CHELATION AS A MECHANISM OF PHARMACOLOGICAL ACTION

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One of the properties of a variety of types of organic molecule is the ability to form internal complexes with metals. These are known as chelate ("Key late") compounds, from the Greek "chele", ($\chi\eta\lambda\eta$) the crab's claw, a name proposed by Morgan (11). This phenomenon has received scant attention from pharmacologists. *Biological Abstracts'* 1951 index published in 1954, carries the **chelation** rubric for the first time while the first chemical treatise was published in 1952 (7). Most of the potent chelating compounds described in it have not been examined pharmacologically.

Among the metals most sensitive in this regard are those which are also of biochemical significance, such as Fe, Co, Mn, Mg, Cu, and Zn. Understanding of the absolute necessity for small amounts of these specific metals to the normal operation of cellular affairs has recently greatly increased. Furthermore, it is becoming clear that these metals exert most, if not all, of their effects while in a bound form (63, 140, 141, 148, 181, 200, 201, 208, 241, 248).

In a period when pharmacology is directing special attention to the ultimate mechanisms by which drugs act on cells, cognizance of a phenomenon which enables unrelated chemical species to exert a similar influence upon the metals involved in metabolism is desirable. It is hoped this review will promote understanding of the chemical factors involved and identify certain drug actions which appear to relate to chelation.

I. SYNOPSIS OF CHELATION CHEMISTRY

The chemistry of metal complexes is usually avoided in the formal training of most biologists, for it is, itself, complex. Although some practical applications

[1] The author is especially grateful to Drs. R. W. Parry and Adrien Albert for their advice and to Dr. D. R. Bennett for assistance with the references.

are traditional in biological sciences, for example, in Benedict's solution or in use of citrate to prevent Ca^{++}-activated blood clotting, some systematic understanding is needed for progress to occur and is valuable for comprehension of this review. Closely guided by Professor R. W. Parry of the Department of Chemistry of the University of Michigan, the reviewer has attempted to formulate a qualitative synopsis of complex chemistry especially oriented toward the needs of biologists.

Chelate compounds are a subdivision of the broad group of coordination compounds first studied in detail by Werner. The familiar ammonia addition compounds of copper sulfate, $CuSO_4 \cdot 4NH_3$, and even the hydrates of familiar metal salts such as $MgSO_4 \cdot 7H_2O$ can be classed as coordination compounds. Such materials are typified by the presence of the coordinate bond. According to the most widely accepted concepts of present day chemical theory, such coordinate bonds arise from the sharing of an electron pair between the ion or atom of a metal and an atom or ion in the complex-forming structure, the ligand. If both electrons are furnished by the complex-forming ligand (*i.e.*, NH_3 in $CuSO_4 \cdot 4NH_3$), then the bond is usually classed as coordinate covalent and is frequently represented as $M \leftarrow X$, where M is the metal and X is the ligand. If on the other hand, one electron is furnished by the metal and the other by the ligand, then the resulting bond is classed as normal covalent and the conventional representation is M—X. It should be emphasized that no real difference in properties can be attributed to differences in the sources of the binding electrons. The different modes of representation are convenient only as an aid to electron bookkeeping and charge balancing. The terms, ionic or covalent character, are frequently used to indicate the extent of charge transfer in the final structure, but their exact meaning is far from clear and varies with different investigators (13, 14, 15, see p. 90). Classification of complexes on the basis of sound experimental criteria would appear to be preferable to the ionic-covalent designation.

The most common electron donating or electron sharing atoms in ligand molecules are nitrogen, oxygen, and sulfur although others are known. The strength of the bond between sulfur and metals is usually so great that substances like diethyl dithiocarbamate, which cannot chelate, form complexes with metals just as stable as the chelated complexes formed by the oxygen- and nitrogen-containing groups found in Table I. Typical coordinating groups are listed in Table I. In the so-called acid groups listed in part A, a proton is replaced by a metal when coordination takes place to give a normal covalent bond ($M^{++} + HOR \rightarrow MOR^{+} + H^{+}$). The groups listed in part B are attached by the coordinate bond.

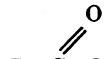
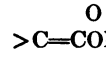
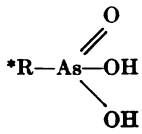
When two bonding groups are present with proper spatial orientation in a single molecule, a heterocyclic ring involving the metal ion may be formed. Such rings were called chelate rings by Morgan; the modern term chelation arises from this designation. Throughout the body of this review "chelation" is used only when ring formation is well established. "Complex" describes cases of uncertainty or definitely no ring structure.

If the ligand molecule contains only two electron donating groups, it is classed as "bidentate" and only a single ring can be formed, I; if it contains three groups

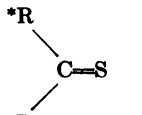
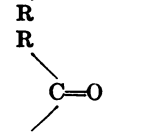
TABLE I

Common coordinating groups

A. Typical acid coordinating groups: One H^+ replaced by metal ion in coordination

$R—CONH_2$	Primary amido	*R═NH	Imine
R—CONHR	Secondary amido	*R—SH	Thiol
	Carboxyl	$*R—SO_2H$	Sulfinic
	Oxime	$R—SO_3H$	Sulfonic
	Enolic hydroxyl	$*R—As(OH)_2$	Arsinic
			Arsonic

B. Typical neutral coordinating groups: H^+ not lost in coordination.

R—OH	Alcoholic hydroxyl	R═N—OH	Oxime
$R—NH_2$	Primary amine	R—S—R	Thio ether
R—NH—R	Secondary amine	*R—O—R	Ether
$R—NR_2$	Tertiary amine		Thionyl
	Cyclic tert. amino		Carbonyl
		*R—C═C—R	Olefinic

* Distinctly less frequent.

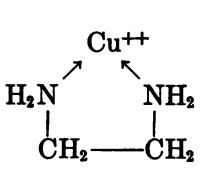
it is "tridentate" and two interlocked rings can be formed if proper spatial orientations obtain, II. In some "polydentate" structures such as the porphyrins, III, many interlocked ring systems may be formed and the resulting structure has amazing stability.

Formation of a chelate ring confers behavioral patterns upon both the chelated metal and the ligand not previously available to either alone. Color changes, solubility changes, variations in chemical reactivity and catalytic functions, increased stability or instability, and the appearance of optical activity are especially pertinent to biology.

The size of the bidentate heterocyclic rings which may be formed has been the subject of much study and an expression of general rules is possible.

1) Three-membered rings have not been proven to exist.

2) Four-membered rings are well known, the strain which might be expected



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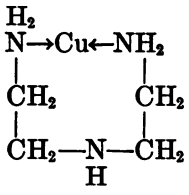

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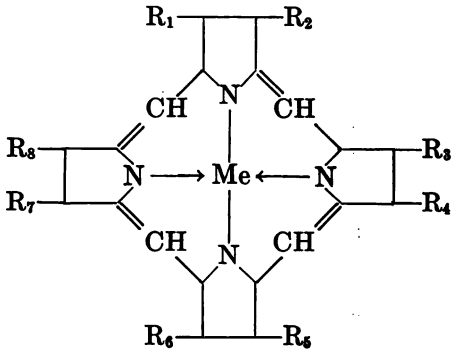


III

from carbon chemistry being alleviated by the difference in bond angles involving metals as well as the difference in sizes of atoms.

3) Five and six-membered chelate rings are the most frequently encountered and, in general, the most stable.

4) Five-membered rings are usually most stable if the ring is saturated.

