The effect of desferrioxamine on transferrin receptors, the cell cycle and growth rates of human leukaemic cells

Adrian BOMFORD,*† Jane ISAAC,* Stephanie ROBERTS,* Andrew EDWARDS,† Stephen YOUNG*§ and Roger WILLIAMS*

*The Liver Unit, King's College School of Medicine and Dentistry, Denmark Hill, London SE5 8RX, and †The Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, U.K.

The effect of the iron chelator, desferrioxamine, on transferrin binding, growth rates and the cell cycle was investigated in the human leukaemic cell line, K562. At all concentrations of the chelator (2–50 μM) binding of ^{125}I -transferrin was increased by 24 h and reached a maximum at 72–96 h. Maximum binding (6–8-fold increased) occurred in cells treated with 20 μM -desferrioxamine, in contrast with control cells which, at 96 h, showed a 50% decrease over initial binding. Scatchard analysis at 4 °C showed that this increased binding was due to an increase in the number of receptors, as the K_d was similar in induced (1.8 nM) and control (1.5 nM) cells. After 96 h cells, cultured with 20 and 50 μM -desferrioxamine accumulated ^{59}Fe from bovine transferrin at over twice the rate found with control cells, reflecting the increase in transferrin receptors. Although iron uptake was unimpaired by the chelator there was a dose-dependent inhibition of cell growth, with control cells completing three divisions in 96 h and those in 10 μM -desferrioxamine only two divisions. At the highest concentration (50 μM), cell division was abrogated although cell viability was maintained (85%). In contrast, DNA synthesis was not markedly affected, except at 50 μM -desferrioxamine when incorporation of [^3H]thymidine was 52% of that in control cells. Flow cytometry revealed that there was a progressive accumulation of the cells in the active phases of their cycle (S, G₂ + M). Desferrioxamine may increase transferrin receptors in two ways: (1) by chelating a regulatory pool of iron within the cell, and (2) by arresting cells in S phase when receptors are maximally expressed.

INTRODUCTION

Transferrin is required by cells proliferating in culture (Hutchings & Sato, 1978) to transport iron into the cell (Rudland *et al.*, 1977; Cherington *et al.*, 1979; Perez-Infante & Mather, 1982). Cells acquire iron from transferrin by the process of receptor-mediated endocytosis (Karin & Mintz, 1981) in which, following binding to a specific cell-surface receptor, iron-rich transferrin enters a population of acidic vesicles (van Renswoude *et al.*, 1982; Dickson *et al.*, 1983) where iron is released from the protein. The iron-depleted but undegraded protein is then recycled to the plasma membrane and released from the cell (Bomford *et al.*, 1985).

Several factors appear to be involved in the regulation of these receptors. Receptor numbers are related to cell proliferation so that their density decreases as a population of cells moves from rapid cell division to confluence (Larrick & Cresswell, 1979; Hamilton *et al.*, 1979; Sutherland *et al.*, 1981; Trowbridge & Omary, 1981). This is also reflected in their modulation during the cell cycle, with high receptor density on cells in S-phase and low density on G₁ cells (Chitambar *et al.*, 1983; Musgrove *et al.*, 1984). The iron available to the cells in the culture medium is also important because the number of receptors falls on cells grown in iron-supplemented media (Ward *et al.*, 1982a,b; Louache *et al.*, 1984). Conversely, when the availability of iron to cells is limited by the presence of the iron chelating agents desferrioxa-

mine (Mattia *et al.*, 1984; Bridges & Cudowicz, 1984) or picolinic acid (Louache *et al.*, 1984) there is a 3–4-fold increase in transferrin receptors.

Clearly there is a complex relationship between expression of transferrin receptors and cell growth on one hand and availability of iron on the other. To investigate further the regulatory role of iron on cell growth as well as expression of the transferrin receptor we have cultured the human leukaemic cell line, K562, in the presence of the iron chelating agent desferrioxamine for periods up to 96 h. Experiments were performed to determine whether transferrin iron in the culture medium was taken up by the cells under these conditions. Transferrin receptors were measured by the binding of radiolabelled human transferrin to intact cells and analysis of the cell cycle performed by flow cytometry.

