# Structure/Red Blood Cell Permeability. Activity of Iron(III) Chelator Complexes

GEORGE J. KONTOGHIORGHES

*Department of Haematology, Royal Free Hospital Medical School, Pond Street, Hampstead, London NW3 2QG, U.K.*

(Received June 23, 1987)

## Abstract

Chelators which form charged or hydrophilic iron complexes (e.g. desferrioxamine, 1,4-dihydroxypyrid-2-one and 1,2-dimethyl-3-hydroxypyrid-4-one) were shown following 0–3 h of incubation to inhibit the incorporation of iron ($^{59}$Fe) into red blood cells (RBC), but those forming lipophilic iron complexes (e.g. 1-hydroxy-4-methoxypyrid-2-one, omadine and 8-hydroxyquinoline) were shown to cause an increase of iron ($^{59}$Fe) transport in RBC. The differences observed between hydrophilic and lipophilic chelators in the transmembrane cellular transport of low molecular weight iron increase the prospects for the design of specific chelators with different modes of action which could be used in the treatment of diseases of iron imbalance and toxicity and also as probes in iron metabolic studies.

## Introduction

The requirements for iron for the normal growth and development of living organisms is almost universal. In man iron is transported in the serum by transferrin and stored intracellularly by ferritin/-haemosiderin. Iron also exists as a low molecular weight chelator complex in the gut lumen, intracellularly [1] and when is released [2, 3] or donated [4] to iron-containing proteins. Micro-organisms synthesize natural chelators called siderophores which acquire iron from the surrounding medium for growth [5] and also from the transferrin of the infected host [6]. Synthetic chelators like A23187, 8-hydroxyquinoline (oxine) and pyridoxal isonicotinoyl hydrazone were shown previously to form lipophilic iron complexes and to transport iron across the red cell membrane [7], tied off jejunal segments of normal rats [8] and into reticulocytes [9], respectively. Furthermore, oxine caused iron overload in rats [10] and with two other chelators, namely omadine and tropolone, caused death to leukaemic cells because of the lipophilicity of their iron complexes [11, 12]. Iron transfer to reticulocytes was also previously shown to occur with the lipophilic members of a large number of carboxylic acid derivatives [13].

In contrast to lipophilic chelators, hydrophilic iron chelators could be used to increase iron excretion and prevent iron absorption from the gut. For example, desferrioxamine, which is the only clinically available chelator, is used for the treatment of iron overload and acute gut iron poisoning [14]. This chelator was also shown to redistribute iron from the reticuloendothelial system in the anaemia caused by chronic disease [15]. In this work several chelators including some new heteroaromatic derivatives [16] have been tested for their ability to transport iron into matured red blood cells (RBC), which are not metabolically active in iron uptake, in order to identify the structure/membrane permeability properties of their iron complexes and also mechanisms of transport of low molecular weight iron chelator complexes in cells. This information could increase the prospects for the design of new, specific chelators for the treatment of iron metabolic disorders and also for the use of chelators and their iron complexes as probes in iron metabolic studies.

## Experimental

### *Chelators and other Materials*

1,2-Dimethyl-3-hydroxypyrid-4-one ($L_1$) and 1,4-dihydroxypyrid-2-one ($L_3$) were prepared using previously described methods [16–18]. Two other compounds were also prepared, namely 1-methyl-3-hydroxypyrid-2-one ($L_2$) [19] and 1-hydroxy-4-methoxypyrid-2-one ($L_6$) [20]. The following compounds were obtained from Aldrich U.K.: 1-hydroxypyrid-2-one ($L_4$), maltol ($L_5$), kojic acid, catechol, pyrogallol, caffeic acid, 2,3-dihydroxybenzoic acid (2,3-DHB), EDTA, NTA and silicon oil (*d*: 1.05). Omadine (1-hydroxypyridine-2-thione), 8-hydroxyquinoline (oxine), 8-hydroxyquinoline-5-sulphonic acid (OX-sulphonate), tropolone, mimosine, ferric chloride and n-octanol were obtained from Sigma, U.K. $^{59}FeCl_3$ was obtained from Amersham, U.K.; phosphate buffer saline (PBS) from Oxoid, U.K.; and desferrioxamine (DF) from Ciba Geigy, Horsham, U.K.

