proteins may bind varying amounts of detergents. However, the linear behavior of the reference proteins in the semilogarithmic plot of molecular weight vs. K_d lends credence to the reliability of gel filtration with Ultragel AcA 22 in estimating the size of the complex of transferrin and a membrane constituent.

The possibility that the 445 000 mol wt complex is an artifact of transferrin aggregation is excluded by several considerations. Firstly, free transferrin migrates as a single peak on the same column, much more slowly than the heavier complex from which it is well resolved. Then, when labeled free transferrin is incubated with solubilized membranes of mature erythrocytes, to which binding of transferrin does not occur, there is no evidence of complex formation. However, transferrin does enter into complex formation with a macromolecular component of reticulocyte membranes, which do exhibit transferrin-binding activity (Figure 2). Finally, the complex shows a definite subunit structure on sodium dodecyl sulfate gel electrophoresis (Figure 3B). For these reasons, we feel that the 445 000 mol wt fraction from the membranes of reticulocytes incubated with transferrin represents the combination of transferrin and its primary membrane receptor.

The ratio of ^{59}Fe to ^{125}I in the retarded peak of the chromatogram in Figure 1 is only about half that in the doubly labeled transferrin with which reticulocytes were incubated. Some of the transferrin in this peak, therefore, has already yielded its iron to the reticulocyte. Apotransferrin has a substantially lower affinity for reticulocyte receptors than does iron-saturated transferrin (Jandl and Katz, 1963). Since gradual dissociation of the transferrin–receptor complex occurs during manipulation (van Bockxmeer et al., 1975), it might be expected that iron-depleted transferrin would be preferentially dissociated from the receptor, in accord with the observations in Figure 1.

On sodium dodecyl sulfate gel electrophoresis the transferrin–receptor complex yields three protein-staining bands, with molecular weights of 75 000, 95 000, and 176 000, respectively, and each giving a strong PAS reaction for carbohydrate (Fairbanks et al., 1971). The first of these, containing over 95% of the radioactive iodine applied to the gel, must be transferrin. Either or both of the other components may derive from the receptor; our results do not permit a definite judgment about this. Since little, if anything, is known about the stoichiometry of dye binding to membrane glycoproteins, which is apt to be unpredictable in general (Maizel, 1971), it is also not possible to deduce anything about the relative numbers of transferrin molecules and membrane components in the transferrin–receptor complex from the electrophoretic patterns of Figure 3.

Neither mature intact erythrocytes, nor their solubilized membranes, display transferrin-binding activity, yet the 400 000 mol wt fraction of their solubilized membranes shows both the 95 000 and the 176 000 mol wt bands on sodium dodecyl sulfate gel. A critical difference between mature erythrocytes and reticulocytes, however, is the absence of a PAS reaction in the 176 000 band of the former. We suggest, therefore, that the mol wt 176 000 glycopeptide is a component of the reticulocyte receptor, and that its carbohydrate moiety, which is lost as the cell matures, has a critical role in the binding of transferrin in accordance with the receptor hypothesis of Furthmayr and Marchesi (1976). Whether the mol wt 95 000 subunit is also part of the receptor molecule cannot be stated with confidence on the basis of our experiments. It may be, for instance, that the receptor consists of two 176 000 subunits, for an aggregate weight of 350 000, which is consistent with the gel filtration studies (Figures 1 and 2) indicating a molecular weight of 350 000–400 000 for the receptor. An equally acceptable explanation, however, is that the receptor consists of two 95 000 subunits and one 175 000 subunit, for a combined weight of 365 000. The procedures for electrophoresis, which involve heating of membrane fractions to 100 °C in the presence of sodium dodecyl sulfate and dithiothreitol, are such that complete dissociation of globular proteins into their constituent subunits should occur (Maizel, 1971). With membrane glycoproteins, however, dissociation may be incomplete under such conditions, particularly when large concentrations of proteins are used (Furthmayr and Marchesi, 1976). It is also possible, therefore, that the 176 000 component is an undissociated dimer of two 95 000 subunits. We believe this unlikely, however, with the low concentrations used in our work. The effects of Pronase are compatible with any model, since neither the 95 000 nor the 176 000 band is left intact after treatment with the enzyme.

