The mechanism of in vivo iron chelation by 3-hydroxy pyridin-4-ones (CP compounds) was studied in hypertrans fused rats in which the major storage iron pools in hepatocytes and in the reticuloendothelial (RE) system have been labeled by selective radioiron probes. Both dimethyl-3-hydroxy pyridin-4-one (CP 20 or L1) and diethyl-3-hydroxy pyridin-4-one (CP 94) have an identical and very high (log β, 38) binding constant and selective affinity to iron(III), but the lipid solubility of CP 94 is considerably higher than that of CP 20. Both chelators induced an increase in the fecal excretion of hepatocellular iron with no effect on urinary excretion. In contrast, about one third to one half of the iron mobilized from RE cells was excreted in the urine. The chelating efficiency of CP 20 was comparable with that of deferoxamine (DF), whereas CP 94 was up to eight times more effective than DF. Unlike DF, which had no effect by the oral route, the oral and parenteral effectiveness of both CP compounds was identical. These findings indicate that: (1) lipid solubility is an important determinant of in vivo chelating efficiency; (2) urinary iron excretion induced by the CP compounds is derived from RE cells; (3) part of the iron mobilized from RE cells and all of the iron derived from hepatocytes is excreted through the bile; and (4) contrary to previous observations in cell cultures, there is no in vivo evidence for a diminishing chelating efficiency at the lowest doses used.

Iron toxicity is one of the most important life-limiting complications of thalassemia major and other chronic anemias requiring long-term blood transfusion support. Although deferoxamine (DF) treatment is able to induce a negative iron balance and to prolong life by preventing the accumulation of iron in vital organs, such treatment is far from satisfactory. The high cost of DF, its limited oral effectiveness, and the consequent need for its parenteral administration by portable pumps underline the need for the development of new, orally effective iron chelating drugs.

The 3-hydroxy pyridin-4-ones (CP compounds) are a family of bidentate orally effective iron chelators designed by Hider and Kontoghiorghes as potentially useful drugs for the management of clinical iron overload. These compounds have been shown to be effective iron chelators in vitro in hepatocyte and heart cell cultures and in vivo in a number of animal species. One of these compounds, 1,2-dimethyl-3-hydroxy pyridin-4-one (L1 or CP20), has already been subjected to clinical evaluation at a number of centers in patients with transfusional iron overload, and was found to have comparable oral efficiency to subcutaneous DF in promoting urinary iron excretion.

Although limited data on the pharmacokinetics of the CP compounds are available, very little is known on the source and fate of iron chelated in vivo by these compounds. Results of some animal studies appear to indicate enhancement of urinary excretion without any evidence of fecal iron excretion, whereas others have found a predominant fecal excretion. This in turn appears to contrast with human studies in which all chelated iron was limited to the urine. Concern has also been expressed regarding the risk of iron mobilization from one internal compartment into an other without increasing net iron excretion. None of these studies offered any information on the direct source of iron chelated in vivo by the CP compounds.

The chelatable iron compartment represents only a small fraction of the total body iron pool. Thus, it was calculated that the percent of iron removed per day in animal models using iron dextran and Fe-lactoferrin injections is less than 0.05% of the total administered iron load. To improve the sensitivity and specificity of our animal model for studying in vivo iron chelation, we have used selective radioiron probes to introduce tracer iron into the two major storage iron pools available for chelation: the hepatic parenchyma and the reticuloendothelial (RE) system. A distinction between these compartments of iron stores is important because RE iron accumulation is believed to be innocuous whereas parenchymal siderosis may result in serious organ dysfunction. In the text that follows we shall describe the difference in the availability of these iron pools for interaction with various chelators, and the difference in their respective routes of excretion.

MATERIALS AND METHODS

Female Wistar rats of the Hadassah strain weighing 170 to 200 g were used throughout. Hypertransfusion was performed by two intravenous injections of 2 mL packed cells per 100 g body weight on days 4 and 1 before storage iron labeling. The mean hematocrit on the first day of study was 69% ± 1%, the serum iron 459 ± 18 µg/dL, and the unsaturated iron binding capacity less than 10 µg/dL. The distribution and excretion of radioiron-labeled iron chelates (10 mg/animal) was assessed after intraperitoneal injec-
tion of the chelator mixed with trace amount of $^{59}$FeCl$_3$. Prelabeling of the iron stores was accomplished via intravenous injection of radioiron labels through a tail vein. Animals were killed under ether anesthesia by exsanguination through the abdominal aorta into heparinized syringes.