0020-1693/88/$3.50 

*Partition Coefficients*

The n-octanol/water partition coefficients ($K_{par}$) of the coloured chelator iron complexes were determined as previously described [12, 16] by mixing for 5 min 4 ml of the chelator iron complex ($1 \times 10^{-4}$ M) dissolved in PBS, pH 7.3, with 4 ml of n-octanol. The mixture was then centrifuged for 5 min at 2200 g and the absorbances of both the aqueous and n-octanol layers were measured at selected wavelengths of the visible region, in the vicinity or at the $\lambda_{max}$ of each chelator iron complex by using an SP 1700 Unicam spectrophotometer.

$$K_{par} = \frac{[\text{iron complex}]_{\text{n-octanol}}}{[\text{iron complex}]_{\text{PBS}}}$$

*Red Blood Cell Studies*

RBC were prepared from fresh heparinised blood and iron incorporation was studied as previously described [12]. In brief, an aqueous solution containing iron and traces of $^{59}FeCl_3$ was mixed with the chelators (in PBS) which were present in a molar excess over iron, made isotonic by adding two times strong PBS and finally mixed with an equal volume of packed RBC. The RBC suspensions (1.5 ml) containing the chelator iron (0.2 μCi $^{59}Fe$) complexes were then mixed gently and samples (200 μl) removed at different time intervals and spun for 3 min in the presence of silicon oil (200 μl) in a microtube using a Beckman Microfuge B. Two different methods of cell separation were used for estimating the incorporation of $^{59}Fe$ in RBC and the supernatant. In the first method (A) the cell suspension was spun through silicon oil, the microtube was frozen in liquid nitrogen, then cut and separated into the cell part (bottom) and supernatant/oil part (top). In the second method (B) the supernatant was removed and placed in another tube, the area above the oil washed twice with PBS and then both washings and the oil were placed in the tube containing the supernatant. The $^{59}Fe$ activity of each solution was measured using an LKB-Wallac 1280 Ultragamma Counter and the total count (RBC + supernatant/oil/washings) taken as 100%. Several experiments using different chelator-to-iron molar ratios and variable times of incubation were carried out. In one experiment the effect of haematocrit and chelator concentration on $^{59}Fe$ incorporation into RBC was studied using one highly hydrophilic (DF) and one highly lipophilic chelator (oxine).

## Results

The structure of the chelators and their physicochemical properties are shown in Fig. 1 and Table I. Charged chelators such as DF and mimosine have low $K_{par}$ and are highly hydrophilic, but neutral chelators could be either hydrophilic (e.g. $L_1$ and $L_3$) or lipophilic (e.g. tropolone and oxine).

TABLE I. Physicochemical Properties of the Chelators and their Iron Complexes[a]

| Chelator | Molecular weight | Charge | $K_{par}$ [11] | Iron complex | |
|---|---|---|---|---|---|
| | | | | Charge | $K_{par}$ |
| $L_1$ | 139 | neutral | 0.18 | neutral | 0.05 |
| $L_2$ | 125 | neutral | 0.40 | neutral | 0.14 |
| $L_3$ | 127 | zwitterionic[b] | 0.09 | neutral | 0.04 |
| $L_4$ | 111 | zwitterionic[b] | 0.09 | neutral | 0.95 |
| $L_5$ | 126 | neutral | 1.23 | neutral | 0.32 |
| $L_6$ | 141 | zwitterionic[b] | 0.37 | neutral | 4.85 |
| DF | 561 | positive | 0.02 | positive | 0.02 |
| Mimosine | 198 | zwitterionic | 0.01 | zwitterionic | 0.01 |
| Kojic acid | 142 | neutral | 0.23 | neutral | 0.28 |
| Catechol | 110 | neutral | ND | negative | ND |
| Pyrogallol | 126 | neutral | ND | negative | ND |
| 2,3-DHB | 154 | negative | ND | negative | ND |
| Caffeic acid | 180 | negative | ND | negative | ND |
| Tropolone | 122 | neutral | 3.04 | neutral | 4.5 |
| Omadine | 127 | zwitterionic[b] | 0.04 | neutral | 2.67 |
| Oxine | 145 | neutral | 28.30 | neutral | 10.00 |
| OX-sulphonate | 261 | negative | ND | negative | ND |
| NTA | 191 | negative | ND | negative | ND |
| EDTA | 372 | negative | ND | negative | ND |