EXPERIMENTAL

Cell culture

K562 cells were obtained from the Imperial Cancer Research Fund, London, U.K., and were cultured at 37 °C in RPMI 1640 (Gibco, Paisley, Scotland, U.K.) containing 10% (v/v) foetal calf serum (Gibco) and 2 mM-glutamine in 25 cm² plastic flasks (Sterilin, Teddington, Middlesex, U.K.) under CO₂/air (1:19). Growth was maintained in exponential phase by dividing cultures 1:2 with fresh medium every 48 h. Experiments

Abbreviations used: MEM, minimum essential medium; PBS, phosphate-buffered saline.

† To whom correspondence and reprint requests should be addressed.

§ Present address: Rheumatism Research Wing, The Medical School, Birmingham B15 2TJ, U.K.

were initiated by diluting a culture of cells, that had been maintained in exponential-phase growth for at least three passages, to between 0.7×10^5 and 0.9×10^5 cells/ml. After 24 h, when cell densities ranged from 0.9×10^5 to 1.5×10^5 cells/ml, desferrioxamine (Ciba Laboratories, Horsham, Sussex, U.K.) was added from a 1 mM stock solution made up in sterile water to give final concentrations of 2–50 μM . The same volume of sterile water was added to control cultures. In some experiments ferrous ammonium sulphate was added to the chelator to half or fully saturate it with iron before addition to the cells. Cultures were sampled at intervals up to 96 h for the following studies.

Preparation and labelling of transferrin

Transferrin was purified from pooled human plasma by repeated DEAE-Sephadex chromatography and the iron-rich protein (470/410 nm absorbance ratio > 1.3) labelled with ^{125}I (Amersham International) by using solid-phase lactoperoxidase treatment (Bomford *et al.*, 1983; Young *et al.*, 1984). Foetal calf serum was labelled with ^{59}Fe (Amersham International) as previously described (Cavill, 1971) and was passed over an anion-exchange resin to remove non-specifically bound ^{59}Fe (Cavill, 1971).

Transferrin binding and iron uptake by cells

Transferrin binding was measured as described previously (Bomford *et al.*, 1983; Young *et al.*, 1984) by incubating 0.5×10^6 – 1.5×10^6 washed cells with up to 310 nM- ^{125}I -transferrin in MEM containing 1% bovine serum albumin and 25 mM-Hepes, pH 7.5, under CO_2 /air (1:19). Incubations at 4 °C were stopped after 75 min or at 37 °C after 30 min by the addition of ice-cold PBS. Cells were recovered by centrifugation (800 g) and washed three times in PBS at 4 °C. Non-specific binding was estimated by adding a 100-fold excess of unlabelled transferrin to a parallel incubation. Cells were counted for radioactivity in a γ counter. Iron uptake was measured by incubating cells (15×10^6 – 20×10^6) at 37 °C in MEM containing 25 mM-Hepes, pH 7.5, and 10% (v/v) foetal calf serum in which the transferrin was labelled with ^{59}Fe . Non-specific binding was estimated in the presence of 62.5 μM human transferrin and cell harvesting and counting was performed as above.

Uptake of [^3H]thymidine

DNA synthesis was measured by incorporation of [^3H]thymidine (21 Ci/nmol; Amersham International) into acid-precipitable materials as described previously (Testa *et al.*, 1982).

Cell growth and cell cycle analysis

Cells were counted in a haemocytometer and viability was assessed by exclusion of 0.2% Trypan Blue. The distribution of cells through the cell cycle was measured by flow cytometric analysis of fixed cells stained with mithramycin (Pfizer, Sandwich, Kent, U.K.) as described by Crissman & Tobey (1974). Cells were fixed in ice-cold 70% ethanol and stained for 1 h in mithramycin (25 $\mu\text{g}/\text{ml}$) in 25% (v/v) ethanol made up in PBS containing 25 mM- MgCl_2 at 22 °C. Analysis was performed at 457 nm with a FACSI (Beckton-Dickinson Immuno Cytometric system).

Table 1. Effect of desferrioxamine on the binding of transferrin to human leukaemic cells at 4 °C and 37 °C

Desferrioxamine was added to cells growing in exponential phase under the conditions given in the text. After 96 h the specific binding of ^{125}I -transferrin to washed cells was determined at 4 °C and 37 °C as described in the text. The results are expressed as means $\pm$ s.d. of three independent experiments. The values in parentheses show the binding expressed as a percentage of control.