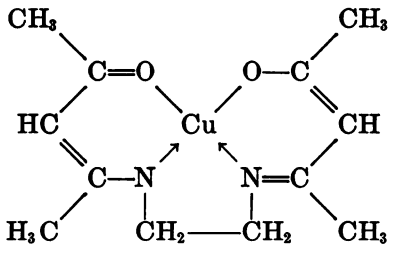
5) Six-membered rings are usually most stable if the ring contains two double bonds.

6) Five or six-membered rings appear to be about equally stable if the ring contains one double bond.

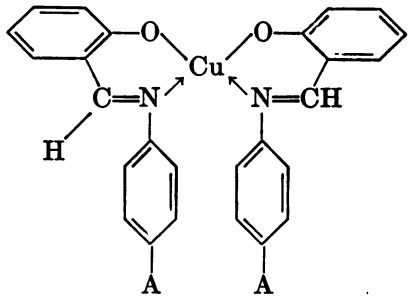
7) Some rings of 7, 8 and 9 members have been reported, but dimers or polymers may be formed in preference to large rings.

Exceptions to rules 4) and 5) are known.

Factors which influence the formation and stability of chelate rings are many, apart from the energy of the coordinate bond between metal and ligand atoms. A chelate ring is inherently more stable than a metal complex without ring formation for the probability is great that dissociation of both ends of the ligand will not occur simultaneously and, therefore, the "free end" may fall back into place more readily. When the chain length between the two ligand groups is excessively long, the chance for this to occur declines and large rings frequently degenerate into polymeric chains. Fused rings in which positioning is rigid have often incredible stability. Copper^{II} ethylene diamine-bis-acetyl-acetone, IV, which contains three interlocked rings may be heated to redness without decomposition (12).



IV



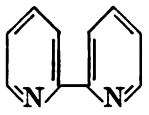
V

A ligand group can, in theory, be bound to any electron acceptor, hence the hydrogen ion may be bound to the electron pair of the ligand in place of a metal cation. As the basic strength of an electron donor group measures its ability to bind the proton, the basic strength might also give some indication of ability of the ligand to bind a metal cation. Thus, a correlation between the basic strength of a coordinating group (*i.e.*, NH_3) and its coordinating ability is not unexpected, although it is obvious that a strict parallelism would be too much to hope for.

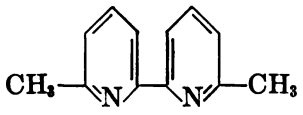
$H_3N \rightarrow H^+$	$H_3N \rightarrow Cu^{++}$
Ammonia bound to H^+ to give NH_4^+. Measures basic strength of ammonia.	Ammonia bound to Cu^{++} to give coordination compound. Measures coordinating ability of NH_3.

However, when systems of sufficient structural similarity are compared, a linear relationship between the stability of the metal complex (as measured by the negative log of its dissociation constant), and the stability of the hydrogen ion adduct (as measured by the negative log of the basic dissociation constant) is obtained. For example, taking V, and making suitable substitutions at A, Calvin and Bailes (1) found, in general, the greater the electron attracting power of the substituent group, the greater the tendency to remove electrons from the nitrogen and hence lower the basic strength of the amine and the stability of the copper chelate complex. In most cases, the relative influence of the various groups appears to follow the usual organic chemistry observations, which fact may be helpful in planning new structures. Further, in any given series the basic strength of a ligand may be useful in the prediction of its chelation characteristics.

Steric hindrance, the clashing of groups on two coordinated ligands, will result in a distortion of bond angles and a decrease in stability (or no ring formation at all in extreme cases). Thus, α,α'-dipyridyl, VI, chelates Fe^{++} but 2,2′-dimethyl-α,α'-dipyridyl, VII, will not, despite the increased basicity of the N induced by the methyl substitution. This phenomenon has been used to provide selective analytic reagents.



VI



VII

Solvation energy has not yet received the attention it should have had for proper evaluation of its role in the formation of chelates involving the large, hydrophobic molecules of biochemical and pharmacological interest. Solubilities of the metal ion, the ligand and the complex as well as the anions present in a multiphase biological system must greatly influence the ring stability, but quantitative data are not abundant.

The two most important determinants of the formation of a complex are the pH of the solution, which determines the amount of ionization of the ligand, and the stability constant, which expresses the tenacity of the particular bonds in the complex. Because the bond energies are essentially immutable absolutes, they

are not discussed here and the references should be seen for details. From a biochemical point of view it is convenient to consider stability constants in relation to glycine chelates, the stability constants of the complex in question being described as less than, equal to, or higher than those of glycine.

The effect of pH on the comparative efficiency of ligands is considerable. If at a given pH value two ligands were equally well ionized, their success in competing for traces of metal ions at that pH would be proportional to their stability constants alone. However, it is often the case that the degree of ionization of two molecular species is quite different at a given pH. Thus, at pH 7.3, the amount of anion (the chelating form) varies from 0.04 per cent for proline to 2.45 per cent for asparagine, or sixtyfold. Asparagine will compete with proline five times as successfully for Fe^{++} and seven times as successfully for Mn^{++} at that pH. The fraction of the amount of complex theoretically possible which is actually formed at a given pH can be obtained from the "formation function", $\bar{n}$. (See (7) for discussion.)

Under physiological conditions, enormous concentrations of assorted metals or ligands may be present relative to the specific ones of interest. A trace of powerful ligand competing *in vivo* for a metal ion against a weaker ligand present in relatively huge amounts will be much less successful than might be supposed from comparison of the specific affinities (stability constants) of the two ligands for the metal determined *in vitro*. As an example, despite clinical usefulness, the efficiency of ethylene diamine tetraacetate in removing lead from poisoned individuals is vastly below expectations based upon *in vitro* measurements of relative binding constants.

When examined using a variety of ligands, there is a tendency for metals to form an order of descending stabilities, generally about the same for each ligand, though not always so. The relative affinity of bivalent cations of the first transition series has been shown by Irving and Williams (6) to be as follows: Mn < Fe < Co < Ni < Cu > Zn, irrespective of the nature of the coordinated ligand or the number of ligands involved. Chapman (2) notes that this sequence closely parallels the electronegativity sequence. The series obtained by Mellor and Maley (9, 10) with salicylaldehyde complexes has been much quoted and, in the order of decreasing chelate stability, is Pb^{++}, Cu^{++}, Ni^{++}, Co^{++}, Zn^{++}, Ca^{++}, Fe^{++}, Mn^{++}, Mg^{++}. This arrangement includes electronically dissimilar metals such as Pb^{++}, Ca^{++}, and Mg^{++} and for this reason does not have the universal application of the Irving-Williams series. On the whole, the pharmacologist will be most interested in those unusual ligands capable of forming chelates with only one or a few of the biologically or toxicologically important metals. So far, very few substances of this kind have been discovered. The existence of some are indicated in analytical works (4, 5, 8, 16).

Determination of the presence of chelation may require several methods. Change in the chemical properties of the metal and ligand, usually in the direction of decreased activities, but occasionally the reverse; changes in color or absorption spectra, electrical conductance (usually decreased, but strikingly increased in the boron-1,2-dihydroxy chelates); and development of optical

activity with previously inactive reagents are all useful methods. Bio-assay tests are provocative but not conclusive. Isolation of the pure compound may be deceptive because crystal lattice energies stabilize many complexes that are too weak to exist in solution.

The interested reader will find much more extensive consideration of the chemistry of chelation in the review by Diehl (3), the treatise by Martell and Calvin (7) and a forthcoming A.C.S. monograph edited by J. C. Bailar.