The N-alkyl-3-hydroxypyridin-4-one chelators (CP compounds) were synthesized and purified as previously described and their purity was confirmed by $^1$H nuclear magnetic resonance, reverse-phase high performance liquid chromatography (HPLC), and elemental analysis. Dexteroxamine B (DF) was supplied as the methane-sulfonate salt (Desferal) by Ciba-Geigy Ltd (Basel, Switzerland). The chemical structure and partition coefficients of the CP compounds used in the present study are described in Table 1.

**Counting Methods**

The radioactivity of spleen, kidney, weighed portions of the liver, and 1-mL samples of blood was determined in an automatic well-type scintillation counter (Auto-Gamma, Model 5260; Packard Instrument Co, Inc, Downer's Grove, IL). Whole body counts were performed in a small animal counter (Packard Model 446, Armac liquid scintillation detector). To measure the excretion of radioactivity after $^{59}$Fe labeling, the animals were confined in solitary metabolic cages with stainless steel grid bottoms and urine and stool were collected separately. Radioiron excretion was also determined by whole body counts on the first and last days of the study, and corrections were made for decay and differences in geometry. Recovery of radioactivity in the excreta compared with the reduction in whole body radioactivity was over 90%.

In preliminary studies using $^{51}$Cr-labeled erythrocytes, the blood volume was found to be 6.4 mL/100 g body weight, and the proportion of blood trapped in the liver was 2.4%. These factors were used in subsequent studies to calculate net hepatic and total blood radioactivity. Residual tissue radioactivity was calculated from total body activity minus the combined radioactivity of liver, spleen, blood, and kidneys.

**Preparation of Radioiron Labels**

Heat-damaged erythrocytes ($^{59}$Fe-DRBC). In vivo $^{59}$Fe-labeled erythrocytes were prepared in rats by injecting 100 to 200 µCi of $^{59}$Fe-citrate intravenously 5 days or more before harvesting the cells. Repeated injections of 50 to 100 µCi $^{59}$Fe were administered as required to maintain a specific activity of 0.05 to 0.1 µCi of $^{59}$Fe per milligram of hemoglobin. Blood was removed in volumes of 0.5 to 1.0 mL, and after three washes in cool normal saline, cells were suspended in 5 vol of ACD formula B and incubated at 40°C for 15 minutes. After two more washings, the cells were resuspended in saline to a final concentration of 5 mg/mL hemoglobin and injected intravenously without delay in aliquots of 1 mL/animal.

Soluble ferritin ($^{59}$Fe-ferritin). In vivo $^{59}$Fe-labeled ferritin was prepared by injecting 100 to 200 µCi of $^{59}$Fe-citrate into rats that had been given 12 mg of iron dextran the preceding week. The animals were killed 24 hours later, and purified radioactive ferritin was prepared by the method of Bjorklid and Helgeland. Acrylamide gel electrophoresis of the ferritin preparation at pH 8.5 showed a single protein band, and a single precipitin line was obtained on immunodiffusion against antiferritin serum. The specific activity of $^{59}$Fe-ferritin was 5 to 10 µCi/mg iron. Shortly before injection, the purified ferritin concentrate was dissolved in sterile normal saline to a final concentration of 0.2 to 0.4 µCi/mL, representing about 40 µg ferritin iron, and injected intravenously in aliquots of 1 mL/animal.

$^{59}$Fe-citrate. $^{59}$FeCl$_3$ (specific activity 10 to 15 µCi/µg; Amer sham Radiochemical Centre, Amersham, England) that had been diluted in 0.005 mol/L HCl was mixed with sufficient sterile 4% sodium citrate to ensure a molar ratio of citrate to iron in excess of 50:1. The final concentration of $^{59}$Fe used for in vivo tracer studies was 0.5 µCi/mL. $^{59}$Fe citrate diluted in normal saline. One-milliliter aliquots were injected intravenously or intraperitoneally into the animals.

**Initial processing of storage iron labels.** This has been described in detail in previous communications. Briefly, 89% ± 1% of soluble ferritin and 81% ± 2% of DRBC were located to the liver and spleen within 1 hour of intravenous injection. After administration of DRBC, the cellular distribution of radioactivity in the liver determined by a quantitative autoradiographic method was 100% in RE cells and 0% in parenchymal cells. In contrast, both soluble ferritin and $^{59}$Fe-transferin were located primarily to parenchymal liver cells (97% to 100%), with little labeling of RE cells (0% to 3%). Thus, with one or the other source of radioiron, it was possible to selectively label either RE or parenchymal cells. The T1/2 in plasma of intravenously injected soluble ferritin was 16 ± 1 minute and that of DRBC was 2 ± 1 minute. Within 1 day of injection, 72% of $^{59}$Fe-ferritin in the liver could be recovered in the ferritin fraction of the tissue homogenate, and the rest was soluble nonferritin iron. Hemoglobin in $^{59}$Fe-DRBC was completely catabolized within 24 hours of injection.