[Desferrioxamine] (μM)	Transferrin binding (ng/ 10^6 cells)	
	4 °C	37 °C
0	18 $\pm$ 6 (100)	45 $\pm$ 17 (100)
2	20 $\pm$ 7 (111)	49 $\pm$ 26 (109)
5	55 $\pm$ 12 (305)	123 $\pm$ 49 (273)
10	106 $\pm$ 46 (589)	262 $\pm$ 69 (582)
20	139 $\pm$ 35 (772)	312 $\pm$ 98 (693)
50	62 $\pm$ 32 (344)	159 $\pm$ 40 (353)

Fig. 1. Effect of desferrioxamine on the binding of transferrin to human leukaemic cells between 0 and 96 h

Desferrioxamine was added to cells growing in exponential phase under the conditions given in the text to give a final concentration of 20 μM . At intervals 0.5×10^6 – 1.5×10^6 cells were withdrawn and washed and the binding of ^{125}I -transferrin to treated (●) and control (○) cells was estimated by incubating the cells with 310 nM-transferrin at 37 °C for 30 min. Non-specific binding was estimated in the presence of a 100-fold excess of unlabelled ligand. Cells were washed in PBS at 4 °C and the cells counted for radioactivity in a γ counter. Values shown are means $\pm$ s.d. of four independent experiments.

RESULTS

Effect of desferrioxamine on binding of ^{125}I -transferrin

Cells growing in exponential phase were cultured with different concentrations of desferrioxamine (2–50 μM) for 96 h and the binding of ^{125}I -transferrin was measured at

Fig. 2 Scatchard analysis of the binding of transferrin to desferrioxamine-treated and control cells at 4 °C

Washed cells (6×10^5) were incubated with up to 310 nM- ^{125}I -transferrin for 75 min at 4 °C and then washed three times at 4 °C in PBS. Non-specific binding was estimated in the presence of a 100-fold excess of unlabelled ligand. In this experiment cells were cultured with 20 μM -desferrioxamine for 96 h (●) and compared with control cells growing in exponential phase (○).

the end of this period. Increased binding as compared with control cells was found at all concentrations of the chelator tested, with the maximum (6–8-fold increase) found with 20 μM -desferrioxamine (Table 1). A comparison of the binding of ligand at 4 °C (surface binding only) with that at 37 °C (full complement of receptors; Klausner *et al.*, 1983) indicated that the drug had little effect on the distribution of receptors between internal and cell-surface locations. In control cells and at all

concentrations of the chelator, surface bound transferrin was $42 \pm 3\%$ (mean $\pm$ s.d.) of total binding (Table 1).

The kinetics of the increase in transferrin binding was investigated by culturing cells in the presence or absence of 20 μM -desferrioxamine and measuring the specific binding of ^{125}I -transferrin every 24 h. As shown in Fig. 1 there was a small increase by 24 h with maximum binding occurring between 72 and 96 h. Binding of transferrin to the control cells gradually diminished with time, falling by 96 h to 50% of the amount bound at the start of the incubation. This reduction in transferrin binding as cells grow to 'plateau-phase' has been previously noted for K562 cells (Frazier *et al.*, 1982) and other types of cultured cells (Larrick & Creswell, 1979). Thus, although in the experiment shown in Fig. 1 there was a 6-fold increase in binding compared with control cells, at 96 h the increase over initial binding was approx. 3-fold.

The affinity of binding of ^{125}I -transferrin to the induced receptors was calculated by Scatchard (1949) analysis of an equilibrium binding experiment performed at 4 °C. The induced cells (K_d 1.8 nM) bound transferrin with similar affinity as control cells (K_d 1.5 nM). In this experiment cells induced with 20 μM -desferrioxamine for 96 h were compared with control cells maintained in exponential-phase growth. From the value of the intercept with the abscissa (Fig. 2) the induced cells had 8×10^5 surface receptors as compared with 2.5×10^5 on control cells, a 3-fold increase. The possibility that desferrioxamine or ferrioxamine, the iron-saturated form of the chelator, influenced the binding of ^{125}I -transferrin to its receptor was also examined. Concentrations of both forms of the chelator up to 100 μM added to an equilibrium binding experiment failed to affect the binding of transferrin to control cells (results not shown).

Ferrioxamine, in concentrations ranging from 2 μM to 100 μM , had little inductive effect on transferrin receptors (Table 2) and when the chelator was only 50% saturated with iron, so that the effective concentration of desferrioxamine was reduced to half the original concentration, the remaining agent had the inductive effect on transferrin receptors expected from the results in Table 1. Thus, in the absence of ferrioxamine, 20 μM -desferrioxamine produced a 560% increase in transferrin binding at 96 h while, in the presence of the 25 μM -desferrioxamine remaining in the half-saturated 50 μM culture, the increase was 700% (Table 2).