II. PHARMACOLOGICAL ASPECTS OF CHELATION

A. Determination that chelation is involved in drug action

1. Isolation of the complex. The isolation and characterization of chelate complexes of ethylene diamine tetraacetic acid (q.v.) (EDTA) and radioactive or otherwise toxic metals from urine has not been reported. The metals are found in an increased concentration in urine following administration of EDTA and the presumption that they are present in the chelated form is easily accepted. The literature with regard to the complexes of dimercaprol (2,3-dimercapto propanol) is similarly void. As explained above, isolation of the metal complex from urine or tissue may prove unprofitable.

In Bodine and Fitzgerald's studies (42) on the effect of copper reagents on grasshopper embryos it was possible to extract a copper diethyldithiocarbamate (DEDTC) complex. This complex is so stable it could not be stabilized by isolation. Satisfying as this experiment was, it still leaves open the possibility that in the process of analysis, copper ions were freed from the tissue and *then* reacted with the reagent, rather than during the life of the cells.

2. Chemical relationships (*"Structure-Activity-Relations"*). When a variety of chemical structures exert a single pharmacologic effect, and are found to bind metallic cations *in vitro*, the possibility should be considered that chelation is the ultimate mechanism of action, even though the other properties of the molecules involved may be very important for stability, penetrations, etc. Some examples of this type of study may be recognized.

a. Diabetogenic action. A number of relatively simple organic molecules are known which will occasion a syndrome typical of diabetes mellitus under suitable experimental conditions. Several are powerful chelating agents.

Diphenyldithiocarbazone ("Dithizone"), a sulfur complex forming molecule, has been reported to induce damage to the beta cells of the pancreatic islets, resulting in classical diabetes mellitus within one or two days after intravenous injections of 50–150 mgm./kgm. (31, 98, 119, 132, 189, 252). Others have not been impressed with its action (26, 52, 64). (The rate of injection of alloxan has a great deal to do with the subsequent development of diabetes (175). Such a consideration may apply here.)

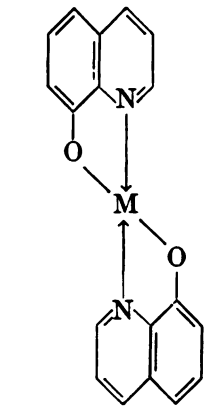
The study of Wolff *et al.* (252) seems particularly instructive. Following dithizone injection, the serum zinc levels of dogs were noted to increase and later, urinary zinc levels increased. Islet cells were found to be damaged upon histological examination and histochemical tests indicated a loss of zinc paralleling dithizone dosage. It would be desirable to establish that the zinc was present in

urine as the dithizone complex, although the possibility must always exist that the reaction occurred secondarily, *i.e.*, not in the pancreas.

8-Hydroxyquinoline ("oxine"), a chelating substance, has been found effective in inducing diabetes mellitus, particularly in rabbits (130, 189). In a study of structure-activity-relationship in this phenomenon, Matsuo *et al.* (160) showed that oxine derivatives such as 8-methoxyquinoline which could not chelate under physiological conditions were not active as diabetogenic agents. Root and Chen (189) and Kadota and Abe (131) have likewise indicated that the presence or absence of diabetogenic activity parallels the ability of various analogs to form metal chelates.

Iwamoto and Adams (129) comparing diabetogenic activity with structural variations encouraging or opposing chelation in a quinoline series and in a series of hematoxylin compounds, concluded in a preliminary paper that the "hypothesis (*i.e.*, chelation) appears promising." It would be of interest to study the actions of a wider variety of structures complexing zinc, particularly as the zinc complex, which should not be active.

b. Antibacterial action. Work of A. Albert and his colleagues on the antibacterial action of oxine and related compounds is an outstanding exposition of the relations between structure, chelation, and biological activity. Beginning with the observation that 8-hydroxyquinoline forms precipitates with a variety of heavy metals, it was first shown under physiological conditions of temperature and pH, that of the seven hydroxyl-isomers possible, only 8-hydroxyquinoline would form metal chelates, VIII, IX (24).



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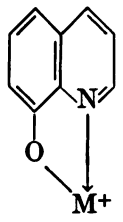

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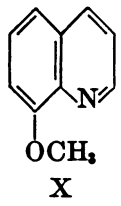

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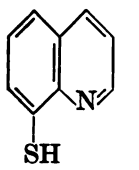

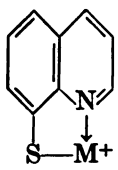

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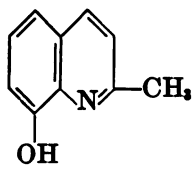

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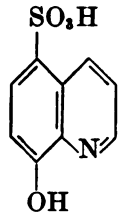


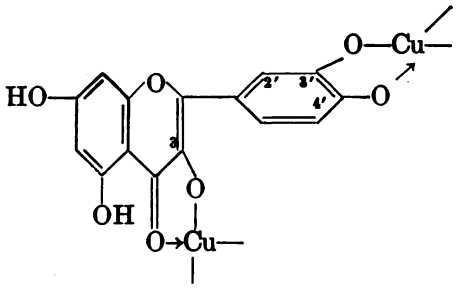
XIII

Quantitative antibacterial studies were made on six species of bacteria, and in particular, *Staphylococcus aureus* (25). That the antibacterial activity of an oxine structure which actively chelates is not coincidental, but directly related, was re-emphasized by studying such other derivatives as 8-methoxyquinoline, X, which cannot chelate under physiological conditions and which have no antibacterial effect. Shchukina and Savitskaya (204) state that 8-alkoxyquinolines are antibacterial and conclude that the antibacterial action of 8-hydroxyquinoline does not involve metals, but they give no quantitative data. Related structures which readily chelate, such as 8-mercaptoquinoline, XI, are actively bacteriostatic. When substitutions such as might interfere with chelation *at a surface* by steric hindrance were made, as in 2-methyloxine, XII, although iron chelation was essentially as vigorous *in vitro* as with oxine, antibacterial activity declined appreciably. Structures which formed hydrophilic complexes unlikely to penetrate the cell such as the 5-sulfonic acid analogue, XIII, were inactive as antibacterials although *in vitro* chelation was vigorous. It has been shown (22) that 8-hydroxyquinoline is not toxic itself to bacteria, but it becomes so when traces of iron are present. Thus the active agent is the oxine-iron complex, and actually only the 1:1 complex (*i.e.*, type IX) is bactericidal.

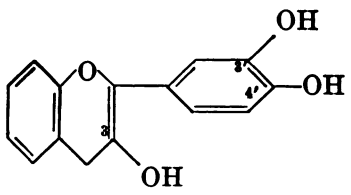
Throughout this study two important concepts appear. 1) The *stability constant* (q.v.), may not indicate the *biologically* most important metal. Thus, nickel and cobalt oxines are more stable than iron oxine, yet it is the latter which appears to be functional. 2) It is well brought out that chelation properties observed *in vitro* cannot be the sole factor in predicting the activity of a drug which may theoretically act through chelation, for the rest of the molecule may exert forces influencing the penetration, steric hindrances to approximation to the metal *in vivo*, etc.

c. Flavonoids. After studying the "Vitamin-P" activity of a series of flavonoids upon the isolated rabbit intestine response to epinephrine, Clark and Geissman (58) concluded that the observed potentiation of the action of epinephrine by a variety of compounds was directly related to the stability and insolubility of the copper chelates formed. They added 1 μgm./ml. $CuSO_4$ to swamp variations in copper concentration in the bath fluid to increase the reproducibility of their results. This also prevented extreme potentiations otherwise noted.

