

Metals and Chelation in Medicine

12.1 DEPENDENCE OF BIOLOGICAL GROWTH ON THE CONCENTRATION OF ESSENTIAL AND TOXIC ELEMENTS

Fig. 12.1.1 illustrates the effect of both essential (a) and toxic (b) elements on growth. The essential element shows toxicity if its uptake is very high. For a representative essential element [Fig. 12.1.1(a)], if its concentration is less than B, the system experiences its deficiency but if its concentration exceeds C, its beneficial role decreases. Thus the deficient concentration range (A—B) is responsible for growth retardation; the optimal concentration range (B—C) stands for optimal growth; the excess concentration range (C—D) again exerts growth retardation and the concentration exceeding the limiting value D induces deleterious effect (i.e. toxic effect). The range of B—C gives the *tolerance limit*, and for the most common essential elements, these tolerance limits are fairly high. For the toxic elements like Cd, As, Hg, Pu, etc. the effect is represented by the curve (b) in Fig. 12.1.1. During the evolution process, life did not meet these toxic elements and this is why it did not evolve any efficient mechanism to cope with these elements. Consequently, the tolerance limit given by the *threshold value* for a toxic element is generally very low and toxicity, i.e. deleterious effect, starts even for a minuscule concentration.

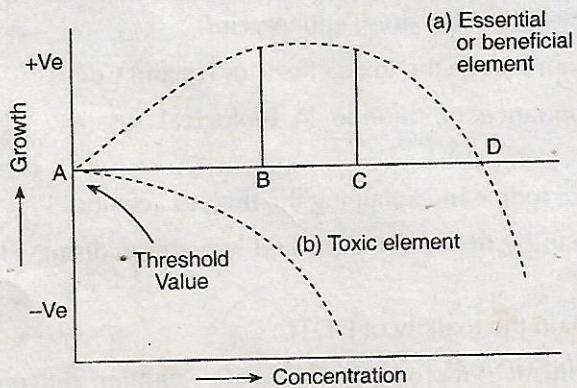


Figure 12.1.1 : Dependence of growth on the concentration of an essential (a) and a toxic (b) element

For the different essential elements, the concentration values denoted by A, B, C and D are different. The amount of requirement of some representative essential elements has been discussed in Sec. 1.1. The distribution of different elements in normal diet is given in Table 12.1.1. The recommended daily doses of some essential elements for a healthy adult human being (~ 70 kg) are as follows : Fe (10 mg for male, 15 mg for female), Zn (15 mg), Cu (2-3 mg), Mn (3-5 mg), F (1.5-3 mg), Mo (150-450 µg), I (150 µg), Cr (50-150 µg), Se (50-150 µg), Co (3 µg, vit. B₁₂), Na (5-10 g), K (3-4 g), Ca (0.8-1.0 g), P (0.8-1.0 g; i.e. Ca : P = 1:1), Mg (0.3-0.4 g).

Table 12.1.1
Distribution of some elements in human diet (mg/day)

Element	Normal	Toxic*
As	0.04 — 1.4	5 — 50
Al	2 — 45	5000
Br	0.8 — 20	3000
Ca	600 — 1500	—
Cd	0.007 — 0.3	3 — 330
Co	0.005 — 1.8	500
Cr	0.01 — 1.2	200
Cu	0.5 — 6	—
Fe	6 — 45	200
F	0.3 — 5	20
Hg	0.004 — 0.02	0.4
Li	0.1 — 2	100 — 200
Mn	0.4 — 10	—
Se	0.006 — 0.2	5

* Toxicity starts in this range.

Table 12.1.2
Effects of some elements on human health

Metal	Disease due to deficiency	Disease due to excessive accumulation
Li	Maniac depressive psychosis.	CNS disorder; nephrotoxicity.
F	Poor bones (i.e. osteoporosis) and dental caries.	Fluorosis; mottled teeth; bone sclerosis.
I	Hypothyroidism; goiter.	Hyperthyroidism
Na	Addison's disease; hyponatremia (reduced blood pressure); Stokes' cramps.	Hypernatremia (increase in blood pressure).
K	—	Addison's disease; cardiac failure.
Mg	Neuromuscular problem like convulsion.	Anaesthesia; cardio-vascular problems.
Ca	Abnormalities in bone (e.g. rickets, osteomalacia and osteoporosis), nerve function, muscle contraction, blood clotting; retarded growth; hypocalcemia.	Cataracts; stones in gall bladder and kidney; calcification of tissues; inhibits the absorption of other essential metals; hypercalcemia.

