ACTIVE IRON. I

THE STRUCTURE OF WERNER COMPLEXES OF METALS OF THE IRON FAMILY IN RELATION TO THE CHEMICAL, PHYSICAL, AND BIOLOGICAL BEHAVIOR OF THEIR CENTRAL METAL ATOMS

OSKAR BAUDISCH AND LARS A. WELO

Sterling Chemistry and Sloane Physics Laboratories, Yale University, New Haven, Connecticut

Gestaltung, Umgestaltung,
Des ewigen Sinnes ewige Unterhaltung.—Faust II. Teil.
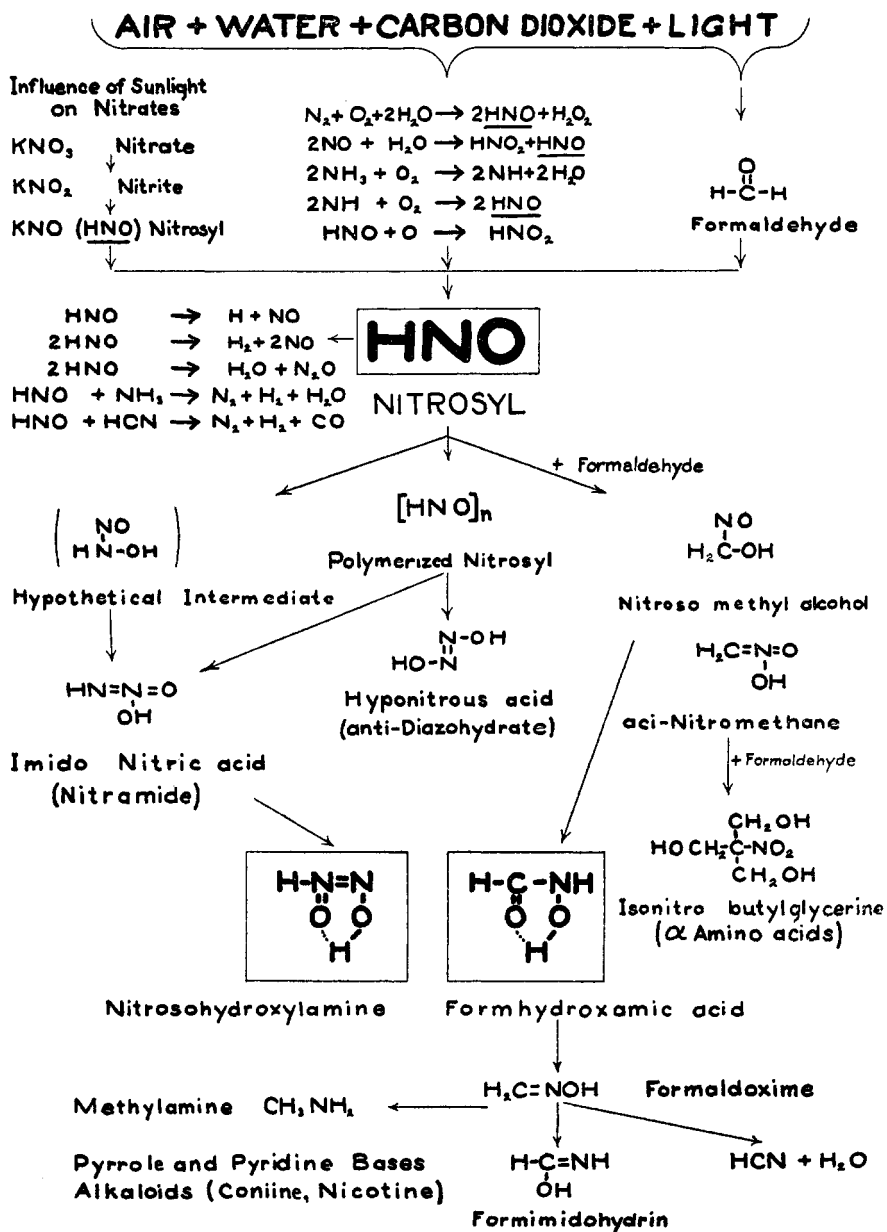
I. INTRODUCTION

In the present review we shall include the work carried out to investigate in detail the Werner complex salts (84) with organic addenda and a central metal ion, especially the so-called inner complex salts (11). The compounds and complex-forming groups which we have used for this purpose were not chosen freely but were retained from the study of fundamental photochemical processes, which were carried out to synthesize organic compounds from inorganic compounds by photochemical methods. In this, we were especially interested in the nitrogen cycle; but the simplest inorganic compounds which are concerned in the building up of organic matter—nitrogen, ammonia, and nitrates—were chosen. As the source of carbon we used formaldehyde chiefly, without consideration of the question of its photochemical formation from carbon dioxide.

In table 1 the results of our photochemical investigations are summarized.[1] Nitrosyl, HNO, functions as the most important compound, as far as the nitrogen in the synthesis of simple carbon- and nitrogen-containing materials is concerned. It acts as a branching-point, leading to several series of compounds. The most important organic compound synthesized by light, which we have isolated and analyzed as an iron and copper complex copper salt, is formhydroxamic acid. From it by the action of light we obtained its labile isomer, acinitromethane, as well as formaldoxime. Formaldoxime in light undergoes a Beckmann rearrangement and gives formimidohydrin or formamide. We note in the table the

[1] A separate bibliography of the publications on which this table is based appears at the end of this article.

1

TABLE 1

Formation of complex-forming groups and of organic compounds from inorganic by the influence of light

AIR + WATER + CARBON DIOXIDE + LIGHT

Influence of Sunlight on Nitrates

KNO_3　Nitrate

KNO_2　Nitrite

KNO (HNO) Nitrosyl

$$N_2 + O_2 + 2H_2O \rightarrow 2HNO + H_2O_2$$
$$2NO + H_2O \rightarrow HNO_2 + HNO$$
$$2NH_3 + O_2 \rightarrow 2NH + 2H_2O$$
$$2NH + O_2 \rightarrow 2HNO$$
$$HNO + O \rightarrow HNO_2$$

$$H-\overset{\overset{O}{\|}}{C}-H$$

Formaldehyde

$$HNO \rightarrow H + NO$$
$$2HNO \rightarrow H_2 + 2NO$$
$$2HNO \rightarrow H_2O + N_2O$$
$$HNO + NH_3 \rightarrow N_2 + H_2 + H_2O$$
$$HNO + HCN \rightarrow N_2 + H_2 + CO$$

HNO

NITROSYL

+ Formaldehyde

$$\left(\begin{matrix} NO \\ H\overset{\cdot\cdot}{N}-OH \end{matrix} \right)$$

Hypothetical Intermediate

$[HNO]_n$

Polymerized Nitrosyl

$$\begin{matrix} NO \\ H_2C-OH \end{matrix}$$

Nitroso methyl alcohol

$$\begin{matrix} HN=N=O \\ OH \end{matrix}$$

Imido Nitric acid
(Nitramide)

$$\begin{matrix} N-OH \\ \| \\ HO-N \end{matrix}$$

Hyponitrous acid
(anti-Diazohydrate)

$$\begin{matrix} H_2C=N=O \\ OH \end{matrix}$$

aci-Nitromethane

+ Formaldehyde

$$\begin{matrix} CH_2OH \\ HOCH_2-C-NO_2 \\ CH_2OH \end{matrix}$$

Isonitro butylglycerine
(α Amino acids)

$$\begin{matrix} H-N=N \\ \overset{\cdot\cdot}{O}\ \ \overset{\cdot\cdot}{O} \\ H \end{matrix}$$

Nitrosohydroxylamine

$$\begin{matrix} H-C-NH \\ \overset{\cdot\cdot}{O}\ \ \overset{\cdot\cdot}{O} \\ H \end{matrix}$$

Formhydroxamic acid

Methylamine CH_3NH_2

Pyrrole and Pyridine Bases
Alkaloids (Coniine, Nicotine)

$H_2C=NOH$　Formaldoxime

$$\begin{matrix} H-C=NH \\ OH \end{matrix}$$

Formimidohydrin

$HCN + H_2O$

following characteristic atom groupings synthesized by light: NO, HNO,

$$-N=N-,\quad -\overset{\overset{O}{\|}}{N}=N-,\quad -C(OH)=NOH,\quad =C=NH,\quad -\overset{\overset{H}{|}}{C}=N-,\quad HCN.$$
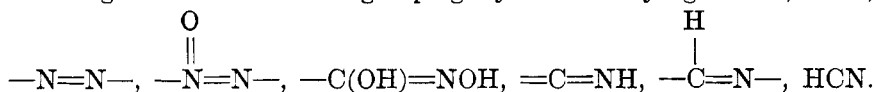
We obtained compounds and atom groups all of which are capable of complex formation with iron and copper, and of which a great number of characteristic iron and copper complex salts have been described in the literature of organic chemistry (72, 80). This fact led us to include in our investigations the metal complex salts, especially the iron and copper salts, of these characteristic complex-forming groups. It is of especial significance that simply by exposing mixtures of alkali nitrites and formaldehyde to sunlight, or by irradiation of aldoximes in the presence of formaldehyde, highly polymerized, dark colored, syrupy substances were obtained. The presence of the pyrrole and also the pyridine ring was established with certainty by characteristic color reactions and by the isolation of nicotine. This fact is of interest from the point of view of plant physiology. The great affinity of the pyridine ring, and in general of nearly all nitrogen-containing rings for the iron atom, is not, we believe, conditioned only by the dipole nature of the pyridine, but is to be attributed chiefly to the special chemical and physical character of nitrogen linked in a ring. We shall later describe experiments dealing with this point.

The particular affinity of nitrogen compounds for iron, especially of the nitrogen–oxygen compounds, is noteworthy even in the simplest nitroso compound, nitric oxide, NO. All organic nitroso compounds, furthermore, the hydroxylamines and the oximes, show characteristic affinity for iron, which we will treat in detail in this review. In compounds of nitrogen with carbon, e.g., in hydrocyanic acid and in complex organic compounds which contain a $=C=NH$, or a $-CH=N-$ group, the selective affinity for divalent iron especially is exceptionally notable. We were able by this means to split, by oxidation, members of the purine, pyrimidine, and hydantoin series (50).

Early in our investigations we emphasized the fact that organic addenda which are drawn into the inner sphere of a metal atom are activated and thereby become more easily available for oxidative cleavage or chemical replacement (14, 15, 21, 64, 65). The linkage to the central metal ion—in the special cases considered here, to the iron ion or atom—is dependent on the chemical composition of the linked organic compounds. The central metal ions together with the addenda form "complex compounds" which possess a characteristic geometric crystal structure. The atoms or molecules attracted into the sphere of the central atom exert a so-called "field force," and substances with similar fields may replace them isomorphously in the crystal lattice, i.e., such substances are "perceived" as

identical by the growing crystal. The field similarity depends essentially
on the number and the arrangement of the outer electrons in the atoms or
molecules. In trying to trace the appearance of isomorphism in a broader
sense back to a fundamental lattice constant, Langmuir (55, 56; see also
62) was the first to apply atomic theory to the problem. Langmuir
expressed the thought that the similarity of the crystallographic ratios in a
series of chemically unrelated substances, to which Barker (7; also 44) had
first called attention, depended on *isosterism*. This means that in the unit
cells of the substances compared, the outer electrons must be the same in
number and arrangement.