[a]The charge of the chelators and their iron complexes at pH 7.4 was estimated from the p$K$ of the ionizable groups of the molecules [16]. The $K_{par}$ is the concentration of the chelator or its complex in n-octanol divided by their concentration in PBS. [b]Neutral, tautomeric 1-hydroxypyrid-2-one ($L_3$, $L_4$, $L_6$) and 1-hydroxypyrid-2-thione (omadine) molecules are also formed. ND = not done. Charged chelators and iron complexes are expected to have a $K_{par}$ similar to that of DF.

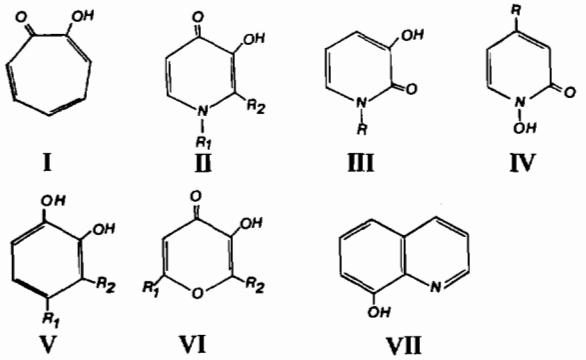

Fig. 1. The structure of the chelators. I = tropolone; II ($R_1$ = $R_2$ = $CH_3$) = 1,2-dimethyl-3-hydroxypyrid-4-one; III (R = $CH_3$) = 1-methyl-3-hydroxypyrid-2-one; IV (R = OMe) = 1-hydroxy-4-methoxypyrid-2-one ($L_6$); IV (R = OH) = 1,4-dihydroxypyrid-2-one ($L_3$); IV (R = H) = 1-hydroxypyrid-2-one ($L_4$); V ($R_1$ = $R_2$ = H) = catechol; V ($R_1$ = H, $R_2$ = OH) = pyrogallol; V ($R_1$ = H, $R_2$ = CH=CHCOOH) = caffeic acid; V ($R_1$ = H, $R_2$ = COOH) = 2,3-dihydroxybenzoic acid (2,3-DHB); VI ($R_1$ = H, $R_2$ = $CH_3$) = maltol ($L_5$); VI ($R_1$ = $CH_2OH$, $R_2$ = H) = kojic acid; VII = 8-hydroxyquinoline (oxine).

TABLE II. The Effect of Haematocrit and Chelator Concentration on $^{59}Fe$ Uptake in Red Blood Cells[a]

| | Percentage $^{59}Fe$ in RBC | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Desferrioxamine | | | | Oxine | | | |
| | 30 min | | 60 min | | 30 min | | 60 min | |
| | A | B | A | B | A | B | A | B |
| Haematocrit % | | | | | | | | |
| 55.5 | 4.0 | 7.0 | 7.0 | 7.0 | 89 | 87 | 86 | 89 |
| 37.0 | 4.0 | 1.0 | 3.0 | 5.0 | 83 | 89 | 84 | 87 |
| 18.5 | 1.0 | 3.0 | 1.0 | 0.8 | 85 | 87 | 84 | 85 |
| 9.2 | 0.5 | 0.6 | 0.9 | 0.5 | 82 | 82 | 83 | 83 |
| 3.7 | 0.5 | 0.5 | 0.1 | 0.6 | 76 | 76 | 71 | 69 |
| [Chelator] $\times 10^{-5}$ M | | | | | | | | |
| 90.0 | 2.0 | 3.0 | 3.0 | 2.0 | 91 | 90 | 92 | 90 |
| 45.0 | 3.0 | 3.0 | 3.0 | 3.0 | 87 | 88 | 90 | 88 |
| 9.0 | 4.0 | 4.0 | 3.0 | 3.0 | 80 | 74 | 80 | 71 |
| 4.5 | 3.0 | 4.0 | 3.0 | 4.0 | | 45 | | 47 |
| 0.9 | 3.0 | 3.0 | 5.0 | 3.0 | 60 | 46 | 54 | 49 |