Metal	Disease due to deficiency	Disease due to excessive accumulation
Cr	Impaired glucose and lipid metabolism.	Cr(VI) causes cancer and ulceration.
Mn	Skeletal abnormalities; gonadal failure; inhibited growth; impaired glucose metabolism.	Ataxia and damage to CNS.
✓ Fe	Anemia.	Hemochromatosis (bronze diabetes); hemosiderosis; lesions in gastrointestinal tract; liver damage.
✓ Co	Pernicious anemia	Coronary failure; polycythemia (increased RBC); thyroid dysfunction.
Ni	—	Dermatitis (sweating leads to complexation of Ni from Ni-plated jewelry with the skin protein keratin); gastrointestinal discomfort.
✓ Cu	Anemia; kinky-hair syndrome; poor bone and connective tissues; pigmentation problem.	Wilson's disease; stomach irritation and nausea; reduced growth; liver damage.
✓ Zn	Dwarfism; gonadal failure; delay in wound healing; affects lactation in woman.	Metal-fume fever due to inhaled Zn-fumes (pulmonary distress); may cause Cu-deficiency and anemia; impaired bone development.
Se	Liver necrosis; cancer; white muscle disease.	Cancer; alkali disease; hair and hoof loss; blind staggers.
Cd	Not known.	Nephritis; <i>itai-itai byo</i> (wrong bone metabolism)
✓ Pb	Not known.	Impaired kidney function, multiple sclerosis; anemia; neurological problem; encephalitis.
✓ Hg	Not known.	Encephalitis; impaired kidney function.

12.2 DISEASE DUE TO METAL DEFICIENCY AND TREATMENT

12.2.1 Iron Deficiency and Anemia and its Treatment

Several Fe-dependent metalloproteins and metalloenzymes are known to occur (see Table 3.1.1.2, 3), but its deficiency is immediately reflected in terms of the appearance of anemia. In fact, 65 – 70% of total body iron exists in Hb (hemoglobin) in human. The anemic condition may also arise for other reasons. Vitamin B₁₂ deficiency (*pernicious anemia*; Hb synthesis is hampered, see vitamin B₁₂), erratic Cu-metabolism (Cu-containing protein ceruloplasmin controls Fe-metabolism, cf. Sec. 3.1.3), Pb-poisoning (preventing Hb-synthesis, cf. Sec. 12.4.5), etc. are known to cause anemia. Sometimes the genetic disorder produces anemia, e.g. *sickle cell anemia* (mutant Hb-S where hydrophobic valine residue is present instead of hydrophilic glutamic acid at the 6th position of the β-chain; deoxy Hb-S polymerises into fibrous structures, cf. Sec. 5.5.8), *Cooley's anemia* (insufficient production of β-chain), *atransferrinemia* (genetic inability to synthesise transferrin), *thalassaemia* (genetic disorder in Hb-structure; in β-thalassaemia the decreased rate of β-chain production leads to precipitation of α-chains in the RBC and it causes the premature destruction of Hb), etc.

12.6 REQUIRED THERMODYNAMIC AND PHARMACOKINETIC PROPERTIES OF THE CHELATING DRUGS IN METAL ION DETOXIFICATION

Organic poisons (even DDT) are biodegradable, but the toxic metals, if entered in the food chain, remain unchanged for ever. Thus, if a metal can poison the food chain at any stage, the poisoning activity will continue throughout the food chain, regardless the length of time. To detoxify these toxic metals from the living system, it requires the *chelation therapy* which utilises the administration of some suitable chelating agents to remove the toxic metal from the living body. To select a chelating antidote, the following aspects are to be considered.