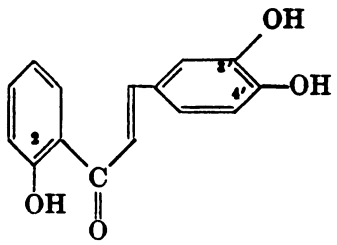
The groupings of importance in this example of chelation are shown as in



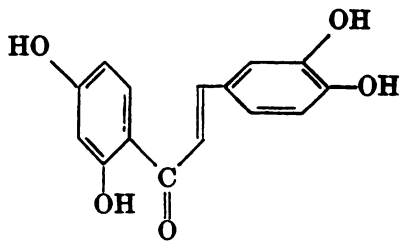
XIV



XV



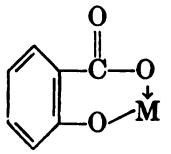
XVI



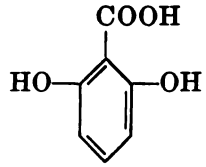
XVII

quercetin, XIV. They predicted "that 3,3′,4′-trihydroxyflavone, XV, would form a complex stabilized to a greater degree (with respect to the uncomplexed compound) than quercetin, since in the latter substance resonance stabilization of the uncomplexed substance would involve not only the 3- and 3′,4′-hydroxyl groups, but those in the benzo ring as well." Therefore, it should be more active. This was found to be the case. A similar variation was noted in the case of 2,3′,4′-trihydroxychalcone, XVI, which was found to be more active than butein, XVII, from which it differs only in the absence of a non-chelatogenic hydroxyl group. Wide differences in activity were shown by pairs of compounds having identical ligand functions but differing in solubility (*e.g.*, gossypin and quercetin, rutin and xanthorhamnetin), but in such pairs the compound which was more soluble was least active. This suggests that copper must be desolubilized, rather than simply sequestered, for maximum protection of epinephrine, which fits with certain of their other observations.

d. Salicylates. Metal chelates of salicylic acid, XVIII, are well known, which



XVIII

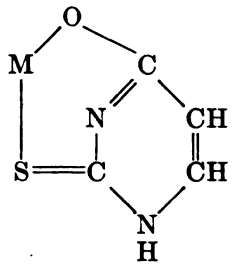


XIX

suggests that salicylic acid may exert some, or all, of its classical actions through its chelating function. In studies of rheumatic fever patients, it was reconfirmed that the nonchelating *meta* and *para* hydroxy benzoic acids were ineffective as compared to *ortho* hydroxy benzoic acid (salicylic), either in relieving the inflammation (183), or in increasing the metabolic rate (59). Because it "chelates to a greater degree," γ-resorcylic acid, XIX, was investigated in these patients. It was reported to be nearly ten times as effective as salicylic acid. Bergel (36), in an interesting discussion of antirheumatic action, ascribes all benefits to a reduction of free heavy metal ions. Studying the depletion of adrenal ascorbic acid in hypophysectomized rats by various salicylate analogues, Cronheim (68) could not find a relation between the chelating ability and this activity.

e. Thiouracil. Thiourea and thiouracil have essentially the same qualitative effect upon the thyroid gland and the familiar reaction between thiourea and copper suggests that it may be of importance in controlling its actions. In the

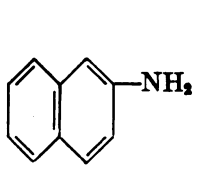
thiouracils, a relation between the ability to form chelate complexes and antithyroid activity has been reported by Libermann (151, 152), who suggests the following formulation for the chelate, XX,



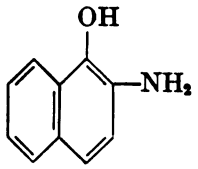
XX

which appears untenable on steric grounds. To exist, the C—O and C—S bonds would have to bisect the angles of the pyrimidine ring. This is not acceptable and it is more likely that these substances form the usual, very firm, non-chelate bonds with metals, just as many other —SH substances do. The 4-thiouracils are inactive upon the thyroid and do not form stable complexes, but rather, form a bisulfide. Various relations between metals and thyroid behavior are reported which are interesting (65, 117, 118, 150, 235).

f. Carcinogens. Recent studies have shown that β-naphthylamine, XXI, is



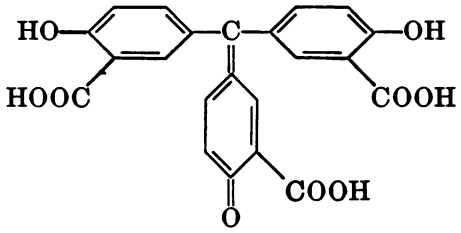
XXI



XXII

metabolized to the chelating o-hydroxy derivative, XXII, in mammals and that this latter compound is a potent cause of bladder tumors. Following direct application, the unconverted amine is inactive, while the metabolite is active (44). Kojahl and Lucas (143) have also shown a close correlation between the tendency toward formation of silver complexes of polycyclic aromatics and their carcinogenicity.

g. Beryllium antidotes. Earlier experiments established the value of aurin tricarboxylic acid (A.T.A.), XXIII, as an antidote for acute beryllium poisoning

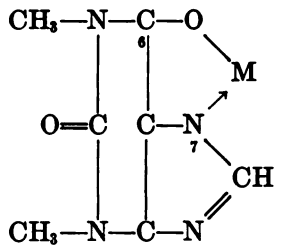


XXIII

(199, 245). The probability that this action involved chelation of the beryllium led Lindenbaum, White and Schubert (153) to examine a variety of closely related salicylates, phenols, catechols, etc. as well as assorted chelating agents. Compounds were first studied *in vitro* against beryllium inhibition of rat plasma alkaline phosphatase. It was found that *ortho* hydroxyls or hydroxyls *ortho* to a carboxyl group on a benzene nucleus were notably active. Further, compounds capable of forming 5 or 6-membered chelate rings with N and O as ligand atoms were also effective. More than 70 chelating compounds were so examined.

When these compounds were examined for their ability to antagonize acute beryllium poisoning in mice, only those compounds actively chelating beryllium *in vitro* were effective antidotes. Naturally, many compounds active *in vitro* were inactive *in vivo*. Frequently this situation had a conventional pharmacologic explanation. Of interest here, the sulphonated compounds, active *in vitro* but inactive *in vivo*, tend to be so highly hydrophilic that cell penetration would be impaired. Indeed, evidence was later added that *in situ* chelation of beryllium in the cell is of importance (246), for A.T.A. does not modify the tissue content of Be^7.

h. Negative correlation. Negative structure-activity correlations may effectively deny the probability that chelation is the critical event. Thus, although theophylline (1,3-dimethylxanthine), XXIV,



XXIV

chelates copper (173), chelation between the carbonyl oxygen at the 6 and the reactive hydrogen on the 7 position cannot occur in caffeine (1,3,7-trimethylxanthine). Hardman *et al.* (111) found caffeine to be only slightly weaker than theophylline in stimulating the hypodynamic heart-lung preparation, while 6-desoxytheophylline was also quite active, rendering further consideration of chelation between positions 6 and 7 unnecessary in this series.

A negative correlation was also reported by Crescitelli (66) for the effect of oxine and related compounds on nerve conduction.

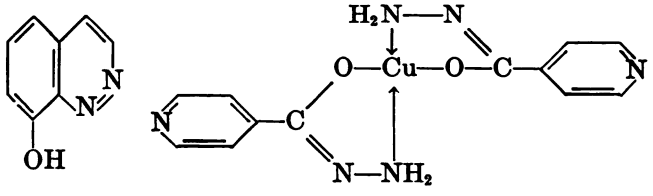
3. Variation of metal ion levels. a. Action of pre-formed chelates. Action of pre-formed chelates is usually studied only after it is known that the drug chelates *in vitro* or it is strongly suspected to involve metals in its action. (Optical activity with its customary effects on pharmacologic responses is also noted in chelate complexes (80).)