[a]The chelator ($4 \times 10^{-4}$ M) iron ($4 \times 10^{-6}$ M, 0.2 $\mu$Ci $^{59}Fe$) mixtures of DF and oxine were incubated with different RBC suspensions and the $^{59}Fe$ incorporation into cells was estimated using method A. The haematocrit in each incubation was estimated using a microhaematocrit centrifuge. In the experiment where chelator concentrations varied, the haematocrit (37%) and iron concentration ($4 \times 10^{-6}$ M) were constant. Duplicate samples were used for each experiment at 30 and 60 min incubations, denoted as A and B respectively.

The effect of the haematocrit and chelator concentration on $^{59}Fe$ incorporation into RBC is shown in Table II. $^{59}Fe$ incorporation in the presence of DF was small in all incubations. Substantial amounts of $^{59}Fe$ (70–90%) were incorporated in RBC in the presence of oxine and small decreases were observed only at low haematocrit (3.7%) and also at low chelator concentrations ($4.5–0.9 \times 10^{-5}$ M).

The time course of $^{59}Fe$ incorporation in RBC by the various chelators using two different methods, A and B, is shown in Figs. 2 and 3, respectively. The results obtained using either methods were similar in most cases. In general, the transport of $^{59}Fe$ into RBC varied and it depended on the charge and the $K_{par}$ of the chelator iron complex. Chelators which form neutral, lipophilic iron complexes ($K_{par} > 0.3$) seem to cause the incorporation of iron into RBC at different rates and at least in two cases using lipophilic chelators the uptake was maximal already at time zero (tropolone and oxine, Fig. 2). In contrast, neutral hydrophilic chelators were found to inhibit iron incorporation into RBC (e.g. $L_1$ and $L_3$, Figs. 2 and 3). The transport of iron into RBC was also inhibited by chelators which form charged iron complexes (e.g. DF, mimosine and 2,3-DHB) (Table III). Iron transport into RBC seems to continue after 3 h of incubation in some cases (e.g. $L_5$ and $L_2$ (Fig. 3)) but to reach a steady state in almost all the others.