12.6.1 The Requirements of a Chelating Antidote in Metal Ion Detoxification

- (a) **Conditional stability constant** : Conditional stability constant gives the measure of stability of a complex (i.e. metal-ligand interaction) under the actual conditions, i.e. biological conditions in the present case. Thus conditional stability constant is different from the thermodynamic stability constant. The toxic metal-species remains bound with the biogenic ligands and consequently the selected chelating drug should successfully compete with the biogenic ligands to snatch away the bound metal. To achieve this, the conditional stability constant between the toxic metal and chelating drug must be greater than that of the competing bioligand involved. The administered chelating drug may undergo complexation with the biologically abundant essential metal ions like Ca^{2+} or Zn^{2+} and this is why, the toxic metal to be removed should be able to displace these essential metals bound with the chelating drugs.
- (b) **Lipophilicity of the chelating drug** : In the case of chronically intoxicated organs by Cd(II) , $\text{CH}_3\text{Hg(II)}$, radionuclides, etc., the chelating drug should be sufficiently lipophilic to penetrate the cellular membrane to reach the target toxic metal. In such cases, by introducing a lipophilic moiety in the chelating drug, the activity may be remarkably improved. For example, if the organism is chronically intoxicated by Pu, then *puchel* having lipophilic moieties in *dtpa* (diethylenetriaminepentaacetate) is a much better drug than the *unsubstituted dtpa* (Sec. 12.7.2). Similarly to detoxify $\text{CH}_3\text{Hg(II)}$, N-acetyl D-penicillamine is more promising than the unsubstituted D-penicillamine.
- (c) **HSAB (hard and soft acids and bases) theory and selection of chelating drug** : According to this theory, to remove a hard toxic metal ion, a chelating drug with the hard donor sites is preferred, and to detoxify a soft metal, the chelating drug should have the soft binding sites. The recommended chelating drugs satisfy this criterion of HSAB theory (Table 12.6.1.1).

Table 12.6.1.1

Chelating drugs and HSAB theory

Metals to be removed	Chelating drug (binding sites)	HSAB matching
Fe(III)	Desferrioxamine B (several O)	Hard-Hard
Pu(IV)	Puchel, dtpa (5O, 3N)	Hard-Hard
Be(II)	Aurintricarboxylic acid (several O)	Hard-Hard

Metals to be removed	Chelating drug (binding sites)	HSAB matching
Pb(II)	Edta (O, 2N)	Borderline-Borderline
Cu(II), Cu(I)	D-Penicillamine (S, N, O)	Borderline-Borderline, Soft-Soft
RHg(II)	N-Acetyl-D-penicillamine (S, N, O)	Soft-Soft
As(III), Hg(II)	British Antilewisite (2S), Unithiol (2S)	Soft-Soft

(d) **Designing of antidotes with the binding sites mimicking the endogenous binding sites** : If the binding sites of the chelating drug are similar to those of the endogenous binding sites trapping the target toxic metal, then the drug can yield better results. Thus for detoxification of Pb^{2+} , the chelating drug should have the features : polypeptide of multidentate character, the amino acid residues of L-configuration should be selected from cysteine, histidine and aspartic acid.

(e) **Toxic effects of the chelating drug** : The administered chelating drugs should not be toxic and the drugs should not be metabolised in performing the scavenging action. Thus the drugs with higher LD_{50} values are preferred. Sometimes the toxic metal-drug chelate may enhance the toxicity due to the translocation of the chelate. In detoxification of $\text{CH}_3\text{Hg(II)}$, if BAL is introduced then the neutral chelate, $(\text{CH}_3\text{Hg})_2\text{BALH}_2$ can easily pass through the blood brain barrier resulting in an excessive accumulation of $\text{CH}_3\text{Hg(II)}$ in brain by redistribution. Thus it enhances the toxicity. LD_{50} values of some common chelating drugs are given in Table 12.6.1.2.

Table 12.6.1.2
 LD_{50} values of some common chelating drugs

Chelating drugs (a)	Species	Mode of Administration (b)	LD_{50} (g/kg)
$\text{Na}_2\text{Ca(edta)}$	mouse, rat	ip	4.6
$\text{Na}_3\text{Ca(dtfa)}$	mouse, rat	ip	2.4
BAL	mouse	ip	0.1-0.2
Dmsa	mouse	oral	4.3
Dmps	mouse	ip	1.1-1.4
DPA	mouse	ip	0.34
DFO	rat	iv	0.52
L1	mouse, rat	ip	0.6-1

(a) Names of the ligands are given in Sec 12.7. (b) ip (intraperitoneal), iv (intravenous).

(f) **Urinary and biliary excretion** : Urinary excretion is favoured for the water soluble complexes of low molecular weight, while the biliary excretion is favoured for the high molecular weight complexes of a very limited water solubility.

12.6.2 Summary of the Basic Requirements of a Chelating Drug

All the essential requirements of a chelating antidote are summarised (May and Williams, 1979) below.