It is of particular interest that the compounds and atom groupings
synthesized by us, using light energy, form such isosteric groups, as may be
seen in the following:

$$\begin{array}{lll}
O{=}O & -N{=}N- & C{=}NH \\
HN{=}O & -N{=}CH- & C{=}O \\
H_2C{=}O & -CH{=}CH- & C{=}CH_2
\end{array}$$
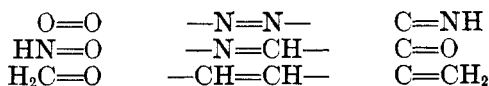
TABLE 2

Diamagnetic susceptibility values for different atomic groups

GROUPS	DIAMAGNETIC SUSCEPTIBILITY VALUES
O= (aldehyde)	+17.5
O= (nitroso)	+79.5
C=C	+54.5
N=N—	+18.5
C=N—	+81.5

Furthermore, it is interesting that the groups given above show a para-
magnetic behavior[2] and thereby reveal a certain unsaturated condition in
the molecule (table 2, from Pascal (63)). That there is also in the metal
complex salts a particular state of affairs is shown by the magnetic meas-
urements of metal salts, which have been extensively investigated by
Welo (81).

The following types of complexes can be distinguished:

(1) Those which show the magnetism of the central ion. Examples are
benzohydroxamic acid iron salt and the iron salt of nitrosophenyl-
hydroxylamine (cupferron iron). These point to the concept that
between the central ion and the addendum only ions or dipoles are
attracted. The symmetry relations which determine the magnetism
are not disturbed here, and one may suppose that no inner fusion of the
electron structure of the addenda with the central ion has taken place.

[2] See however, E. C. Stoner, Magnetism and Atomic Structure, p. 36, E. P. Dut-
ton and Co., for another interpretation of this effect.

(2) Those complexes which show a fundamentally different magnetism than that of the central atom or ion. Here it is presumably the case that a part of the electron structure of the addendum is attracted so close to the central ion that a complete rearrangement has occurred.

The complexes of the first class were called by W. Biltz (33) "normal;" those of the second class were called by P. Ray (69) "(penetration) complexes." Table 3, by W. Klemm, H. Jacobi, and W. Tilk (52), shows that there is here a sharp distinction. The normal complexes possess the magnetic moment of the Fe^{++} or Fe^{+++} ions; the penetration complexes are in part diamagnetic and in part are paramagnetic to be sure, but their

TABLE 3

Weiss magneton numbers for some complex compounds of iron (to nearest unit)

Fe^{++} IONS	MAGNETON NUMBERS	Fe^{+++} IONS	MAGNETON NUMBERS
A. Normal complexes			
$[Fe(H_2O)_4]Cl_2$	26	$Na_3[FeF_6]$	29
$[Fe(H_2O)_6]SO_4 \cdot H_2O$	26	$Na_3[Fe(SO_4)_3]$	29
$FeCl_2$	27	$Fe_2(SO_4)_3$	29
$FeSO_4$	26	$FePO_4$	28
B. Penetration complexes*			
$K_4[Fe(NC)_6]$	0	$[Fe(CO)_5]$	0
$K_3[Fe(NC)_6]$	12	$[Fe_2(CO)_9]$	0
$Na_3[Fe(NC)_5 \cdot NH_3]$	0	$[Fe_3(CO)_{12}]$	0
$Na_3[Fe(NC)_5NO]$	0	$[Fe(dipyridyl)_3]Br_2$	0
$Na_3[Fe(NC)_5NO_2]$	11	$Na_3Fe(NC)_5SO_3 + 8.8 H_2O$ . . .	0

* To differentiate between the valences in these compounds would have no meaning.

magnetic moments are very much smaller than those of the simple ions. Therefore, in this case, one can with certainty assign a substance to one or the other of the two classes on the basis of the magnetic measurements. It is therefore shown by this table that the magnetic behavior of the complex is the most definite criterion for the differentiation of the two different types of complexes.

According to W. Biltz (34; also 67), the theory of the diamagnetic cobalt salts requires a close linking of the nitrogen with the central atom, exhibited by the sharing of two of the nitrogen electrons by the two atoms, similarly to the non-polar bond between carbon and its addenda which we assume to exist in organic substances. X-ray study of luteo iodide yields a direct measurement of the shortest cobalt-nitrogen linkage. As a consequence

of this diminution of the cobalt-nitrogen distance, there is a possibility of assigning a smaller space to the ammonia molecule. This possibility is realized in the luteo salts, all of which have equal cobalt-nitrogen spacing. Chemical results are in agreement with this structural concept, which is equivalent to that of organic molecules.

Exactly as in the case of cobalt, the complex compounds of iron either retain the moments of the Fe^{++} or Fe^{+++} ions—in which case the central ion and the addendum remain apart—or they undergo a profound change in which the iron and the added constituent couple to form a single system. There are, for example, complex iron compounds which are more weakly paramagnetic the firmer the complex bond of the iron, in other words, the higher the degree of complexity. According to a theory introduced by Welo and Baudisch (82), it is assumed that the paramagnetism, which is due to unpaired electrons of the central atom, is reduced by the linking on of complex addenda so that new electron pairs are formed with consequent lowering of the number of unpaired electrons. Ladenburg, like Klemm (53) and his coworkers, assumes that, by the linking of the unpaired outer electrons of the iron with electrons of the addenda, an "intermediate layer" is produced about the central atom, and that diamagnetism is obtained whenever this intermediate layer contains sixteen or eighteen electrons, the latter being a rare gas configuration.

According to experiments of Cambi and Szegö (38), the entrance of electrons into this intermediate layer, the third level of iron, occurs only with addenda with weak dipole properties. On the other hand, addenda with strong dipole properties are deformed by the central ion without leading to the formation of the intermediate layer. One finds, therefore, in this class of complexes the same paramagnetic moments as in the simple ionic compounds of iron.

We have found that iron compounds which are chemically very complex—i.e., form a stable complex, as for example the iron cupferron salt— retain the high paramagnetism of the Fe^{+++} ion. Iron benzohydroxamate behaves similarly. In this case the chemical behavior contradicts the magnetic behavior. In this connection, interesting and important work has been done recently by Felix Haurowitz and Hubert Kittel (46; see also 19), who have investigated the magnetic properties of some hemoglobin derivatives. From the magnetic properties of the three hemins investigated, it is demonstrated that here also the iron atom has retained the high paramagnetism of the Fe^{+++} ion in combination in the molecule and that, therefore, no stable complex linkage in the sense of the previously discussed observations of Ladenburg, Klemm, and Cambi has been formed. Chemically, the iron in hemin is very firmly bound, for hemins can be treated with boiling hydrochloric acid or alkalies without removal of the iron. Hauro-

witz and Kittel consider that the iron in hemins is indeed linked by only weak forces, but that it is protected against the dissociating action of water and the influence of acids and alkalies by the characteristic structure of the porphyrin nucleus.

Our work is concerned with the clearing up of the special type of linkage of metals, particularly iron and copper, with organic nitrogen-containing addenda and we have sought to relate the chemical and biological functions of these metals with their special complex linkage and with their magnetic properties. With this in view, Welo (81) has measured magnetically a great number of complex metal salts.

For our purpose, we have used especially nitroso and oxime compounds, since it is known that the NO and HNO groups have a great influence on the magnetic susceptibility of iron ions, and since the manner of linkage of the HNO or of the oxime group to the metal appears to be determining for its complex-chemical behavior. The following simple metal compounds with the $=$NOH group were found:

$$\overset{\displaystyle O}{\underset{\displaystyle N-Me}{\overset{\displaystyle \|}{}}}$$

$=$N$\cdot$OMe   N—Me   Me . . . .N$\cdot$OH
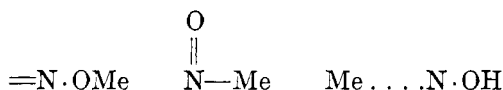
It is of determining significance for the chemical and also for the magnetic character of the oxime complex, whether the metal is linked by primary valence to *oxygen* or to *nitrogen*. Moreover, linkage through secondary valences to nitrogen of the . . . . NOH group is quite characteristic of certain complexes. However, there can also be combinations of all of these types of linkage, and in such cases it is difficult to determine the fine structure of such complexes. The character of the metal (8) itself, i.e., its position in the periodic system, is also very important in governing the chemical and physical properties of its complexes, as we shall see later.

II. GENERAL DISCUSSION OF COÖRDINATION VALENCY AND COÖRDINATION COMPOUNDS

Werner (84) developed the concept of coördination valency as applied to compounds which are formed by addition of ammonia to certain heavy metal salts, the metal ammonia compounds. The stability of the ammonia complexes was thoroughly studied by Ephraim (42) and by W. Biltz (32) and collaborators. Their results showed that the stability of these compounds was increased as the charge on the central ion increased and as its size decreased. The same rules apply to the linking of water of crystallization; with the increase in size of the cation the tendency for the formation of hydrates diminishes. Thus calcium chloride crystallizes with six, and barium chloride crystallizes with two, molecules of water.

Elements do not possess the ability to exert coördination valence in all compounds. Thus, for example, nitrogen can develop a coördination valence in ammonia and all of its derivatives but not in ammonium compounds. The nitrogen of nitrites exerts coördination valence toward iron, but that of nitrates does not.

Nitric oxide (NO) exerts coördination valence toward iron, e.g., in the compound[3]

$$[Fe^{II}(NO)]SO_4$$

Those compounds are decidedly more stable in which the NO is linked to a metal atom along with other types of neutral fragments, such as NH_3 or CN:

$$\left[Co^{II} {NO \atop (NH_3)_5}\right] X_2 \quad \text{and} \quad \left[Fe^{II} {NO \atop (NC)_5}\right] Na_2$$
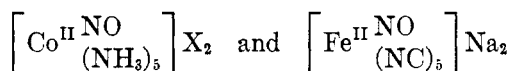
Oxygen and also sulfur can exert coördination valence in certain compounds (metal oxides, ketones, aldehydes, carbonyl oxygen atoms, thiourea, etc.).

According to the Werner theory, coördination compounds can be divided into three classes.

The *first class* consists of those compounds which arise by coördinative linkage to atoms, the principal valences of which are partially or completely saturated. The first group of this class is made up of those in which the union has taken place between two or more molecules by coördination valences, e.g.:

$$[Cl_2Pt\text{---}(PCl_3)_2]$$

In this compound the phosphorus is so firmly bound by coördination valences to the platinum that water will hydrolyze the chlorine atoms and form platinum dichloro-diphosphorous acid.