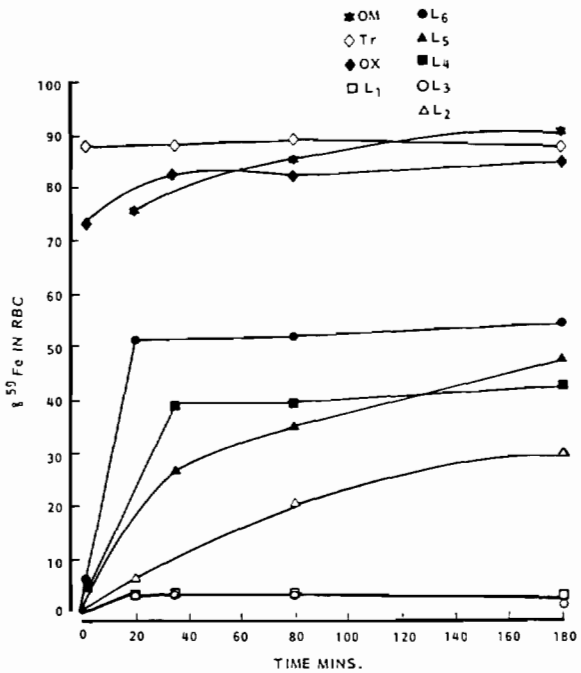


Fig. 2. The effect of chelators on iron transport in red blood cells (method A). Chelator ($3 \times 10^{-4}$ M) iron ($2 \times 10^{-5}$ M) complexes containing traces of $^{59}Fe$ (0.2 $\mu$Ci) in PBS were incubated at 26 °C with equal volumes (750 $\mu$l) of packed red blood cells and samples (200 $\mu$l) were withdrawn at different time intervals, separated into cells and supernatant/oil (method A) and the percent $^{59}Fe$ incorporated into RBC was estimated from the total $^{59}Fe$ count in the 200 $\mu$l mixture (100% $^{59}Fe$ $1.4 \times 10^3$ cpm) [OM = omadine, Tr = tropolone, OX = 8-hydroxyquinoline (oxine)].

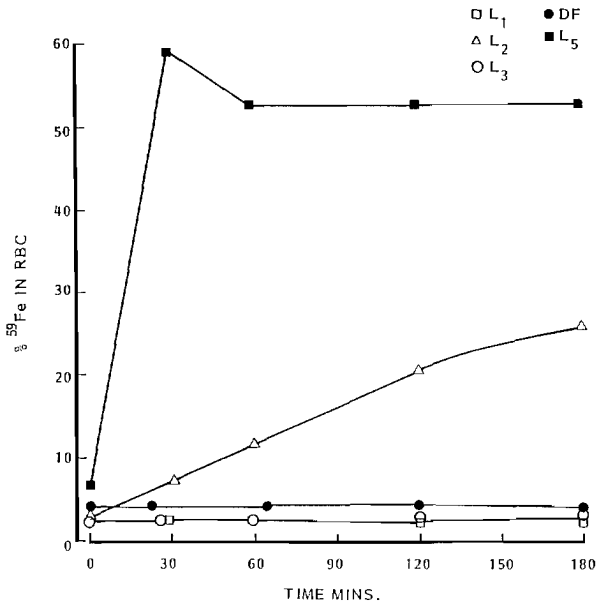


Fig. 3. The effect of DF and α-oxohydroxy heteroaromatic chelators on iron transport in red blood cells (method B). DF ($5 \times 10^{-4}$ M) iron ($1.4 \times 10^{-6}$ M) and $L_1$, $L_2$, $L_3$, $L_5$ ($5.2 \times 10^{-4}$ M) iron ($6.9 \times 10^{-6}$ M) complexes containing traces of $^{59}Fe$ (0.2 μCi, $1.4 \times 10^3$ cpm) in PBS were mixed with RBC and studied for $^{59}Fe$ incorporation as described in Fig. 2 but using method B.

The amount of iron incorporated into RBC within 3 h of incubation varied amongst the chelators. The highest iron uptake was caused by tropolone, omadine and oxine (>70%) but less iron was incorporated by $L_4$, $L_5$ and $L_6$ (40–55%) and even less by $L_2$ (30%). In all the other incubations with chelators which form charged or hydrophilic iron complexes, the amount of iron incorporated was less than 13% ($L_1$, $L_3$, mimosine, kojic acid, catechol, pyrogallol, 2,3-DHB, caffeic acid, OX-sulphonate, NTA and EDTA) (Table III).

TABLE III. The Effect of Chelators on $^{59}Fe$ Uptake in Red Blood cells[a]