Table 2. Effect of ferrioxamine on the binding of transferrin to human leukaemic cells

Desferrioxamine was added to cells growing in exponential phase under the conditions given in the text. In some experiments the desferrioxamine was half-saturated with iron and in others fully saturated before it was added to the culture medium. After 96 h the specific binding of ^{125}I -transferrin to washed cells was determined at 37 °C as described in the text. The values are mean $\pm$ s.d. for $n = 3$.

[Desferrioxamine] (μM)	Binding (% of control)		
	Unsaturated desferrioxamine	Half-saturated desferrioxamine	Fully saturated desferrioxamine
0	100	100	100
2	118 $\pm$ 15	97 $\pm$ 25	102 $\pm$ 18
10	298 $\pm$ 103	178 $\pm$ 58	84 $\pm$ 33
20	564 $\pm$ 161	256 $\pm$ 119	108 $\pm$ 18
50	197 $\pm$ 82	702 $\pm$ 154	117 $\pm$ 31
100	—	210 $\pm$ 51	90 $\pm$ 27

Fig. 3. Effect of desferrioxamine on cellular uptake of ^{59}Fe

Desferrioxamine was added to cells (15×10^6 – 20×10^6) growing in exponential phase under the conditions given in the text to give final concentrations of 20 μM and 50 μM . After 96 h the cells were washed three times at 37 °C in MEM containing 25 mM-Hepes and resuspended in this medium containing 10% (v/v) ^{59}Fe -labelled foetal calf serum. The incubations were performed at 37 °C and contained desferrioxamine at the original concentration. At intervals cells (1×10^6 – 2×10^6) were removed and washed three times in PBS at 4 °C and counted for radioactivity in a γ counter. Shown is the uptake of ^{59}Fe by control cells and desferrioxamine-treated cells. Radioactivity was corrected for non-specific binding which was estimated in the presence of 62.5 μM -human transferrin. ▲, control; ■, 20 μM -desferrioxamine; ●, 50 μM -desferrioxamine.

Effect of desferrioxamine on uptake of ^{59}Fe

We investigated whether prolonged culture with the chelator interfered with the cellular uptake of iron by incubating cells that had been cultured with desferrioxamine for 96 h with foetal calf serum in which the transferrin was labelled with ^{59}Fe . As shown in Fig. 3,

Fig. 4. Concentration-dependent inhibition of cell growth by desferrioxamine

Desferrioxamine (2–50 μM) was added to cells growing in exponential phase under the conditions given in the text. At intervals cells were counted with a haemocytometer and viability was assessed by exclusion of 0.2% Trypan Blue. ●, Control; desferrioxamine at: ■, 2 μM ; ▲, 10 μM ; □, 20 μM ; ○, 50 μM .

uptake of ^{59}Fe was linear over a period of 3 h with the rate of uptake by cells cultured with 20 and 50 μM -desferrioxamine increased 2-fold over that found with control cells. This probably reflects the increase in functioning transferrin receptors on induced cells.

Effect of desferrioxamine on cell growth and DNA synthesis

The experiment in Fig. 4 in which cells cultured with increasing concentrations of the chelator were counted at intervals up to 96 h shows that the inhibition of cell growth was dose-dependent. At the end of the experiment the control cells had completed three divisions, increasing to 800% of initial numbers, while in 10 μM -desferrioxamine two divisions were completed, resulting in a 400% increase; 50 μM -desferrioxamine abolished cell proliferation. Cell viability was maintained at 98% up to 20 μM -desferrioxamine, but at the maximum concentration the cultures contained 10–15% non-viable cells at 96 h. In contrast, the chelator produced little reduction

Table 3. Effect of desferrioxamine on cell growth and [^3H]thymidine incorporation into DNA in human leukaemic cells

Desferrioxamine was added to cells growing in exponential phase under the conditions given in the text. After 96 h cells were counted and pulsed with [^3H]thymidine for 1 h to determine its incorporation into DNA. The results shown are means $\pm$ S.D. for three independent experiments.