The chelating antidote should (i) bind the toxic metal sufficiently strongly to compete with the endogenous biological ligands such as proteins, (ii) discriminate against the relatively abundant Ca^{2+} and Zn^{2+} ions which are essential *in vivo*, (iii) be sufficiently lipophilic to penetrate the lipid membranes to reach the body compartment where the toxic metal is accumulated, (iv) possess a high LD₅₀ value.

To remove the toxic metal from an interior compartment, the drug must form a lipophilic complex in the body compartment, then it must change to a hydrophilic complex upon reaching the blood plasma so that elimination of the metal complex is possible through the urinary excretion rather than its redistribution back into the tissues. Sometimes all these conditions are not satisfied by a single ligand and then double ligand therapy is recommended.

12.6.3 Characteristics of Double Ligand Therapy

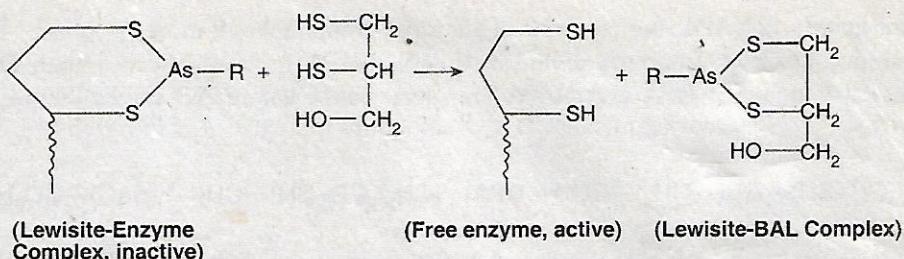
All the requirements summarised in the above sections, particularly for removing the toxic deposits in the interior body compartments, are not satisfied by a single chelating drug. For chronic Pu-intoxication, the two drugs dtpa and puchel can act synergistically. Puchel being more lipophilic can mobilise the radionuclides from the sites of Pu-deposition and transfer it into the plasma where dtpa (hydrophilic polyaminocarboxylate is mainly confined in the extracellular space) can trap plutonium to excrete in the urine. In another view, it is proposed that the mixed ligand complex (i.e. ternary complex) formation is the important step. The stability of the mixed ligand complexes is higher than that of the corresponding binary complexes. In Pu-detoxification, dtpa + SA (salicylate), enhances the excretion to the level much above than that induced by dtpa alone and SA alone shows no activity. The lipophilicity or hydrophilicity of the metal complex depends on the total dentateness of the ligands and ligancy of the metal ion. If the total dentateness is greater or less than the ligancy of the metal ion, then the complex is hydrophilic [e.g. Pu(dtpa)(SA) of total dentateness 10; Pu(edta) of total dentateness 6; cf. the metal ligancy = 8] and the complex is lipophilic if the total dentateness is equal to the ligancy of the metal ion. Thus, the binary complex, Pu(dtpa) or the ternary complex Pu(edta)(SA) is lipophilic.

12.7 SOME REPRESENTATIVE CHELATING DRUGS USED IN METAL ION DETOXIFICATION

12.7.1 Chelating Drugs Having —SH Groups for Detoxification of Heavy Metal Ions (i.e. Soft Centres)

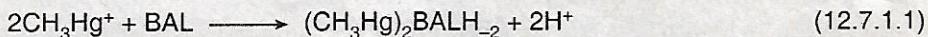
According to HSAB theory, such chelating drugs will preferably bind the soft and heavy metals. Consequently, depletion of Ca^{2+} (hard centre) ion from the body fluid by these chelating ligands does not occur. This is an advantage. Some drugs in this group in clinical uses are discussed below.

(a) **BAL (2,3-dimercapto-1-propanol, or dimercaprol)** : This drug was developed by the British scientist, Sir Rudolph Peters during the Second World War to treat the patients affected by the poison gas, Lewisite ($\text{ClCH}=\text{CHAsCl}_2$). Lewisite can block the —SH groups of different enzymes to cause the toxicity (cf. Scheme 9.4.3.8, Sec. 12.4.3), but BAL can remove the enzyme bound arsenic compound to restore the activity of the enzymes, and arsenic-BAL complex is excreted through urine. The detoxification process is outlined in Scheme 12.7.1.1.



