CHAPTER 1

1.1 General Introduction

The word 'metal complex' seems to have been first used in late nineteenth century for the products formed by metal ions like Cr$^{3+}$, Co$^{3+}$ and Pt$^{2+}$ with ammonia, chlorine, etc. Tassaert's chance discovery (1978) of hexamine cobalt chloride, CoCl$_3$.6NH$_3$, is the beginning of the chemistry of metal complexes.

Early work centered around Co$^{3+}$, Pt$^{4+}$ and Pt$^{2+}$ complexes with amines. Towards the end of the nineteenth century, the field was dominated by the Danish Chemist S.M. Jorgensen and the Swiss Alfred Werner (1).

1.1.1 Ligands, Complexes and Chelating Agents

With the development of electronic theory of valency in the beginning of the present century, much progress in understanding of the structure and behavior of metal complexes has been made.

A complex compound consists of a central metal atom or cation to which several anions and/or neutral molecules are bonded. The latter are called ligands. The ligands are disposed about the central metal ion in a regular geometric manner. The total number of donor groups directly bonded to the central metal ion is called the coordination number of the central metal ion.

With few exceptions, free ligands have at least one atom with lone pair of electrons. In complex formation, the lone pair of electrons may be considered to be donated by the ligands to the electron deficient central metal ion.
Certain ligands are capable of coordinating to the central ion through two or more donor atoms appropriately situated in the molecule leading to the formation of ring structures. The coordination of such ligands is called chelation.

The term 'Chelate' from Greek word 'Chele' (meaning crab's claw) was introduced in 1920 (2) to describe those complexes that result from a combination of an electron donor with a metal ion to form a ring structure. The compounds capable of forming a ring structure with a metal are designated as ligands. Ligands having the potential to form the rings are called chelating agents. They are often associated with additional complex stability, particularly when the rings are five membered (3). If the metal is not in a ring, the compound is called simply a metal complex.

A ligand molecule containing only two electron donating groups is designated as "bidentate" and is able to form only a single ring; if it contains three electron donating groups, it is "tridentate" and may form two rings in an interlocked complex; the prophyrine are able to form a number of interlocked ring systems with metals and are designated as "polydentate" structures.

The size of the rings in chelate compounds is of interest with respect to both the stability and occurrence. The 5- and 6-membered chelate rings are most common and usually show the greatest stability. If the complex forming ligand supplies both electrons for chelation, then the bond is classified as a
coordinate covalent bond and by convention is represented as \( M--X \), where \( M \) is the metal and \( X \) is the ligand. If one electron is supplied by the metal and one by the ligand (normal coordinate bond), the bond is shown as \( M-X \).

### 1.1.2 The Metal-Ligand Bond

Werner (1), in 1893, suggested that the complex forming elements possess an "auxiliary" or a "secondary" valency, now termed as coordination number, in addition to "primary" valency termed as oxidation number, or oxidation state. Coordination numbers are simply the number of donor groups attached to the central metal ion of the complex. Lewis (4) gave an electronic basis to Werner's views of secondary valency. Sidgwick (5) suggested that the central metal ion has tendency to add sufficient number of electrons by coordination, so as to acquire an "effective atomic number" (E.A.N.) of the next noble gas.

With the advance of quantum mechanical theory, a new era in the field of coordination compounds is opened. The four different theories of treating the bonding in complexes developed during the 1930's are: (1) Valence Bond Theory (6,7), (2) Crystal Field Theory (8), (3) Molecular Orbital Theory (9), and (4) Ligand Field Theory (10).

### 1.2 Bio-Inorganics

The term bioinorganic sounds rather paradoxical because of the dictionary classification of inorganic matter as "non-living" (11). However, the definition is misleading because life is as
dependent on inorganic chemistry as it is on organic chemistry, e.g., the provision of bone structure, the role of polyphosphate functions in cellular energetic and the central roles of transition ions in biological redox processes.

The remarkable development of molecular biology has had its counterpart in an impressive growth of a segment of biology that might be described as atomic biology. The past several decades have witnessed an explosive increase in our knowledge of the many elements that are essential for life and maintenance of plants and animals (12). This field of research also includes the subject area which is frequently identified as bio-inorganic chemistry and trace element research. Out of 109 known elements, 30 elements are believed to be essential for the survival of living organisms. Among these, 6 are bulk or structural elements, 5 macrominerals and 19 trace elements. The dominance of the 11 common structural elements accounts for over 99% of the atoms (13). The trace elements occur in much lower concentration. The 19 essential trace elements include the three prominent biologically active metals iron, zinc and copper. The remaining elements are considered ultratrace. Classification of the essential elements is given in Table 1.

Knowledge of the biological function of the trace elements has lagged far behind our understanding of their chemistry. We can account for the biological activity of about one-half of the essential trace elements which function in metalloenzyme,
including Fe, Zn, Cu, Mn, Mo, Co, Ni and Se. However, we cannot explain physiological role with certainty of V, Cr, Cd, Pb, Sn, Li, F, Si, As and B. The treatment of anaemia with iron and the association of iodine deficiency with goitar marked these as the only two trace elements recognised as essential for animals.

In the twentieth century, there are two major periods of activity in biological trace element research (13). In an early classical period, 1925-1956, serendipity contributed to the discovery of essentiality of copper, zinc, cobalt, manganese and molybdenum in animals. A more active modern period, from 1957 onwards, is based on the experimental induction of trace elements deficiencies. These efforts have resulted in evidence supporting the essentiality of selenium, chromium, tin, vanadium, fluorine, silicon, nickel, lead, cadmium, arsenic and lithium.

Table 1: Classification of the Essential Elements

<table>
<thead>
<tr>
<th>Category</th>
<th>Elements</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Microminerals</td>
<td>Na, K, Mg, Ca, Cl</td>
</tr>
<tr>
<td>3. Trace elements</td>
<td>Fe, Zn, Cu</td>
</tr>
<tr>
<td>Ultratrace elements</td>
<td>F, I, Se, Si, As, B</td>
</tr>
<tr>
<td>Non-metals</td>
<td>Mn, Mo, Co, Cr, V, Ni, Cd, Sn, Pb, (Li)</td>
</tr>
<tr>
<td>Metals</td>
<td></td>
</tr>
</tbody>
</table>
An element is considered essential when its deficient intake produces an impairment of function. The restoration of that element relieves the impaired function or prevents impairment. The organism can neither grow nor complete its life cycle without the element in question. The elements have a direct influence on the organism and are involved in its metabolism. The effect of the essential element cannot be wholly replaced by any other element. The criteria for essential elements are summarised in Table 2.

Table 2: Criteria for Essential Elements

1. A physiological deficiency appears when the element is removed from a purified diet.
2. The deficiencies can be relieved by the addition of one specific element.
3. A specific biochemical function is associated with a particular element.

The additional criteria for essential elements are as follows:
(i) The element is present in tissues of different animals at comparable concentration. (ii) Its withdrawal produces similar physiological or structural abnormalities regardless of species. (iii) Its presence reverses or prevents these abnormalities. (iv) These abnormalities are accompanied by specific biochemical changes that can be remedied or prevented when the deficiency is checked.
1.2.1 Physiology of Metal ion

The study of transition metal ion has received fresh impetus in recent years because of the interest in their essential biological roles and concern with their potentially toxic effects as environmental contaminants.

In particular, the results may be entirely spurious whenever the effects of complexation are neglected in the design, execution or interpretation of results of such research. For example, early work on the mechanisms of intestinal iron absorption concluded that active transport processes were involved (14); and the complex formation within the mucosal cell was not adequately appreciated. It has now been demonstrated that movement across the membrane occurs by passive diffusion (15).

1.2.2 Metal ion Toxicity

It is well established that the toxicity of metal ions can be ascribed to their interference in certain critical biochemical processes (16). Coordination of transition metal ions to donor groups on biological macromolecules (particularly those belonging to the cysteine and histidine residues of proteins) is primarily responsible for their toxicity. All heavy metal ions are liable to poison membranes and inhibit sulphhydryl enzymes.

However, the discovery that lethal elements such as arsenic might also be physiologically essential makes it clear that no genuine distinction can be drawn between toxic and beneficial metals. Bertrand (17) observed that all the trace elements are
poisonous, if ingested in sufficient amount. Subsequently, Venchikov (18) has described the general effect of increasing metal ion concentrations on physiological well-being as, first, having a metabolic function (if any) and then a pharmacotoxicological action.

The transition elements fall into the categories, according to whether they have a major biological function or not. The metal ions like Fe$^{2+}$, Cu$^{2+}$, Zn$^{2+}$ and Mn$^{2+}$ are having very efficient homeostatic control mechanisms. Such elements display toxic symptoms only when their homeostatic processes fail or are temporarily impaired. Zn$^{2+}$ and Mn$^{2+}$ are, accordingly, amongst the least toxic metals and relatively massive of Fe$^{2+}$ and Cu$^{2+}$ should be ingested before any permanent damage is done.

The non-essential elements, particularly those associated with pollution such as lead, mercury, cadmium and plutonium, tend to accumulate in the body in specific tissues and may persist in their detrimental effects long after the period of assimilation and give rise to irreversible biochemical damage at relatively low levels of exposure, e.g. Cadmium binding by metallothionein (19), the incorporation of lead into bone and the immobilization of plutonium in erythropoietic marrow.

The toxic effect in man arising from various kinds of exposure to different metals is shown in Table 3.
Table 3: Toxicity of Metals (20)

<table>
<thead>
<tr>
<th>Element</th>
<th>Acute effects</th>
<th>Chronic effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>N</td>
<td>O</td>
</tr>
<tr>
<td>As</td>
<td>G, N</td>
<td>N, J, L, C, M</td>
</tr>
<tr>
<td>Cd</td>
<td>G, P, K</td>
<td>P, K, L, O</td>
</tr>
<tr>
<td>Cr</td>
<td>G</td>
<td>D, C</td>
</tr>
<tr>
<td>Cu</td>
<td>B, L</td>
<td>N, L</td>
</tr>
<tr>
<td>Fe</td>
<td>G, S, L</td>
<td>H, C, L</td>
</tr>
<tr>
<td>Hg</td>
<td>G, K, P*, N**</td>
<td>G, N, M</td>
</tr>
<tr>
<td>Mn</td>
<td>P*</td>
<td>N, M</td>
</tr>
<tr>
<td>Ni</td>
<td>N***</td>
<td>D, C, M***</td>
</tr>
<tr>
<td>Zn</td>
<td>N*, G*, P*, K*</td>
<td></td>
</tr>
</tbody>
</table>

B = Haematological complication  
C = Carcinogenic risk  
D = Dermatitis  
E = Encephalopathy  
G = Gastroenteritis  
H = Cardiac involvement  
K = Kidney failure  
*Vapour or fumes, **Methyl mercury, ***Nickel carbonyl

Very little concrete information about the actual free concentration of metal ion occurring within biological fluids is available. Using non-selective electrodes, values for Ca$^{2+}$ of about $10^{-3}$M in plasma and $10^{-7}$M in cells have been measured (21). The non-protein bound Mg$^{2+}$ levels in plasma can also be determined. Williams (22) reported that the concentration of Mn$^{2+}$ is likely to be around $10^{-6}$M in vesicular spaces. Yet, this conclusion undermines the implications of numerous studies in which enzymes activation by milimolar concentrations of Mn$^{2+}$ has been demonstrated (23).
The four cations, Na\(^+\), K\(^+\), Mg\(^{2+}\) and Ca\(^{2+}\) are widely distributed in all living organisms. The uptake of these elements by biological systems has been reviewed recently. The extra and intracellular concentration of Na\(^+\), K\(^+\), Mg\(^{2+}\) are very different (the values for Ca\(^{2+}\) being quite similar) (Table 4).

Table 4: Concentration of cations in extracellular and intracellular fluids (in m eq per litre) (24)

<table>
<thead>
<tr>
<th>Cations</th>
<th>Extracellular</th>
<th>Intracellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^+)</td>
<td>145</td>
<td>10</td>
</tr>
<tr>
<td>K(^+)</td>
<td>5</td>
<td>150</td>
</tr>
<tr>
<td>Mg(^{2+})</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Ca(^{2+})</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

1.2.3 Metalloenzymes:

Most of the facts available about the function of the trace elements focus on their role as metalloenzymes. The trace elements are essential because they serve as required prosthetic groups in active sites and/or as coenzyme for indispensable metalloenzymes or metal-ion activated enzymes.