[Desferrioxamine] (μM)	Cell growth (% of control)	[^3H]thymidine incorporation	
		(d.p.m.)	(% of control)
0	100	60808 $\pm$ 2720	100
2	94 $\pm$ 8	52908 $\pm$ 5351	87 $\pm$ 8
10	48 $\pm$ 8	49054 $\pm$ 4769	81 $\pm$ 8
20	38 $\pm$ 12	54697 $\pm$ 1194	90 $\pm$ 2
50	0	32514 $\pm$ 6390	52 $\pm$ 11

Fig. 5. Effect of desferrioxamine and ferrioxamine on cell growth

Desferrioxamine was added to cells growing in exponential phase under the conditions given in the text to give final concentrations of 20 μM ($\blacktriangle$) and 50 μM ($\circ$). To other cultures was added 100 μM -ferrioxamine ($\bullet$) or a mixture of 25 μM -ferrioxamine and 25 μM -desferrioxamine ($\square$). Control cells ($\blacksquare$) received no additives. At intervals cells were counted with a haemocytometer and viability was assessed by exclusion of 0.2% Trypan Blue.

in incorporation of [^3H]thymidine into DNA (Table 3) except at the highest concentrations (50 μM) which reduced the incorporation by nearly 50% compared with control cells. Lederman *et al.* (1984) have previously shown that at these concentrations the chelator does not inhibit the cellular transport of thymidine.

The iron-saturated form of the chelator had no effect on growth, since control cells and cells cultured in 100 μM -ferrioxamine grew at the same rate (Fig. 5). Fig. 5 also shows that cells cultured with 20 μM -desferrioxamine, and with 50 μM -desferrioxamine which had been half-saturated with iron to reduce the effective concentration of the chelator to 25 μM , grew at similar rates and were 98% viable at 96 h. In agreement with the experiment in Fig. 4, cells cultured in 50 μM -desferrioxamine did not grow and were 85% viable at 96 h.

Cell cycle analysis

The cells from the experiment shown in Fig. 4 were harvested at 96 h and the DNA content was analysed by flow cytometry (Fig. 6). In the control cultures approx. 53% of cells were in G_1 phase, 30% in S phase and 17% in $G_2 + \text{M}$ phases. As the concentration of desferrioxamine was increased there was a progressive accumulation of cells in S phase and a corresponding fall in the proportion of cells in G_1 . This effect was most marked at 50 μM -desferrioxamine (Fig. 6, panel c) when 28% of the cells were in G_1 and 39% and 32% in S and $G_2 + \text{M}$ respectively.

DISCUSSION

Our results show that desferrioxamine can inhibit the growth of K562 cells while increasing the binding of transferrin. This does not represent an increased affinity

Fig. 6. Distribution of DNA in human leukaemic cells

Cells were cultured under control conditions (a) or with 20 μM - (b) or 50 μM - (c) desferrioxamine for 96 h. The cellular DNA content was measured by flow cytometry on fixed cells stained with mithramycin. The relative fluorescence intensity is proportional to the DNA content of the cells. The phases of the cell cycle are given by the values for relative fluorescence intensity shown in parentheses: G_1 (52–75), S (76–109) and $G_2 + \text{M}$ (110–150).

of the interaction between the ligand for its receptor but an increase in the number of transferrin receptors, a membrane protein which is usually maximally expressed on proliferating cells (Trowbridge & Omary, 1981; Sutherland *et al.*, 1981). A similar increase in transferrin receptors has been previously reported for K562 cells induced with either desferrioxamine (Mattia *et al.*, 1984; Bridges & Cudkowicz, 1984) or picolinic acid (Louache *et al.*, 1984). We found that ferrioxamine, which crosses cell membranes only slowly (Bottomley *et al.*, 1985) was without effect and since desferrioxamine does cross the

cell membrane and accumulates within the cell it has been suggested (Bottomley *et al.*, 1985) that it acts by chelating a pool of intracellular iron involved in the regulation of transferrin receptor biosynthesis. Our results showing that iron uptake from transferrin was unimpaired in cells exposed to the chelator for 96 h are in agreement with a previous report in which iron uptake was studied after 24 h (Bridges & Cudkowicz, 1984) and provide support for the interpretation that intracellular iron is made unavailable by conversion to ferrioxamine rather than by prevention of uptake. In contrast picolinic acid, which appears to reduce membrane transport of iron at the concentration required to induce transferrin receptors (Louache *et al.*, 1984), may reduce the regulatory pool. A contributing mechanism by which iron could be made unavailable is its removal from transferrin by the chelator in the extracellular environment. This transfer occurs relatively slowly in simple buffers (Pollack *et al.*, 1976) and further work will be required to determine whether this could be quantitatively important in the enriched media used for cell culture.