Total nonheme iron in the liver and spleen was determined by the method of Torrance and Bothwell.

**Design of animal studies.** All studies were performed in hypertransfused animals. Hypertransfused rats were housed in metabolic cages and daily stool and urine was collected for 7 consecutive days. One hour after intravenous injection of the radioiron label on day 0, a single dose of chelator was administered either intraperitoneally or by gastric gavage. In all experiments each subgroup of control or treated animals consisted of four subjects. All animals were killed on day 7 and the organ distribution of radioiron was determined as described previously. Whole body radioactivity was determined on day 0 (representing 100% initial radioactivity) and day 7 to determine total radioiron excretion. This measurement was used as an independent control for total radioiron excretion and portion of blood trapped in the liver was established.

**RESULTS**

The fate of tracer amounts of radioiron preincubated with 10 mg each of DF, CP 20, or CP94 before injection to hypertransfused rats is shown in Table 2. Control animals received identical amounts of radioiron in the form of ferric...
citrate diluted in normal saline. All animals were killed on
day 7. The cumulative spontaneous excretion of radioiron
in untreated animals was 10% of the injected dose, re-
stricted entirely to the stool. Hypertransfusion resulted in
the suppression of radioiron incorporation into circulating
erthrocytes (11%), and the diversion of 31% of the
radioiron into the hepatic parenchyma.

Preincubation with each of the three chelators resulted in
a sharp increase in the rate of radioiron excretion amount-
ing to 82% or more of the injected dose. With DF, radioiron
excretion was mostly in the urine, whereas with CP 20 and
CP 94 fecal excretion was higher than or equal to urinary
excretion. Of all the compounds tested, residual whole body
radioactivity was lowest and radioiron excretion highest
(P < .001) with CP 94, with total radioiron excretion
exceeding 92%.

In contrast to the first study in which radioiron has been
chelated before injection, in the next two studies tissue iron
stores have been prelabeled with either 59Fe-ferritin (he-
patic parenchyma) or 59Fe-DRBC (RE cells), and the
chelator was administered as a single dose of 200 mg/kg
intraperitoneally following storage iron labeling. Table 3
describes the distribution in various organs and cumulative
excretion within 7 days of radioiron injected intravenously as
59Fe-ferritin and localized initially to hepatic parenchymal
cells. In untreated hypertransfused control animals
85% of the injected radioactivity was still located to the
liver 7 days later, and 10% were excreted in the stool
representing spontaneous biliary excretion. Less than 5% were
found in other organs. Treatment with a single parenteral
dose of 200 mg/kg DF, CP 20, or CP 94 resulted in a
significant (P < .001) reduction in hepatic radioactivity
of 20%, 25%, and 65%, respectively, and an almost identical
reciprocal increase in fecal radioiron excretion. Urinary
radioiron excretion was negligible.

Table 4 describes the effect of chelating therapy on
hypertransfused rats in which RE iron stores have been
labeled with 59Fe-DRBC. Comparison of radioiron distribu-
tion in the control group in Table 4 with the controls in
Table 3 shows that in contrast to the parenchymal label
59Fe-ferritin, the RE label 59Fe-DRBC was located to both
spleen and liver. Hypertransfusion was quite effective in
preventing the redistribution of 59Fe-ferritin into other
organs and the reutilization of its radioiron by newly
formed RBC. In contrast, with 59Fe-DRBC there was some
redistribution of radioiron initially localized to RE stores.
This is indicated by the fact that only 70% of the injected
activity was still in the liver and spleen by the end of
the study, and by an 11% reutilization of the injected
radioactivity by newly formed erythrocytes, similar to
the rate of utilization of ferric citrate in hypertransfused rats
(Table 2).

The pattern of radioiron excretion in 59Fe-DRBC labeled
animals after iron chelating therapy was different from that
obtained with 59Fe-ferritin. A significant proportion of the
total excretion has been recovered in the urine. This was
most impressive with CP 94 where about one third of the
total excretion of 63% was urinary excretion. In both Tables
3 and 4 the reduction in whole body radioactivity and
increase in fecal and urinary excretion induced by CP 94
were significantly higher than with the other two chelators
(P < .001).