There is not a single enzyme catalyzed reaction in which the enzyme itself, the substrate or the product is not directly and specifically influenced by the nature and concentration of the inorganic ions which surround it.
In the metalloenzymes, a fixed number of specific metal atoms (usually, Fe$^{2+}$, Zn$^{2+}$, Cu$^{2+}$, Mo, Co$^{2+}$, Ni$^{2+}$, etc.) are firmly associated with a specific protein. This combination produces a unique catalytic function.

The prime examples of metalloenzymes are the proteins that contain iron, zinc or copper ions in their active sites. Zinc enzymes have been identified for all six major classes of enzymes, viz., oxido-reductases, transferases, hydrolases, lyases, isomerases, and ligases. The zinc ion serves as a super acid active center in proteins which are capable of promoting hydrolysis of a variety of susceptible chemical bonds. Metal ions can also play a major role in the geometry of the protein, the positioning of the substrate, and the formation of the active site.

Metals such as iron and copper have another dimension that of readily changing their oxidation states so that they can also serve as catalytic electron carriers. They produce oxidized substrates which then can fit into a variety of metabolic cycles. The deficiency symptoms and specific functions of essential ultratrace elements are recorded in Table 5.
Table 5: Properties of the essential Ultratrace Metals

<table>
<thead>
<tr>
<th>Ultratrace metal</th>
<th>Deficiency symptoms</th>
<th>Specific function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese</td>
<td>Growth depression, bone deformities, membrane abnormalities, connective tissue defects</td>
<td>Carbohydrate metabolism. Pyruvate carboxylase, etc.</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>Growth depression</td>
<td>Oxidases; aldehyde, Sulfite, Xanthine, Molybdopterin</td>
</tr>
<tr>
<td>Cobalt</td>
<td>Anaemia, growth retardation</td>
<td>Constituent of Vitamin B&lt;sub&gt;12&lt;/sub&gt;</td>
</tr>
<tr>
<td>Chromium</td>
<td>Insulin resistance</td>
<td>Potentiation of insulin, action on carbohydrates and lipids, active as a bioinorganic chromium complex</td>
</tr>
<tr>
<td>Vanadium</td>
<td>Growth depression</td>
<td>Control of sodium pump, inhibition of ATPase, Phosphotransferases</td>
</tr>
<tr>
<td>Nickel</td>
<td>Growth depression, reduced nitrogen utilisation, reduced iron metabolism</td>
<td>Constituent of urease, reduced homopoiesis</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Growth depression, reduced reproduction</td>
<td>Stimulates elongation factors in ribosomes</td>
</tr>
<tr>
<td>Tin</td>
<td>Growth depression</td>
<td>Interaction with Riboflavin</td>
</tr>
<tr>
<td>Lead</td>
<td>Growth depression, anaemia</td>
<td>Many enzyme effects</td>
</tr>
<tr>
<td>Lithium</td>
<td>Growth depression, reduced reproduction</td>
<td>Control of sodium pump</td>
</tr>
</tbody>
</table>
In nitrogenase, the metal is present in a unique cluster containing Fe$^{2+}$, Mo and S$^-$. In plant and microorganisms, nickel is known to function in several metalloenzymes in urease, several dehydrogenases and carbon monoxide dehydrogenase.

The role of cobalt, despite its complexity, is best understood of any of the essential ultratrace metals. Both vitamin B$_{\text{12}}$ and coenzyme B$_{\text{12}}$ have cobalt ion complexed in their equatorial positions by four nitrogens of a macrocyclic ligand called corrin. These compounds serve as cofactor in various enzymatic reactions.

Thus far, metalloenzymes have provided the best model for determining how the trace metal ions operate. Chromium functions in vivo as an organic chromium complex. Its biological role is to potentiate insulin activity. Vanadium compounds inhibit numerous enzymes, particularly ATPases and phosphotransferases. This has suggested a role for the Vandate ion in the control of sodium pump. Vanadium also serves as a biocatalyst for oxidation of substrates. The deficiency signs and specific function of ultratrace non-metals are given in Table 6.
### Table 6: Properties of the Essential Ultratrace Non-metals

<table>
<thead>
<tr>
<th>Ultratrace non-metal</th>
<th>Deficiency signs</th>
<th>Specific functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorine</td>
<td>Growth depression, dental caries</td>
<td>Structure of teeth and bones, replaces OH&lt;sup&gt;-&lt;/sup&gt;, inhibits enolase, pyrophosphate</td>
</tr>
<tr>
<td>Iodine</td>
<td>Goiter, reduced thyroid function</td>
<td>Constituent of thyroid hormones T&lt;sub&gt;3&lt;/sub&gt;, T&lt;sub&gt;4&lt;/sub&gt;</td>
</tr>
<tr>
<td>Selenium</td>
<td>Muscle and pancreas degeneration, hemolysis</td>
<td>Constituent of glutathione peroxidase and other enzymes, protector against oxidation of erythrocytes</td>
</tr>
<tr>
<td>Silicon</td>
<td>Growth depression, bone and matrix deformities</td>
<td>Structural role in connective tissue and osteogenic cells</td>
</tr>
<tr>
<td>Arsenic</td>
<td>Impairment of growth, reproduction, heart function</td>
<td>Increased arginine, metabolism of methyl compounds</td>
</tr>
<tr>
<td>Boron</td>
<td>Growth of angiosperms impaired, nitrogen fixation</td>
<td>Control of membrane function, nucleic acid biosynthesis; lignin-biosynthesis.</td>
</tr>
</tbody>
</table>

### 1.2.4 Metallo-drugs

Thirty elements are known to be essential for mammalian life. Metals control many biological events (Fe<sup>2+</sup> and Cu<sup>2+</sup> in oxygen metabolism) and fundamental ones too (e.g. Mg<sup>2+</sup>, Fe<sup>2+</sup> and Zn<sup>2+</sup> in DNA biochemistry). They often turn out to be intimately involved in the pharmacology of organic drugs either directly, e.g. Ca<sup>2+</sup> or an amplifier of organic messages.
Although metallodrugs are well known in medicine and are used to treat a diverse range of complaints and diseases (Table 7), few have been designed to have a specific therapeutic effect (25), after, their pharmaceutical properties were discovered by chance. This is particularly true of the gold drugs that are currently used to treat rheumatoid arthritis. The history of gold in medicine can be traced back over many centuries and include the treatment of alcoholism to syphilis and tuberculosis. Gold (I) thiolates were found to be effective against arthritis in the 1920s. Inorganic pharmaceuticals are summarised in Table 7.

Table 7: Some Inorganic Pharmaceuticals

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Therapeutical uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Lithium carbonate</td>
<td>Treatment of Manic depressive psychoses</td>
</tr>
<tr>
<td>2. Magnesium sulphate</td>
<td>Laxative (Epsom salt)</td>
</tr>
<tr>
<td>3. Magnesium oxide, hydroxide,</td>
<td>Antacids</td>
</tr>
<tr>
<td>4. Magnesium carbonate,</td>
<td></td>
</tr>
<tr>
<td>trisilicate</td>
<td></td>
</tr>
<tr>
<td>5. Aluminium hydroxide</td>
<td>Antacids</td>
</tr>
<tr>
<td>6. Aluminium silicate</td>
<td>Antidiarrhoeal (Kaolin)</td>
</tr>
<tr>
<td>7. Ferrous sulphate, succinate,</td>
<td>Iron supplement</td>
</tr>
<tr>
<td>fumarate, gluconate, glycin</td>
<td></td>
</tr>
<tr>
<td>8. Sodium nitrite</td>
<td>Antihypertensive</td>
</tr>
<tr>
<td>9. Vitamin B_{12} (Co)</td>
<td>Treatment of pernicious anemia</td>
</tr>
<tr>
<td>10. Zinc oxide</td>
<td>Skin healing</td>
</tr>
<tr>
<td>11. ZnCO_3/Fe_2O_3</td>
<td>Athletes foot</td>
</tr>
<tr>
<td>12. Selenium sulfide</td>
<td>Calamine lotion</td>
</tr>
<tr>
<td>13. Silver Sulfadiazine</td>
<td>Antiséborrhoeic</td>
</tr>
<tr>
<td>14. Stannous fluoride</td>
<td>Antibacterial (burn, wounds)</td>
</tr>
<tr>
<td>15. Antimony gluconate</td>
<td>Tooth-protectant (toothpaste)</td>
</tr>
<tr>
<td>16. Cis-PtCl_2(NH_3)_2</td>
<td>Treatment of leishmaniasis (Pentostam)</td>
</tr>
<tr>
<td>17. Gold thiomalate</td>
<td>Anticancer drug (Cisplatin)</td>
</tr>
<tr>
<td></td>
<td>Antiarthritic Myocrisin)</td>
</tr>
</tbody>
</table>
Progress in the design of inorganic drugs has been slowed because of problems of substitution and hydrolytic equilibria, redox and polymerisation reactions which are uncommon for organic drugs, are often encountered with metal complexes.

1.3 Chelation Therapy

Since the discovery that metal ions are active constituents of many enzymes, the use of metal chelation to explain biological and pharmacological effects of chelating agents has grown importance (26). The necessity for small amounts of specific metals for the operation of many metabolic reactions has also given rise to postulation involving chelation chemistry.

Among the metal ions which have been found to exert these catalytic or otherwise "activating" effects are nineteen essential "trace metals" for the humans. These metal ions are effective in a bound form rather than as free ions (27). On the other hand, chelates of riboflavin and pyridoxal function as enzyme model.

The mode of action of chelating agents are as follows:

(a) Transient combination of a chelating agent occurs with the metal of an enzyme without removal of the metal from the enzyme.

(b) Combination of a chelator with metal ions in the cellular environment results in removal of the metal ions.

(c) Combination of a chelating molecule with metal ions to form complex which has suitable polarity to transport either a metal ion or the molecule to or through a cell membrane.
(d) Chelation results in stabilisation of either a chelating molecule or a valence state of a metal ion.

(e) Combination of a chelating molecule and metal ions to give an "unsaturated" chelate which exerts a toxic or catalytic effect per se.

(f) Combination of a chelator and metal ion may alter the oxidation potential of metal.

The use of chelating agents for the removal of toxic metal ions from the body is well documented (28). The role of chelation in pharmacological action and antimicrobial action have been reviewed. The modification of toxic as well as medicinal effects by metal chelation are discussed. Further, chelation effects in plant cells have also been reported (29).

There are evidences that metal ion chelation or complexation may be involved somewhere in the course of a medicinal substance through a living organism. The stability constants for some medicinal substances with biologically important metal ions are summarised in Table 8. They are compared with those found for cellular chelators Table 9.

Some chelates have been found effective and provide good indication of the importance of metal chelation, e.g. combination of some fungicides with metal such as 8-hydroxyquinoline and sodium dimethylthiocarbamate, streptomycin complex as a growth factor, and zinc-bacitracin as antibacterial agent etc.
Moreover, when metal ions are intended for administration, their chelates are frequently preferred, e.g. Fe$^{1+}$, Co$^{2+}$, Cu$^{2+}$, Pb$^{2+}$, Sb$^{3+}$ and Bi$^{3+}$ are given as their chelates (30) with citric, lactic, tartaric and thioglycolic acids and 1,2-dihydroxybenzene.