At least two informative results may be obtained when a pre-formed chelate complex is added to a biological system. 1) The characteristic action of the ligand molecule is suspended, suggesting that (a) the complex *per se* is not the active

form and chelation of a fixed, intracellular cation may be required to produce the action, or (b) the inappropriate metal was used. This latter possibility can be eliminated by testing many metals, leaving (a) as the usual interpretation. 2) The characteristic action of the ligand molecule is (a) unaffected or is (b) enhanced. If there is no enhancement it suggests that the system already contains sufficient metal ion, and enhancement may only be expected if the critical metal is reduced below an optimum. When enhancement is noted it would appear to indicate the chelate complex *per se* as the active molecule.

Such considerations led Adrien Albert and his co-workers to examine the bactericidal action of 8-OH quinoline ("oxine") in the absence of trace metals such as Fe^{++} and Cu^{++} (22). Under such conditions oxine was inactive until traces of Fe^{++} or Cu^{++}, inactive alone, were added. Further, although M/100,000 oxine was active in ordinary media, at M/800 it was inactive. The dependence of oxine activity upon either Cu^{++} or Fe^{++} has been confirmed (211, 236). These findings could only lead to the conclusion that an Fe^{++} or Cu^{++} oxine chelate is the active bacteriocidal factor, particularly since it had already been shown that only those derivatives of oxine which can chelate can be active (see II, A. 2. b.). Failure of high concentrations to act could be overcome by addition of more Fe^{++} and from these findings, strongly bolstered by chemical measurements, they concluded that when Fe^{++} is in excess a 1:1 chelate is formed, IX. As this complex is unsaturated and reactive chemically, it is probably the active bacteriocide. When oxine is in excess, the 1:2 chelate is formed, VIII. As this is non-polar and lipid soluble it is probable that it is the form which penetrates along with uncomplexed oxine to the susceptible centers of the bacterium. By application of the Law of Mass Action it could be shown that conversion of the penetrating 1:2 complex to the toxic 1:1 complex in the cell is possible. The inactivity of great excess of oxine is thus explained as failure to allow the slightest conversion. A curious finding, not exactly explicable as yet, is the complete antagonism which small amounts of Co^{++} can exert against iron-oxine. Ni^{++} combines thirty times more firmly with oxine than Co^{++}, yet exerts no such antagonism. Vajda and Nógrádi (232) have recently suggested that Cu^{++}-oxine replaces Co^{++} in a prosthetic group of a *Mycobacterium* enzyme, based upon their finding of a correlation between the quotient of complex stabilities $KI_{Co^{+++}}/KI_{Cu^{++}}$ and antituberculous activity.

The question of diffusion into the cells was explored further (23) by synthesis of aza-oxines such as, XXV,



XXV XXVI

Just as in the case of oxine, only those derivatives of aza-oxine which could chelate were bacteriocidal and the action was dependent upon the relative con-

centrations of aza-oxine and iron. Further, there is a close relation between activity and lipophilic properties of the molecule. These data were offered as additional evidence for the concept of the necessity for penetration "into" the cell before bacteriocidal action could occur.

Culture filtrates of *Fusarium oxysporum f. lycopersici* contain lycomarasmin, a dipeptide which causes a characteristic wilting of tomato leaves. It is of interest here because its effect is enhanced tenfold by addition of ferrous ions. Maximum toxicity was obtained by addition of equimolar amounts of ferrous salts to the lycomarasmin before testing on plants (100). It has been shown that the iron chelate must be formed in order for lycomarasmin to act. Copper appears to form a more stable chelate and interferes with the toxicity of lycomarasmin. In a system containing a limited amount of iron, 8-hydroxyquinoline prevents the action of lycomarasmin, presumably by chelating all the iron (239). It seems likely that the iron-lycomarasmin complex may act intracellularly as described for 8-hydroxyquinoline on bacteria, and in contradistinction to the latter's action on fungi (254), for unlike this latter case, excess metal will not reverse the toxicity of lycomarasmin.

It has been reported that the activity of isonicotinic acid hydrazide (INH) against tubercle bacilli is increased tenfold by the presence of copper (211). This was found to be the case for many compounds capable of forming chelates (82, 108, 210). Studies of isonicotinic acid hydrazide have shown (84, 85) that a chelate complex, XXVI, was formed with copper, but its affinity for metals is lower than most amino acids (21) and the significance of these observations remains unclear.

b. Controlled trace metal content. The usual implication in this approach is that uncertain concentrations of heavy metals have been eliminated in the media. It is well adapted to microbiologic and isolated tissue studies, although difficulties multiply when very low metal concentrations are required. Even analytical grade reagent chemicals may add surprising amounts of heavy metals which must be removed (76, 116, 127).

At its technical best, this approach cannot establish chelation as the key reaction involved. However, if a compound known to chelate *in vitro* is found to have an obligatory relation to metal concentration or to a specific metal, it surely implies it as in the observation of Zentmeyer (254) that 8-hydroxyquinoline did not act upon fungi in the presence of zinc.

Numerous *in vitro* studies of the antibiotics and their relation to trace metals have been made. They generally have not been performed on media in which trace metal content was definitely known, or exceedingly low. Perhaps as the result of this omission, the conclusions drawn have sometimes been completely different. Chemical instability and inactivation of penicillin in the presence of heavy metals (56) is difficult to reconcile with the potentiation reported (134, 251), etc. Chloramphenicol is usually reported as unaffected by metal ion variations. Although aureomycin and terramycin form heavy metal chelate complexes (20, 168, 242), specific inactivation by metals in low concentrations, *e.g.*, magnesium (48, 209), manganese (196) makes interpretation of such experiments

difficult. The interesting possibilities in these compounds and other antibiotics in relation to chelation such as aspergillic acid (103), polymyxin (72) streptomycin (96) and the iron chelate, albomycin (46), calls for a careful examination of their actions in the presence of precisely known concentrations of ions. Procedures such as adding extra copper to conventional media merely add confusion, for such media are rich in trace metals.

B. Mechanisms by which a chelating compound may influence cells

1. Sequestration of unwanted metal ions. a. Metal buffers. The buffer systems employed to control pH *in vitro* have frequently served to regulate cation concentration generally. The concept of pM, where M(metal ions) is the equivalent of H^+ has only recently been introduced but it may be expected that separate control of metal ion concentrations will become much more common.

Numerous differences in responses of systems at the same pH have been reported, probably reflecting the metal binding capacity of different hydrogen ion buffers popular in different laboratories. Thus, the use of a phosphate buffer allowed effects quite unlike those obtained by the bicarbonate-carbon dioxide system (27) while a glycylglycine system as an hydrogen ion buffer may be expected to exert a marked effect upon other cations, as well. Glycylglycine provides a convenient pH buffer, while its chelating potency for zinc and cadmium, although known to be less than glycine (75, 171), is still such that quite low effective ionization of heavy metals may be expected. It is, however, susceptible to attack by a metal-activated peptidase present in many crude cell preparations (37). Tris(aminotrishydroxymethylmethane) is a useful hydrogen ion buffer which does not control pM at pH 7 to 8 to any greater degree than ammonia, a degree that is for many purposes negligible, and always preferable to citrates and phosphates (18). Addition of traces (*e.g.*, 10^{-6} M) of EDTA which has been found resistant to enzymic attack (207), to the usual pH buffers seems practical, and has recently been described in detail (55).