| Chelator[b] | Percentage $^{59}Fe$ incorporation into RBC ($\bar{x}$ ± SE) | | |
|---|---|---|---|
| | 30 min | 80 min | 180 min |
| $L_1$ | 2.7 ± 0.3 | 3.7 ± 1.2 | 3.7 ± 0.9 |
| $L_2$ | 5.0 ± 0.6 | 21 ± 3.0 | 31 ± 4.9 |
| $L_3$ | 2.9 ± 0.1 | 2.4 ± 0.2 | 1.9 ± 0.4 |
| $L_4$ | 45.3 ± 4.9 | 51.0 ± 5.9 | 48.3 ± 6.8 |
| $L_5$ | 28.6 ± 2.2 | 43.7 ± 6.8 | 58.3 ± 5.8 |
| $L_6$ | 50.0 ± 4.4 | 49.8 ± 4.7 | 53.7 ± 4.9 |
| DF | 2.3 ± 0.3 | 2.7 ± 0.9 | 6.0 ± 2.5 |
| Mimosine | 2.7 ± 0.3 | 3.7 ± 0.7 | 3.0 ± 0.6 |
| Kojic acid | 4.3 ± 0.3 | 6.7 ± 0.7 | 8.0 ± 0.6 |
| Catechol | 2.7 ± 0.3 | 7.0 ± 2.5 | 6.3 ± 1.3 |
| Pyrogallol | 6.6 ± 0.9 | 8.6 ± 0.9 | 13.5 ± 3.2 |
| 2,3-DHB | 3.1 ± 0.1 | 3.2 ± 0.6 | 5.4 ± 0.7 |
| Caffeic acid | 10.7 ± 2.4 | 12.7 ± 0.7 | 12.0 ± 1.5 |
| Tropolone | 76.3 ± 3.4 | 79.3 ± 4.7 | 78.3 ± 3.2 |
| Omadine | 87.7 ± 4.4 | 79.3 ± 4.7 | 84.3 ± 4.2 |
| Oxine | 89.7 ± 1.7 | 90.7 ± 4.3 | 87.6 ± 4.2 |
| OX-sulphonate | 2.7 ± 0.7 | 2.7 ± 0.3 | 3.0 ± 0.6 |
| NTA | 5.3 ± 1.2 | 6.3 ± 1.2 | 6.0 ± 1.0 |
| EDTA | 2.8 ± 0.6 | 2.4 ± 1.3 | 5.5 ± 1.8 |

[a]The chelators ($3.3 \times 10^{-4}$ M) were mixed with iron ($2.2 \times 10^{-5}$ M, 0.2 μCi $^{59}Fe$) and the $^{59}Fe$ incorporation into RBC was estimated at 30, 80 and 180 min using method A. Triplicate samples were used for each chelator iron complex incubation with the RBC. [b]Abbreviations: $L_1$ = 1,2-dimethyl-3-hydroxypyrid-4-one; $L_2$ = 1-methyl-3-hydroxypyrid-2-one; $L_3$ = 1,4-dihydroxypyrid-2-one; $L_4$ = 1-hydroxypyrid-2-one; $L_5$ = maltol; $L_6$ = 1-hydroxy-4-methoxypyrid-2-one; OX-sulphonate = 8-hydroxyquinoline-5-sulphonic acid; DF = desferrioxamine; PBS = phosphate buffer saline; RBC = red blood cells. $\bar{x}$ ± SE = mean ± standard error.

## Discussion

Several molecular structural properties seem to effect the lipid/water solubility of the chelators and their iron complexes. Since almost all the chelators in Table I form a 3:1 chelator:iron(III) complex at physiological pH [16], the overall charge of the complex would depend on the molecular composition of the iron binding site and the side-chain substituents. For example, chelators $L_1$, $L_2$, $L_3$ and $L_5$ loose a $H^+$ from the hydroxyl group when binding to $Fe^{3+}$ and the overall complex is neutral, whereas the catechol derivatives loose two $H^+$ from their two –OH groups and form a negative iron complex (3–). Other chelators like DF, mimosine and 2,3-DHB have charged side-chains and their iron complex is also charged. Charge and lipophilicity seem to be the main determinants effecting the diffusion of iron into RBC by these low molecular weight iron complexes. Neutral and lipophilic iron complexes ($K_{par} > 0.3$) incorporated iron into RBC (Figs. 2 and 3) at different rates, which were related to their lipophilicity. The most lipophilic iron complexes (i.e. those of omadine, oxine, tropolone and $L_6$) caused the highest rates of incorporation of iron, in contrast to the less lipophilic ones ($L_2$, $L_4$ and $L_5$) (Figs. 2 and 3, Table III). Thus neutral lipophilic iron complexes could be easily incorporated and partitioned into lipophilic compartments of the RBC. Although oxine, tropolone, omadine and their iron complexes were shown previously to be cytotoxic in leukaemic cells [11], no major damage was observed in the incubations with RBC as detected by haemolysis. It seems that leukaemic cells are more susceptible to damage by these chelators and their iron complexes than RBC. Similar observations were previously reported for the anti-