Polynuclear compounds, which play a part in the aging of metal hydrates, are formed among other ways by coördinative linking to the oxygen atoms of hydroxyl groups, e.g.:

$$\left[(H_3N)_4Co^{III} {\diagup O \diagup \atop \diagdown O \diagdown} Co^{III}(NH_3)_4 \right] (SO_4)_2$$

$$\begin{array}{c} H \\ O \end{array}$$
$$\begin{array}{c} O \\ H \end{array}$$
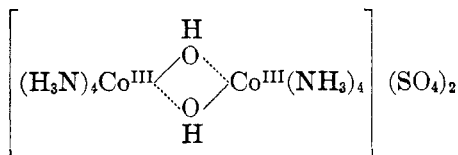
Octammino-μ-diol-dicobaltisulfate

[3] NO as a monovalent positive radical is called nitrosyl, while as a neutral fragment, as in the above compound, it is called the nitroso group.

Weinland (80) has prepared a great number of metal salts of organic acids, e.g., the deep red ferric acetate is the acetate of a polynuclear tri-ferri-hexaacetato-dihydroxo cation with the formula

$$\left[Fe_3 \frac{(CH_3COO)_6}{(OH)_2} \right] CH_3COO + 1H_2O$$
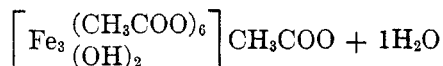
The linkage of the iron atom takes place through coördination valence which is located on the carbonyl oxygen atom of the carbonyl group.

$$Fe^{III}\!-\!O\!-\!\underset{\underset{CH_3}{|}}{C}\!=\!O \ldots\ldots\ldots Fe^{III}\!-$$
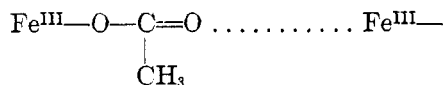
Welo (81) has measured a large series of the Weinland salts magnetically and found that in almost every case they show the normal magnetic moment of the Fe^{+++} ion.

The second group is made up of the compounds characterized by the operation within a single molecule of both principal and subsidiary valences. The salts of this type belong to the *inner complex salts* discussed earlier. An example is:

Ferri-acetylacetonate   Acetylacetone
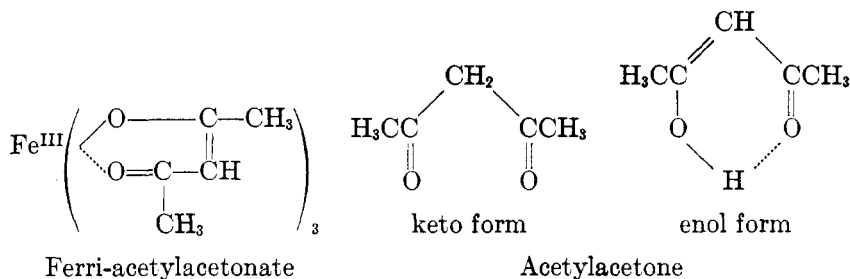
This iron compound represents chemically a highly complex compound, in which the properties of the ferric ion are completely masked. However Welo's measurement of it shows it to be derived from the iron ionogen in a magnetic sense, for it shows 29 Weiss magnetons, as does the iron in ordinary ionogenic salts.

The blood pigment and chlorophyll belong to the inner complexes. It is interesting physiologically that the iron in hemin, according to the most recent magnetic measurements of Haurowitz and Kittel (46), has retained the high paramagnetism of the Fe(III) ion. It completely resembles in this respect the ferri-acetylacetonate or cupferron iron. Magnetically they are ionogenic salts; chemically they appear to be strong complex salts in which the chemical properties of the central metal have been lost.

The third group are the organic molecular compounds, such as the

quinhydrones, choleic acid, and addition compounds of polysaccharides and proteins.

The *second class* of coördination compounds consists of those in which an uncombined atom is linked to one the principal valence of which is satisfied, as in the yellow silver subfluoride.

$$Ag_2F = Ag[F\text{---}Ag]$$

The ammonia compounds of the alkali and alkaline earth metals are examples of compounds of elements with molecules; thus the lustrous red compound (35):

$$[Ca(NH_3)_6]$$

Further illustrations are oxy- and carbon monoxy-hemoglobin, and peroxo-ferro-aquo salt (30):
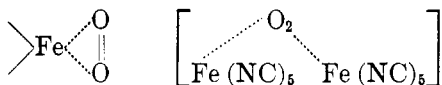
The *third class* of coördination compounds is formed by the amalgams, such as KHg_{12} and $CaHg_{12}$.

The number of coördination valences which an element possesses is always one in the case of the non-metals of low atomic weight, as nitrogen, oxygen, sulfur, fluorine, and chlorine. These are in part elements whose principal valences vary greatly.

When simple negatively charged ions are linked to a central atom the most frequent coördination number met with is six; only four negatively divalent ions can generally be arranged around a central atom. Many researches have been conducted recently to study the factors which govern this number. The maximum coördination number in every case depends not only on the charge of the complex addendum, but also on the size of the central ion (59); when the same charges are concerned, the coördination number is smaller with a small central ion than with a larger one. Thus only four fluoride ions can be arranged around the small, trivalent positive boron ion (KBF_4), while around the larger aluminum ion six can be arranged (K_3AlF_6). The pentavalent nitrogen atom can add only three oxygen ions, as in $K\begin{bmatrix} O \\ NO \\ O \end{bmatrix}$; phosphorus on the other hand can add four, as in K_3PO_4 (77).

Also, the distance of approach to the same central ion will vary with the charge on this ion (47, 60, 85). In

$$[Co^{II}(NH_3)_6]Cl_2$$

the distance of approach of the NH_3 is 2.5 A.U., while in

$$[Co^{III} (NH_3)_6] Cl_3$$

it is at a distance of 1.9 A.U. from the trivalent cobalt ion. This diminution of the distance indicates a very strong polarization; one might therefore assume that in this case the single electrons of the ammonia molecules are drawn so far toward the Co^{+++} ion that they belong equally to the coördinated constituent and to the central ion. Such compounds particularly concern us here, because they are either only very weakly paramagnetic or else diamagnetic.

Dipoles or polarization in complexes

In some complex ions, the coördinated ions are not uniformly surrounded by particles of opposite charge, but rather are subjected to a one-sided attraction to the central atom. We would therefore expect a one-sided polarization of this coördinated ion, which would give rise to dipoles, so that the bond between central ion and coördinated ion would be strengthened. For example, if one dissolves potassium ferrocyanide in water, the cyanide ions remain united with the central atom, while the potassium ions split off.

$$[Fe^{II^+} (NC)_6]^{----} + 4K^+$$
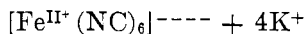
Judging from the magnetic data on this compound, the cyanide ions are linked to the central ion quite differently than, for example, are the fluoride ions in the compound

$$\begin{bmatrix} F & & F \\ F & Fe^{III} & F \\ F & & F \end{bmatrix}^{---} + Na_3$$
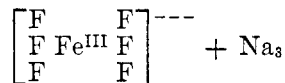
in which the iron ion shows the magneton number 29. The ferrocyanides themselves show no sign of the magnetism characteristic of the Fe^{++} (4.9 magnetons for the spin alone). A quite different type of linkage must therefore be present. From further experiments we shall see that one cyanide group of the hexacyanides can easily be attacked and replaced by neutral atoms, radicals, or molecules. If we had assumed an ionic linkage of the CN group as exists, for example, with the fluoride ions of Na_3FeF_6, this fact would be practically inexplicable. The assumption of a "true valence linkage" makes the matter less complicated. Unfortunately there is still lacking a definite explanation of the linkage in all of these complexes. In the case of potassium ferrocyanide we have clearly a case of "true valence linkage" giving rise to shared electron groups. In this substance we have the special case in which loss of the chemical properties

of the central ion occurs simultaneously with the loss of its paramagnetic quality. The same condition obtains in nickel dimethylglyoxime.

In complexes formed by addition of an uncharged dipole molecule to a central ion, this dipole molecule becomes polarized in one direction by the central ion, and the dipole moment is increased. Consequently the permanent electrical moment of this molecule is not alone sufficient to determine the strength of the linkage between a dipole molecule and a central ion. So, for example, the great affinity of pyridine for iron is not entirely due to the dipole nature of pyridine, but depends greatly upon the *specific nature of the pyridine nitrogen*. Again, the dipole moment of water is greater than that of ammonia. Were the binding energy determined only by the permanent dipole moment, then with any central ion the hydrate would always be more stable than the ammonia complex, and the latter with water would react to give ammonia and the hydrate. Experiment shows that though this often does occur, in many cases it does not.

As we have already mentioned, it is especially interesting that paramagnetic central ions of many complexes behave magnetically like simple ions, although chemically they are characterized as very strong complex compounds. So, for example, we have the iron salt of nitrosophenylhydroxylamine or iron benzohydroxamate and others, in which the iron ion is completely masked. On the other hand, there are other compounds in which the magneton number is low, thus in (33)

$$[CO(N_2H_4)_2]^{++}$$

the Weiss magneton number is 4.3. The copper ammonia compounds show smaller susceptibility than the hydrates, a matter of interest, since because of the smaller dipole moment of the NH_3 it was formerly expected to have less effect on the central ion. Then some salts such as $K_4Fe(NC)_6$, $K_2Ni(NC)_4$, and K_2PtCl_4 are diamagnetic, that is, the paramagnetic character of their metal ions has completely vanished.

From these few data, it may be seen that knowledge of the magnetism is of great importance in chemistry of the complexes, a fact strongly supported by recent work of Cambi and Szegö (38). Iron compounds can in general be divided into two groups: (1) ionized ferrous and ferric salts, and (2) complex iron compounds. The former are strongly paramagnetic with 27 and 29 Weiss magnetons, respectively. The complex iron compounds are weaker paramagnetically the more firm the complex linkage to iron is, i.e., the more they lose their residual affinity. In our investigation two compounds can be chosen as chemically and magnetically characteristic, viz., cupferron iron and nickel dimethylglyoxime.
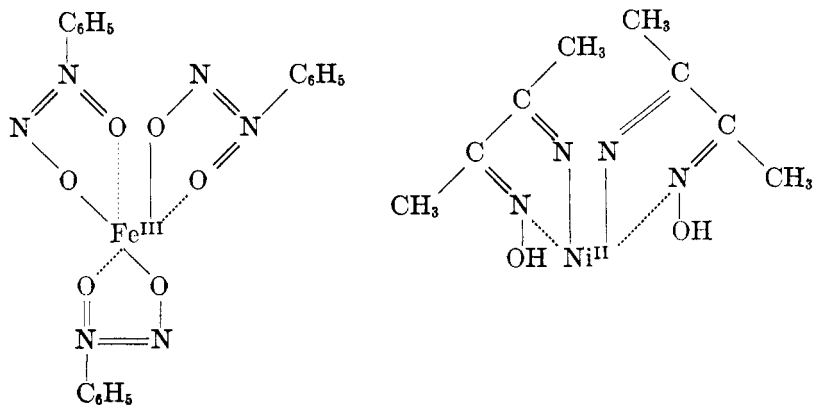
In both compounds the paramagnetic metal ion is linked to HNO groups as an inner complex salt. Both are strong complexes from a purely chemical point of view,—compounds in which the ionic nature of the metal, chemically speaking, is completely lost. By the study of their constitution, i.e., by exact knowledge about the linkage of the metals to the HNO group, we hope to be able to draw conclusions about their magnetic behavior. In cupferron iron, the metal ion is linked by principal valency to oxygen, since the hydrogen of the =NOH group is replaced. The iron is also joined by partial valences to oxygen since the pentavalent nitrogen
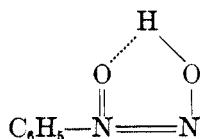
has no partial valences to be utilized. On the other hand, the doubly bound oxygen exerts a field force. The iron in this compound behaves magnetically like a ferric ion—its magneton number is 29.