At the low concentrations of desferrioxamine (10 and 20 μM) used here we found that the chelator apparently selectively inhibited cell division while producing little inhibition of DNA synthesis (Table 3). A similar observation has been made by Rudland *et al.* (1977) who reported that cell division was selectively inhibited by iron deficiency caused by omitting transferrin from the culture medium. The addition of various growth factors and hormones to quiescent 3T6 fibroblasts stimulated DNA synthesis, but the cells failed to divide. Transferrin and iron salts were required if cell division was to proceed. The results of Cuthbert & Lipsky (1984) provide further evidence that cell proliferation can be selectively inhibited. They found that low density lipoproteins could inhibit the proliferative response made by T lymphocytes to mitogens by interfering in some unknown way with transferrin metabolism. In their experiments the addition of low density lipoproteins in concentrations that had no significant effect on the incorporation of [^3H]thymidine into mitogen-stimulated lymphocytes at 96 h inhibited cell proliferation by nearly 40%.

It is apparent that high concentrations of desferrioxamine are required to inhibit DNA synthesis in established cell lines since we found there was little inhibition (10–20%) at low concentrations, while 50 μM -desferrioxamine reduced synthesis by only 50% (Table 3). Complete inhibition has been achieved in previous studies using millimolar concentrations (Robbins & Pederson, 1970; Hoffbrand *et al.*, 1976; Ganeshaguru *et al.*, 1980). Under these conditions there is probably inhibition of ribonucleotide reductase which is essential for DNA synthesis (Thelander & Reichard, 1979). This enzyme, which provides the cell with a balanced supply of the four deoxyribonucleotides, contains non-haem bound iron and a tyrosyl free radical on which enzyme activity depends (Graslund *et al.*, 1982).

Lederman *et al.* (1984) have reported that mitogen-stimulated human lymphocytes accumulate in S-phase when exposed to micromolar concentrations of desferrioxamine, and we likewise find that the cells of an established human leukaemic line are prevented from traversing S-phase during 96 h culture. Other conditions that have been reported to induce S-phase arrest include the use of a monoclonal anti-(transferrin receptor) antibody to block transferrin binding to a human

T-leukaemia cell line (Trowbridge & Lopez, 1982) and mitogen-stimulated human lymphocytes (Mendelsohn *et al.*, 1982). Cherington *et al.* (1979) found that hamster fibroblasts did not enter G₂ if transferrin and iron salts were omitted from the culture medium and the inclusion of transferrin saturated with gallium in the culture medium of human leukaemia cells produced a concentration-dependent accumulation of cells in S-phase (Chitambar *et al.*, 1983). The additional finding, in the latter study, of increased transferrin receptors on cells arrested in S-phase means that the effects of transferrin-gallium are very similar to those of desferrioxamine described in the present report.

In the short term, iron deprivation may thus increase transferrin receptors by chelating a regulatory pool of iron and also by arresting cells in the active phases of the cell cycle when receptor expression is maximal (Musgrove *et al.*, 1984; Chitambar *et al.*, 1983). An explanation of why cells are particularly sensitive to iron depletion in S-phase could be provided by the recent observation that ribonucleotide reductase, which is iron-dependent, is also cell-cycle dependent and is synthesized in large amounts in S-phase (Eriksson *et al.*, 1984).

In conclusion, the results of the present study, showing that it is possible to inhibit cell proliferation selectively while leaving DNA synthesis intact, indicate that iron is needed at several points in the cycle of cell maturation and division, but that the requirement for iron varies for the different components involved.

