

A SYMPOSIUM ON
COPPER METABOLISM

Edited by

William D. McElroy and Bentley Glass

COPPER METABOLISM

A SYMPOSIUM ON
ANIMAL, PLANT AND SOIL RELATIONSHIPS

sponsored by

THE
MCCOLLUM-PRATT INSTITUTE
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THE JOHNS HOPKINS UNIVERSITY

Edited by

WILLIAM D. MCELROY AND BENTLEY GLASS

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PREFACE

A Symposium on Copper Metabolism was held at Johns Hopkins University under the sponsorship of the McCollum-Pratt Institute on June 14, 15 and 16, 1950. This publication consists of the papers and discussions presented at these meetings.

Copper was selected as the primary topic in order to allow a more thorough coverage of the numerous studies which have been conducted in the fields of soil science, animal and plant nutrition and biochemistry. However, nutritionally the micronutrients cannot always be considered individually. This fact is brought out by several papers in the present symposium. We hope that future symposia will further emphasize the important interrelationships of the micronutrients.

In the selection of speakers an attempt was made to bring together those specialists who, on the basis of their own research, best represented a given area of investigation. In addition to the speakers, a small group of scientists from various fields was asked to participate in the symposium. Thus an opportunity was afforded for a greater exchange of ideas in the various fields of micronutrient research related to copper. It is hoped that the published papers and discussions will be of value to those interested in the general field of micronutrients.

The McCollum-Pratt Institute is indebted to many specialists in the field for their valuable suggestions in planning the Symposium. Thanks are also due to the Scientific Advisory Committee of the Institute for their suggestions and assistance.

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THE FORMATION OF COPPER COMPLEXES

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EVERYONE INTERESTED in micronutrients is aware of the contribution which the analytical chemists have made to the field. By investigating vegetable ash they have established which mineral constituents are present and have given the biologists those leads which it is necessary to follow to determine the influence of these materials on plant nutrition and health. They have then provided means, more or less satisfactory, for following the amounts of the micronutrients in various soils and in isolated portions of plant materials, and have thus provided useful tools for following the distribution of these substances. In addition to these major services, numerous others could be cited.

I believe it to be true that biologists are somewhat less aware of the part which biochemists trained in the techniques of organic reaction mechanisms might play in a field such as that of the micronutrients. This is undoubtedly because most of the contributions which can be made from this field are still in the future instead of being accomplished facts, and many of them will require further exploratory biological experimentation as a necessary background for the initiation of sound research programs. It is my purpose here to attempt to point out some of the contributions which might be made to the field by chemists, choosing the reactions of copper as the focal point of attention for purposes of illustration.

Let us suppose that the analytical chemist has established the presence of a metallic constituent such as copper in the ash of plants. A plant physiologist would then be in a position to experiment with plant diets deficient in this element and to determine the effect of its exclusion upon the metabolism of a large variety of plants. He would then be able to proceed with diagnostic and therapeutic measures, and one practical goal would have been achieved.

To secure a greater degree of control, much more knowledge would be essential. A plant biochemist taking this information would be in a position to search for the active enzymatic constituent of the living tissues in which the metallic element functions. If he were successful in finding a suitable assay method for the enzymatic action in question, he would, in most cases, be able to isolate the active enzyme in pure form. He would then be in a position to study its action in more detail, to learn the nature of materials which poison it, to find what substances it acts upon, to find where it fits into a biological chain of synthesis or degradation, to study the effects of other metals in activating or deactivating it, and in general to provide a wealth of background information for the intelligent handling of substances concerned with its most effective functioning. Thus the stage would be set for the achievement of another practical goal.