We find quite a different valence situation in nickel dimethylglyoxime. As Pfeiffer (66) has irrefutably shown, the nickel is linked with principal valences to the central ion
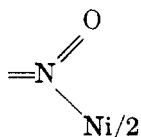
and with coördination valences to the =N—OH group. Since the =N—OH group contains trivalent nitrogen, this nitrogen atom still has partial valency to be utilized. The compound is diamagnetic and there-

fore, in the magnetic sense, a true complex salt. Both chemically and magnetically, nickel dimethylglyoxime, then, is a strong complex. In the undistorted complex the central ion can be recognized neither by ionic reactions nor by paramagnetism.

A thorough magnetic study of the pentacyano ferrous and ferric compounds led us to the observation, new in principle, that sodium nitroprusside (sodium pentacyano-nitroso-ferriate) possesses outstanding diamagnetic properties, while all other penta- and hexa-ferricyanides studied magnetometrically by Welo (81) were paramagnetic. Therefore, although it contains iron in the trivalent condition, it belongs magnetically to the complex ferrocyanides. In alkaline solution it is known that sodium ferropentacyanide-nitrite

$$\left[Fe^{II} \begin{array}{c} NO_2 \\ (NC)_5 \end{array} \right] Na_4$$
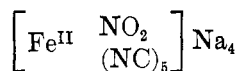
is formed (49), which by oxidation by permanganate goes over to the ferri compound.

$$\left[Fe^{III} \begin{array}{c} NO_2 \\ (NC)_5 \end{array} \right] Na_3$$
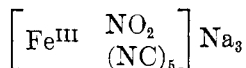
This in contrast to sodium nitroprusside is paramagnetic (14). Ferro-aquo salt

$$\left[Fe^{II} \begin{array}{c} (NC)_5 \\ OH_2 \end{array} \right] K_3$$
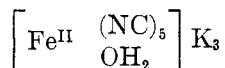
links nitric oxide, NO, similarly to carbon monoxide, CO, to the divalent iron through its coördination valences. A secondary oxidation results, however, and ordinary sodium nitroprusside with trivalent iron is formed.

We assume that molecular oxygen, O_2, also can be linked to ferro-aquo salt by chemical valence. The magnetic relations involved in molecular oxygen are much more complicated than they are in NO. Both, however, are paramagnetic gases. In the oxygen molecule two outer electrons behave extraordinarily like, although not to so great an extent as, the single electron of nitric oxide, which exists outside a system of ten electrons, and therefore behaves like a valence electron. We see, therefore, that the two gases bear a certain similarity in electron configuration as well as in magnetic behavior.

Sodium nitroprusside, because of the reactivity of its complex-bound NO group, is a valuable reagent in analytical chemistry. In particular, aldehydes and ketones can be recognized by vivid color reactions. Cambi (37) and collaborators have shown that reaction occurs with all substances having an activated —CH_2— group, and have succeeded in isolating a
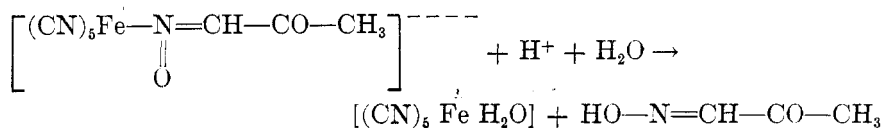
number of strongly colored compounds and explaining their structure. According to Cambi the organic compounds with sodium nitroprusside form deep red complexes; in the case of acetone,

$$\left[(CN)_5 - Fe^{II} - \underset{\underset{O}{\|}}{N} = C = R_1 R_2\right]^{----}$$

formed by the reaction

$$\left[Fe^{III} \begin{array}{c} NO \\ (NC)_5 \end{array}\right]^{--} + CH_3 - CO - CH_3 + 2OH^- \rightarrow$$

$$\left[(CN)_5 Fe^{II} \underset{\underset{O}{\|}}{N} = CH - CO - CH_3\right]^{----} + 2H_2O$$
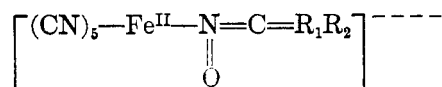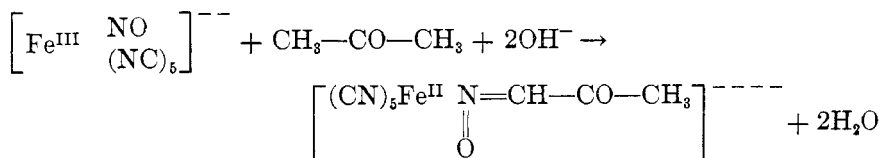
Thus we have occurring here a transformation quite similar to change from nitroprussi salt to nitroprusso salt in alkali; the deeply colored complexes, generally not very stable, are split by dilute acids to form isonitroso compounds and ferro-aquo salt:

$$\left[(CN)_5 Fe - \underset{\underset{O}{\|}}{N} = CH - CO - CH_3\right]^{----} + H^+ + H_2O \rightarrow$$

$$[(CN)_5 Fe H_2O] + HO - N = CH - CO - CH_3$$

which proves their structure.

We may see from all of these examples what special interest sodium nitroprusside, and indeed all metal compounds of the iron family which link NO coördinatively, claim both practically and theoretically. It is very interesting further to note that NO can be displaced from most compounds by CO. We know that the iron in the blood pigment possesses this ability in the highest degree. In the metal carbonyls $(Fe(CO)_5)$ and nitrosyls, NO and CO are joined to the metal atoms by coördination valences without regard to the valence ("electrovalence") of the metal.

When the principal valences are satisfied the radicals CO and NO are linked differently, as they are when a keto group $(R=CO)$ or a monovalent nitroso group $(R-NO)$ results from the saturation of principal valences, e.g., in nitrosobenzene. In spite of this, however, the characteristic coördinative linkage of the nitrogen to iron still operates, as we have proved by the synthesis (12) of the following compound:

$$\left[\begin{array}{c} O \\ \| \\ N - C_6H_5 \\ Fe^{II} \quad (NC)_5 \end{array}\right] Na_3 \cdot 3H_2O$$
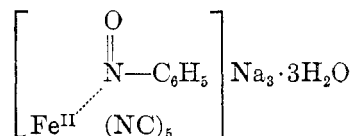
### III. DETERMINATION OF STRUCTURE OF NITROSOARYLHYDROXYLAMINES AND THEIR COMPLEX METAL SALTS

It has been found that nitrosoarylhydroxylamines can exist in two tautomeric forms. In some cases two isomeric esters have actually been isolated, for which the following structural formulas have been given.
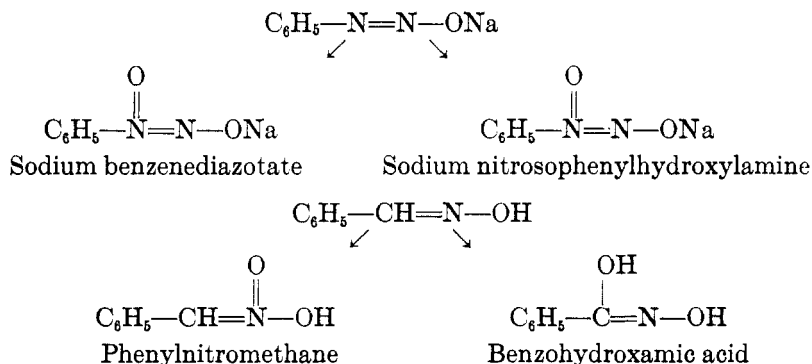
$$R \cdot N \begin{array}{c} \nearrow NO \\ \searrow OR_1 \end{array} \qquad and \qquad \begin{array}{c} R \cdot N = N \cdot R_1 \\ \| \\ O \end{array}$$
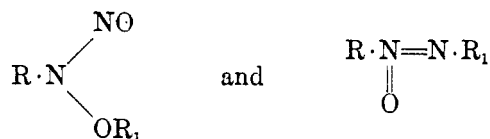
The work of Angeli (2) on the structure of the isomeric azoxy compounds forms the basis for the determination of the constitution of the nitrosoarylhydroxylamines. Angeli showed that azoxybenzene compounds of the general formula $Ar—N_2O—Ar'$ exist in two isomeric forms which are represented by

$$\begin{array}{c} O \\ \| \\ Ar—N=N—Ar' \end{array} \qquad and \qquad \begin{array}{c} O \\ \| \\ Ar—N=N—Ar' \end{array}$$
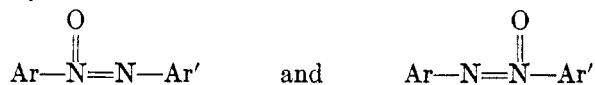
He formulated the diazo acids and the nitrosoarylhydroxylamines as follows:

$$\begin{array}{c} O \\ \| \\ R \cdot N = N \cdot OH \end{array} \qquad and \qquad \begin{array}{c} R \cdot N = N—OH \\ \| \\ O \end{array}$$
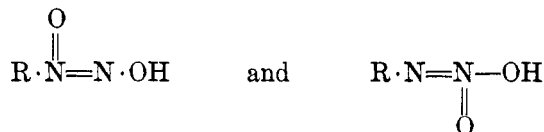
This structural representation was supported further by the work of Bamberger and Baudisch (6) on the oxidation of diazohydroxides and azo compounds. At the same time the analogy between oximes and diazo compounds, first recognized by Hantzsch, was broadened.

$$C_6H_5—N=N—ONa$$
$$\swarrow \qquad \searrow$$

$$\begin{array}{c} O \\ \| \\ C_6H_5—N=N—ONa \end{array} \qquad\qquad \begin{array}{c} O \\ \| \\ C_6H_5—N=N—ONa \end{array}$$

Sodium benzenediazotate   Sodium nitrosophenylhydroxylamine

$$C_6H_5—CH=N—OH$$
$$\swarrow \qquad \searrow$$

$$\begin{array}{c} O \\ \| \\ C_6H_5—CH=N—OH \end{array} \qquad\qquad \begin{array}{c} OH \\ | \\ C_6H_5—C=N—OH \end{array}$$

Phenylnitromethane   Benzohydroxamic acid

The oxidation of an azo group to a nitrosohydroxylamine

$$—N=N— \quad \rightarrow \quad —N=N—OH$$
$$\underset{O}{\overset{\|}{}}$$
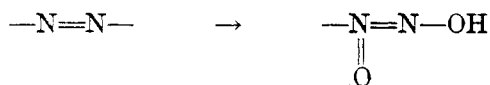
was first observed by Bamberger and Baudisch (6). It forms a direct proof for the structural relation between the azoxy and nitrosohydroxylamine compounds. We shall later use this relation in explaining the fine structure of the nitrosoarylhydroxylamines and their complex metal salts.