Table 8: Stability Constants of Some Drugs with Metal Cations

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Cu$^{2+}$</th>
<th>Zn$^{2+}$</th>
<th>Co$^{2+}$</th>
<th>Fe$^{2+}$</th>
<th>Mn$^{2+}$</th>
<th>Fe$^{3+}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-Hydroxyquinoline</td>
<td>12.2</td>
<td>-</td>
<td>9.1</td>
<td>8.0</td>
<td>6.8</td>
<td>12.3</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>10.6</td>
<td>6.9</td>
<td>6.8</td>
<td>6.6</td>
<td>5.9</td>
<td>16.4</td>
</tr>
<tr>
<td>Gentisic acid</td>
<td>7.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.8</td>
</tr>
<tr>
<td>p-Aminosalicylic acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>16.1</td>
</tr>
<tr>
<td>Methyl salicylate</td>
<td>5.9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>9.7</td>
</tr>
<tr>
<td>Isonicotinyl hydrazide</td>
<td>8.0</td>
<td>5.4</td>
<td>4.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>7.8</td>
<td>4.9</td>
<td>5.4</td>
<td>5.3</td>
<td>4.4</td>
<td>9.9</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>7.6</td>
<td>4.5</td>
<td>4.8</td>
<td>5.7</td>
<td>4.3</td>
<td>9.4</td>
</tr>
<tr>
<td>Dimethyldithiocarbamic acid</td>
<td>11.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2-Mercaptoethylamine</td>
<td>-</td>
<td>10.2</td>
<td>7.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 9: Stability constants of some cellular chelators with metal cations

<table>
<thead>
<tr>
<th>Ligand</th>
<th>( \log K_1 ) (water, 20-30°C) (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{Cu}^{2+} )</td>
</tr>
<tr>
<td>Glycine</td>
<td>8.5</td>
</tr>
<tr>
<td>Cysteine</td>
<td>-</td>
</tr>
<tr>
<td>Histidine</td>
<td>10(^a)</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>-</td>
</tr>
<tr>
<td>Histamine</td>
<td>9.6</td>
</tr>
<tr>
<td>Pteroylglutamic acid</td>
<td>4.4(^a)</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>7(^a)</td>
</tr>
<tr>
<td>Hypoxathine</td>
<td>6.2</td>
</tr>
<tr>
<td>Guanosine</td>
<td>6</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>1.8(^a)</td>
</tr>
<tr>
<td>Oxalic acid</td>
<td>6</td>
</tr>
</tbody>
</table>

(a) Values for \( \log K_1 \) were considered to give a better comparison than \( \log K_8 \) (overall) values, which are not always available.

(b) Estimated from \( \log K_8 \) values.
Metal-containing protein species can be classified into two groups. First group consists of the complexes formed by proteins which pre-exist in the biofluid. The second group includes all those metalloproteins which have their metal ions incorporated as part of their biochemical synthesis.

Often the second type of metalloprotein has a specific biological role that needs to be fulfilled regardless of the local concentrations of free metal ion or its associated low-molecular weight complexes. On the other hand, many metal protein interactions are thermodynamically reversible. As the free metal ion concentration falls, the metal-protein complex tends to dissociate.

These generalisations are fundamental of any chelation therapy. The ability of a chelating agent to promote heavy metal decorporation mainly depends upon the type of metal-protein complex.

The transition metal storage proteins within cells represent another substantial reservoir of metal ions which could serve as the target for chelating pharmaceuticals. They possess an intriguing set of properties which combine with a certain degree of thermodynamically reversible binding with the induction of synthesis as a response to increasing metal ion concentration (31,32). In this respect, there are distinct parallels between the two most prominent metal storage proteins, ferritin and metallothionein. The primary purpose of both would seem to be concerned with the homeostasis of essential elements (33). Yet, both readily
incorporated polluting heavy metals, sometimes even at the expense of the natural cation (34). Metallothionenin, containing an extraordinary percentage of thiol functional group (35), avidly sequester Hard and Soft acids and bases, 'Soft' metals such as Cd$^{2+}$ and Hg$^{2+}$, whilst 'harder' ions like Pu$^{5+}$ are incorporated by ferritin.

1.3.1 Multicompartmental Distributions

Essential transition metal ions appearing in plasma as a result of intravenous injection or absorption from the GI tract or discharge of the lymphatic system are generally removed by liver (36), except iron bound to transferrin, which is transported to erythropoietic marrow (37). Redistribution to the other organs may subsequently occur depending on the particular metal, but excretion to the bile is one typical pathway that is followed.

Electrically neutral and non-polar molecules tend to be best absorbed across the GI tract. On the other hand, agents which exist in solution are highly charged species. They cannot diffuse through biological membranes.

A therapeutically effective chelator must pass through the gastrointestinal epithelium when administered orally. In man, the pH of the GI tract rises from pH 2 in the stomach, through pH 6 in the small intestine, to pH 8 in the colon. If the drug remains fully ionised over the whole pH range, it cannot diffuse passively through lipid cell membranes and hence, does not penetrate the gastrointestinal epithelium. On the other hand,
if at some stage, the drug occur in an unionised or partially ionised form, the neutral component can cross into plasma. The partitioning of the drug between the plasma and the GI tract is thus determined by the acid dissociation constants of the drug. In the classic study of the antibacterial properties of 8-hydroxyquinoline, it has been shown that the activity is due to the passive diffusion of the neutral complex through the cell membrane. It has also been shown that the more lipophilic the complex formed by Fe$^{3+}$, the greater amount of metal is delivered to reticulocytes. Further the lipophilicity of injected complexes determines their route of excretion: polar complexes are confined to extracellular space until they are excreted in the urine, whereas less polar species tend to be taken up by the liver and emerge in the bile (38).

The metal ions and their highly polar complexes have a restricted ability to move from one body compartment to another unless there are specific transport mechanisms. Therefore, the charged transition metal complexes are generally confined to the biofluid in which they are formed, or into which they are administered. This is a major obstacle in most chelation therapies.

A great deal of effort has been devoted to develop agents which can specifically enter intracellular space. Esterification of polar functional groups was an obvious starting point. Other methods of getting chelating agents inside cells include drug delivery systems using liposomeencapsulation (39) or red cell ghost (40).
1.3.2 Synergistic Chelation Therapy

When chelating activity depends on a large number of binding groups, sequestration tends to be a slow process. This is particularly so when the metal has to be acquired from another ligand, such as a protein, e.g. Desferrioxamine does not acquire Fe$^{3+}$ from transferrin. Similarly, the macrocyclic tetramines do not induce a significant cupriuresis in spite of their high copper-binding formation constants.

One way of overcoming these restrictions is to administer not one but a combination of chelating agents. This concept has been termed synergistic chelation therapy.

A combination of Diethylenetriaminepenta acetate (DTPA) and Desferrioxamine (DFOA) has proved to be better treatment of intramuscularly deposited plutonium than either agent alone. It is also known that a variety of low molecular weight ligands are able to increase the rate of iron exchange between transferrin and DFOA (41). Nitrilotriacetic acid (NTA) is effective in vivo. From the rates of dissociation of the mixed ligand complexes formed by transferrin, it has been calculated that synergistic chelation therapy might be capable of removing gram of iron per month (42). The current treatment of lead poisoning employs EDTA in combination with either British Antilewisite (BAL) or penicillamine.
1.3.3 Side effects of Drugs

Though complex formation may have no direct relation with the major action of the drug, it may be responsible for significant side effects. For example, the antitubercular agent, thiacetazone, produces an onset of diabetes mellitus. Similarly, diphenylthiocarbazone, oxine and alloxan may be considered to produce a diabetogenic effect. The anaemia produced by administration of the hypotensive agent, hydralazine, has been attributed to its ability to complex with iron (43). Dimercaprol and isonicotinic acid hydrazide tend to induce histamine-like actions. It has been suggested that this may be due to complexing with a copper-catalysed enzyme responsible for the destruction of histamine. On the other hand, boric acid chelates with epinephrine and related catecholamines, but does not alter the pharmacological properties.

1.4 Biological Chelating Agents

Chelating agents play an important role in medical sciences. They are employed to prevent or treat the harmful effects of metals, to ensure adequate dietary supplies of essential elements and as diagnostic aids.

A number of naturally occurring chelates are present in biological systems. The amino acids, proteins and amides of tricarboxylic acid cycle are the main ligands. The metals involved are iron, magnesium, manganese, copper, cobalt, zinc, etc. A group in which iron is present, is the hemoprotein such as
haemoglobin, which is involved in oxygen transport. Other hemo-
proteins are myoglobin, catalase, peroxidases and cytochromes.

Copper-containing enzyme include the oxidase, ascorbic acid
oxidase, tyrosinase, and polyphenol oxidase. Magnesium is present
in chlorophyll. It is involved also in the action of some proteo-
lytic enzymes, phosphatases and carboxylases. Zinc is present
in insulin. It also activates some carboxylases, proteolytic
enzymes and phosphatases. Cobalt activates some enzymes belonging
to each of the above classes and is present in Vitamin B_{12}.

The fact that a number of biologically important compounds
are chelates, opens up other approaches to chemotherapy. In order
to reduce or eliminate the toxic effects of a metal, the unnatural
chelating agent (ligand) must effectively compete with the chemical
system in the body to which the excess metal is bound. The chela-
ting agent, because of its greater affinity for the metal, forms
a more stable chelate, thereby decreases the concentration of the
toxic metal ion in the tissues. Soluble chelate thus formed is
excreted by the kidney.

The role of iron in oxygen transport by the blood gives
rise to a multiple of disorders. The treatment of these disorders
necessitates chelation therapy to reduce iron stores. Secondly,
the rare complication of copper metabolism (Wilson's disease) has
implicated for geneticists, neurologists and ophthalmologists.
Recently, there is renewed interest about the sociological and
psychological impact of lead (Pb) from petrol. Further, the
effect of copper in reducing the inflammation associated with
rheumatoid arthritis (44), the role of trace elements in the
mechanisms of the immune response system (45), their antibacterial
and antiviral (46) properties, the suggestion that surplus iron
may be a fundamental cause of heart disease (47) and that metal
complexes may be used to control infections and cancerous growth
are all matters of topical research interest.

1.4.1 Selectivity of chelating agents

Improved selectivity has been the aim pursued in the search
for better chelating agents. There are two disadvantages with
compounds such as EDTA, which strongly sequester a variety of
metal ions. The side effects caused by interference with the
metabolism of essential trace elements may limit their usefulness
(48). In addition, any interaction with abundance metals in vivo
(e.g. calcium ions) clearly act to suppress their affinity for
toxic target ions (49).

There is a characteristic variation in the stability of
complexes which depends on where the metal and ligand are located
in the periodic Table. The majority of metals in their common
oxidation states form their most stable complexes when ligand
donor atoms come from the first row of Groups V, VI, and VII
(i.e. nitrogen, oxygen and fluorine). The alkali and alkali
earth metals, the earlier transition elements as well as zinc
belong to this group. The transition elements from Group VI
onwards, i.e. Cadmium, Mercury and heavy metals, Thallium, Lead,
Bismuth and Polonium form their most stable complexes when the ligand donor atoms come from the second raw in the Periodic Table (i.e. phosphorus, sulphur, or chlorine). It is also noteworthy that the first set of metals displays an order of affinity for the halide ions (F $\gg$ Cl $>$ Br $>$ I) which is reversed by the second set.

The relative strength of bonding between a particular metal ion and a single electron donor site on a ligand depends on the electronic character of the atoms concerned. Those combinations in which both atoms have a similar preference for either ionic or covalent bonding, are favored. These facts have been embodied in two common classifications of metals and ligands according to which Lewis acids (electron acceptors) and Lewis bases (electron donors) are subdivided into two main groups. These have been called 'hard' and 'soft' (50). Small, highly charged atoms that are not easily polarised belong to 'hard' category. On the other hand, larger, more polarised atoms fall in 'soft' category.

The usefulness of these concepts depends on an ability to divide both metals and ligands into two different groups. Pearson's concept (51) of 'hard' and 'soft' acids and bases (HSAB) has been widely recognised as having considerable application. A large group of metals cannot satisfactorily be described as either 'hard' or 'soft' and hence, have been termed 'Intermediate'.
The term 'intermediate' is something of a misnomer since, rather than lying between the two extremes, they exhibit an ambivalent character. In spite of attempts to quantify the HSAB approach (51), little further has emerged to provide more theoretical insight to improve the selectivity. This can possibly be attributed to uncertainties in respect of certain fundamental definitions.