It is approximately at this point that the biochemist who leans to the organic chemical approach would be able to start his contributions. If he knew the nature of the substrate for the enzymatic action in question, he would be able to modify this chemically and study the effectiveness of the enzyme in dealing with the modifications. He would thus be in a position to learn something about the shape of the active part of the enzymatic molecule and the nature of its chemical interaction with the substrate. On the side of the enzyme itself, he would be in a position to make structural investigations on the active group, to learn the nature of its combination with the metallic element under investigation, and finally to undertake the synthetic production of model substances to duplicate one or more of the properties of the naturally occurring material. As his understanding of structure, activity, and their interrelationships grew, he could hope to achieve a goal which has so far eluded us, the production of an entirely synthetic material capable of duplicating the action of a naturally occurring enzyme. Having achieved this goal, he would be in a position to modify the activity of his synthetic enzyme model by modifying its structure and to substantiate his hypotheses concerning the relationships between chemical structure and biological activity.

It would be useless to speculate about the degree of control over plant physiology and pathology that knowledge of the type just indicated would be capable of yielding. It is certain that such information would give much more precise control than is now possible over therapeutic measures to be taken on soils. Substances capable of neutralizing the effects of overdosage with metallic elements would become available and compounds of micronutrient elements less prone to produce toxic effects by overdosage would certainly result. This would greatly broaden our knowledge of the field which we might call "soil chemotherapy." Past experience indicates that the most useful applications of this type of knowledge, however, would be those that we cannot now foresee.

In requesting a brief review of the chemistry of copper to provide a background for a biological discussion, the organizer of this symposium assigned a peculiarly difficult task. Our chemical knowledge of the copper-containing materials having biological activity is so meager as to provide the chemist with but the slimmest toehold for a pointed discussion. In this situation, two methods of approach are open. The first is descriptive. If this were adopted, a broad survey of the chemistry of copper complexes could be given, in the hope that it would later be discovered that the biologically active compounds resemble some known compound to a sufficient degree to permit attention to be withdrawn from the 999 substances that did not fit and be focused on the one that did. It seems to me that even this long chance would probably fail. For example, the hemocyanins are copper compounds which are capable of combining reversibly with oxygen. Our known copper complexes do not have this power. The chances of finding a known compound without this power whose chemistry could serve as a model for that of the hemocyanins seems remote. Because of the unfavorable nature of this approach, I shall not adopt it.

The second method of approach is mechanistic. We may study the forces at work in various types of chemical combination and learn something of how their interactions are capable of yielding varied results under varied circumstances. While we may not be assured that all the forces which are at work in the biologically

interesting cases have yet been catalogued, we may have complete assurance that some of the forces at work are now understood, and we may see how their combinations and extensions could lead to the production of new materials with unique properties.

The field of complex compounds was first exploited systematically by A. Werner (10), over fifty years ago. Pioneering studies in the field were also made by P. Pfeiffer (6). Numerous modern investigators have contributed to our understanding of the forces which determine the phenomena of the chemistry of complex reactions. Parts of this work will be reviewed.

The first force which we shall discuss may be called "polarity" because of the fact that the term is familiar to biologists. The polarity of the chemist is fundamentally related to the positive and negative electrostatic charges of the physicist and is responsible for the familiar phenomenon of the periodicity of the elements, as well as for the somewhat less familiar phenomenon of atomic size. We shall be interested in both of these phenomena in connection with our investigation of the chemistry of copper.

If we imagine a central positively charged particle attracting a group of negatively charged particles which are constrained from colliding with the positive nucleus, the resultant figure will have a shape determined by the resultant of the attractive force of the nucleus and the respective repelling forces of the negatively charged particles. A two-dimensional model of such a system was investigated by R. W. Wood (11) in 1898. The interesting feature of this model is that regular polygons are formed, up to a certain point. As the rings surrounding the central nucleus expand, however, the negatively charged particles tend to recede too far from the center for stability to be maintained. At this point one of them wanders into the center, and the remainder start to build a smaller ring about it. The building process can then be repeated until a third period is formed, and so on. From this model one learns that the resultants of these forces of opposite polarity are not rings of increasing size but instead a series of rings of differing numbers of constituents, resembling in this respect the rows of the periodic table.