The effect of chelating therapy for 5 consecutive days at a
dose of 200 mg/kg/d intraperitoneally on tissue iron stores
is described in Table 5. Similar to radioiron excretion, CP
94 treatment resulted in a greater reduction in both hepatic
(P < .01) and splenic (P < .05) nonhem iron stores than
either one of the other compounds tested.

Figure 1, A and B, compares the pattern of fecal iron
excretion after a single dose of the various chelators given
to 59Fe-ferritin labeled animals, and the effectiveness of
parenteral versus oral treatment. Total fecal radioiron
excretion after parenteral treatment was 10.5% ± 2.1% in
controls, 31.0% ± 4.9% with DF, 36.1% ± 3.6% with CP
94, and 76.1% ± 4.9% with CP 94. Fecal iron excretion
was largely completed within 4 days. Peak excretion was
observed on day 2 for CP 94 and DF and on day 1 for CP 94.
After oral treatment, total fecal excretion was 38.3% ±
4.9% with CP 20 and 74.4% ± 3.0% with CP 94. However,
with DF total excretion after oral therapy did not exceed
the excretion found in untreated controls.

Finally, dose-response relations were studied with CP 94,
the most powerful iron chelator in the present series, and
compared with DF given under identical conditions to

### Table 2. Radioiron Distribution After Injection of Chelated Iron (% of injected 59Fe)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Liver</th>
<th>Spleen</th>
<th>Blood</th>
<th>Kidneys</th>
<th>Whole Body</th>
<th>Stool</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>30.6 ± 3.4*</td>
<td>1.0 ± 0.2</td>
<td>11.1 ± 4.0</td>
<td>2.1 ± 0.7</td>
<td>90.0 ± 1.6</td>
<td>10.0 ± 0.6</td>
<td>0</td>
</tr>
<tr>
<td>DF</td>
<td>4</td>
<td>4.7 ± 1.3</td>
<td>0.2 ± 0.1</td>
<td>2.3 ± 0.9</td>
<td>0.6 ± 0.1</td>
<td>16.4 ± 1.5</td>
<td>19.2 ± 0.9</td>
<td>64.4 ± 1.5</td>
</tr>
<tr>
<td>CP 20</td>
<td>4</td>
<td>3.7 ± 0.8</td>
<td>0.2 ± 0.1</td>
<td>3.7 ± 0.8</td>
<td>0.6 ± 0.0</td>
<td>18.2 ± 3.4</td>
<td>53.2 ± 2.0</td>
<td>28.4 ± 6.1</td>
</tr>
<tr>
<td>CP 94</td>
<td>4</td>
<td>1.0 ± 0.2</td>
<td>0.1 ± 0</td>
<td>1.7 ± 0.3</td>
<td>0.1 ± 0</td>
<td>7.5 ± 1.1</td>
<td>47.0 ± 8.5</td>
<td>45.5 ± 0.8</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD.

### Table 3. 59Fe-ferritin Distribution After Iron Chelating Treatment (% of injected 59Fe)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Liver</th>
<th>Spleen</th>
<th>Blood</th>
<th>Kidneys</th>
<th>Whole Body</th>
<th>Stool</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>85.1 ± 7.8*</td>
<td>0.8 ± 0.1</td>
<td>3.4 ± 0.6</td>
<td>0.4 ± 0.2</td>
<td>89.5 ± 3.8</td>
<td>10.5 ± 0.2</td>
<td>0</td>
</tr>
<tr>
<td>DF</td>
<td>4</td>
<td>64.8 ± 3.7</td>
<td>0.5 ± 0.1</td>
<td>3.7 ± 0.8</td>
<td>0.4 ± 0.1</td>
<td>69.0 ± 4.9</td>
<td>31.0 ± 2.2</td>
<td>0</td>
</tr>
<tr>
<td>CP 20</td>
<td>4</td>
<td>59.6 ± 2.9</td>
<td>0.4 ± 0.1</td>
<td>4.5 ± 1.1</td>
<td>0.3 ± 0.1</td>
<td>63.6 ± 3.6</td>
<td>36.1 ± 2.6</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>CP 94</td>
<td>4</td>
<td>26.1 ± 6.5</td>
<td>0.4 ± 0.1</td>
<td>3.2 ± 1.0</td>
<td>0.2 ± 0</td>
<td>23.6 ± 9.2</td>
<td>76.1 ± 2.4</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD.
*59Fe-ferritin labeled hypertransfused rats. As shown in Fig 2, at all doses studied, the reduction in hepatic radioiron content (except for 50 mg/kg/d) and the corresponding increase in fecal radioiron excretion were higher with CP 94 than with DF (P < .05 to < .001). This was particularly striking at the lowest dose studied: fecal excretion after 25 mg/kg CP 94 was equal to that observed with 200 mg/kg DF, implying an eightfold higher in vivo chelating efficiency of the former drug.