REFERENCES

- Bomford, A., Young S. P., Nouri-Aria, K. & Williams, R. (1983) *Br. J. Haematol.* **54**, 93–101
- Bomford, A., Young, S. P. & Williams, R. (1985) *Biochemistry* **24**, 3427–3478
- Bottomley, S. S., Wolfe, L. C. & Bridges, K. R. (1985) *J. Biol. Chem.* **260**, 6811–6815
- Bridges, K. R. & Cudkowicz, A. (1984) *J. Biol. Chem.* **259**, 12970–12977
- Cavill, I. (1971) *J. Clin. Pathol.* **24**, 472–474
- Cherington, P. V., Smith, B. L. & Pardee, A. B. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3937–3941
- Chitambar, C. R., Massey, E. J. & Seligman, P. A. (1983) *J. Clin. Invest.* **72**, 1314–1325
- Crissman, H. A. & Tobey, R. A. (1974) *Science* **184**, 1297–1298
- Cuthbert, J. A. & Lipsky, P. E. (1984) *J. Clin. Invest.* **73**, 992–1003
- Dickson, R. B., Hanover, J. A., Willingham, M. C. & Pastan, I. (1983) *Biochemistry* **22**, 5667–5674
- Eriksson, S., Graslund, A., Skog, S., Thelander, L. & Tribukait, B. (1984) *J. Biol. Chem.* **259**, 11695–11700
- Frazier, J. L., Caskey, J. H., Yoffe, M. & Seligman, A. (1982) *J. Clin. Invest.* **69**, 853–865
- Ganeshaguru, K., Hoffbrand, A. V., Grady, R. W. & Cerami, A. (1980) *Biochem. Pharmacol.* **29**, 1275
- Graslund, A., Ehrenberg, A. & Thelander, L. (1982) *J. Biol. Chem.* **257**, 5711–5715
- Hamilton, T. A., Wada, H. G. & Sussman, H. H. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6406–6410
- Hoffbrand, A. V., Ganeshaguru, K., Hooton, J. W. L. & Tattersall, M. H. N. (1976) *Br. J. Haematol.* **33**, 517
- Hutchings, S. E. & Sato, G. H. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 901–904
- Karin, M. & Mintz, B. (1981) *J. Biol. Chem.* **256**, 3240–3252
- Klausner, R. D., Ashwell, G., van Renswoude, J., Harford, J. & Bridges, K. (1983) *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2263–2266

- Larrick, J. W. & Cresswell, P. (1979) *Biochim. Biophys. Acta* **583**, 483–490
- Lederman, H. M., Cohen, A., Lee, J. W. W., Freedman, M. H. & Gelfand, E. W. (1984) *Blood* **64**, 748–753
- Louache, F., Testa, U., Pelicci, P., Thomopoulos, P., Titeux, M. & Rochant, H. (1984) *J. Biol. Chem.* **259**, 11576–11582
- Mattia, E., Krishnamurthy, R., Shapiro, D. S., Sussman, H. & Klausner, R. (1984) *J. Biol. Chem.* **259**, 2689–2692
- Mendelsohn, J., Trowbridge, I. & Castagnola, J. (1982) *Blood* **62**, 821–826
- Musgrove, E., Rugg, C., Taylor, I. & Hedley, D. (1984) *J. Cell Physiol.* **118**, 6–12
- Perez-Infante, V. & Mather, J. P. (1982) *Exp. Cell. Res.* **142**, 325–332
- Pollack, S., Aisen, P., Lasky, F. D. & Vanderhoff, G. (1976) *Br. J. Haematol.* **34**, 231–235
- Robbins, E. & Pederson, T. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **66**, 1244–1251
- Rudland, P. S., Durbin, H., Clingan, D. & Jimenez de Asua, L. (1977) *Biochem. Biophys. Res. Commun.* **75**, 556–562
- Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
- Sutherland, R., Delia, D., Schneider, C., Newman, R., Kemshead, J. & Greaves, M. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 4515–4519
- Testa, U., Thomopoulos, P., Vinci, G., Titeux, M., Bettaieb, A., Vainchenker, W. & Rochant, H. (1982) *Exp. Cell. Res.* **140**, 251–260
- Thelander, L. & Reichard, P. (1979) *Annu. Rev. Biochem.* **48**, 133–158
- Trowbridge, I. S. & Omary, M. B. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3039–3043
- Trowbridge, I. S. & Lopez, F. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1175–1179
- van Renswoude, J., Bridges, K. R., Harford, J. B. & Klausner, R. D. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6186–6190
- Ward, J. H., Kushner, J. P. & Kaplan, J. (1982a) *Biochem. J.* **208**, 19–26
- Ward, J. H., Kushner, J. P. & Kaplan, J. (1982b) *J. Biol. Chem.* **257**, 10317–10323
- Young, S. P., Bomford, A. & Williams, R. (1984) *Biochem. J.* **219**, 505–510

Received 21 August 1985/20 December 1985; accepted 15 January 1986