However, in accord with the HSAB theory, certain thumb rules can be established. 'Soft' metals such as Hg\(^{2+}\), Ag\(^{+}\), Cd\(^{2+}\) and Au\(^{+}\) favor interaction with 'soft' sulfur donor atoms, while the complexation of Ca\(^{2+}\), Mg\(^{2+}\), Fe\(^{3+}\) and plutonium ions is likely to be dominated by 'hard' oxygen donors. Metal ions such as Cu\(^{2+}\), Ni\(^{2+}\) and Zn\(^{2+}\) will bond strongly to both 'hard' and 'soft' donors. They often prefer to mixture of both. The corresponding formation constants of Zn\(^{2+}\) are generally smaller than those of Cu\(^{2+}\) and Ni\(^{2+}\).

Differences in metal ions selectivity between ligands depend on the fact that certain donor atoms enhance d-orbital splitting more than others. For example, nitrogen generally exhibits a greater effect than oxygen. So ligands which contain many nitrogen donor sites or which have shortened nitrogen-metal interatomic distances show a marked preference for Cu\(^{2+}\) and to a lesser extent Ni\(^{2+}\).

Another possible means of establishing selectivity is to exploit the ionic size differences between various metal ions. Chelating sites or ligands with limited flexibility have a
natural preference for those cations which fit most comfortably into their molecular architecture. For example, monocyclic molecules can be synthesised such that their ion-binding cavities space a range of sizes. Since only small differences in the diameters of the cavity and the coordinating cation can be tolerated, extra-ordinary specification for almost any chosen metal can be accomplished (52).

This phenomenon is demonstrated particularly well by chelating agents known as ionophores. The synthetic ionophores include the two-dimensional crown ethers such as benzo-8-crown-6, the three-dimensional cryptends and the acyclic polands. The latter is sufficiently flexible to wrap themselves around the central cation (53). Naturally occurring ionophores are of two types: (i) the cyclodepsipeptides, e.g. Valinomycin and (ii) the cyclic and acyclic polyethers, e.g. Nonactin and Nigericin (53). Most of these ionophores are known for their ability to complex and discriminate between the alkali metal ions. However, some of them exhibit considerable specificity for other metals (54).

1.4.2 Computer Simulations

The affinities between a given metal and ligand in isolation is substantially altered in the presence of other metals and ligands (14). For example, EDTA forms more stable complexes with Hg$^{2+}$ than with Zn$^{2+}$, yet removes zinc from the body without affecting mercury (55). This phenomenon was explained by Perrin (56).
Attempts to investigate metal complex formation in biological systems by the mathematical approaches outlined earlier are faced with a number of specific complications, e.g. (i) the lack of quantitative thermodynamic data concerning association between metal ions and biological macromolecules such as protein and (ii) the uncertainties of the composition of many biofluids, both in respect of constituents and concentrations. Since all of these depend on computers to assess the representative concentrations of various chemical species in their biological environment, they collectively are known as computer simulations.

The first computer simulations of metal ion equilibria in blood plasma was based on a relatively small number of low molecular weight components (57). These early models served a very useful purpose (58,59). Perrin extended his original model to include Ca$^{2+}$ and Mg$^{2+}$ in addition to Cu$^{2+}$ and Zn$^{2+}$ ions (60).

This information is especially valuable when it comes to understanding the physiological effects of chelating agents. In particular, the polyaminocarboxylic acids cause urinary excretions of heavy metals which follow the order displayed by the PMI (Plasma Mobilising Index). This means that their ability to sequester the metal in competition with the natural components of plasma is the dominant factor for the determination of their therapeutic activity.
1.4.3 Therapeutically active chelating agents

In chelation therapy, it is desirable to administer selective agents which bind the target metal ion powerfully, and it should not interfere the metabolism of essential elements. It is also clear that an ideal agent should penetrate into the body compartment where the offending metal ion has been largely deposited. In addition, the drug must be cheap, non-toxic, and resistant to metabolic degradation. It can be conveniently (i.e. orally) administered.

The chelating agents in general clinical use today had been introduced by the mid 1960s. The first was 2,3-dimercapto propanol (BAL) to treat arsenical gas poisoning. Ethylenediaminetetraacetic acid (EDTA) was administered to humans to remove lead (61). Then, through the 1950s and the early part of next decade, there followed a period of intense research into the properties of chelating agents from a chemical and physiological point of view. This resulted in the introduction of penicilliamine (PEN) by Walshe in 1954 (62), the identification of diethylthiocarbamic acid (DDC) as a drug for the specific treatment for nickel carbonyl poisoning in 1958 (63), and the discovery of desferrioxamine (DFOA) as the iron complex in 1960s. During this period, diethylenetriaminepentaacetic acid (DTPA) was also established as a possible alternative to EDTA, particularly for radionuclide decorporation.
With the exception of 2,2,2-triethylenetetramine (TRIEN) (64) and 2,3-dimercaptopropanesulphonic acid (DMPS), no new chelating agents have been accepted into the clinic for nearly 20 years.

(a) **British Antilewisite (BAL)**

It is, perhaps, surprising that BAL, which has been used for about 40 years, has not been superceded as antidote for some half-a-dozen elements including As$^{3+}$, Au$^{+}$, Hg$^{2+}$, Pb$^{2+}$, Sb$^{3+}$, Bi$^{3+}$, Cr$^{3+}$, Se$^{4+}$ and Te poisoning. As it has limited solubility in water and is prone to decompose, it is administered parenterally as 10% solution in peanut oil with 20% benzyl benzoate added as a stabiliser. Ability of BAL to complex the metal ion even after its distribution into tissues is its advantage. However, BAL is contraindicated in the treatment of patients who have been chronically exposed to cadmium (65). There is also a great risk associated with its use against alkyl mercury poisoning (66).

(b) **The Polyaminocarboxylic acids**

**Ethylenediaminetetraacetic acid (EDTA)**

It forms water-soluble stable chelates with many metals. It is used as antioxidant for the stabilisation of drugs which rapidly deteriorate in the presence of trace metals, e.g. ascorbic acid, epinephrine and penicillin; for the removal of lead arsenants spray residues from fruits and radio-active contaminants and as an antidote for heavy metal poisoning.
The EDTA and its sodium salt when administered to mammals, produce an excessive loss of body calcium and are quite toxic. The calcium-disodium salt (edathanil) is comparatively non-toxic and serves as an effective antidote for the treatment of lead, copper, chromium, iron and nickel poisoning (67).

The stability constant of EDTA complex varies with each metal. For the divalent ions the order is as follows (68):

\[ \text{Cu}^{2+} > \text{Ni}^{2+} > \text{Pb}^{2+} > \text{Co}^{2+} > \text{Zn}^{2+} > \text{Fe}^{2+} > \text{Mn}^{2+} > \text{Mg}^{2+} > \text{Ca}^{2+} \]

EDTA is recommended as an antidote for acute lead toxicity. However, it does not have sufficient selectivity to offset the relatively high concentrations of Ca\(^{2+}\) in blood plasma. DTPA is generally more effective for HSAB 'hard' metals, while BAL and PEN are preferred for the treatment of 'soft' metals poisoning. DTPA has proved better than EDTA in the decorporation of many radionuclides. Since DTPA is octadentate and EDTA is only hexadentate, the former is superior with metal ions having coordination number of 8 or more.

Absorption of polyaminocarboxylic acids from the GI tract is about 5% of the dose which has precluded its oral administration. When the compounds are administered parenterally, they are assimilated rapidly and completely.

It is noteworthy that when the agents are injected intraperitoneally as the Ca\(^{2+}\) salts, EDTA (with an LD\(_{50}\) value in mice of 17.4 mmol/kg\(^{-1}\)) is less toxic than DTPA (LD\(_{50}\) is 12.5 mmol/kg\(^{-1}\)).
Moreover, Zn\(^{2+}\) and Mn\(^{2+}\) are implicated by computer simulation studies, cell culture (69) and animal experiments as susceptible metal ions. Urinary excretion of Zn\(^{2+}\) and Mn\(^{2+}\) is significantly enhanced by both the polyaminocarboxylic acids (70). Furthermore, Planas-Bohne and Olinger (71) have shown that loss of Mn\(^{2+}\) correlates well with the lethality of DTPA in mice. Contilena and Klassen have recently compared the excretion of endogenous metal ions from mice injected with a variety of chelating agents. Both DTPA and EDTA promote substantial losses of Fe\(^{2+}\), Mg\(^{2+}\) and Zn\(^{2+}\) (72).

The studies in the toxicity of Zn-DTPA compared with its Ca\(^{2+}\) counterpart show that Ca-DTPA is much more toxic than Zn-DTPA. EDTA and DTPA infusates supplemented with a mixture of appropriate metal ions were substantially less toxic than the solutions of calcium sodium salts presently administered. Calculations concerning trace element supplements for the fluids used in total parenteral nutrition (73) can also be applied to chelating agent infusions (74). Such an approach promises to improve various aspects of the treatment for lead and plutonium poisoning.

Among the other polyaminocarboxylic acids, N-hydroxyethylene diaminetriacetic acid was tested for iron removal. Further, triethylenetetraminehexaacetic acid and its analogs seem to be effective chelating agents for a whole range of metal ions. However, in clinical applications, these agents have tended to be eclipsed by EDTA and DTPA.
(c) Penicillamine (PEN)

Penicillamine is one of the hydrolysis products of penicillin. It is effective in the treatment of Wilson's disease (75). It is administered orally. It produces 20-fold increase in the urinary excretion of copper.

PEN has become a wonder drug. It is used to chelate other heavy metals such as Pb^{2+}, Hg^{2+} and Au^{+} (76). It is effective against cystinuria (77). The compound is employed in the treatment of rheumatoid arthritis (78-80). It may exhibit some benefit in the treatment of progressive systemic sclerosis, primary biliary cirrhosis (81,82) and ketoids (83). About 10% of patients with Wilson's disease develop an absolute intolerance (84). The most serious side effects include nephrotic syndrome, autoimmune disturbance and bone marrow depression (85,86). Some still consider pyridoxine supplements as necessary to prevent PEN-induced neuropaths (87). Visual dysfunction due to induced loss of zinc is the another potential complication.

(d) Desferrioxamine (DFOA)

Recognition of the therapeutic potential of naturally occurring iron chelators of microbial origin goes back to 1952 when ferrichrome was isolated from the smut fungus, Ustilago sphaerogena (88). Most of these siderophores are highly specific for Fe^{3+} although there are some reports of Fe^{2+} chelators (89).
Desferrioxamine was isolated from streptomyces pilosus. It combines with Fe$^{3+}$ to form a water soluble chelate. DFOA enjoys the distinction of being the most selective of all the chelating agents in clinical use. Whilst the formation constant of its iron complex (log $B = 31$) is modest as compared to reported values for other siderophores such as enterobactin (log $B = 52$), the binding to iron is greater than to the other essential transition metals (Cu$^{2+}$, Zn$^{2+}$, Mn$^{2+}$, Mg$^{2+}$ and Ca$^{2+}$) (90).

However, it does not mobilise or enhance the extraction of any of the above mentioned essential elements. It seems that the extraordinary selectivity is one of the reasons why it is relatively free from toxicity (91). Apart from a few patients who are allergic to the compound itself, there are few side-effects. Doses of 10 mg kg$^{-1}$ hr$^{-1}$ are commonly administered for extended periods without any signs of toxicity (92).

The use of DFOA in the treatment of iron overload is first reported in 1962 (93). It has revolutionized the treatment of acute iron poisoning. In addition, it was soon recognised as a very potent and safe iron-chelating agent in a series of clinical trials for chronic overload (94,95).

The lethality of mice, induced by oral administration of FeSO$_4$ (96) is lowered by early oral administration of DFOA, diethylenetriaminepentacacacetate (DTPA) and sodium hexaacyanoferrate (CF). The following protective efficiency was established: DFOA $<$ DTPA $<$ CF.
DFOA is employed in the treatment of iron poisoning (97,98). Diagnostic application of the differential ferrioxamine tests include idiopathic hemochromatosis, cirrhosis with siderosis, idiopathic pulmonary hemosiderosis and hypersiderosis (99). Other clinical investigations include its use in sideropenia, hemolytic anaemia and renal function. It induced urinary iron evaluated as a test for measurement of iron stores (100).

(e) 8-Hydroxyquinoline (Oxine)

It has been established that 8-hydroxyquinoline and its analogs act as antibacterial and antifungal agents by complexing iron or copper. 8-Hydroxyquinazoline in absence of these metals, is non-toxic to microorganism.