First of all it is necessary to determine the correct arrangement of the principal and subsidiary valences between the organic addenda and the central metal ion. Although in the free organic acid the fine structure hardly needs to be investigated, it plays an important rôle in the metal complex compounds in determining the properties of the metal. By making use of the Angeli nitrosohydroxylamine formula, we may represent the general formula of the metal complexes schematically as follows.

$$C_6H_5—N=N—OMe \qquad or \qquad C_6H_5—N=\!=\!=\!=N$$

I   II
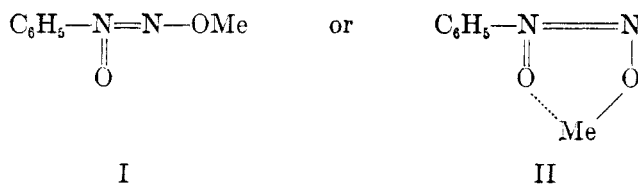
In formula II, which was first proposed by Bamberger and Baudisch (6), the metal is joined to the oxime group $=NOH$ by principal valency, and is joined by subsidiary valency to the doubly bonded oxygen attached to the pentavalent nitrogen atom. Depending on the nature of the metal (e.g., iron) this cyclic linkage can produce a chemical complex so strong that the ionic properties of the metal may be entirely lost.

First we sought to support by experimental evidence the analogy between azoxy compounds and nitrosohydroxylamines, and to study the influence on the complex-forming group of side chains in the benzene ring. If we were able to influence the nitrosohydroxylamine group by side chains, then a great field of new possibilities would be opened up with regard to the use of the nitrosohydroxylamine group in analysis.

As so often before, the finding of isomeric compounds has led to new discoveries, so here also the isomeric o-hydroxyazoxybenzenes pointed out the way. Bamberger (4, 5), who discovered them, first considered them stereoisomers. Baudisch (10) found that they are structural isomers and that the properties of the isomers are best explained by coördination influence of the ortho phenolic group on the trivalent nitrogen atom.

normal I   iso II
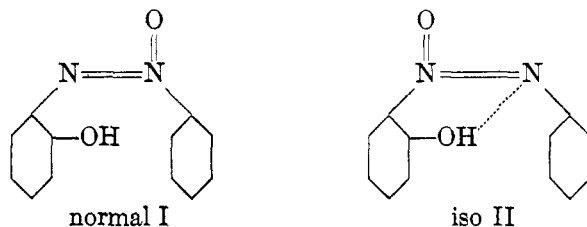
The "normal" o-hydroxyazoxybenzene, because of the free phenolic hydroxyl, is easily soluble in dilute alkali; the iso compound however is not, since the hydroxyl is anchored by subsidiary valences to the nitrogen. Compound I dyes wool and silk, but compound II does not.

If the above relations are applied to the nitrosoarylhydroxylamines we should expect the character of the nitrosohydroxylamine group to be modified when the linking ability of the trivalent nitrogen atom is changed by the influence of an o-hydroxyl group. This influence should be diminished in m- and p-hydroxy compounds, since these no longer offer the possibility for the formation of a six-membered ring. With this in mind we prepared the copper salts of the following three acids (24, 27, 28):

I   II   III

(clear gray)   (greenish gray)   (grayish green)
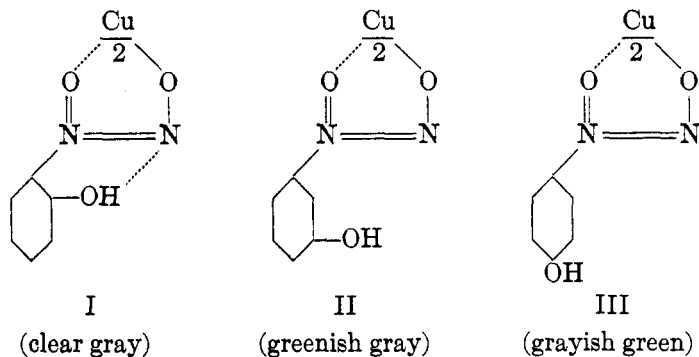
The copper salt I is basically different chemically from II and III. Although II and III are stable salts insoluble in water, the clear gray copper salt I even on standing in the air splits HNO and is changed into the copper salt of o-quinone oxime, which is deep red in color (25).

I   IV
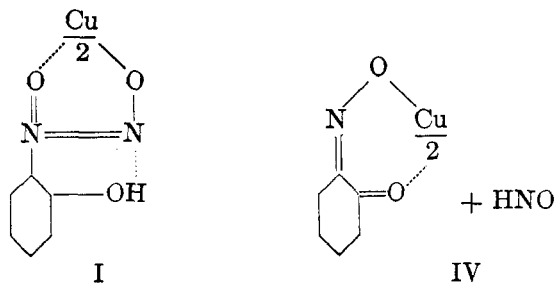
As the formula shows, the o-hydroxyl group in the salt I is coördinatively linked to the trivalent nitrogen atom. Because of this, the nature of the nitrosohydroxylamine group is completely changed, and there results the spontaneous decomposition into the quinone oxime copper salt and HNO. The formation of HNO is evidenced by the evolution of red-brown vapors of N_2O_3.

When the o-hydroxyl group is methylated, the corresponding copper salt (23, 29)

V
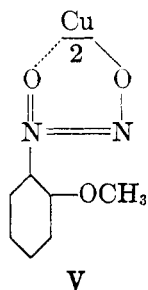
is a silver-gray metal compound, unchanged by boiling in benzene solution. Although the copper salt I is easily soluble in either water or organic solvents, the copper salt V is soluble only in benzene and a few other organic solvents.

The differences in the solubility of the copper, cobalt, and nickel salts of the o-, m-, and p-hydroxynitrosophenylhydroxylamines (I, II, and III above) are particularly striking. While the ortho salts of the three metals are equally soluble in water and ordinary organic solvents, the meta salts are soluble only in acetone, and the para salts are insoluble in either water or organic solvents. Our assumption of the coördination linkage between the phenolic hydrogen and the trivalent nitrogen is also borne out by the work of Feigl (43) on the inner-complex formation by salicylaldoxime.
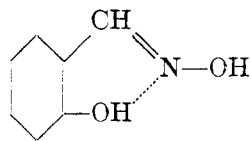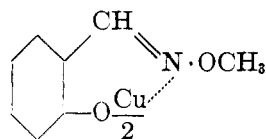

Free acid
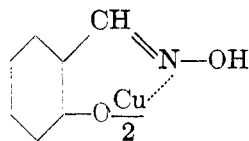
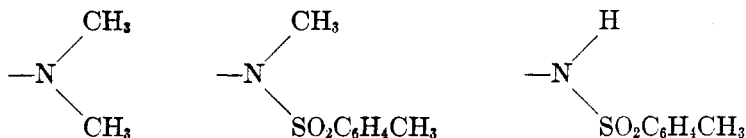Clear green copper salt
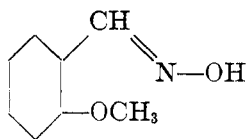
Forms no copper salt

Dark brown copper salt

The analogy between the oximes and the nitrosohydroxylamines comes to light here.

In order to obtain more exact insight into the character of the influence exerted by side chains, above exemplified by the *o*-, *m*-, and *p*-hydroxy-nitrosophenylhydroxylamines, we employed an amino group meta to the nitrosohydroxylamine group and varied only the properties of the nitrogen by substitution of the hydrogen by other residues:

$$-N\begin{matrix} CH_3 \\ \\ CH_3 \end{matrix} \qquad -N\begin{matrix} CH_3 \\ \\ SO_2C_6H_4CH_3 \end{matrix} \qquad -N\begin{matrix} H \\ \\ SO_2C_6H_4CH_3 \end{matrix}$$

It was expected that the trivalent nitrogen atom in I would retain the coördination valence properties of the amino group, —NH_2, since in it the hydrogen atoms have been replaced only by methyl groups. However in II and III the group —SO_2—C_6H_5—CH_3 is attached, which in itself possesses coördination valences, so that the coördination valence of the nitrogen should be partially neutralized and the characteristic properties of the nitrogen should disappear. These expectations are borne out by the properties of the following metal complex salts. The copper salts are again, in the case of these three acids, the most characteristic, therefore we can compare them to the copper salts of the hydroxy-substituted nitroso-phenylhydroxylamines.


I	II	III
(yellowish green)	(bright blue)	(bright blue)
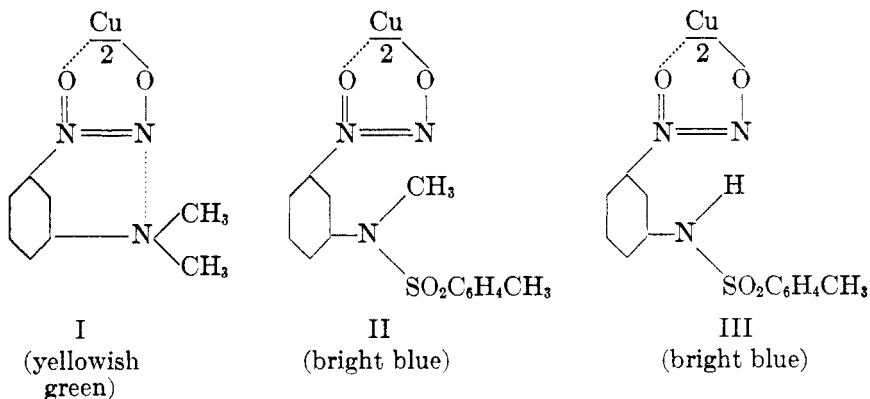
The influence of the nitrogen of the —$N(CH_3)_2$ group of salt I on the trivalent diazo nitrogen is appreciably greater than in salts II and III, as shown by the green color of the salt I. For, according to Pfeiffer, hand in hand with the increase in the saturation of the subsidiary valences, there is observed a shifting of color from blue (color of the copper ions) through

violet and red to brown.   The copper salt of o-hydroxyazobenzene, which is colored brown, is to be considered a strong complex (5).
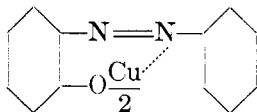

In the copper salt of o-hydroxyazobenzene the coördination linkage goes from copper to nitrogen, and affects the chemical and physical nature of the central copper ion.   However, with the salts I, II, and III, we have made the new important observation that the inner-complex forming nitroso-hydroxylamine group can be changed in its properties if the subsidiary valence of the side chain group is not linked to the central metal ion itself, but to the diazo nitrogen atom instead.   We have already assumed a similar constitution for the o-hydroxynitrosophenylhydroxylamine salt:
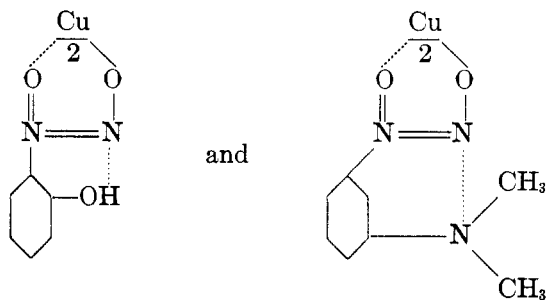

Not only the colors of the copper salts I, II, and III, but also the colors of the iron salts differ.   While the iron salts of the acids from II and III are red-brown, and outwardly resemble cupferron iron salt, the iron salt of m-dimethylaminonitrosophenylhydroxylamine, I, is brownish black (with violet luster).   The copper salt II is easily soluble in hot chloroform and benzene, difficultly soluble in hot alcohol, and insoluble in acetone, ether, and water.   It dissolves in acids with a dark red color.