The radius of the outer ring in any case will be determined by the magnitude of the oppositely polarized forces at work. In the case of the chemical elements in the periodic table, the addition of each valence electron is accompanied by the addition of a single positive charge on the nucleus. Thus the central force increases as we move up the periodic table. The effect of this on the atomic radius is most readily grasped by reference to Pauling's so-called "isoelectronic series" (4), that is, the series across the periodic table in which the number of outer electrons is maintained constant in each row while the nuclear charge increases, thus systematically increasing the overall charge on the resulting ion. It will readily be seen that the size of the resulting ion will diminish systematically along each row. For example, in the first row the values calculated are Li^+ , 0.60; Be^{++} , 0.31; B^+ , 0.20; C^{++} , 0.15; N^{++} , 0.11; O^+ , 0.09; F^{++} , 0.07.

The sizes to be expected when the normal complement of electrons is permitted to remain on each atom are not so readily apparent. For our present purposes it is sufficient to know that the nature and magnitude of the forces of attraction and repulsion at work are such that the forces of attraction predominate increasingly as one proceeds along a row of the periodic table, so that each succeeding element has a smaller radius than the preceding one. Thus in the series C, N, O, F, the covalent radii in Angstrom units are: C, 0.77; N, 0.70; O, 0.66; F, 0.64.

Each successive ring of electrons that is added to the atom increases its radius, as would be anticipated. Thus the atomic radius increases as one goes down a column of the periodic table. For instance, in the halogen family the radii as one goes down the column are: F, 0.64; Cl, 0.99; Br, 1.14; I, 1.33.

These relationships can be combined to give a general, rough relationship which is useful, namely, that atomic radius increases in the periodic table when one proceeds from upper right to lower left.

Atomic radius by itself has an important bearing on the formation of complexes. If we have a small central atom and wish to place around it a number of large atoms, it immediately becomes

apparent that the number that can be introduced is limited by the size of the outer atoms. If too many large atoms were introduced, they would tend to overlap and crowd each other away from the center. This principle was recognized by Magnus (3). Limiting radius ratios can be calculated by inscribing outer spheres of suitable diameters tangent to the central coordinating sphere at the proper angles and finding the interferences graphically (9). For symmetrical distribution of three groups around a central atom we find a radius ratio of 0.155. This means that if the outer spheres are approximately six times as large as the central sphere three of them can just be fitted on to the surface. If they are larger than this, only two would fit. For symmetrical distribution of four groups, the limiting radius ratio is 0.225. Roughly, this means that outer spheres must be smaller than four times the radius of the central sphere to permit four of them to cluster in it. For symmetrical distribution of six groups, the radius ratio figure is 0.414, meaning that spheres only two and a half times as large as the central sphere can be accommodated in this arrangement. For eight atoms around a central sphere, the limiting radius ratio is 0.732, meaning that in this distribution the outer spheres can only be approximately one and a third times as large as the central sphere to be accommodated. Thus we see that the common sense rule which we would expect is followed. The larger the groups you wish to place next to the central atom, the fewer such groups can be accommodated.

Stripped of its valence electrons, copper in the form of its cuprous ion has a radius of 0.96 Å. With its bonding electrons in place, the cuprous atom has a radius of 1.35 Å. Each of these figures represents an atom of moderate size. This size turns out to be sufficient, in comparison with the largest in the periodic table, that the radius ratio effect would never limit the number of atoms that could be placed around copper to a number below four, and only in extreme instances would any limitation be placed on having six groups around the copper. Since the ordinary coordination number of copper is four and only few compounds with higher coordination are known, this means that copper is large enough to coordinate about itself as many atoms of as large size as its other properties

will permit. This gives us an important element of freedom in dealing with copper complexes.

The next important consideration with respect to coordination complexes is the overall shape of the complex. An important factor in determining this shape is the included angle between the bonds of the central complexing atom. In general, it may be stated that the bonds, being made up of electrons which have a like charge, tend to repel each other and to assume positions in space as far apart as possible. The exceptions to this statement will appear as we proceed (8a).

If we have a central atom with only two atoms linked to it, the included angle between the bonds will tend to be 180° . It is probable that most of the copper complexes with a coordination number of two, such as the diammines, have this structure.