The antimicrobial action of chelating agents has been reviewed extensively. Oxines are the foremost among the chelating agents used for this purpose. Weinberg has discussed the mutual effects of antimicrobial compounds and metallic cations and the possible metal chelation mechanisms involved (29). Albert showed that the six isomers of oxine incapable of chelation are devoid of antibacterial action (101). For the antibacterial action of oxines, chelation is necessary.

The stability constants for the reaction of oxine with the trace metals are higher (102) than those with potential cellular chelators, particularly amino acids and purines (103). This indicate the ability of oxine chelates to exist under biological
conditions. As chelation, either in vitro or in vivo, is controlled by the ionisation constant of the chelator as well as by the stability constants of the chelates, it is necessary for the ligand to be ionised before chelation can take place.

Zentmyer (104) suggested that the activity of oxine is due to its ability to remove essential metals from the microorganisms. In both the bacteriostatic (105) and bactericidal (106) action of oxine, the latter seems to be operative. Experiments conducted in metal depleted media against *staphylococcus* and *streptococcus* revealed that neither oxine nor iron ions were harmful to the organism. However, equimolar concentrations of the two were lethal at high dilution (M/100,000). Albert (106) interpreted that the unsaturated 1:1 or 2:1 chelates were toxic, whereas the 3:1 chelates were not toxic. When sufficient iron was added to 3:1 chelates to shift the equilibrium in favour of the 1:1 chelate, bactericidal action was restored.

For Gram positive bacteria, Fe$^{2+}$, Fe$^{3+}$ and Cu$^{2+}$ ions gave toxic chelates with oxines. Trace amounts of Co$^{2+}$ ions suppressed the activity. Gram negative bacteria are much less susceptible to oxine. Others have also reported the necessity for Fe$^{2+}$ or Cu$^{2+}$ ions for the antibacterial action of oxine (107). Toxicity to fungi and yeasts by oxine apparently requires only Cu$^{2+}$ ions. (108)

Introduction of a highly hydrophilic group, the 5-sulphonic acid, (8-hydroxyquinoline-5-sulphonic acid) removed antibacterial properties altogether, although the metal-binding ability remained
equal to that of oxine (102). The aza-oxines were found to have antibacterial effects which decreased as the liposolubility decreased (109). Introduction of short alkyl groups was effective in resorting both liposolubility and antibacterial properties. The two effects ran parallel as long as the chelate stability constants remained sufficiently high.

Albert suggested that the less polar 3:1 oxine-iron or 2:1 oxine-copper chelates penetrate the cell wall, and then are converted into the more toxic 1:1 or 2:1 chelate within the cellular environment.

Other evidences exist that oxine acts at the cell wall or cytoplasmic membrane. Beckett (110) has shown that 1:1 oxine-iron chelate reacts with the *Staphylococcus aureus* cell and liberates oxine rather than iron. Greathouse et al. (111) has shown radioactive oxine to be taken up by the *Aspergillus niger*.

Bernheim and Bernheim (112) found that iron and oxine together catalyze the oxidation of -SH groups in nucleoproteins, whereas iron alone is ineffective. Traces of cobalt can inhibit iron or copper catalyzed oxidation of mercapto compounds. The phenomenon explains the antagonism between Co$^{+2}$ and iron-oxine or copper-oxine. Vajda and Nogradi (113) have suggested that copper-oxine replaces Co$^{+2}$ in the prosthetic group of an enzyme.
(f) Ionophores

With the development of X-ray diffraction methods, the discovery of alkali cation and alkaline earth cation complexing agents in living systems opened a new field of investigations (114).

Biochemical studies showed the dramatic effects of some of antibiotics on mitochondria. Valinomycin is capable to induce the transport of $K^+$ across the lipophilic membrane of the mitochondria (115). As many antibiotics are available, this area of research expands quickly. Many groups work on the subject including Kilbourn et al. (116) and Ovchinnikov et al. (117) among others (118).

All the naturally occurring ion carriers share some common characteristics. The most important originate from the problem of transporting a very hydrophilic ion across a lipophilic membrane. They usually transport only one specific cation. Ionophores having high specificity for divalent cations are rare. Calcimycin is the one example, which transports calcium across membranes. It can form 1:1 or 1:2 cation-ligand complexes, the latter is neutral (119).

Ionophores have long range implications in many areas such as cation separation, isotope enrichment, extraction of harmful metals, supply of required metals, ion sensitive electrodes, formation of alkali anions, studies of reaction mechanisms, anionic reactivity, etc.
(g) Salicylates

Salicylic acid is known to chelate metal ions. The stability constants for salicylic acid with a number of metals have been recorded (120). Stability with only three metals Fe\(^{3+}\), Al\(^{3+}\) and Cu\(^{2+}\) are greater than those of the amino acids and trace metal ions (121).

Reid et al. (122) found \(\gamma\)-resorcylic acid to be nearly ten times as effective as salicylic acid in relieving rheumatic fever. A greater analgesic effect has been reported by O'Brien and Thomas (123) from a dialkyl substituted derivative of o-thymolic acid than from acetylsalicylic acid. o-Thymolic acid have a lower affinity for Fe\(^{3+}\), Al\(^{3+}\), and Cu\(^{2+}\) ions than does salicylic acid. Gentisic acid was also found to have less affinity for metal ions than salicylic acid (120), so it appears that strength of metal binding is not an important criterion for salicylate cations.

A series of substituted salicylates and other structurally unrelated compounds having a common property of metal ion complexation have been examined for the ability to inhibit the incorporation of sulfate ion into mucopolysaccharide. The course of mucopolysaccharide sulfate formation is definitely affected by metal-binding agents (124).
1.4.4 Use of Chelating Agents in Therapy

The chelating agents may be used for following purposes:

(1) sequesteranation of metals to control concentration of metal ion e.g. buffer systems; (2) stabilisation of drugs e.g. epinephrine; (3) Elimination of toxic metals from intact organism, e.g. EDTA as an antidote for the treatment of lead poisoning; (4) Improvement of metal absorption, e.g. EDTA-iron complex increases uptake of iron in plants; (5) Increasing the toxic effects of a metal, e.g. antibacterial activity of the unsaturated oxine-iron chelates; and (6) as diagnostic agents.

(a) In treatment of Wilson's disease

Wilson's disease is an autosomal recessively inherited disorder. The finding of unusually high copper concentration in liver and blood led to the conclusion that this metal might be responsible for the histological damage (123). This finding was confirmed when BAL was shown to increase urinary copper output and that the chelate therapy could yield some chemical benefits (126,127).

In most of the cases removal of excess of copper can be achieved by long term PEN (9) or TRIEN (64,128) chelation therapy.

(b) In Metal Poisoning

(i) Lead Toxicity

Lead toxicity represents the most serious problems in occupational medicine that is associated with poisoning by the heavy metals. When the amount of lead consumed exceeds 1 mg/day,
clinical symptoms are likely to develop (129). Severe and permanent brain damage occurs in up to 50% of them who develop lead encephalopathy. Otherwise, the most prominent complications are haematological; up to 95% of assimilated lead deposits in R.B.C. where it interferes with heme-biosynthesis by blocking the incorporation of iron into protoporphyrin (130).

The treatment involved prompt institution of chelation therapy using a combination of BAL and EDTA. The advantage of using BAL is primarily due to its ability to release lead from erythrocytes in a way that EDTA alone cannot (131).

Patients with lead concentration greater than 50 mcg/100 ml of whole blood were treated successfully with chelating agents calcium disodium edetate and penicillamine (132).

(ii) Cadmium Toxicity

No essential biological function has been established for cadmium (118), but its adverse effects have been reported extensively. It is a carcinogenic and a teratogen (133). It can also lead to the development of hypertension (28). Although the kidney is the critical organ in chronic toxicity, it causes Japanese 'itai itai' disease (134). It is reported that BAL can remove cadmium from the liver without affecting its deposition in the kidney (107,108). DMPS is adjunctive therapy with BAL which promises well for the unsatisfactory area of chelation therapy (109-112).
(iii) Mercury Toxicity

The extremely lipophilic nature of methylmercury makes it almost completely absorbable from the GI tract. It can readily cross the blood brain barrier or the placenta. It thus interferes with nervous system leading to sensory disturbancy, visual constriction, ataxia dysarthria and ultimately involuntary spasms. Sodium mercaptosuccinate is found to be an effective chelating agent for lead and mercury poisoning (135).

(iv) Miscellaneous Metal Toxicity

Thallium poisoning (136) is treated with Prussian blue. Penicillamine was found most effective among commercially available products as antidote for the treatment of acute bismuth intoxication (137).

Monocalcium-disodium salt of bis (dicarboxy-aminoethyl) ether was found to be one of the most effective agents among ten chelating agents tested for the removal of strontium from the body (98).

1.5 Chelation in Drug Action
(a) Antimicrobials

The antimicrobial action of oxines are mentioned earlier. Recently, phenanthrolines have replaced hydroxyquinolines as the most intensively investigated lipophilic chelating agents possessing antimicrobial action (138,139). 3,4,7,8-Tetramethyl-1,10-phenanthrolinate Nickel has been used as a disinfectant in the cleansing of new born babies (140).
Weinberg (29) discussed the effects of metal ions in the bacterial growth inhibition caused by tetracycline. Chelation mechanisms have been proposed for the action of the tetracyclines on bacterial growth and enzymes systems (141). The stability constants of the tetracyclines with divalent and trivalent metals are sufficiently high for the tetracyclines to compete for metal ions with the amino acids and proteins of the cells.

In the various enzyme systems, the inhibitions may be reversed by Fe$^{2+}$, Mg$^{2+}$, Mn$^{2+}$ and Ce$^{2+}$ (63), which points to possible chelations of these metals or metalloenzymes by the tetracyclines. The tetracyclines, however, have been found active in iron-depleted media against *staphylococcus aureus*. Saz and Stie (142) have shown inhibition of nitro reductase by tetracyclines. They have postulated that the inhibition takes place due to the binding of Mn$^{2+}$ ion by tetracycline, since Mn$^{2+}$ stimulated the reducing ability of the enzyme.

Other antibiotics and antibacterials where metal ion activities and reversals have been observed include penicillin (143-145) bacitracin (143), polymyxin (146), novobiocin (147,148), 1-nitroso-2-naphthol (149), kojic acid (150), actidions (150), juglone (150), picolinic acid (150), cycloserine (150), morin (150), patulin (150), neomycin (151) and aspergillic acid (152). Cycloserine chelates with Cu$^{2+}$, Zn$^{2+}$, Co$^{2+}$, Cd$^{2+}$, Ni$^{2+}$, Fe$^{2+}$, Fe$^{3+}$, Mn$^{2+}$ and Mg$^{2+}$ (150-154).
The complex formation capacity of penicillin with Cu\(^{2+}\) has been measured by Weiss et al. (155). Comparison of the stability constants with those for related heterocyclic carboxylic acids and Cu\(^{2+}\) ions indicate that penicilloic acid is responsible for the chelation of Cu\(^{2+}\).

The chlorinated bisphenol antibacterial appears to act via a chelation mechanism. 2,2'-Thiobis(4,6-dichlorophenol) was found to chelate Fe\(^{2+}\), Fe\(^{3+}\), Cu\(^{2+}\), Mn\(^{2+}\) and Co\(^{2+}\) ions, while hexachlorophene chelated Fe\(^{2+}\), Fe\(^{3+}\) and Cu\(^{2+}\) ions (156).

The thiobisphenol was bactericidal to *Staphylococcus aureus* in distilled water and metal depleted broth at 1 ppm. It was found to be independent of the presence of trace metals in the medium for activity, in contrast to oxine (157). Both these Cu\(^{2+}\) and Fe\(^{2+}\) chelate showed the same activity as the thiobisphenol. Both phenols were suppressed in activity by Fe\(^{2+}\) alone among the metals tried. The retention of activity of these phenols in mild alkaline medium, or soap solutions was attributed to iron chelation.