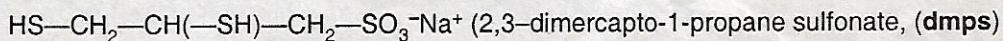
Scheme 12.7.1.1: Antidotal activity of BAL in As-poisoning by Lewisite ($R = -CH=CH-Cl$)

In acute Cu-poisoning and in Wilson's disease, BAL can show its antidotal activity. In fact, BAL is recommended for the treatment of poisoning by different heavy metals like Hg, Au, Tl, Bi. But in detoxification of CH_3Hg^+ , the neutral chelate, $(\text{CH}_3\text{Hg})_2\text{BALH}_2$ formed can pass through the biological membrane to enhance the toxicity by its redistribution. BAL is found to increase the toxicity of Cd and Pb in experimental animals.

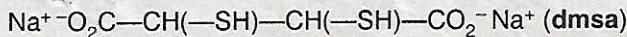


Though BAL is recommended in As-poisoning, it has some serious limitations. Its aqueous solution is unstable with respect to aerial oxidation. It is to be taken in a solution of vegetable oil and it is to be injected intramuscularly. It has an objectionable odour and the site of injection experiences a painful irritation. This is why, local anaesthesia is required for BAL administration. Sometimes, it may lead to hypertension, vomiting, sweating, etc. *Thus BAL is not at all a good chelating drug.* In fact, with the advent of *dmsa* and *dmps* (which are safer), the clinical use of BAL is going to be phased out.

(b) Unithiol (2,3-dimercapto-1-propane sulfonic acid, dmpts) : This water soluble drug has several advantages compared to BAL. Because of the clinical advantages such as water solubility, stability, high LD₅₀ value (i.e. less toxicity), strong complexing power, unithiol is more promising to detoxify the soft metals like As, Hg, Tl, etc. In detoxification of CH₃Hg⁺, BAL can lead to enhance the toxicity but unithiol can be safely used. The corresponding CH₃Hg-unithiol complex being charged cannot pass through the biological membrane. The structural formula of the drug is shown below.

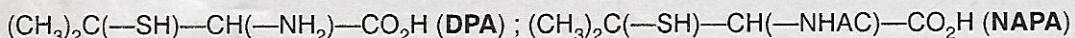


(c) Disodium meso-2,3-dimercaptosuccinate (dmsa) : It is soluble in water and it can be given in drinking water. It is of low toxicity and its LD₅₀ value is about 30 times higher than that of BAL. In detoxification of CH₃Hg⁺, it is quite promising. It can also detoxify many other soft metals. Its structure is given below.



(d) D-Penicillamine (DPA) and N-acetyl D-penicillamine (NAPA) : D-penicillamine is 3-mercaptopvaline (i.e., 3, 3'-dimethyl cysteine). The D-isomer is therapeutically active but the L-isomer is toxic. By using the binding sites, S, N, O it can bind with Hg(II), $\text{CH}_3\text{Hg}(\text{II})$, Cu(II), Au(I), Pb(II), etc. Its LD₅₀ value is much higher than that of BAL and it can be orally

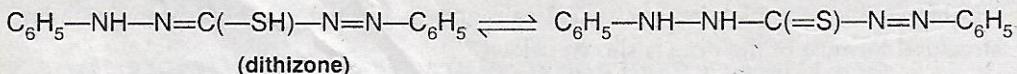
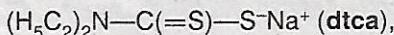
administered. In NAPA, the presence of an acetyl group makes it more lipophilic. This is why to remove H_3CHg^+ from the erythrocyte cells, NAPA is more effective than DPA. The structural features of DPA and NAPA are given below where AC stands for acetyl group, $\text{CH}_3\text{CO}-$.



In DPA therapy, there is a possibility of depletion of essential elements like Zn and Cu. The Cu-depletion is indicated by the *loss of taste sensitivity* and it can be restored by an oral administration of copper salts.

In Wilson's disease (*hepatolenticular degeneration*), D-penicillamine is clinically recommended. With Cu(II), DPA forms an *intense purple coloured multinuclear complex*. It is interesting to note that the complex formation does not occur unless chloride or bromide ions are present in the reaction media. The isolated complex is found to contain a small amount of halide. The structural analysis indicates that the complex contains the halide ion at the centre and it is surrounded by eight Cu(I) centres bridged by S-sites. Cu(I) centres are produced through the reduction of Cu(II) by DPA having -SH groups. The S-sites coordinate six Cu(II) centres whose residual sites are occupied by N-sites (i.e. each Cu(II) centre binds with 2S and 2N sites). The stoichiometric formula of the complex ion is $[\text{Cu}_8^{\text{I}}\text{Cu}_6^{\text{II}}(\text{penicillaminato})_{12}\text{Cl}]$.