The next common coordination number is four. The arrangement which permits greatest separation of four groups on a spherical surface is the tetrahedral, with included angles of approximately 109.5° . This is the bond angle found in carbon compounds, such as methane, with four substituent atoms on the central atom. It is also the arrangement in the cuprous complexes, which have an electronic structure essentially similar to that of the carbon compounds.

An interesting case is that of ammonia. This molecule might conceivably be arranged with all four atoms in the same plane, or it might be pyramidal, with the three hydrogens in one plane and the nitrogen in another. In the latter case the molecule could be regarded as a truncated tetrahedron, or as a tetrahedron in which the top point is occupied by an electron pair instead of by another atom. In fact, it is found that the latter case is the correct one—ammonia is pyramidal.

Another interesting case is that of water. At first it might appear that the bond angles should be 180° . If we consider water to be similar to the case of ammonia, however, we soon perceive that it also should be a truncated tetrahedron with points occupied by electron pairs and two by hydrogen atoms. Again this is the structure found. From these cases we may conclude that electron pairs

should be taken into account in deciding on bond angles much as if the positions they occupy were occupied instead by atoms.

Another case that we need to consider is that in which six atoms surround the central atom. In this case again, the bonds will seek the positions in space which permit them to be farthest apart. This turns out to be the octahedral arrangement (Fig. 1). If a sphere is placed at the center of a regular octahedron and bonds are

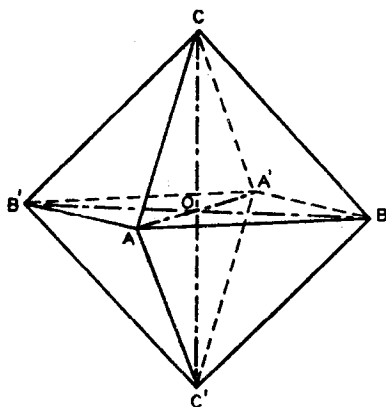


Fig. 1. Octahedral Arrangement of Atoms as in Cupric Ammine Complexes.

drawn from it to each of the six corners of the octahedron, it will be found that the included angles between bonds are all 90° . This is the case in the well-known ferro- and ferri-cyanide complexes. This bond type also appears in the 6-covalent cupric ammine complexes.

The last case which we shall consider is that of the 4-covalent cupric complexes. On examination it will be found that this bond type bears closer resemblance to the ferrocyanides than it does to the cuprous complexes. It has a relationship to the ferrocyanides similar to the relationship between methane and water. If we look upon the cupric ammonia complex as an octahedron truncated to a plane, with an electron pair in the top position and a single electron at the bottom, we have an arrangement in which the central atom is at the center of a square and the included angles between the

bonds are 90° . This is the arrangement actually found in the cupric complexes.

We are now in a position to combine two of the pieces of information which we have obtained. If we consider a five-membered ring in which each of the atoms is carbon, with a normal bond angle of 109.5° , we see that there is very little strain in bending all the bonds to the angle of 108° required to form a regular pentagon. If we now substitute a much larger atom, such as copper, for one of the carbons, the effect would be to open up the ring if the bond angles were left unaltered. However, in the case

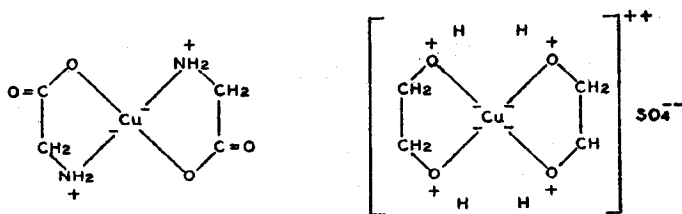


Fig. 2. 90° Cupric Bond Angles in Five-Membered Rings.

Left, Copper glycinate. Right, Ethylene glycol.

of the cupric complex, the bond angles are altered and in precisely the way required to bring the bonds back toward their original positions. The 90° cupric bond angle almost exactly compensates for the extra size of the copper atom, with the result that the five-membered ring is again extraordinarily stable. This is illustrated by the formation of copper glycinate and the copper complex of ethylene glycol (Fig. 2).