A few examples from the literature may serve to emphasize the desirability of pM control along with pH. In a series of studies on sea-urchin spermatozoa, it was observed in 1950 (228) that amino acids or ammonia (a mild complex-forming compound) prolonged the fertilizing capacity of sea-urchin spermatozoa better than proteins and it was suspected that conversion of the amino acids to ammonia was important. Ammonia was credited with a metabolic effect, for it was determined that the amino acids (glycine, alanine, histidine) were not metabolized (229). But by 1953 (30, 230) it had become evident that a variety of chelating and complexing agents exerted this effect simply by sequestration of heavy metals in the sea water media. Had pM control been instituted from the first, much labor would have been saved.

A particularly high activity of histidine or cysteine among the amino acids found to influence a given phenomenon (172) suggests that sequestration by chelation is at the root of the phenomenon (for example, 50, 166, 197). Staehelin (213) duplicated an *in vitro* effect of thyroxine by the otherwise more nearly inert EDTA and such a control should be part of all such type experiments.

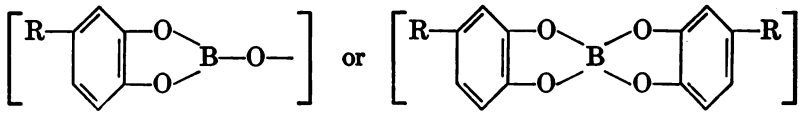
However, caution is necessary, because metal complexes are sometimes more active chemically than their separate constituents. Indeed, acceleration, by a two-phase process, of destruction of glutathione in the presence of EDTA has been described (176) and acceleration of the reaction on some basis occurs in other systems such as ATPase (97, 105, 162). It is evidently not always related to simple sequestration of depressant heavy metals (45).

Although in 1939 it was shown (124) that citrate, oxalate, and particularly malonate in low concentrations increased the Q_{O_2} of succinate-succinic dehydrogenase systems by removal of copper, it is rediscovered regularly. It should also be recalled that phosphate, cyanide, azide, fluoride, etc. form very poorly dissociated heavy metal salts (148), while amino acids will always present an attractive pitfall for the unwary.

In the case of isolated enzyme systems, when chelating agents have been added, their sequestrating action has usually been desired, but occasionally a catalytic effect is noted. Thus, Isaka and Ishida (128) noted that the folic acid copper chelate (see Albert (19) for details of this and similar chelates) accelerated blackening of dihydroxyphenylalanine solutions over either constituent alone.

b. Protection of unstable drugs against destruction. Few drugs are more unstable than epinephrine, which is susceptible to attack through several reactions including oxidation by metals, especially copper, with which it forms a highly oxidizable complex (57).

Chelation between the 3,4-hydroxyls of epinephrine has been shown to occur with boron, XXVII,



XXVII

stabilizing the epinephrine, but not altering its pharmacodynamic potency (227). This may be the mode of attachment in the pharmacologically active ferrous chelate described by Vogeler (237).

Substances potentiating the effects of epinephrine, particularly upon isolated organs, are usually chelating or complexing agents, or actively reduce metal ions to a lower valence state, and the details of such potentiation may most often be attributed to such effects.

Thus, sensitization of pre-capillary sphincters to epinephrine by ethylenediamine tetraacetic acid was ascribed to iron-binding (67). It was shown that small amounts of plasma added to Ringer's solution were effective in potentiating the inotropic response to epinephrine of the isolated papillary muscle of the cat heart. This effect, and that of cysteine, ascorbic acid, cystine and tyrosine were attributed to sequestration of copper which otherwise would have catalyzed destruction of epinephrine, for addition of copper nullified their protective effect (138).

Potentiation, or prolongation of the actions of epinephrine on isolated intestine by "Vitamin P-like" flavonoid structures was shown by Clark and Geissman (58) unequivocally to be the result of chelation of copper by the flavonoids. In addition, 8-hydroxyquinoline and diethyl dithiocarbamate, both powerful copper sequestrants, were active in this system.

Potentiation by trihydroxy-N-methylindole both *in vivo* and *in vitro* (87) has been reported as the result of copper binding by this ligand. Similar potentiation by salicylates might be explicable in a similar fashion (154). However, the classic effects of cocaine and ephedrine do not appear to be explicable in this way.

It would appear that the inhibitory effects of ferrous ions on the action of epinephrine and histamine *in vivo* require more study (107). Still, an explanation derived from the similar blocking effect of ferrous ion on histamine action, that the action of ferrous ion is on the smooth muscle itself, ignores the chelation potentialities of histamine (167, 172).

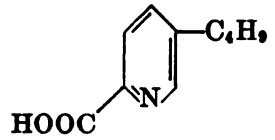
2. Removal of metals from intact organisms. a. Toxic metals. Old clinical observations such as that nickel "itch" (eczema) is reduced by a diet rich in fresh fruits (citric acid) (53), no doubt find their explanation in the formation of excretable chelate complexes. At present, two chief chelating agents are employed for removing toxic metals from mammalian organisms by producing a less toxic chelate. Dimercaprol (2,3-dimercaptopropanol, "BAL") has been considered in detail in a review by Stocken and Thompson (218) and more recently discussed by Kensler (137). Little can be added at this point. Ethylenediamine tetraacetic acid (EDTA) forms chelate complexes with an even wider variety of metals and reference to Table II and Section III will suffice here.

b. Normal metals. It might seem that a persisting nuisance in the use of chelating agents would be unwanted losses of cellular trace metals. Yet it is surprising how little such interference has been noted, perhaps because metals in foods are already firmly chelated, or because *in situ* they are well insulated in the cell structure. Studies of this sort are considered in Section III.

The accessibility of pancreatic islet tissue zinc to complexing agents has already been described (II, A. 2. a.). It may be more sensitive than other body metals for overall losses of body zinc to ethylenediamine tetraacetic acid chelate have been noted in rats (163, 188) and man (187). During therapy of lead poisoning diuresis was noted paralleling the excretion of zinc, and it was suggested that the zinc came from renal carbonic anhydrase (186). Zinc in the tapetum lucidum, the reflecting membrane of carnivore eyes, is particularly accessible to dithizone (244).

Reduction of tissue metal levels by antithyroid substances (q.v.), and by cardiac glycosides (169) are perhaps pertinent. Albinism of plants induced by the antifungal agent, bisthiocarbamylhydrazine, is restored by Cu^{++} (184). Administration of α,α'-dipyridyl, an avid iron chelator, renders guinea-pigs alcaptonuric (excretion of homogentisic acid) when tyrosine is administered in excess. *In vitro*, tissues from these animals show a diminished homogentisicase activity raised by addition of Fe^{++} ions (221).

3. Reaction with fixed intracellular metals. Fusarinic acid (3-butylpyridine-6-



XXVIII

carboxylic acid), XXVIII, has been found to exert an injurious effect on plants because of its ability to chelate with the porphyrin iron (Fe^{+++}) in catalase (224) by increasing the coordination of the iron without removing it from the enzyme. This effect could be reversed or prevented by addition of Fe^{+++}, as shown by plant growth experiments and studies *in vitro* on the properties of purified catalase. Other chelating agents such as 8-hydroxyquinoline and 2-methyl-4-pyridine carboxylic acid exerted similar effects while non-chelating homologs did not. There was no indication that Fe^{+++} or other metals in the various media were required for the action to occur, in contradistinction to lycomarasmin.

It would seem likely that many more such examples will be found. Extension of the use of water-soluble chelating agents as histochemical "stains" should prove interesting (133, 157). The conclusions of White and Schubert (246) regarding the chelation of beryllium *in situ* by aurin tricarboxylic acid constitute a case in point with a toxic metal known to exist intracellularly.