A few additional instructive examples will be given showing the effect of coördinative linking with respect to copper salts of nitrogen and oxygen compounds.
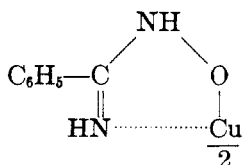

Benzamide oxime copper salt
(brown)
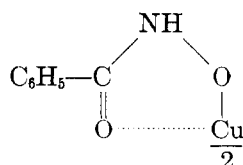
Copper benzohydroxamate
("grass" green,
bright green)

The nature of the complex depends upon whether the subsidiary valences of the central metal ion are linked to nitrogen or oxygen atoms; judging by the color, the nitrogen complexes are much stronger.   Magnetic measurements of these salts would be of special interest, particularly of the iron, cobalt, and nickel salts.

Another very interesting illustration is the comparison of the copper salt of nitrosophenylhydroxylamine with that of nitrosophenylhydrazine:

I
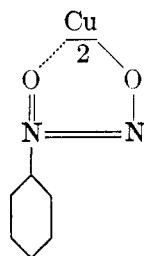
Nitrosophenylhydroxylamine
copper salt
(grayish blue)
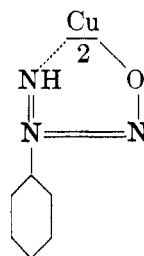
II

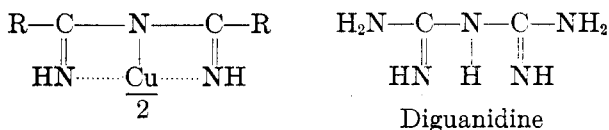Nitrosophenylhydrazine copper
salt
(deep red-brown)

In all cases where the coördinative linkage is strengthened, the color of the copper salt is shifted toward red and brown.   The basis for representing the salts I and II with analogous structures is the fact that salt II, by simple treatment with a mixture of acetic acid and alcohol, is converted into the grayish blue salt I, i.e., the imino group is replaced by an oxygen atom. While the salt I is relatively stable, the nitrosophenylhydrazine copper salt decomposes explosively, often without cause, with deposition of metallic copper.

The inner-complex salts of 8-hydroxyquinoline are also interesting.   For example, the zinc salt has the following structure (41):
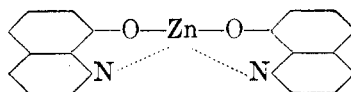

Coördination valences link the cyclic nitrogen of quinoline to zinc, while the metal is linked by principal valences to oxygen.

Other interesting copper salts in which the metal is linked to nitrogen by principal valences are, according to Ley and Müller (57), those of the amidines, which possess the following structure:

$$R-C-\!\!\!-N-\!\!\!-C-R \qquad H_2N-C-N-C-NH_2$$
$$HN\cdots Cu\cdots NH \qquad\qquad HN\ \ H\ \ NH$$
$$\underset{2}{} \qquad\qquad\qquad\qquad Diguanidine$$

If we replace the $>\!\!C\!\!=\!\!NH$ group of diguanidine by carbonyl, we produce dicyandiamidine,

$$H_2N-C-N-C-NH_2$$
$$NH\ \ H\ \ \ O$$
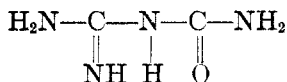
and the important biuret (76):

$$H_2N-C-N-C-NH_2$$
$$O\ \ \ H\ \ \ O$$
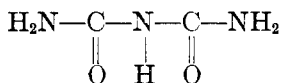
In concluding the discussion of coördination involving the nitrogen atom and of ways of affecting the reactivity of nitrogen complexes, we should like to mention a few additional examples which illustrate chemical changes of the complex-forming group. We have found that the coördination valences of the nitrogen in pyridine, which show great affinity for divalent iron, are very much weakened by the presence of an o-methyl group. We have explained this as a diversion or neutralization of the coördinative valence (13):

Pyridine   α-Picoline
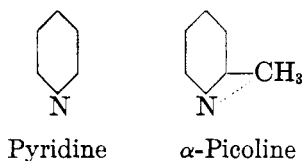
Quite similarly we explain the unreactivity of the o-methyl tertiary amines (51):
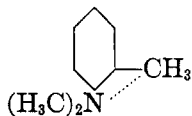
The nitrosophenylhydroxylamine ammonium salt, which was introduced to analytical chemistry under the name "cupferron" by Baudisch (9, 26), has served for many uses in the quantitative separation of metals, as the great number of publications testify. In neutral solution it precipitates

all metals, but only those which form inner complexes produce salts soluble in organic solvents. The stability of these salts toward acids varies enormously. A great number of new practical methods for separating metals depend upon this property. Without going into the processes, only the names of the metals will be mentioned here. Besides iron and copper, zirconium, vanadium, tin, thorium, columbium, niobium, tantalum, tetravalent uranium, aluminum, gallium, and hafnium can be precipitated and quantitatively determined with this useful analytical reagent. One may not speak of a "specific affinity" of the nitrosohydroxylamine group; according to the conditions of precipitation the most different metals form more or less strong complexes, and we find representatives of all stages from the most typical complex compounds to ordinary ionized salts of this acid. This property is intensified by side chains in the benzene nucleus. So, for example, the cadmium salts of the acids
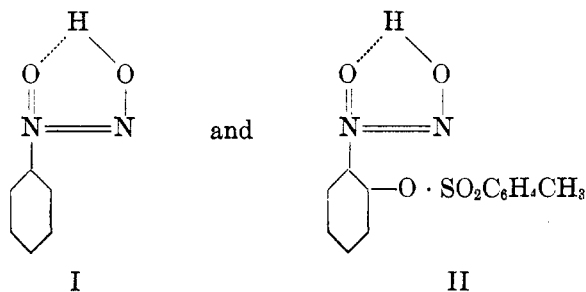
show completely different behavior. While the cadmium salt of acid I is an ordinary salt, soluble in water and acids, but insoluble in organic solvents, the cadmium salt of acid II is easily soluble in benzene, but insoluble in water. Acid II is especially suited for preparation of metal complexes which are easily soluble in ordinary organic solvents and insoluble in water. We have prepared salts of the following metals, and analyzed them: Fe, Cu, Co, Ni, Cr, Mn, U, Pb, Cd, Hg, Al, Bi, Ce, La, Th, In, Nd, Pr, and Er. Of these metal salts, the rare earth salts are of outstanding interest, because of the technical importance of their easy solubility in benzene and also petroleum ether.

IV. PROOF OF THE STRUCTURE OF NICKEL DIMETHYLGLYOXIME FROM EXPERIMENTS OF MEISENHEIMER, PFEIFFER, AND THILO

Nickel dimethylglyoxime is of special interest with respect to our investigations of the structure of complexes in relation to their chemical and magnetic properties. This red complex salt is diamagnetic in contrast to cupferron iron, which shows the paramagnetic moment of ordinary iron ions,

although chemically the properties of the central iron ion have completely disappeared, and in acetone solution it does not conduct the electric current. There still remain unexplained relations between the complex addenda and the central metal ion which influence the electron configuration and especially the spin of the electrons.

A series of nickel salts with dimethylglyoxime are known which have red, blue, green, and yellow colors. We shall discuss their constitution here.
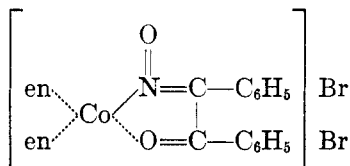
The color-determining portion of the molecule in the nickel glyoximes is the atom grouping
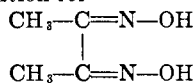
$$-C{=}N\diagdown\quad\diagup N{=}C-$$
$$\text{(O above both N)}\quad Ni$$
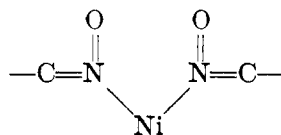
in which it makes no difference whether the two $-C{=}N-$ (with O double bond) groups belong to the same dioxime, or to two different dioximes. The atom configurations of the differently colored nickel dimethylglyoxime compounds have been cleared up by the work of Meisenheimer, Pfeiffer, and Thilo (75).

T. Meisenheimer has shown that the α-dioximes, formerly expressed in the *syn* form, possess the *anti* configuration. Therefore on the basis of purely steric relationships, it must be incorrect to assume that the nickel atom is generally linked to *oxygen*, if we maintain that the DH_2[4] possesses in complex salts two places of coördinative linkage. The important experimental proof of the duplexity of the dioxime in inner-complex compounds was furnished by P. Pfeiffer and I. Richarz. They prepared[5]

$$\left[\begin{array}{c}O\\en\diagdown\quad\diagup N{=}C-C_6H_5\\\quad Co\\en\diagup\quad\diagdown O{=}C-C_6H_5\end{array}\right]\begin{array}{c}Br\\Br\end{array}$$

----

[4] DH_2 is used as the abbreviation for
$$CH_3-C{=}N-OH$$
$$CH_3-C{=}N-OH$$

[5] "en" is used as the abbreviation for ethylenediamine.
$$CH_2-NH_2$$
$$CH_2-NH_2$$
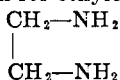
an orange-yellow compound from α-benzil monoxime in the following way: α-benzil monoxime was reacted with *cis*-hydroxo-aquo-diethylenediamine with formation of the compound II:

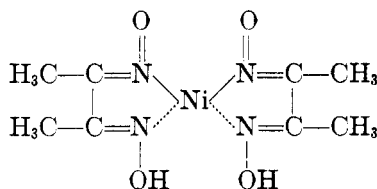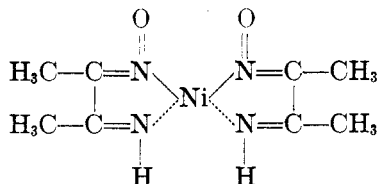$$Br_2 \begin{bmatrix} en \diagdown \\ \diagup Co \diagup OH \\ en \diagup \diagdown OH_2 \end{bmatrix} + \begin{matrix} O \\ \| \\ H—N=C—C_6H_5 \\ | \\ O=C—C_6H_5 \end{matrix} \rightarrow Br_2 \begin{bmatrix} \begin{matrix} O \\ \| \\ en \diagdown \quad N=C—C_6H_5 \\ \diagup Co \diagup \quad | \\ en \diagup \quad O=C—C_6H_5 \end{matrix} \end{bmatrix}$$

II
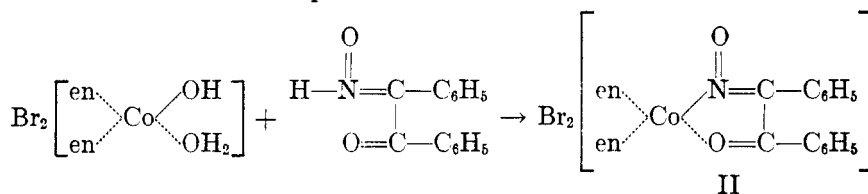
However, the β-benzil monoxime under exactly the same conditions gave no such salt. In salt II the monovalent rest $R—CO—C(R):\overset{\cdot}{N}:O$ quite certainly contains two positions of coördinative linkage: only so can the coördination number six of cobalt be fulfilled. II is therefore a true inner-complex salt.