We have seen how the interactions of polar forces account for the periodicity of the elements, their sizes, and their bond angles. We shall now examine the effect of polarity as one of the factors influencing the strength of chemical binding and the mutual affinities of atoms in chemical compounds.

It will be recalled that the increasing attractive forces exerted by the nucleus as we go across the periodic table from left to right result in steadily closer approach of the electrons to the nucleus and

diminishing atomic radius. This information is of immediate use in the study of chemical affinities.

In biological systems the elements whose affinities for copper will be of greatest interest are nitrogen, oxygen, and sulfur. Let us first consider their affinities for copper in air, that is, in the dry state. We may consider each bond between one of these elements and copper as a tripole with the positive nucleus of the element under consideration at one end, the negative electrons in the middle and the positive nucleus of the copper at the other end. If we now imagine a process by which the positive charge of the first nucleus is increased, it will be seen that the attraction for the electrons will be increased and the repulsion for the copper nucleus will not be increased nearly so much, since it operates at a much greater distance. The net effect of this increase of charge on the nucleus of the element bound to copper will be to increase the strength of binding. This effect is that which is actually observed in going from nitrogen to oxygen. In the dry state, nitrogen has only moderate affinity for copper and oxygen has much, in line with the greater attracting power of the oxygen nucleus.

Let us consider the same process taking place in a column of the periodic table, moving from top to bottom. In this case, it is true that the nuclear charge has increased. However, because of the interposition of extra rings of electrons, the distance from center to circumference has also increased, and the outer electrons are screened from the effect of the nucleus by the inner electrons. The combined effect of distance and screening overbalances the effect of the increased attraction of the nucleus, with the result that electron-attracting power diminishes as we go from top to bottom in the table. We may thus formulate a rough guide to electron-attracting power which will usually work going directly across the periodic table or directly up and down but will not necessarily work when moving diagonally across the table. This rule is that the larger the atomic radius the less the attracting power of the nucleus for valence electrons. In our particular case, this means that the affinity of sulfur for copper, in the dry state, should be less than that of oxygen. This is also found to be true

(1, 2). In general, this relationship may be expected to hold whenever a medium of very low dielectric constant is substituted for air. A biological example would be fat, which has a very low dielectric constant.

When we change to aqueous solutions, these affinity relationships undergo a profound modification. This may be largely charged to the tremendous increase in dielectric constant accompanying the change in medium. The effect of the increase in dielectric constant is to make it progressively easier to separate ions. In air, ions tend to stick together. In water, they tend to move apart. Hence, in water, increasing polarity results in increasing ionization and therefore in diminished affinity, in contrast to the case in air.

A simple illustration of this effect may be found in the formation of the copper-ammonia complexes. Because of the strong electron-attracting power of the oxygen nucleus H_3O^+ is a strong acid, it being the main species responsible for the acidity of water solutions of mineral acids. In losing its proton and thus ionizing, the oxygen of H_3O^+ exerts its electron-attracting power in such a manner as to monopolize an electron pair. In this case, then, electron-attracting power no longer parallels bond strength. In the case of the ammonium ion, the attraction of the nitrogen for its electrons is less than that of oxygen in the hydronium ion, with the result that the ammonium ion does not part with its hydrogen as readily and is thus a weaker acid than the hydronium ion. This is shown by the well-known fact that ammonium chloride is a weaker acid than a water solution of hydrochloric acid. The converse argument is also true. Because of its lesser affinity for the hydrogen ion, just demonstrated, water is a weaker base than ammonia.

Let us apply this information to the case of copper. Just as the stronger base, ammonia, has greater affinity for the positively charged hydrogen ion, so it has greater affinity for the positively charged copper ion. In water solution, then, competition between H_2O and NH_3 for copper will result favorably to NH_3 , with ammine formation taking place (8b). As acidity is increased, however, NH_3 is converted to NH_4^+ long before conversion of H_2O to H_3O^+ becomes appreciable. Because the systems under considera-