(e) Other related chelating drugs : Some important examples are : sodium diethyldithiocarbamate (dtca) (used in Ni-poisoning), diphenyl thiocarbazone (dithizone) (used in Tl-poisoning), potassium methyl and ethyl xanthates, polythiolated ion-exchange resin (used to reduce the $\text{CH}_3\text{Hg}^{\text{II}}$ load by interrupting the *enterohepatic cycle*; the resin fed orally can chelate much of the mercury in the bile and ultimately excreted in the feces), prussian blue (to remove the toxic metals from the gastrointestinal tract). The role of thionein protein as the body's own protective chelating agent has been already discussed (Sec. 12.5). The structural features of dtca and dithizone are given below.

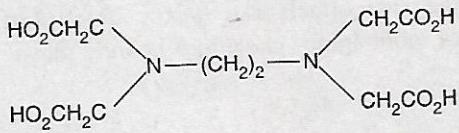


12.7.2 Polyaminocarboxylic Acids as Chelating Drugs

Generally, the polyaminocarboxylic acids (e.g. edta, cdta, dtpa, puchel, cf. Fig. 12.7.2.1) are administered intravenously or intramuscularly as solutions of their calcium salts. These ligands form strong chelates with the Ca^{2+} ions. Hence, if their Na-salts are administered, then they induce *hypocalcaemic tetany* through Ca-depletion. In fact, to avoid the depletion of the essential metal ions like Ca^{2+} and Zn^{2+} , they are used as : $\text{Na}_2\text{Ca}(\text{edta})$, $\text{Zn}_2(\text{edta})$, $\text{Na}_3\text{Zn}(\text{dtpa})$, etc.

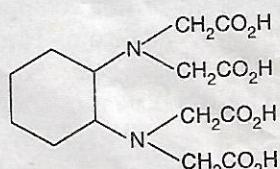
In Pb-poisoning, $\text{Na}_2\text{Ca}(\text{edta})$ is administered intravenously. It leads to excretion of Pb(edta) along with $\text{Na}_2\text{Ca}(\text{edta})$ through urine. Here it should be mentioned that, $\text{Na}_2\text{Ca}(\text{edta})$ cannot be administered orally to reduce the Pb-burden. The lead already excreted into the gut will be reabsorbed as Pb(edta) complex. In general, $\text{Na}_2\text{Ca}(\text{edta})$ is administered through slow intravenous injection as its intestinal uptake is very low. Its detoxifying activity is mainly confined in the extracellular region. $\text{Na}_2\text{Ca}(\text{edta})$ can also detoxify Co- and Cd-poisoning. To remove the stones

(made of Ca-phosphate and Ca-oxalate) from urinary tract, edta may find its application. The drug may also deplete Zn-content. The ligand dtpa and its derivative like puchel (having additional lipophilic moieties) are used in Pu-detoxification. Puchel can pass across the cell membrane into the interior part of the cell to invade the Pu-deposition in chronic intoxication.



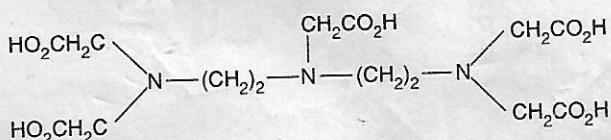
(edta)

Ethylenediaminetetraacetic acid



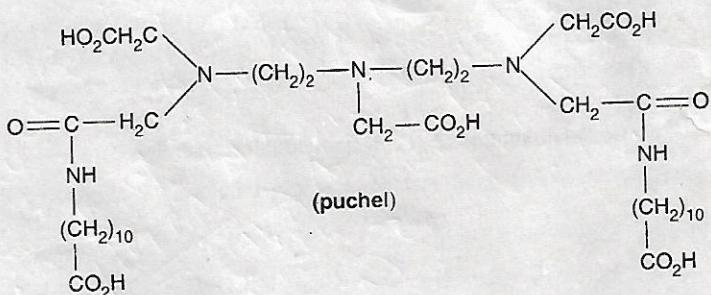
(cdta)