bacterial activity of the iron complexes of omadine and oxine which, however, was diminished in the presence of RBC, probably because of substantial incorporation into these cells in contrast to bacterial cells [21]. It could also be suggested that lipophilic chelators may redistribute iron in cells or tissues resulting in toxicity. Deprivation or diversion of iron from the normal metabolic pathways or pools and chelator iron complex compartmentalization into cell constituents susceptible to damage could also lead to toxicity.

The small incorporation of iron observed with oxine at low concentrations where iron was not fully chelated and also at low haematocrit (3.7%) indicates that chelator-to-iron molar ratios and tissue/cellular mass-to-chelator iron concentration ratios could effect the extent of iron complex uptake into cells during the incubations. Other additional factors, however, may prevail *in vitro* and *in vivo* which could also modify the effect of the chelator on iron transport, such as biotransformation, binding to other metals or proteins and competition with natural occurring chelators such as ATP etc. [22].

It could be envisaged that the chelators and their iron complexes may have many uses related to the cellular biochemistry and physiology of iron. For example, neutral, lipophilic iron complexes may have a use in replacing transferrin in the transport of iron into cells (e.g. cells in culture [23] and possibly *in vivo*) for the treatment of atransferrinaemia [24]. These chelators may also have a use in the radiolabelling of cells with $^{59}Fe$ and also with other metals such as $^{67}Ga$ and $^{111}In$ for the diagnosis of diseases, as shown previously with oxine [25]. The transport of iron into RBC and also through rat intestinal sacs [16] with some of these chelators which are acid stable (e.g. $L_5$ and $L_6$) increases the prospects for their use in the treatment of iron deficiency anaemia. Maltol ($L_5$) is particularly promising because it has already been used in foods [26].

In contrast to the lipophilic chelators, the neutral/-hydrophilic (e.g. $L_1$ and $L_3$) and charged chelators (e.g. DF and mimosine), some of which are effective in mobilising iron from transferrin [3] and ferritin [27, 28] *in vitro* and also from animals *in vivo* [29–32], inhibited the incorporation of iron into RBC (Table III). Most of the chelators tested, including the well-known carboxylic acid derivatives NTA and EDTA, the charged oxine derivative (OX-sulphonate) and the naturally occurring chelators caffeic acid, kojic acid, catechol and 2,3-DHB, are hydrophilic. The hydrophilic iron complexes of these chelators did not mix with n-octanol and did not seem to diffuse or be incorporated into the RBC even after long-term incubations (3 h). Instead, these remained in the supernatant of the RBC suspensions which are aqueous and do not contain lipophilic constituents. It is suggested therefore that in the treatment of transfusional iron overload hydrophilic chelators and iron complexes would be rapidly excreted without being accumulated in the tissues, where they may cause toxic side effects. Such hydrophilic chelators may also decrease iron absorption by inhibiting iron transport into cells of the gut and may have a use in the treatment of diseases of iron overload arising from increased gastrointestinal iron absorption (e.g. idiopathic haemochromatosis and thalassaemia intermedia). Further work is required to explore the specific effects of chelators in iron and related metal transport in cells, their use in iron metabolism and as clinical diagnostics.

Cell studies could also be of great importance in predetermining the toxicity of chelators intended for clinical use such as $L_1$ [17], which was shown to be orally effective in increasing iron excretion and non-toxic in iron-loaded patients [33].

## Acknowledgements

The author would like to thank the United Kingdom Thalassaemia Society for financial support, S. Chambers for technical assistance and J. Martin for the word processing.