4. Catalysis. Although the literature of organic and biological chemistry is replete with examples of catalytic effects involving chelate complexes, the pharmacological parallels are sparse indeed. Tenuous examples might be the iron: dihydroxy maleic acid synthetic spreading factor described by Daubenmerkl (71) or a cobalt-histidine chelate (226) reported to increase survival of rats at 200 mm. Hg from 7 minutes to 37 minutes by virtue of its ability to function in oxygen transport. (Such O_2-cycling chelate compounds are well known (7).)

5. Tissue ligands and extraneous metals. Suitable metals can form chelates interfering with the normal function of cellular ligands as in the familiar cases of mercury and arsenic. Reaction with *α,β-cis* hydroxyls in many compounds (43) is said to account for the toxic properties of boron (146, 257, 259). Vitamin deficiency has been described which may result from this sort of effect in metal poisonings (126). The hypothyroid effect of cobaltous chloride (144) may be due to reaction with thyroxine. Pernicious anemia refractory to Vitamin B_{12} has resulted during chromium poisoning and it was suggested that substitution of chromium for cobalt in the Vitamin B_{12} occurred (215). The effectiveness of zirconium salts in the treatment of *Rhus toxicodendron* dermatitis is ascribed to chelation of the zirconium by the toxin (69, 219) presumably through the catechol groups present in urushiol, etc. (223).

6. Improvement of absorption. Very large amounts of ferrous chelates, ethylenediamine tetraacetate notably, are employed to add iron to soils, particularly of citrus groves. A greater uptake of iron by the plant from the chelate than from $FeSO_4$ is found. It is probable that the action is concerned with better approximation of soluble iron salts to the absorbing surfaces (217), although actual transport of the chelate is not excluded (240, 243).

A parallel situation is found in the mammalian gut. However, in anemic rats no greater regeneration of hemoglobin followed oral Fe^{+++} EDTA than $FeSO_4$; while intravenously, Fe^{+++} EDTA was less effective than saccharated iron oxide (202). Will and Vilter (247) reported essentially the same negligible difference between Fe^{+++}Na EDTA and $Fe^{++}SO_4$ using radioactive iron to measure the absorption in normal and iron deficient patients. A radioactive copper chelate, copper allyl thioureabenzoic acid sodium salt, was found to be less toxic on intravenous injection and to contribute more copper to the liver, with less to other organs, than copper acetate or copper glycine (115). It was suggested that the complex was destroyed selectively by the liver, while other organs could not arrogate copper from this molecule and so passed it on to the liver.

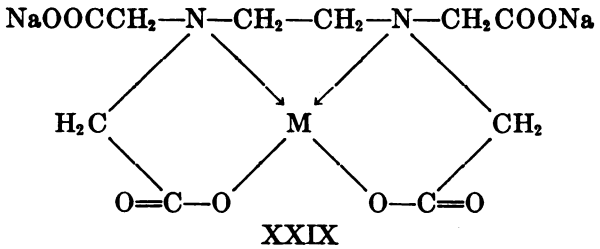
A boron-sucrose chelate may be of importance in the translocation of sucrose in plants (99). This latter phenomenon appears to be the only present illustration of the possible improvement of transport of ligands by making chelates.

Plasma transport of iron, copper and probably other metals is commonly regarded as occurring through mediation of special proteins such as ferritin, caeruloplasmin, etc. Warner (241) has distinguished between certain of these which are coordinations with a single functional group and those which are true chelates.

7. Miscellaneous effects of chelation. When it is evident that the major action of some drug is not exerted by its ability to chelate, it may be possible to explain some side effects of the compound by this ability. Occurrence of diabetes mellitus in tuberculous patients receiving amithiozone (83) may fit into the effects of chelating agents on the pancreas. Dimercaprol (1,2-dimercaptopropanol) induces histamine-like actions. Like isonicotinic acid hydrazide (220), it may inhibit histamine destruction by a copper catalyzed enzyme (49). It is possible that the fatty livers produced by aureomycin may be occasioned by its ability to chelate (48, 256). The mild anemia produced by 1-hydrazinophthalazine therapy of hypertension has been ascribed to its affinity for iron (174).

III. SPECIAL PHARMACOLOGY OF ETHYLENEDIAMINE TETRAACETIC ACID

Many of the current investigations which deliberately make use of a chelating substance take advantage of the singular properties of ethylenediamine tetraacetic acid (EDTA) and its salts. When calcium, magnesium, or the metal ions still more likely to be chelated are present in solution, the water-soluble metal chelate form, XXIX



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may usually be expected to exist. As this is the case under most biological conditions, unless calcium chelation is desired, EDTA is usually introduced as the

calcium disodium salt ($CaNa_2EDTA$) (edathamil N.N.R.), thus avoiding excessive sequestration of calcium.

As might be expected, the sodium salts are appreciably toxic by parenteral routes, and apparently owe most of their acute actions to the sequestration of serum calcium, for injections of calcium chloride counteract the effect (81), especially upon the blood pressure (234). Neither the calcium chelate, nor various heavy metal chelates cause the hypotensive response produced by the sodium salt in normotensive dogs (110, 149), although somewhat dissimilar results were obtained in hypertensive rats (198). The heavy metal chelates are usually of low toxicity *per se* (47), for they are so poorly ionized as to release little toxic metal ion. The therapeutic uses of EDTA are based upon this fact, so extreme as to permit experimental (193, 194) and clinical (195) use of the lead chelate as an x-ray contrast material.

While it might have been hoped that EDTA would be a particularly suitable agent for the control of hypercalcemia (178), Spencer *et al.* (212) found, to the contrary, it would not lower the serum calcium in man and Holland *et al.* (121) likewise found it useless and of some hazard. Certain pathologic changes which were ascribed to the drug occurred in two patients critically ill with hypercalcemia who were treated with sodium ethylenediamine tetraacetate (79). The calcium complex was reported to be a useful calcium source (120), but this was not confirmed (73).

EDTA inhibits the organism often accused of producing dental caries, *Lactobacillus acidophilus*, by sequestration of manganese, essential for its growth (54). However, addition of the free acid to a cariogenic diet in concentrations of 0.2 per cent increased the frequency and severity of smooth surface caries in the rat (216). When added at the 0.5 per cent level (258), the caries was notably worsened and a curious inhibition of the yellow pigmentation of the teeth was noted. As the hematocrit also fell decidedly and both effects could be reversed by addition of extra iron to the diet, their origin was ascribed to sequestration of dietary iron. Decalcification was not held responsible for the increased severity of the carious process. Therefore, the chronic effects of calcium EDTA, as well, should be examined closely in this regard.

Animal studies indicating the low acute toxicity of calcium EDTA were reported in 1952 (32, 234). Acute animal experiments indicate an LD_{50} by most routes of administration of at least 1 gm./kgm. in several species. Chronic administration of calcium EDTA results in an impairment of growth and loss of weight. The minimally hazardous chronic doses require careful determination, for it may be expected to find its way into the human food intake. Following oral administration to man, Cotter (62) found minor changes in plasma trace metal levels, but they did not appear to be reflected in the patients' general status. There is little other information as yet on the possibility of serious losses of essential trace elements occasioned by administration of EDTA. However, mammals appear to extract iron readily from EDTA chelates (see II, B. 6.).

The influence of EDTA upon the actions of other drugs has been but little explored. Calcium EDTA is reported to synergize with barbiturates (38). Ad-

ministration of EDTA as the acid to overdigitalized cats is reported to normalize the cardiac irregularities (192). It was suggested that alteration of the K^+/Ca^{++} ratio may be the *modus operandi*. The increase in toxicity of digitalis tincture to animals pre-treated with salicylates (155) suggests caution with chelating agents in this condition.