Counter et al. reported that a metal chelate of a non-bacteriostatic agent can exert a greater growth-inhibitory effect than a molecular equivalent of the metal ion. This was demonstrated by the action of a cobalt complex of methionine and a copper complex of biotin against *E.coli* and *S.aureus*. A comparable experiment with a copper complex of glycylalanine showed the inhibitory effect of the complex to follow that exerted by the free metal ion alone over a period of 8 hrs.
Further indication of this effect was provided by the lack of antibacterial properties of a p-aminosalicylic acid-copper complex of low fat solubility and the possession of antibacterial properties of a p-aminosalicylic acid copper chelate of much greater fat solubility. Presumably, the former was not capable of penetrating the cell whereas the latter could penetrate the cell and liberate metal ions.

The nickel chelate of 3,4,7,8-tetramethyl-1,10-phenanthroline is as effective as hexachlorphene in the prophylaxis of staphylococal infection in the new born and in patients undergoing elective obstetric surgery. The chelate is also beneficial in controlling secondary infection in adolescents with Acne vulgaris of long standing (158).

Platinum and palladium complexes with various 2-alkyl-N-(β-aminoethyl)pyrrolidines possess moderate antibacterial activity (159). Iron complexes with some semi-synthetic penicillin show much lower antibacterial activity than that of the respective penicillins (160). Isatin-benzoxazole-2-hydrazone was complexed with heavy metals. The copper and cobalt complexes show high activity against gram-positive organisms (161).

(b) Antiamoebic drugs

Several chelating agents are known to possess antiamoebic activity. Currently, 5,7-diiodo-8-hydroxyquinoline is most commonly employed as antiamoebic drug.
(c) Antituber drugs

Carl and Marquardt (162) have postulated that there is a direct relation between the activities of antitubercular compounds and their ability to form copper complexes. Practically, all the antitubercular agents have been shown to be influenced by the metal ions.

(i) Isonicotinic acid hydrazide (INH)

Growth inhibition of Mycobacterium tuberculosis by INH is suppressed by the presence of Fe$^{2+}$, Cu$^{2+}$ and Co$^{2+}$ ions in vitro (163). Stimulatory effects by metal ions have been noted in the ability of INH to inhibit respiration of Mycobacterium tuberculosis and to inhibit the action of mammalian or microbial catalase (164). Albert has determined stability constants for INH and a number of the trace metals and found them only slightly less than those of the common amino acids (165). Cymerman-Craig et al have supported chelation hypothesis by showing that INH derivatives incapable of metal chelation were devoid of antitubercular activity in vitro. Rubbo et al (166) reported that the copper chelate of INH was as effective in tuberculotic mice as INH itself.

In other studies carried out in vivo INH chelates of Cu$^{2+}$, Co$^{2+}$, Fe$^{2+}$ and Zn$^{2+}$ having both 1:1 and 2:1 ligand : metal ratio, showed approximately the same antitubercular activity as INH itself.
Equilibrium studies showed the tendency of the 2:1 copper chelate to shift to the 1:1 chelate in aqueous medium over a period of time, which led to the postulation that a 1:1 metal chelate was an active species. However, the 2:1 chelate would be required for transport through cell wall, as shown by the much higher oil/water partition coefficients in comparison to 1:1 chelate.

Further evidence of the liberation of metal ions in vivo by an INH chelate was obtained using the 2:1 cobalt chelate in broth cultures of *E.coli* and *S.aureus*.

Maher (164) suggested that isoniazid metal chelates compete with peroxide for sites on catalase molecules. This would result in accumulation of peroxide and results in death of the bacilli. Coleman (167) has proposed that isoniazid might act by interference with metal pyridoxal catalyzed transamination and with cytochrome-catalyzed respiration through chelation with Fe$^{3+}$ ion.

An increase in the antitubercular activity of INH by the presence of agents forming complexes with copper was noted by Leonardi (168). This suggested that metal chelation might be interfering with the action of INH against the mycobacteria.

(ii) *p*-Aminosalicylic acid (PAS)

Activation by Cu$^{2+}$ or Co$^{2+}$ ions have been shown for the tuberculostatic effect of PAS in vitro. The Cu$^{2+}$ chelate was found to possess antitubercular activity equal to or greater
than that of PAS itself, whereas the Fe$^{2+}$ chelate possesses lower activity. It has been reported that excess Cu$^{2+}$ ion increases the tuberculostatic activity of PAS tenfold (169).

(iii) Miscellaneous

Evidence of chelating possibilities for streptomycin has been reported by Weinberg (151). The suppression of catalase and dehydrogenase activity in E. coli and Shigella organisms by streptomycin indicates the interference by complexation with the metal ion involved.

Streptomycin complexes with Cu$^{2+}$, Co$^{2+}$ and Ni$^{2+}$ are found to have less antitubercular effect but more prolonged blood levels in guinea pigs than streptomycin sulfate (170).

A number of antitubercular thiosemicarbazones have also been shown to be complexing with copper (171). Copper complex of p-acetylaminobenzalithiosemicarbazone is shown to be more active against mycobacteria in vitro. Marquardt (162) suggested that the anaemia observed in human after oral treatment with p-acetylaminobenzalithiosemicarbazone may be due to complexing of copper in the body.

The tuberculostatic activities have been found in number of compounds capable of metal chelation in vitro to be enhanced four to eight hundred fold in the presence of Cu$^{2+}$ or Co$^{2+}$ ions. Cymerman-Craig (172) noted that among a series of
aromatic amines having tuberculostatic properties, the most active compounds were chelating agents. Leonardi (168) observed that compounds capable of chelating heavy metals show an inhibitory effect on \textit{M.tuberculosis} and \textit{M.paratuberculosis}.

Prior to 1940, copper oxide has been successfully used in the treatment of tuberculosis. Later, several additional Cu complexes were reported to have antitubercular activity. Sodium 3-(allyl cuprothioureido)benzoate (allocupreide sodium, cupralene) was suggested to be superior to gold in therapy of tuberculosis (173).

A series of metal chelates of some \(\alpha\)-hydroxyphenylazonaphthols and phenanthrols have exhibited antitubercular effects \textit{in vivo}. Copper chelates of phenylazo-cresols and resorcinols have been reported to be tuberculostatic. The copper chelate of \(p\)-aminophenyl-\(p\)-hydrazidoethylaminophenyl sulfone possesses much greater antitubercular effect in mice than the ligand itself (174).

(d) Antiviral Properties

Aromatic and heteroaromatic thiosemicarbazones are powerful chelating agents and are useful in treating complications of smallpox vaccination. They are also employed as effective prophylactics.

Antiviral activity has been reported for oxine (175), INH (175), DTPA and 1,10-phenanthroline. Phosphoroacetic acid and phosphoroformic acids are active against the herpes viruses and several other viruses (176). The antiviral activity of
$\beta$-diketones has been demonstrated. It might arise from the complexation of iron, copper or zinc (46).

Antiviral activity has also been shown for bleomycin and for Rifamycin. The latter has been modified so that additional chelating sites are introduced (177).

Inhibition of the synthesis of DNA in the cells by DFOA has given impetus to further studies in the action of various iron-specific chelating agents (129). In general, diliphoric hydroxamic acids are the most active agents examined, although salicyl hydroxamic acid was also effective.

The cupridihydroporphyrins have shown anti-infectivity action on the hemagglutinating viruses. Similar inhibition of both influenza virus infectivity and hemagglutination was postulated for the antiviral action of the benziminazoles (178). The cobalt chelate of EDTA was found to give a definite but transitory suppressive effect against influenza A and B (179). The inhibitory effects on the larger viruses shown by some thiosemicarbazones (180) could possibly be connected with metal-binding since the thiosemicarbazones are known to chelate (171).

Metal complexes with 1-(2'-tetrahydrofurfuryl)-2-aminocyclopentane and 2-aminocyclohexane are tested for virucidal activity (181).
(e) Diabetogenic Agents

A number of molecules having strong abilities to produce in animals diabetogenic effects resembling to those by alloxane are reported. Diphenylthiocarbazone damages the beta cells of the pancreatic islets and bring about diabetes mellitus (182). 8-Hydroxyquinoline, has also been found to produce diabetes mellitus (182). Other studies by Root and Chen (182), Kadota and Abe (183), and Iwamoto and Adams (184) have shown metal chelating ability to parallel diabetogenic activity.

Alloxan undergoes chelation with heavy metal ions such as Co$^{2+}$, Ni$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$. Administration of the Co$^{2+}$ and Ni$^{2+}$ chelates to rabbits in non-diabetogenic doses produce increase in the blood sugar level equivalent to, or greater than that from alloxan and the duration of action is much longer (185). Greater increase in blood sugar levels were obtained from administration of cobalt chelates of riboflavin and the dichloro analog of riboflavin.

The antitubercular, p-acetylaminobenzalthiosemicarbazone is also known to be a metal complexing agent, causes diabetes mellitus in tubercular patients (186). 6-Hydroxy-1,7-phenanthroline and 1-hydroxy acridine caused hyperglycemia and destructive changes of the pancreatic islets in rabbit (187).
(f) **Antidiabetic agents**

1-p-Chlorophenyl-4-isopropyl biguanide, amyl biguanide and \( \beta \)-phenethylbiguanide have shown significant antidiabetic effects. The unsubstituted biguanide structure has been found to chelate a number of heavy metals, but \( \beta \)-phenethylbiguanide gives evidence of forming chelates of appreciable stability only with copper and nickel ions (188). The copper chelate of \( \beta \)-phenethylbiguanide was found to exceed slightly the stability of copper-glycine at acid pH.

Other metal-binding agents including the salicylates are known to reduce the fasting blood sugar level and glycosuria of moderately severe diabetic patients.

(g) **Glucogenic Corticosteroids**

Some evidence of metal binding by glucogenic corticosteroids has been reported. ACTH was found to bind \( Zn^{2+} \) and \( Cu^{2+} \) and a relationship was claimed to exist between the extent of binding and adrenocorticotropic activity (189).

The chelating action of cortisone, hydrocortisone, prednisone and prednisolone has been demonstrated (190). These steroids reduce the Cu contents of skin and muscle and increase the amount of \( Cu^{2+} \) in the kidney in rats.

(h) **Cardiovascular drugs**

Thiouracil and cardiac glycosides have been shown to cause reduction of tissue metal levels (191).
The pharmacologic properties of K-strophanthin-B are due to the complex formation of K-strophanthin-B with calcium ions in vivo with an increase in the concentration of labile calcium (192).

Use of chelating agent dipotassium edetate (193) as an antagonist to the cardiac toxicity of Oleandrin. Dipotassium edetate produces its salutary effects by chelating extra-cellular calcium and removing this ion from cellular membrane. This action in turn enhances the influx of potassium which is ultimately responsible for counter-acting the cardiotoxic effect of Oleandrin.

The influence exerted by complex cobalt preparations-Coamide and Cobalt-30 was studied on the minute coronary circulation in dog. With intravenous administration, the compounds are apt to produce coronary dilating and hypotensive action. Co-amide is more effective than Cobalt-30.

1.6 Therapeutical uses of chelates

Recently, the development of non-invasive diagnostic imaging of the body has provided one of the greatest benefits of the 'nuclear age'. The cation of gallium (\(^{67}\text{Ga}\)) (\(^{111}\text{In}\)) and technetium (\(^{99m}\text{Tc}\)) are widely employed radionuclides for this purpose. By carefully selecting chelating agent, it is possible to direct them to predetermined organs or regions of the body. For example, the affinity of tetracyclines for calcium results in their uptake into myocardium and this has been exploited to delineate infarced
areas by the uptake of the $^{99m}$Tc chelate (194). $^{99m}$Tc-tetracycline complexes have also been used to detect tumors in the extremities of man (195).

(a) Diagnostic agents

(i) Hepatobiliary imaging

The clinical value of $^{99m}$Tc-aminodiacetic acid derivatives has been established for diagnosis of a variety of liver dysfunctions in man (196).

Hepatobiliary clearance of technetium can also be achieved by ethylenediamine-N, N'-bis (α-2-hydroxy-5-bromophenyl)acetate (197). $^{99m}$Tc complexes of pyridoxylidene-L-leucine and pyridoxylidene-L-phenylalanine are the most effective derivatives for imaging the gall bladder of the rabbit.