## References

1 A. Jacob, *Blood, 50,* 433 (1977).
2 I. Romslo, in A. Jacobs and M. Worwood (eds.), 'Iron in Biochemistry and Medicine (II)', Academic Press, London /New York, 1980, pp. 325–362.
3 G. J. Kontoghiorhges, *Biochim. Biophys. Acta, 869,* 141 (1986).
4 G. W. Bates and M. R. Schlabach, *J. Biol. Chem., 248,* 3228 (1973).
5 J. B. Neilands, *Science, 156,* 1443 (1967).
6 E. D. Weinberg, *Microbiol. Rev., 42,* 45 (1978).
7 S. P. Young and B. D. Gomperts, *Biochim. Biophys. Acta, 469,* 281 (1977).
8 W. Forth and W. Rummel, *Physiol. Rev., 53,* 724 (1973).
9 P. Ponka, H. M. Schulman and A. Wilzynska, *Biochim. Biophys. Acta, 718,* 151 (1982).
10 R. S. Yamamoto, G. M. Williams, H. H. Frankel and J. H. Weisburger, *Toxicol. Appl. Pharmacol., 19,* 687 (1971).
11 G. J. Kontoghiorghes, A. Piga and A. V. Hoffbrand, *Haematol. Oncol., 37,* 63 (1986).
12 G. J. Kontoghiorghes, A. Piga and A. V. Hoffbrand, *FEBS Lett., 204,* 208 (1986).
13 J. V. Princiotto, M. Rubin, G. C. Schashaty and E. J. Zapolski, *J. Clin. Invest., 43,* 825 (1964).
14 G. J. Kontoghiorghes, in C. Rice-Evans (ed.), 'Free Radicals, Oxidant Stress and Drug Action', Richelieu Press, London, 1987, pp. 277–303.
15 A. A. H. Lawson, E. T. Owen and A. G. Mowat, *Ann. Rheum. Dis., 26,* 552 (1967).
16 G. J. Kontoghiorghes, *'Ph.D. Thesis'*, Essex University, 1982, (British Library Microfilm D66194/86).
17 G. J. Kontoghiorghes and L. Sheppard, *Inorg. Chim. Acta, 136,* L11 (1987).
18 G. J. Kontoghiorghes, *Inorg. Chim. Acta, 135,* 145 (1987).
19 H. Mohler and H. Weber, *Tetrahedron, 26,* 3779 (1970).

20 S. Mizukami, E. Hirai and M. Morimoto, *Ann. Rep. Shionogi Res. Lab., 16,* 29 (1966).
21 A. Albert, C. W. Rees and A. J. H. Tomlinson, *Br. J. Exp. Pathol., 37,* 500 (1956).
22 K. Konopka, W. Leyko, R. Gondko, Z. Sidorczyk, Z. Fabjanowska and M. Swedowska, *Clin. Chim. Acta, 24,* 359 (1969).
23 D. Barnes and G. Salo, *Cell, 22,* 649 (1980).
24 E. H. Morgan, *Mol. Aspects Med., 4,* 1 (1981).
25 S. M. Moerlein and M. J. Welch, *Intern. J. Nuclear. Med. Biol. 8,* 277 (1981).
26 H. H. Rennhard, *J. Agric. Food Chem., 19,* 152 (1971).
27 G. J. Kontoghiorghes, *Biochem. J., 233,* 299 (1986).
28 G. J. Kontoghiorghes, S. Chambers and A. V. Hoffbrand, *Biochem. J., 241,* 87 (1987).
29 G. J. Kontoghiorghes and A. V. Hoffbrand, *Br. J. Haematol., 62,* 607 (1986).
30 G. J. Kontoghiorghes, *Lancet, I,* 817 (1985).
31 G. J. Kontoghiorghes, *Scand. J. Haematol., 37,* 63 (1986).
32 G. J. Kontoghiorghes, *Mol. Pharmacol., 30,* 670 (1986).
33 G. J. Kontoghiorghes, M. J. Aldouri, L. Sheppard and A. V. Hoffbrand, *Lancet, I,* 1294 (1987).