Cyclohexane-1,2-diaminetetraacetic acid



(dtpa)

Diethylenetriaminepentaacetic acid



(puchel)

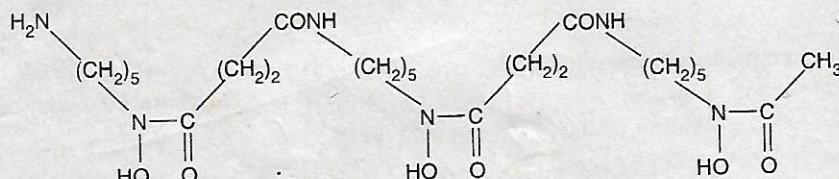
Figure 12.7.2.1 : Structural representation of some polyaminocarboxylic acids used as chelating drugs.

12.7.2 The Desferrioxamines (DFO) as Chelating Drugs

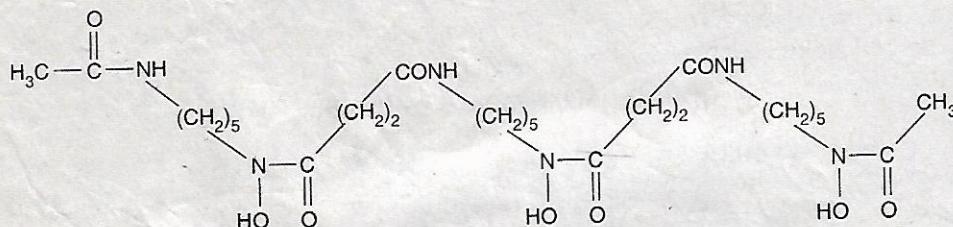
The nonprotein low molecular weight naturally occurring siderophores called desferrioxamines (Fig. 12.7.3.1) having hydroxamic acid groups are highly specific to reduce the Fe-burden. These ligands are known to control the Fe-metabolism in some bacteria (Sec. 3.1.4). These were first isolated from bacteria and subsequently characterised and synthesized. The binding constants with Fe(III) are tremendously high ($\sim 10^{30}$) under physiological conditions. Desferrioxamine-B (commonly known as desferal) injection is now clinically well established to remove the Fe-load from the patients suffering from genetic disorders (e.g. Cooley's anemia, thalassemia, hemochromatosis, hemochromatosis, etc.) or accidental ingestion.

Desferrioxamines are poorly absorbed in the gut and in some cases these may develop allergic responses. The hydroxamates hydrolyse in the acid environment of stomach. As its absorption from the gastrointestinal tract is very poor, it must be administered through *iv* injection. A wide range of side effects have been noted. It may lead to allergic and skin reactions, neurological effects, renal

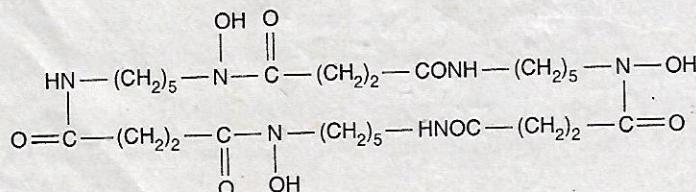
effects. This is why, the scientists are looking for the simpler ligands which will form low molecular weight neutral complexes with iron and the ligands will be absorbed in the gut. The ligand, 1,2-dimethyl-3-hydroxypyridine-4-one known as **L1** (which can be orally taken) is under clinical trial in removing the Fe-load. L1 (Fig. 12.7.3.2) is a promising antidote and it is rapidly absorbed from the gastrointestinal tract. It is commercially cheaper than DFO (desferrioxamines). It can be given for detoxification of aluminium. However, it shows some side effects like gastric discomfort, Zn-depletion, joint pains, etc. Several tris-catecholates containing chelating ligands have been synthesised for this purpose.



Desferrioxamine B (i.e. desferal)



Desferrioxamine D₁ (i.e. N-acetyl desferrioxamine B)



Desferrioxamine E

Figure 12.7.3.1 : Structural representation of desferrioxamines (DFO) having hydroxamic acid groups [i.e. $-C(=O)-N(-OH)-$] as the chelating sites.

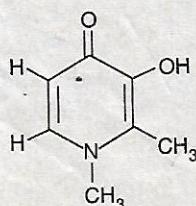


Figure 12.7.3.2 : Structural representation of 1,2-dimethyl-3-hydroxypyridine-4-one (L1).