(ii) Kidney imaging

Low molecular weight HIDA complexes of $^{99m}$Tc undergo renal clearance. Further, the low molecular weight mercaptan complexes are taken up by the kidneys and excreted into the urine (198).

In addition to the development of renal imaging kits based on $^{67}$Ga and $^{111}$In, a renal imaging procedure based upon complexation of cationic form of $^{99m}$Tc by 1,4,8,11-tetraazacyclotetradecane has been reported (199).
(iii) Tumor imaging

A significant development in the detection of tumors had been made by coupling derivatives of 1-phenyl-EDTA to bleomycin which is known to be selectively accumulated in some cancer cells (200). $^{57}$Co-bleomycin is found to be also useful for detecting metastases, especially lungs (201).

The uptake of radionuclides by bone is valuable aid in detecting fractures, primary or metastatic neoplasms in the bones and skeletal disorders such as pedget's disease.

The extensive uptake of Ethane-1-hydroxy-1,1-diphosphoric acid (EHDP) occurs in bone. The 1,1-diphosphorates are used in treatment of Pedget's disease. In recent investigation of $^{99m}$Tc-labelled aminoethane diphosphoric acid (AMDP) derivatives. Unterspann and Fink found the following order of uptake into bone (202).

N-methyl AMDP > N-dimethyl AMDP > N-trimethyl AMDP > AMDP

(b) Technetium radiopharmaceuticals

Because technetium can form a wide range of stable $^{99m}$Tc complexes, its use in nuclear medicine has grown enormously.

Technetium radiopharmaceuticals are diagnostic imaging agents used in the field of nuclear medicine to visualise tissues, anatomical structures, and metabolic disorders (203). After intravenous administration, $^{99m}$Tc radiopharmaceuticals localise in specific target tissues, which can then be imaged using sodium iodide crystal cameras.
Technetium radiopharmaceuticals can be divided into two distinct classes (204). The first is the technetium tagged radiopharmaceuticals, consist of technetium-labelled molecules whose biodistribution is determined entirely by the molecules to which the technetium is attached. This class includes high molecular weight species such as proteins, large particular colloids and cells. The technetium tagged radiopharmaceuticals are reviewed extensively (131,204).

The second group of technetium radiopharmaceuticals is the technetium essential class which consists of small technetium-ligand coordination complexes. The biodistribution of the technetium radiopharmaceuticals of this class is controlled by the physicochemical properties of the technetium complexes themselves. The in vivo distribution of these radiopharmaceuticals can vary with structure of the technetium complexes and is governed by the technetium oxidation state, functional groups of the ligands and technetium core configuration. The technetium essential class includes technetium complexes used to image tissues such as the kidney function, liver function, heart, bone, thyroid and brain.

$^{99m}$Tc-fe-Ascorbic acid complex, the renal scrimming agent, was evaluated with respect to protein binding, red cell binding, and its renal clearance compared with that of insulin (205). There is 56% binding to plasma proteins and 10% binding to red cells. The clearance ratio between the complex and insulin is 0.36. This ratio changes to 0.91 with the protein free complex and insulin.
(c) Radiolabelling of Cellular Blood Elements

Chromate ($^{51}\text{Cr}$) was used earlier for labelling cells in the blood. 8-Hydroxyquinoline was used to radiolabel blood cells with $^{111}\text{In}$ (206). Later, the technique was extended to label platelets (199,207) and leucocytes (199). These labelled cells can be reintroduced into blood pool to locate thrombi and to detect inflammation and abscesses.

(d) Transport across the blood-brain barrier

Oldendorf has suggested that technetium complexes with an octanol-water partition coefficient of 0.5 can be expected to cross the blood-brain barrier (208). The $^{99m}\text{Tc}$ complexes of oxine and 5,7-diiodo-8-hydroxyquinoline with a high partition coefficient have the percentage of protein binding. Their brain uptake index is also high.

(e) Antifungal Agents

Several fungicides offer well-defined examples of the action of metal chelates per se. Reference to the requisite for cupric ion in the fungicidal action of 8-hydroxyquinoline has already been made (108,209). $\text{Zn}^{2+}$ complexes with sodium dimethyldithiocarbamate and tetramethylthiuramdisulfide were found to bind with proteins and succinic oxidase was inhibited by Zn complexes.

Stability constants and solubility products of the copper complexes of the dialkylthiocarbamic acids have been determined. The growth inhibition patterns of these complexes were compared with those of oxine and 2-pyridinethiol-N-oxide copper complexes against Aspergillus niger and Glomerella cingulata.
Dimethyldithiocarbamic acid is used as a fungicide in the form of its sodium salt or zinc or iron complex. Other examples of chelating agents which have shown fungitoxic action have been by Horsfall and Rich (210) in the case of dihydroxy and di-α-aminodiphenylsulfides. Further, metal chelates with fungicidal properties include copper complexes of variously substituted salicylaldehydes and their imines (211) and a number of metal chelates of 2-heptadecylimidazoline (212). Some aromatic dithiocarbamates and their copper complexes showed weak antifungal activity (213).

The antifungal action of N-methyl thiosemicarbazones was studied and a relation was found between ability to form complexes with copper and antifungal activity (214). Antifungal and antibacterial activity was exhibited by some of the transition metal complexes with 3,5-dinitrosalicylic acid.

The zinc pyrithione can be used as antidandruff-cleaner, rinser, conditioner and grooming agent (215). The copper chelate of 3,4,7,8-tetramethyl phenanthroline (cuphan) is especially effective in eradicating chronic monilial infection of the nail and surrounding soft tissues. The compound provides rapid relief of symptoms in patients with chronic monilial or trichomonal vaginitis. However, the copper chelate is more effective in monilial vaginitis (158).
Carcinogens and Anticancer agents

A number of metals are concerned in the cause of cancer, while others are connected with the antitumor activities. Various elements particularly the first row of the transition elements in the periodic chart are capable of exerting either effects. Some of the transition metal ion complexes with aminocarboxylic acids produce a slight inhibition on the growth of Sarcoma (216).

A correlation between the ability to form silver complexes and the carcinogenicity of polycyclic aromatic compounds has been observed (217). Other chelating agents which have been found by Boyland and Watson (218) to produce bladder tumors include the aminonaphthols, 8-hydroxyquinoline and anthranilic acid.

The Fe-ascorbic acid complex increases the tumor growth, whereas the Mn\(^{2+}\) or Cd\(^{2+}\) chelate inhibits the growth.

Zinc and Cadmium complexes of riboflavin phosphate were claimed by Anichini (219) to have curative effect on benzopyrene carcinoma in rats. However, Riboflavin-5-phosphate has been shown to form water insoluble metal complexes with divalent Fe\(^{2+}\), Cu\(^{2+}\), Ni\(^{2+}\) and Zn\(^{2+}\) which are proved to be ineffective on the growth of Sarcoma (220).

A series of oxines were found more effective against Ehrlich carcinoma and Crocker sarcoma in mice in the presence of Cu\(^{2+}\) and Fe\(^{3+}\) ions. Dimethylglyoxime did not exhibit antitumor activity by itself but did so in the presence of Cu\(^{2+}\). Both Cu-dimethylglyoxime and Cu-biacetylmonoxime were found to inhibit dehydrogenase activity in the carcinoma cells (221).
Copper complexes with N-phenyl anthranilic acid derivatives show antineoplastic activity (222).

Platinum complexes, a new class of antineoplastic agents are reviewed with relation to their chemical structure, biological and toxicological effects (223). Antitumor activity in platinum sulphonamides complex is reported (224). Pt\(^{2+}\), Pt\(^{4+}\) and Pd\(^{2+}\) complexes with 1-vinylazoles were tested for their antineoplastic activity in mice (225). Cis-Dichloro bis(oligopeptide ester)-platinum (II) complexes exhibit antitumor activity in in vitro screening (226).

Rh (III) complexes with sulfonamide and thiazole derivatives are screened for antitumor activity. They are found to be active (227).

Co\(^{2+}\), Mn\(^{2+}\) and Zn\(^{2+}\) complexes with amino acids were tested in vitro against Ehrlich ascites tumor. Some of them demonstrated marginal activity (228).

Very few inorganic compounds have undergone extensive clinical trials as anticancer drugs (Table 10), but fortunately the situation is changing. Thanks to Barnett Rosenberg, platinum anticancer agents have made a dramatic entry into the scene.

Cisplatin is the most widely used anticancer agent. A structure activity relation has been established. Square planar Pt\(^{2+}\) complexes containing cis primary or secondary amines and weakly bonded ligands
in the other coordination positions are usually active. Some Au⁺ alkyls e.g. (Au(CH₃Cl₂)⁻ have shown to possess anticancer activity (229).

It was reported that 'soft' phosphine ligands stabilise 'soft' metal. Eventually the thioglucose derivative of triethyl phosphine Au (Auranofin) was selected for extensive clinical trials. Auranofin added to human blood readily enters red cells and some gold binds to the tripeptide glutathione (230). Micromolar concentrations of auranofin could pull tumor cells in culture and prolong the lifespan of mice with P388 leukaemia (231).

Table 10: Some of the metal compounds entered clinical anticancer trials*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Trial began in</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCF</td>
<td>1965</td>
<td>Abandoned</td>
</tr>
<tr>
<td>Cu(NO₃)₃</td>
<td>1973</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Spirogermanium</td>
<td>1981</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Cis-PtCl₂(NH₃)₂</td>
<td>1981</td>
<td>Registered (Cisplatin)</td>
</tr>
<tr>
<td>Pt(1,1-CBDCA)(NH₃)₂</td>
<td>1981</td>
<td>Registered (Carboplatin)</td>
</tr>
<tr>
<td>Pt(IV)Cl₂(OH)₂(i-C₆H₄NH₂)₂</td>
<td>1983</td>
<td>Phase 2 (Iproplatin)</td>
</tr>
<tr>
<td>Pt(Isocitrate)(1,2-dach)Cu</td>
<td>1983</td>
<td>Phase 1</td>
</tr>
<tr>
<td>Pt(TMA)(1,2-dach)</td>
<td>1982</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Pt(SO₄)(H₂)(1,2-damch)</td>
<td>1980</td>
<td>Phase 2</td>
</tr>
<tr>
<td>Pt(Malonate)(en)Cu</td>
<td>1983</td>
<td>Phase 1</td>
</tr>
<tr>
<td>Pt(pyruvate)(1,2-dach)</td>
<td>1982</td>
<td>Phase 1</td>
</tr>
<tr>
<td>PtCl₂(C₅H₉NH₂)₂</td>
<td>1979</td>
<td>Abandoned</td>
</tr>
<tr>
<td>Pt uracil Gluer</td>
<td>1979</td>
<td>Abandoned</td>
</tr>
<tr>
<td>Pt(Malonate)(1,2-dach)</td>
<td>1975</td>
<td>Abandoned</td>
</tr>
</tbody>
</table>

*Compiled with data supplied by National Cancer Institute, Ken.Herap Institute of Cancer Research & Other work (71). Abbreviations: Spirogermanium = B-B-dimethyl-N,N-dinalil-2-aza-B-germa spiro-4,5-decane-2-propanamine dihydrochloride; 1,1-CBDCA=1,1-cyclobutane dicarboxylate, dach=1,2-diaminocyclohexene, TMA=1,2,4-benzactrimethylcarboxylate, 1,1-damch=1,1-diaminomethylcyclohexane, en = 1,2-diaminoethane.
The tetrahedral complex can react via a ring opening mechanism (232) Cu⁺ diphosphine complexes exhibit good anticancer activity (Table 11) (232). Tetrahedral Ag⁺ complexes are also active (232). Analogs of the Au⁺, Ag⁺ and Cu⁺ complexes containing either a three carbon or a cis-ethane bridge are found to be active, but activity was reduced or lost altogether, when the phenyl groups were replaced by ethyls.

Table 11: Anticancer activity of Au(1), Ag(1), and Cu(1) Analogues of (Au(DPPE)₂Cl (232)).