It has been demonstrated (95) that C^{14}, carboxyl-labeled calcium EDTA, is less than 0.1 per cent oxidized by rats and is excreted intact in about one hour. The compound is distributed in about 90 per cent of body water and excreted by a mixed process of tubular secretion and glomerular filtration. Like most polybasic acids, it does not penetrate erythrocytes to any extent. In man, it was reported to behave in a similar manner with the added observations that penetration into the cerebro-spinal fluid was slow (93) and the drug was absorbed only to the extent of 5 per cent from the gut and not at all from the skin.

Sequestration and transportation of a variety of metals may be achieved *in vivo* by calcium EDTA to a very effective degree. (Table II.) The most notable example is lead, a number of acute and chronic lead poisoning cases in man, domestic animals and laboratory animals having been effectively treated with calcium EDTA. Clinically, it appears to be clearly the treatment of choice, although Rieders (185) has totaled up a balance sheet on lead-poisoned rabbits and found the same total amounts of lead at the end of the experiment in both the control animals and the EDTA-treated animals. Certainly, Kehoe (136) is justified in warning that use of EDTA for the prophylaxis of lead poisoning must not substitute for the time-proven methods of industrial hygiene. The effect of EDTA in mercurialism is inferior to dimercaprol (135). It is yet to be tried in cadmium or iron poisoning.

The interim expression of opinion by a number of experienced clinical investigators for a program of treatment has been well summarized by Foreman *et al.* (91). The maximum dose should not exceed 0.5 gm. per 30 lb. body weight (13.6 kgm.) per hour, as an intravenous drip of a 3 per cent solution. The maximum dose per day should not exceed 1.0 gm./30 lb. of body weight. Five days would constitute a course of therapy, following which a week should elapse before repetition. (See also Hughes (125).) Subcutaneous clysis is a convenient way to administer the drug in infants and has proven successful (142). It is reported that 1.8 mgm.Pb is excreted for each 500 mgm. calcium EDTA administered (39). Analysis of body fluids for lead in the presence of EDTA requires special procedures (39).

The new appearance of radioactive isotopes of many metals has propounded toxicological problems but it has offered some interesting theoretical therapeutic applications. Different tissues might be exposed to unusual concentrations of radioactive metals, the distribution of the metal isotope being largely controlled by a chelating agent.

Alternatively, isotopes having a longer physical half-life may be employed with more confidence, removing the metal as a chelate when indicated. Thus, although La^{140} deposits appreciably from EDTA, a mass re-exchange with excess calcium EDTA occurs so that 40 per cent of the La^{140} may be withdrawn

TABLE II

Studies in mammals aimed at accelerated excretion or detoxification in vivo of metals by chelating agents

Metal	Chelating Agent	Species	General Practical Result	References
Beryllium	"Aluminon" (aurin tricarboxylic acid)	Rat	+	199, 245
Cadmium	Dimercaprol	Rabbits	−[1]	218
Chromium	Dimercaprol	Several	+	218
	EDTA $CaNa_2$	Man (Chrome ulcers)	+	158
Copper	EDTA $CaNa_2$	Man (Wilson's Disease, normal)	+	255
	"Cuprelone"	Man (Wilson's Disease)	+	161
	Dimercaprol	Man (Wilson's Disease)	+	161, 170
Gallium	Numerous, EDTA	Several	−[2]	77
Gold	Dimercaprol	Man	+	218
Iron	Diethanolnitriloacetic acid	Rat	+	92
	N-hydroxyethylethylenediamine triacetic acid	Man	+	203
	EDTA $CaNa_2$	Man (Hemochromatosis)	+	249
	EDTA $CaNa_2$	Man (normal)	+	86
Lanthanum[140]	EDTA $CaNa_2$	Rat	+	114, 147
Lead	Dimercaprol	Several	−[3]	218
	EDTA $CaNa_2$	Rabbit	+	91, 190
	EDTA $CaNa_2$	Rabbit	−[4]	185
	EDTA $CaNa_2$	Calves	+	122
	EDTA $CaNa_2$	Horse	+	123
	EDTA $CaNa_2$	Child	+	51, 102, 225
	EDTA $CaNa_2$	Child	±	41
	EDTA $CaNa_2$	Man	+	33, 34, 35, 40, 41, 51, 62, 135, 186, 190, 191, 205, 206, 238
Lead Tetra Ethyl	EDTA $CaNa_2$	Man	±	139
Mercury	Dimercaprol	Several	+	218
	EDTA $CaNa_2$	Man	−[2]	35
Nickel	EDTA $CaNa_2$	Rabbit	+	190
	EDTA $CaNa_2$	Man, (Skin)	+	145
Plutonium	EDTA $CaNa_2$	Rat	±	109
	EDTA $CaNa_2$	Rat	+	88, 94
	Diethanolnitriloacetic acid	Rat	+	88
	EDTA $CaNa_2$	Man	+	89, 90
Strontium[89]	EDTA—Na	Rat	−[2]	60, 156
	EDTA—Na	Rabbit	−[2]	233
	EDTA $CaNa_2$			

TABLE II—*Concluded*

Metal	Chelating Agent	Species	General Practical Result	References
Vanadium	EDTA $CaNa_2$	Mouse	+	164
	EDTA $CaNa_2$	Rat, Dog	+	165
Yttrium[91]	EDTA $CaNa_2$	Rat	±	61
	EDTA $CaNa_2$	Rabbit	+	233
Zinc	EDTA $CaNa_2$	Rat	+	163

[1] Toxic compound formed.
[2] Weak chelation *in vitro*.
[3] Distribution of complex *in vivo* is undesirable.
[4] Mechanism unknown.

from biological systems (114, 147). More recently, Y^{90} appears to be more conveniently manipulated (78, 113).

In connection with radioactivity, radiation may release metal ions from harmless combinations to become toxic. Bacq and Herve (29) found EDTA and several other chelating agents to be effective against radiation poisoning.

One might surmise that EDTA would be found useful as an *in vitro* anticoagulant, and it is indeed the case (106, 250, 260). No effect on *in vivo* clotting mechanisms would seem to be expected at reasonable doses, although Popovici *et al.* (177) noted a diminution of platelets when magnesium EDTA was infused to control hypertensive states in man. No specific effect on platelets seems to occur and EDTA is recommended for optimal collection of platelets (214).

Another evident use of sodium EDTA is to dissolve urinary tract calculi and it was found by Raymond and Gehres (182) that a 3 per cent aqueous solution at pH 7.5 was more effective than sodium citrate, 3 per cent at pH 4. Later, Gehres and Raymond (101) used an isotonic 1.5 per cent solution in seven patients, four of whom were successfully treated, although Marcos (159) was less satisfied with his experience. Abeshouse and Weinberg (17) found calcium carbonate stones most soluble *in vitro*, but even cysteine calculi were partially affected, suggesting a calcium or magnesium matrix.

A similar idea suggested the successful use of EDTA in calcific corneal opacities (104). Calcium chelation may be the cause of the softening of intercellular cement by EDTA in embryonic tissue (261) and the deposition of fed cholesterol in rabbit arteries (231). However, Curran (70) has recently described experiments on conversion of C^{14} labeled acetate to cholesterol in the presence of oxine and EDTA. The former chelating agent decreased, and the latter increased, conversion in liver slices. The relations of oxine to manganese and of EDTA to vanadium which were found, as well as actions of other chelating agents, now suggest a far more complex pattern.

The ability to sequester traces of heavy metals which decrease the stability of solutions of drugs has led to the use of traces of EDTA in sodium acetrizoate

N.N.R. solutions, epinephrine, penicillin (222) etc. It may be determined in urine by a spectrophotometric titration with arsenious acid (253).

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