On the basis of these investigations, Pfeiffer formulated the red nickel glyoxime as the inner complex salt:

$$\begin{matrix} & O & \quad O & \\ & \| & \quad \| & \\ H_3C—C=N \diagdown & \quad & \diagup N=C—CH_3 \\ & \diagdown Ni \diagdown & \\ H_3C—C=N \diagup & \quad & \diagdown N=C—CH_3 \\ & | & \quad | & \\ & OH & \quad OH & \end{matrix}$$

The final proof of this concept was given by P. Pfeiffer by preparation of the difficultly water-soluble nickel diacetyl iminoxime, which crystallizes in red plates:

$$\begin{matrix} & O & \quad O & \\ & \| & \quad \| & \\ H_3C—C=N \diagdown & \quad & \diagup N=C—CH_3 \\ & \diagdown Ni \diagdown & \\ H_3C—C=N \diagup & \quad & \diagdown N=C—CH_3 \\ & | & \quad | & \\ & H & \quad H & \end{matrix}$$

By addition of acids to the Ni(DH)$_2$, blue and green salts are obtained, which are formulated as follows:

$$\begin{bmatrix} & OH & \quad OH & \\ & | & \quad | & \\ H_3C—C=N & \quad & N=C—CH_3 \\ & \diagdown Ni \diagdown & \\ H_3C—C=N & \quad & N=C—CH_3 \\ & | & \quad | & \\ & OH & \quad OH & \end{bmatrix} \begin{matrix} X \\ \\ X \end{matrix}$$

III

(blue)

$$
\begin{array}{c}
\text{OH} \\
| \\
H_3C-C{=}N \\
| \\
H_3C-C{=}N \\
| \\
\text{OH}
\end{array}
\quad {>}Ni{<}^{X}_{X} \cdot nH_2O
$$

IV

(bright green)
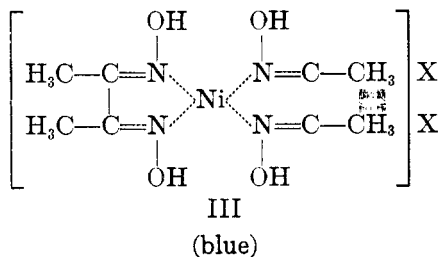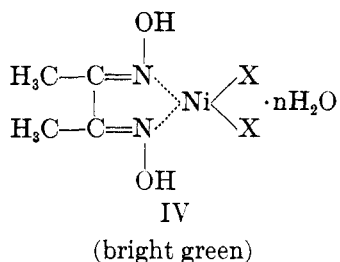
In III, by addition of acids to the $Ni(DH)_2$, the two atom complexes have been transformed back to the original oxime groups. In IV the compound contains a strongly acid residue.

Dimethylglyoxime also gives complex salts with iron. Thus, for example, a neutral ferrous salt solution containing dimethylglyoxime is colored deep red upon addition of ammonia. The solutions are very unstable and decompose on standing in the air, with precipitation of ferric oxide hydrate. From the color, which is very similar to that of the nickel dimethylglyoxime, one might suppose that there is formed in the solution the compound:

$$
\begin{array}{ccc}
\text{O} & & \text{O} \\
\| & & \| \\
H_3C-C{=}N & & N{=}C-CH_3 \\
| & {>}Fe{<} & | \\
H_3C-C{=}N & & N{=}C-CH_3 \\
| & & | \\
\text{OH} & & \text{OH}
\end{array}
$$
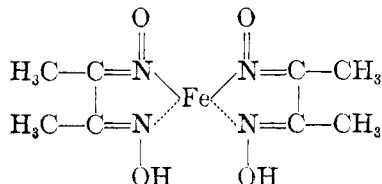
This may be abbreviated as DH—Fe—DH. However, the constitution is probably:

$$
\begin{array}{c}
NH_3 \\
\\
DH \quad Fe \quad DH \\
\\
NH_3
\end{array}
$$
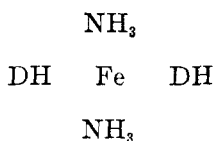
for, as L. Tschugaeff showed, if one treats ferrous salt solutions containing dimethylglyoxime with an excess of pyridine instead of ammonia, after a short time there is precipitated a compound

$$
\begin{array}{c}
Py \\
\\
DH \quad Fe \quad DH \\
\\
Py
\end{array}
$$
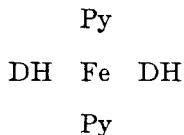
in the form of dark brownish red crystals.

Little is yet known about the compounds of trivalent iron with dimethylglyoxime. From both divalent and trivalent cobalt a great series of characteristic complex compounds with dimethylglyoxime are known.

From the standpoint of the metal atom (iron, cobalt, nickel) it is found that iron occurs only as a hexavalent coördinative central atom. With cobalt the compounds with coördination number six are far more stable; nickel (also copper) gives no compounds containing a hexavalent metal atom, and only those with four coördination linkages are known.

In general, it appears that the chemical behavior of the metals iron, cobalt, nickel, and copper in their complex dioxime compounds is completely similar. All replacements are continuous, yet clearly graduated however. Thilo finds no indication of any specific affinity of dimethylglyoxime for nickel.

Later we shall go more into detail about the magnetic properties of nickel dimethylglyoxime.

Next we are concerned with the chemical configuration and its relation to the linkage of the central atom. It is of fundamental importance, in determining the properties of the metal complex compound, whether the metal is linked directly to nitrogen or to oxygen:
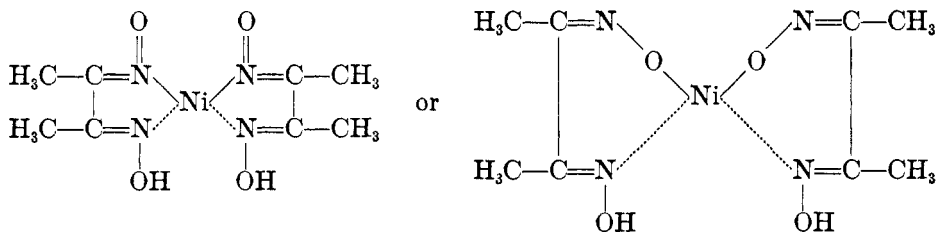
The assumption of the "nitron formula" is necessary to explain the fact that only the α-form (I) of the benzil monoximes and only the α-stereoisomer (II) of the benzil dioximes leads to the formation of inner complexes (68).
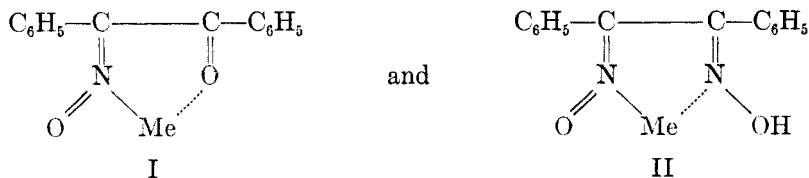
From the investigations on the structure of nickel dimethylglyoxime reported here, it is seen that in the *red* nickel dimethylglyoxime the central ion is linked by principal valency to the complex addendum. In the blue salt on the other hand, the $=NOH$ group is linked exclusively by sub-

sidiary valences to the nickel ion.   Iron behaves in a quite similar manner in the dimethylglyoxime compounds.

V. THE RELATION BETWEEN THE STRUCTURE OF CERTAIN WERNER IRON COMPLEXES AND THE IRON OXIDE HYDRATES AND IRON OXIDES FORMED FROM THEM

It has been demonstrated by the experiments of Welo and Baudisch (83) that under certain conditions in the oxidation of magnetite ($FeO \cdot Fe_2O_3$), the inner lattice structure of the ferromagnetic magnetite is retained and a ferric oxide (Fe_2O_3) is formed which is ferromagnetic.   It is a surprising fact that magnetite after complete oxidation to iron oxide (Fe_2O_3) retains its spinel structure.

They found further that ferrous hydroxide, $Fe(OH)_2$, in a strongly alkaline solution quickly changes by loss of water into ferrous oxide, which through oxidation by air is converted chiefly into the rhombohedral iron oxide, hematite (α-Fe_2O_3), along with small amounts of γ-Fe_2O_3 (31).   In this case, then, during the oxidation, intermediate formation of magnetite does not occur, or occurs only to a slight extent as disclosed by the production of small quantities of ferromagnetic iron oxide with the larger quantities of α-oxide.   The nature of the process of formation of α- and γ-Fe_2O_3 was thereby explained by us through x-ray determination of the crystal structure.   Always in those cases in the oxidation of ferrous hydroxide or oxide in which magnetite is formed as an intermediate, complete oxidation leads to the formation of a ferromagnetic iron oxide. On the other hand, direct oxidation of ferrous oxide to ferric oxide leads to the formation of non-magnetic hematite.

However, there appeared to be an exception to this rule, since, as Albrecht (1) first showed, it is possible by simple dehydration of artificially prepared γ-ferric oxide hydrate (γ-$Fe_2O_3 \cdot H_2O$) to prepare a ferromagnetic γ-Fe_2O_3 which has a crystal structure identical with that of γ-Fe_2O_3 obtained from magnetite.   Here, the intermediate formation of a ferrous–ferric oxide cannot be postulated, since with the γ-hydrate we have from the beginning trivalent iron.

The preparation of γ-iron oxide hydrate in very pure form by Baudisch (17) and by Baudisch and Albrecht (20) by a new method, afforded the opportunity to investigate more closely the inner lattice structure and other properties of this interesting compound.   Two principal factors seemed to favor the production of γ-hydrate by the oxidation of solutions of divalent iron salts; firstly, the pH, and secondly, the tendency of the reacting system to form more or less stable Werner complexes with iron as the central atom. The pH may be of importance only inasmuch as it affects the dissociation equilibria of the intermediate labile complexes.   It was our desire to

demonstrate the existence of such labile complex compounds, which might contain both divalent and trivalent iron. This expectation might be difficult to realize, since such complexes would probably in general exist in solution only as components of a dissociation equilibrium and not be readily obtained for analysis. Our experience with the preparation of γ-Fe_2O_3 pointed to the supposition that the γ-hydrate is formed only when intermediate compounds are formed which have a constitution analogous to that of magnetite. Magnetite itself is ferromagnetic also in the *hydrated* condition and thereby fundamentally different from the γ-ferric oxide hydrate. Further x-ray investigations showed that hydrated magnetite gives the x-ray spectrum of the water-free magnetite ($FeO \cdot Fe_2O_3$), and therefore is entirely different from the Roentgen spectrum of the γ-ferric oxide hydrate which was first determined by Böhm (36).