<table>
<thead>
<tr>
<th>Basic structure</th>
<th>M</th>
<th>n</th>
<th>R₂</th>
<th>R₂'</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Au</td>
<td>2,3 CH=CH</td>
<td>Ph</td>
<td>Ph</td>
<td>Active</td>
</tr>
<tr>
<td></td>
<td>Ag</td>
<td>2 CH=CH</td>
<td>Ph</td>
<td>Ph</td>
<td>Active</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>2*,3CH=CH</td>
<td>Ph</td>
<td>Ph</td>
<td>Active</td>
</tr>
<tr>
<td></td>
<td>Au</td>
<td>2</td>
<td>Ph</td>
<td>C₂H₅</td>
<td>Active</td>
</tr>
<tr>
<td></td>
<td>Cu</td>
<td>2</td>
<td>Ph</td>
<td>C₂H₅</td>
<td>Marginal</td>
</tr>
<tr>
<td></td>
<td>Ag</td>
<td>2</td>
<td>C₂H₅</td>
<td>C₂H₅</td>
<td>Marginal</td>
</tr>
<tr>
<td></td>
<td>Au</td>
<td>2</td>
<td>C₂H₅</td>
<td>C₂H₅</td>
<td>+Inactive</td>
</tr>
</tbody>
</table>

*In the solid this complex contains two CuCl units bridged by a DPPE ligand and each chelated by another DPPE ligand i.e. (CuCl)₂(DPPE)₂.

+ x = PF₆
P = Phosphorus  DPPE = Diphenyl diphosphine cis ethane

Diphenyldiphosphine ligands are cytotoxic to cells and exhibit anticancer activity against P388 leukaemia (233). Activity is maximised by two or three carbon P-P bridge, but only cis-ethane is active.
The diphosphine bridged, digold complexes ClAu(P-P) AuCl are more potent than the free ligands and exhibit a broad range of anticancer activity (233). The activity is maximum when the ligands form strong chelate rings. The observation that DPPE bridged digold complexes are converted to (Au(DPPE)₂)⁺ in blood plasma, suggests that tetrahedral species may play an important role in the activity of these bridged complexes.

Antineoplastic activity related to chemical structure, biological and toxicological effects, methods of preparation, mechanism of action, pharmacokinetics of platinum complexes are reviewed (234).

The bisdioxopiperazines, Razoxane, is used for the suppression of soft metastases and in the treatment of psoriasis.

In addition of cisplatin and cis-dichlorodiamino ethane platinum, other complexes have been shown to possess antineoplastic properties.

(g) Antiarthritic drugs

The first report that a copper complex was effective in the treatment of rheumatoid and other degenerative connective tissue diseases appeared in 1941.

Antiarthritic properties of copper complex has also been reviewed extensively (235,236). The role of copper can be summarised thus: (i) there is a marked increase in total serum copper in rheumatoid arthritis patients; (ii) there is an increased
rate of synthesis and accelerated turnover of ceruloplasmin; (iii) increased levels of copper and of ceruloplasmin are found in synovial fluid of patients during prolonged treatment of rheumatoid arthritis; (iv) the distribution of copper between exchangeable and non-exchangeable forms in biofluids is altered by the disease; (v) the intravenous administration of copper has a marked antiinflammatory effect; and (vi) the drugs such as penicillamine which interact with copper in vivo facilitate remission of rheumatoid arthritis by promoting tissue utilisation of copper. It has shown that other less lipophilic species such as Cu histidine are particularly effective only when given systematically. In 1965, Plantin and Strandberg (237) reported that total whole blood Cu was elevated in rheumatoid arthritis.

A historical review of the use of Cu complexes in the treatment of rheumatoid and other degenerative connective tissue diseases has been published (238). This review provides an account of the therapeutic results obtained with Cu complexes in treatment of acute or chronic rheumatoid arthritis, rheumatic fever, staphylococcal spondylitis, gonococcal spondylitis, arthritis with psoriasis, Reiter's syndrome, lumps erythematous, sarcoidosis arthrosis deformans, erythema nodosum sciatica, cervical spine-shoulder syndrome, lumbar spine syndrome or osteoarthritis.

Fenz (239) used allocupreide to treat rheumatoid arthritis. Further, he reported that allocupreide brought about remission of the anaemia associated with rheumatoid arthritis. The toxic side effects with gold therapy, were not observed with Cu therapy.
Foreistier, Jacqueline and Lenoir (240) reported their results with bis(8-hydroxyquinoline di(ethylammonium sulfonate)-copper (Cuproxoline, Dicuprene, Cuprinyl). Although this compound has been found to be less effective orally, cuproxoline is somewhat superior to allocupreide because it is less irritating and can be administered by both i.v. and i.m. routes.

O'Reilly (241) reported results of a single study with i.m. sodium n(N-allyl cuprothiocarbamide) benzoate, (cuproally-thiourea-sodium benzoate, Alcuprin), and analogue of allocupriide and Cuproxoline.

Hangarter (242) had studied salicylic acid Cu complex (Permalon, SACC) as antiarthritic agent. He attributed the antiarthritic effect to Cu. Therapeutic results with inorganic Cu alone were comparable with those of Au therapy although Cu treatment was associated with fewer side effects.

The Cu-dependent metalloenzyme (Oryotein), has been shown to be safe and effective for the treatment of established osteoarthritis when given intra-articularly into knee and hip joints in single or multiple doses.

Gold thiomalate has been in use as an injectable antiarthritic drug since the 1930s.

In the late 1960s, Sutton (243) made Au(I) phosphine complexes such as (C₂H₅)₃PAuC, and established their oral antiarthritic activity.
(h) **Antiinflammatory agents**

Copper-aspirin chelate is reported to be a more active anti-inflammatory agent than aspirin. Copper complexes with carboxylic acids showed anti-inflammatory activity in rats and antipyretic activity in rabbits at doses of 10mg/kg (244). Complexation of Penicillin G and V and Penicilloic acid with Cu$^{2+}$ ion (245) is reported.

(i) **Anti-ulcerative activity**

The anti-ulcer activity of various copper complexes has also attracted considerable attention, particularly because most of the current drugs used to treat arthritis are prone to be ulcerogenic (246).

Copper (II) complexes with triphosphon and phenylalanine alone and in combination show progressive inhibition of gastric acid secretion (247). The mixed complexes are significantly more potent than the simple complexes. The difference in biological activities of the complexes were directly related to the percentage of neutral species present in solution.

(j) **Miscellaneous activities**

Chelating agents have been used for a variety of medicinal purposes, Co-EDTA is employed in therapy for cyanide poisoning and to dissolve kidney stones (130,248). The chelation of Cu(II) or Zn(II) L-3,4-dihydroxyphenylalanine (DOPA) is reported.

The simultaneous intracerebral administration of calcium chloride reduced the acute toxicity of Neomycin. Further, calcium reduces the peripheral toxicity of Neomycin (249).
Chromium-B-glycerophosphate complex showed absence of acute toxicity even at exceedingly high concentrations (250). Unlike ionic trivalent chromium in the range of concentrations tested, 7.5-625 mg chromium/100ml, chromium-B-glycerophosphate had no precipitating effect on human serum albumin.

Calcium-sucrose phosphate-calcium orthophosphate complex is used in the treatment of dental caries (251). It decreases the rate of dissolution of enamel by acids. Its action is similar to fluoride.

Metal chelates are found to inhibit acetylcholine esterase (252).

Alkaline hydrolysis of the ethyl picolinate is greatly enhanced when copper(II) ion is present due to complex formation between the Cu-ion and the ester. Since the picolinic acid formed in the process is a stronger chelating agent than the ester, the catalytic effect of the copper ion is diminished during the reaction (253).

The effect of different metal ions on the rate of anaerobic degradation of ascorbic acid in aqueous solution has been studied (254). The experimental results indicate that the metal ions form complexes with ascorbic acid.

The efficiency, sequence, specificity and chemistry of DNA cleavage mediated by Fe$^{2+}$ chelates of deglycobleomycin $A_2$ and
decarbamoylbleomycin $A_2$ are reported (255). Both products were found to mediate DNA degradation in the presence of Fe$^{2+}$ and to have the same sequence selection for DNA strand scission as bleomycin $A_2$.

1.7 Chelation in Pharmacy
(a) Stability of Drugs

The stability of thiamine HCl solution in the presence of chelating agents was studied (256). The stabilising efficiency of diethyleneetriaminepentacetic acid and N-hydroxyethylethylene diaminetriacetic acid is markedly superior to that of disodium edetate.

Oxytetracycline forms 1:2 complexes with copper and nickel ions, while 1:1 complexes are formed with these ions and dimethylchlortetracycline (257). Weaker 1:1 complexes were noted with iron, zinc, magnesium and calcium. The potential use of metal complexation in in vivo reduction of photosensitisation is discussed. Stability of vitamins in pharmaceutical formulation is reviewed (258).

Epinephrine chelates with metal ions (62). The chelate with boron did not alter its physiological potency (259). EDTA, cysteine, cystine, ascorbic acid, tyrosine, flavonoids, 8-hydroxyquinoline, diethyldithiocarbamate, trihydroxy-N-methylindole, salicylates, and 1-hydrazinophthalazine potentiate the action of epinephrine.
These potentiators seem to act by chelating copper or iron and may lower or prevent its ability to catalyse the destruction of epinephrine.

Histamine chelates with Cu^{2+} complex cause a weaker contraction of guinea pig ileum than histamine itself (260).

(b) Bioavailability of Drugs

It is shown that the rate of transfer of several model drug compounds through an artificial lipoid barrier can be modified by complex formation.

Complexes that are more lipoid-soluble than the drug itself penetrate the lipoid barrier more rapidly than uncomplexed drug (261). Iron absorption in rats was evaluated with concurrent administration of amino acids and ascorbic acid (262). Asparagine, glycine, serine and ascorbic acid caused a satisfactory significant increase in iron absorption with the greatest effect for asparagine and glycine.

The effect of metal chelation on intestinal absorption of Vitamin B_{12} was studied in man and rats. When a physiological dose of Vitamin B_{12}-Co (60) was instilled through an indwelling tube into the duodenum in man, immediately followed by EDTA in a dilute solution, absorption of Vitamin B_{12} was reduced markedly. Administration of Ca^{2+}, Mg^{2+}, Sr^{2+}, but not Fe^{2+}, Fe^{3+} or Zn^{2+} shortly after EDTA counteracted the inhibition by EDTA (263).
Reduction in oral penicillamine absorption by food, antacid and ferrous sulphate was observed (264). The combination of 1:20 drug aluminium magnesium silicate complex with amphetamine in 1:1 ratio, resulted in recovery profiles of amphetamine resembling those obtained from prolonged-release dosage forms (265). The use of chelating agents in sustained release drug delivery system is reviewed (266). Industrial role of EDTA and other chelating agents is discussed (267).
AIM OF PRESENT WORK

In chelation therapy, it is desirable to administer selective agents which bind the target metal ion very powerfully and do not interfere with the metabolism of vulnerable essential elements. It is also clear that an ideal agent should penetrate into the body compartment where the offending metal ion has been largely deposited. In addition, the drug must be cheap, nontoxic, resistant to metabolic degradation and conveniently (i.e. orally) administered. Folic acid fulfills these requirements. Therefore, it was selected as ligand in the present work.

Folic acid can occur in keto (I) and enol (II) forms. It is known that folic acid exists in enol form (II), which is therapeutically active. The enol form is structurally similar
to oxine (III) (as shown by dotted lines) - a well known chelating agent.

In the present study, the selection of metal ions is based on their biological classification viz., essential bulk structural elements (e.g. Ca and Mg) transitional elements (e.g., Fe, Mn, Cu, Co, Zn, Ni, etc.) and toxic elements (e.g. Ba and Sr).

The aims of the present work are as follows:

(i) To complex folic acid with metal ions in aqueous medium.
(ii) To evaluate stability constant of the complexes from the formation curves.
(iii) To determine the composition of the metal ion complexes.
(iv) To study the physico-chemical properties of the complexes.
(v) To evaluate the stability of folic acid in its metal complexes.
(vi) To study the absorption pattern of folic acid-metal ion complexes in in vitro.
(vii) To study the compatibility of the metal ions with folic acid under physiological conditions.
(viii) To screen the pharmacological properties of the complexes.
(ix) To study the microbial activity of the metals in complexes.