There were no toxic effects observed with any one of the three compounds investigated at the doses used in the present study.

**DISCUSSION**

With the establishment of regular iron chelating therapy, a significant improvement in the life expectancy of patients with transfusional siderosis has been witnessed. This is attributed mainly to the prevention of heart disease in well-treated patients and, in a few, to the reversal of existing heart disease by aggressive intravenous DF therapy.21-22 Despite the proven efficacy of DF in the treatment of iron overload, there is an obvious need for the development of alternative, orally effective iron chelating drugs that would be more convenient for use and therefore available for a larger number of patients who are at present unable to comply with the need for long-term subcutaneous infusion by portable pumps.

The 3-hydroxypyridin-4-ones (CP compounds) are a family of bidentate orally effective iron chelators with an identical and very high (log β36) binding constant and selective affinity to iron (III).23 Previous studies in cell cultures and in vivo studies in a number of animal species have shown that the CP compounds are effective iron chelators, and that chelating efficiency may be improved by increasing lipid solubility by altering the length of the R2 substituent on the hydroxypyridin-4-one ring nitrogen.26-11

The source of iron chelated in vivo by the CP compounds is unknown. Likewise, the relative importance of the urinary and fecal routes of iron excretion induced by these compounds is controversial.10,11,17 Such difficulties are typical of animal models used in previous studies, in which the total body iron pool was labeled nonspecifically, and less than 0.05% of the total administered iron load was available for mobilization by iron chelating treatment.17

The use of selective radioiron probes for labeling the major body iron stores in the hepatic parenchyma (*59Fe-ferritin) and the RE system (*59Fe-DRBC) has greatly improved the sensitivity and specificity of animal models for studying in vivo iron chelation.18 In contrast to radioiron bound to transferrin or lactoferrin, all of the radioiron injected with these compounds is located initially to parenchymal or RE iron stores, resulting in a striking increase in the availability of the injected radioiron for interaction with the chelator. Hypertransfusion simulates the clinical condition of polytransfused patients and further increases the specificity of the model by reducing the rate of storage iron release and of the recycling of the radioiron probes from their initial site of deposition.

**Table 4. *59Fe-DRBC Distribution After Iron Chelating Treatment (％ of injected *59Fe)**

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Spleen</th>
<th>Blood</th>
<th>Kidneys</th>
<th>Whole Body</th>
<th>Stool</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>60.0 ± 10.9*</td>
<td>10.5 ± 3.4</td>
<td>11.3 ± 5.4</td>
<td>2.0 ± 0.9</td>
<td>85.6 ± 1.9</td>
<td>14.1 ± 0.1</td>
<td>0.3 ± 0</td>
</tr>
<tr>
<td>DF</td>
<td>40.7 ± 3.4</td>
<td>15.1 ± 3.7</td>
<td>11.1 ± 4.9</td>
<td>1.1 ± 0.2</td>
<td>71.5 ± 4.3</td>
<td>21.7 ± 0.4</td>
<td>6.8 ± 2.5</td>
</tr>
<tr>
<td>CP 20</td>
<td>42.5 ± 1.5</td>
<td>8.8 ± 3.5</td>
<td>6.9 ± 1.3</td>
<td>1.5 ± 0.2</td>
<td>71.1 ± 1.7</td>
<td>24.7 ± 0.4</td>
<td>4.2 ± 0.6</td>
</tr>
<tr>
<td>CP 94</td>
<td>19.3 ± 7.0</td>
<td>6.1 ± 1.2</td>
<td>7.0 ± 3.5</td>
<td>0.8 ± 0.2</td>
<td>37.5 ± 9.7</td>
<td>43.1 ± 1.8</td>
<td>18.4 ± 0.1</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD.