Our findings differ from those of Speyer and Fielding (1974; Fielding and Speyer, 1974, 1975), who found the transferrin–receptor complex of human reticulocytes to migrate with catalase (mol wt 232 000) on a column of Sepharose 6B, after preliminary fractionation in a column of Sepharose 2B. Our repeated attempts to reproduce these results with rabbit reticulocytes were unsuccessful. However, Sly et al. (1975) were able to isolate a 200 000 mol wt complex of transferrin and a membrane component from acetylphenylhydrazine-induced

rabbit reticulocytes. Since acetylphenylhydrazine is known to alter the interaction of transferrin with reticulocyte membranes (Workman et al., 1975), studies on acetylphenylhydrazine-induced reticulocytes may not be strictly comparable to ours. van Bockxmeer et al. (1975) have also noted an apparent molecular weight of 225 000 for the receptor–transferrin complex of rabbit reticulocytes, but did not explicitly state how reticulocytosis was induced. All of these studies contrast with the recent report by Witt and Woodworth (1975) indicating that the receptor for ovotransferrin (conalbumin) on the chick embryo red cell has a mol wt of about 35 000. We presume, therefore, that species variations, and methods used to induce reticulocytosis, may be important in the characterization of the transferrin–receptor.

The fate of the transferrin–receptor complex during the sojourn of transferrin on the reticulocyte is still poorly understood. The site and detailed mechanism of iron transfer from protein to cell are not known. Since, in our studies, the iron:iodine ratio in doubly labeled transferrin complexed to its receptor is appreciably lower than in the starting protein, it would appear that iron removal from transferrin may occur while the protein is still affixed to the receptor, but where this occurs cannot be deduced from our findings. Having yielded up its iron, the affinity of transferrin for its receptor may be sufficiently diminished so that dissociation of the complex (Kornfeld, 1969) occurs. Free transferrin is then released from the cell to undergo another cycle of iron transport (Jandl and Katz, 1963).

References

Andrews, P. (1965), *Biochem. J. 96,* 595.

Baker, E., and Morgan, E. H. (1969), *Biochemistry 8,* 1133.

Baker, E., Shaw, D. C., and Morgan, E. H. (1968), *Biochemistry 7,* 1371.

Bezkorovainy, A., and Rafelson, M. E., Jr. (1964), *Arch. Biochem. Biophys. 107,* 302.

Dodge, J. T., Mitchell, C., and Hanahan, D. J. (1963), *Arch. Biochem. Biophys. 110,* 119.

Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971), *Biochemistry 10,* 2606.

Fielding, J., and Speyer, B. E. (1974), *Biochim. Biophys. Acta 363,* 387.

Fielding, J., and Speyer, B. E. (1975), *Proteins Iron Storage Transp. Biochem. Med., Proc. EMBD Workshop Conf.,* 121.

Fish, W. W. (1975), *Methods Membr. Biol. 4,* 189.

Fish, W. W., Mann, K. G., and Tanford, C. (1969), *J. Biol. Chem. 244,* 4989.

Furthmayr, H., and Marchesi, V. T. (1976), *Biochemistry 15,* 1137.

Garrett, N. E., Garrett, R. J., and Archdeacon, J. W. (1973), *Biochem. Biophys. Res. Commun. 52,* 466.

Harris, D. C., and Aisen, P. (1975), *Biochemistry 14,* 262.

Hemmaplardh, D., and Morgan, E. H. (1974), *Biochim. Biophys. Acta 373,* 84.

Hemmaplardh, D., and Morgan, E. H. (1975), *Biochim. Biophys. Acta 426,* 385.

Holloway, P. W. (1973), *Anal. Biochem. 53,* 304.

Jandl, J. H., Inman, J. K., Simmons, R. L., and Allen, D. W. (1959), *J. Clin. Invest. 38,* 161.

Jandl, J. H., and Katz, J. H. (1963), *J. Clin. Invest. 42,* 314.

Kornfeld, S. (1969), *Biochim. Biophys. Acta 194,* 25.

Maizel, J. V., Jr. (1971), *Methods Virol. 5,* 179.

Sly, D. A., Grohlich, D., and Bezkorovainy, A. (1975), *Proteins Iron Storage Transp. Biochem. Med., Proc. EMBO Workshop Conf.,* 141.

Speyer, B. E., and Fielding, J. (1974), *Biochim. Biophys. Acta 332,* 192.

Tanford, C., Nozaki, Y., Reynolds, J. A., and Makino, S. (1974), *Biochemistry 13,* 2369.

van Bockxmeer, F., Hemmaplardh, D., and Morgan, E. H. (1975), *Proteins Iron Storage Transp. Biochem. Med., Proc. EMBO Workshop Conf.,* 111.

Witt, D. P., and Woodworth, R. C. (1975), *Proteins Iron Storage Transp. Biochem. Med.,* 133.

Workman, E. F., Jr., Graham, G., and Bates, G. W. (1975), *Biochim. Biophys. Acta 399,* 254.