**Table 5. Effect of Chelating Treatment on Tissue Iron Content (nonheme iron µg/organ)**

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1,287 ± 183</td>
<td>336 ± 105</td>
</tr>
<tr>
<td>DF</td>
<td>1,152 ± 204</td>
<td>567 ± 225</td>
</tr>
<tr>
<td>CP 20</td>
<td>1,160 ± 200</td>
<td>347 ± 190</td>
</tr>
<tr>
<td>CP 94</td>
<td>735 ± 191</td>
<td>166 ± 22</td>
</tr>
</tbody>
</table>

*Mean ± 1 SD.

![Fig 1. Fecal excretion of radioiron after a single dose of chelator administered on day 0 to *59Fe-ferritin labeled hypertransfused rats. Urinary excretion was 0. (A) Parenteral treatment by intraperitoneal injection. (B) Oral treatment by gastric gavage. n = 4; mean ± 1 SD. (-----), Control; (----), DF; (---), CP 20; (- - -), CP 94.](http://www.bloodjournal.org)
In the present study we have examined in detail the in vivo chelating properties of two CP compounds representing the spectrum of activities and solubilities typical of this family of iron chelators. CP 20, with a low partition coefficient, is a relatively inefficient iron chelator under in vivo conditions, whereas CP 94 (Table 1) is a more effective iron chelator with a higher partition coefficient. In this study their in vivo behavior was compared with that of DF.

The results of studies in animals labeled with "Fe-ferritin illustrate the in vivo interaction of iron chelators with hepatocellular iron stores. All chelators induced an increase in fecal excretion without having any effect on urinary radioiron excretion. These findings are in line with results of previous studies and indicate that the CP compounds are able to mobilize iron from both hepatocellular and RE iron stores, about one third to one half of the iron derived from RE stores is excreted directly into the bile. In contrast, about one third to one half of the iron derived from RE stores is cleared by the kidneys, and the rest is recycled into the liver and excreted through the bile.

The CP compounds are bidentate chelators and a 3:1 chelator:iron ratio is required for the formation of their neutral complex with iron. In our previous studies with heart cell cultures, a relatively low chelating efficiency of CP compounds was found at low molar concentrations, raising concerns about the formation of polar [Fe-L]" and [Fe-L,]" intermediate complexes in the presence of excess iron. However, it is reassuring that in the present study there was no evidence of a diminishing in vivo effectiveness of CP 94 at the lowest concentrations used in our dose-response studies. Finally, comparison of oral to parenteral effectiveness of both CP compounds has shown that the total amount of radioiron excreted after both methods of administrations was identical, confirming all previous reports of the oral effectiveness of the CP compounds.

A number of clinical studies are presently underway to determine the safety and efficiency of oral treatment by CP 20 (L1) in patients with transfusional iron overload. Results of these trials and those to be conducted in the future with some of the most promising CP compounds will provide critical information regarding the suitability of these compounds for clinical use. In particular, the toxicity associated with the use of the CP compounds should be an important consideration in view of the observation of Porter et al that in mice improved chelating efficiency may be achieved at the cost of increasing acute toxicity. No toxic
effects have been observed with either CP 20 or CP 94 in the present study, but the doses used by us have been modest, the duration of treatment limited, and these studies were not designed to test the limits of toxicity. Information derived from studies in experimental models, such as the present one, cannot be directly extrapolated to the clinical setting. Nevertheless, our data raise important issues to be examined in future clinical trials by showing that: (1) the CP compounds are able to remove iron directly from both hepatocellular and RE iron stores; (2) urinary iron excretion after treatment with the CP compounds is derived from RE cells; (3) part of the iron mobilized from RE cells and all of the iron derived from hepatocytes is excreted through the bile; and (4) contrary to previous observations in cell cultures, there is no in vivo evidence for a diminishing chelating efficiency at the lowest doses used.

REFERENCES
16. Bartlett AN, Hoffbrand AV, Kontoghiorghes GJ: Long-term trial with the oral iron chelator 1,2-dimethyl-3-hydroxypyrid-4-one (L), II. Clinical observations. Br J Haematol 76:301, 1990
17. Kontoghiorghes GJ: Assessment of oral 1,2-dimethyl-3-hydroxypyrid-4-one (L1) in different ferrokinetic models in animals and humans. Br J Haematol 77:440, 1991
Origin and fate of iron mobilized by the 3-hydroxypyridin-4-one oral chelators: studies in hypertransfused rats by selective radioiron probes of reticuloendothelial and hepatocellular iron stores

S Zevin, G Link, RW Grady, RC Hider, HH Peter and C Hershko