Since the γ-iron oxide hydrate prepared by oxidation of ferrous salts is a true hydrate, the hypothetical intermediate compounds with divalent and trivalent iron could not be hydrated magnetite. We obtained evidence as to the mechanism of the formation of the γ-hydrate only when we studied its synthesis from a simply constituted Werner complex salt. We chose for this study the canary-yellow complex salt easily prepared from ferrous chloride and pyridine, having the following constitution:

$$\begin{bmatrix} Fe^{II} & Py_4 \\ & Cl_2 \end{bmatrix}$$
$$I$$
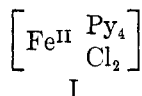
This complex salt was first described by N. Costachescu and G. Spacu (39). Just recently in an extensive compilation of data which relate partly to the dissolved state (conductivity studies), and partly to the crystalline state (x-ray and magnetic studies), it has been shown that complex salts of halides of the transition elements with four neutral amine molecules, contain the halogen in general still in the inner sphere.
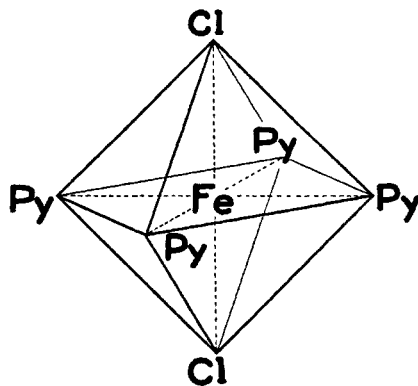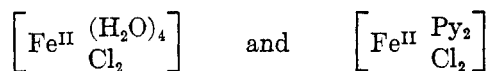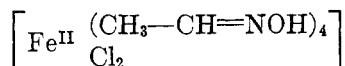

FIG. 1

To these "pseudo salts" (as they are termed by Hantzsch) the compounds

$$\left[Fe^{II}\ \begin{matrix} (H_2O)_4 \\ Cl_2 \end{matrix}\right] \quad \text{and} \quad \left[Fe^{II}\ \begin{matrix} Py_2 \\ Cl_2 \end{matrix}\right]$$

also belong, according to the recent investigations of Stelling (74). The compounds of ferrous chloride with acetaldoxime and other aldoximes also belong to this group (48); thus, for example,

$$\left[Fe^{II}\ \begin{matrix} (CH_3—CH=NOH)_4 \\ Cl_2 \end{matrix}\right]$$

This fact is of interest in connection with our concept of the genetic relationship of hydroxamic acid and aldoximes to pyrrole and pyridine compounds mentioned earlier.

Of the tetraamino complexes mentioned, the thiocyanate compounds especially have been thoroughly investigated recently. The tetrapyridine compound of ferrous thiocyanate,

$$\left[Fe\ \begin{matrix} Py_4 \\ (SCN)_2 \end{matrix}\right]$$

II
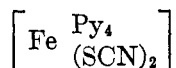
which was first prepared by Grossmann and Hünseler (45), is autoxidizable in solution. A. Rosenheim, E. Roehrich, and L. Trewendt (71) have recently studied the autoxidation of solutions of this compound and found that a bimolecular compound is produced which contains both divalent and trivalent iron, and is constituted analogously to magnetite:

$$[Fe^{II}\ (C_5H_5N)_6]\left[Fe^{III}\ \begin{matrix} (C_5H_5N)_2 \\ (SCN)_2 \end{matrix}\right]_2$$
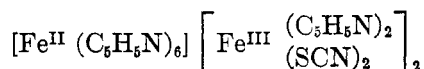
Spacu (73), who repeated the work of Rosenheim and collaborators, obtained in his many experiments analytical values which varied quite widely, yet which were in part in agreement with those of Rosenheim. He expresses the view that in all of these intermediate products from the autoxidation of II, mixtures of tripyridine ferric thiocyanate and closely related ferrous compounds are present. Rosenheim (70) also concurred in this view. At any rate, it is certain that the yellow compound II is autoxidizable and compounds can be formed which are constituted analogously to magnetite.

$$Fe^{II}\ [Fe_2^{III}\ O_4]$$
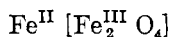
In direct connection with these results stands our earlier work with the pentacyano-aquo compounds. The yellow pentacyano-aquo-ferroate is

autoxidizable in solution and forms polynuclear complexes which contain both divalent and trivalent iron (40).   According to Michaelis (61), a pH of 6.6 furnishes the best condition for the formation of a salt having the structure

$$\left[Fe^{II} \begin{array}{c} OH_2 \\ (NC)_5 \end{array} \right] Na_3 \cdot \left[Fe^{III} \begin{array}{c} OH_2 \\ (NC)_5 \end{array} \right] Na_2$$
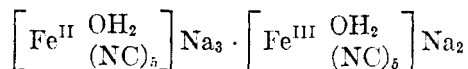
III

If a yellow solution of ferro-aquo salt is mixed with a violet solution of ferri-aquo salt in the molecular ratio 1:1, the new salt, III, is formed which is brownish yellow.   This salt is also formed when potassium ferrocyanide solutions are exposed to light in the presenc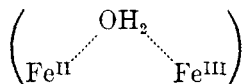e of oxygen.   As we have found, the formation of the bimolecular salt is inhibited when small amounts of sodium azide are added to the ferro-aquo salt solution.   This compound possesses an affinity only for the coördination position occupied by the water molecules, and prevents the water molecule from forming a bridge

$$\left(\begin{array}{c} OH_2 \\ Fe^{II} \qquad Fe^{III} \end{array} \right)$$

and thus joining two molecules so that they can form a bimolecular complex. Therefore, when a yellow ferro-aquo salt solution is oxidized (by oxygen or hydrogen peroxide) in the presence of sodium azide, a deep violet-colored solution is obtained, which contains the ferri-aquo salt in equilibrium with the corresponding ferri-azido salt (15).

If we apply these principles to the autoxidation and hydrolytic decomposition of tetrapyridine ferrous chloride, we reach some useful conclusions as to the mechanism of the formation of γ-oxide hydrate.   These will be discussed below.

We have already shown in earlier publications (20) that from ferrous chloride solutions treated with pyridine, by passage of air or oxygen a certain amount of orange-yellow γ-$Fe_2O_3 \cdot H_2O$ is precipitated.   On the other hand, if ferric chloride is treated under the same conditions with pyridine, a red hydrate is obtained, which on dehydration gives non-magnetic hematite. By varying the conditions of temperature and precipitation, amorphous orange-yellow hydrates are obtained, which on dehydration also give only hematite.

In further experiments we used the yellow pyridine salt,

$$\left[Fe^{II} \begin{array}{c} (C_5H_5N)_4 \\ Cl_2 \end{array} \right]$$
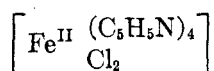
which dissolves to a colorless solution in water. These solutions in the presence of air are extremely unstable and hydrolyze with the splitting-out of pyridine. The colorless solutions become green and turbid, and orange-yellow ferric oxide hydrate is quantitatively precipitated. The filtered clear solution shows the pH 5.6–5.8. If the yellow salt is dissolved in a strong aqueous solution of pyridine, the primary green coloration does not appear, but the solution which is at first colorless, becomes dark brown and finally, after long shaking with air, all the iron is precipitated as an orange-yellow hydrate. The pH of the clear colorless filtrate is 7.1–7.3.

From these experiments it is seen that in both weakly acid and weakly basic solutions, autoxidation produces an orange-yellow hydrate. The dehydration of this hydrate at 260°C. yields a strongly ferromagnetic γ-Fe_2O_3. If, however, the yellow salt is dissolved, instead of in water, in superoxol (H_2O_2), vigorous oxidation ensues and heat is evolved. By dilution with water, a brown hydrate is obtained which is completely non-magnetic. If the hydrate is dehydrated at 260°C., a red oxide (hematite) is formed which is also non-magnetic. Thus, the result of the oxidation of the yellow salt is absolutely different, depending upon whether the oxidation is carried out with hydrogen peroxide or with air.
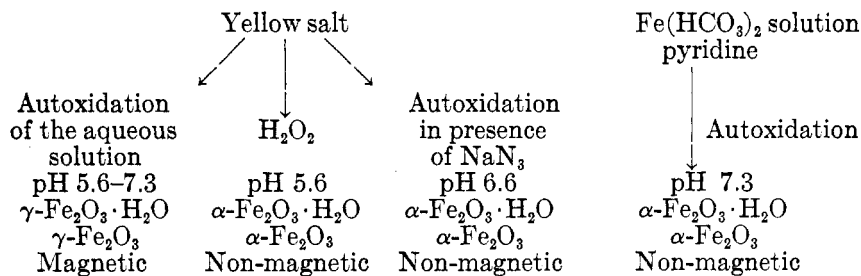
In order to effect oxidation as rapidly as possible with oxygen the salt was added in small portions to water saturated with oxygen, through which oxygen was continuously bubbled. There was formed almost immediately an orange-yellow hydrate. The dehydration of this hydrate also gave an α-oxide (hematite) which contained only small amounts of admixed ferromagnetic oxide. The experiments were often repeated and showed conclusively that slow autoxidation of the yellow salt leads to the formation of the γ-hydrate, while rapid oxidation favors the formation of the α-hydrate.

From the previously mentioned facts, it seems logical to assume that by autoxidation (slow oxidation) bimolecular complexes containing divalent and trivalent iron are formed which possess a structure analogous to that of magnetite, and by hydrolysis and oxidation are converted to the γ-hydrate. With *rapid* oxidation of the yellow salt, such ferrous–ferric complexes cannot be formed, and therefore only the α-hydrate is obtained. Likewise by hydrolysis of the complex pyridine ferric compounds only α-hydrate can be produced.

From some previously mentioned experiments with the pentacyano iron compounds we know that we can inhibit the formation of bimolecular complexes by sodium azide. We can therefore test our assumption of the intermediate formation of such complexes in the case of the tetrapyridine ferrous chloride compound. We would expect that in the presence of sodium azide only an α-hydrate would be obtained by autoxidation of the

yellow salt, since sodium azide prevents the formation of polynuclear compounds and it is known that direct oxidation of divalent iron leads to the formation of the α-hydrate. This is actually what does occur. If the yellow salt is dissolved in a dilute sodium azide solution, the autoxidation occurs in the same way that it does in pure water. The orange-yellow hydrate formed is not a γ-hydrate, however, but is a pure α-hydrate. (The pH of the clear filtrate is 6.6.) Although the oxidation is quite gradual, and all other conditions are as given for the formation of the γ-hydrate, only the α-hydrate is formed. We are thus able to prepare either α- or γ-iron oxide hydrate from the yellow salt, depending upon the conditions of precipitation.

If instead of the yellow salt, a solution of ferrous bicarbonate is used, and it is oxidized slowly with oxygen in the presence of pyridine (pH $=$ 7.3), in all cases α-hydrate alone is formed. The ferrous bicarbonate has no tendency to form complexes with pyridine, therefore oxidation always converts the ferrous ions directly into the α-hydrate.

	Yellow salt		$Fe(HCO_3)_2$ solution pyridine
Autoxidation of the aqueous solution	H_2O_2	Autoxidation in presence of NaN_3	Autoxidation
pH 5.6–7.3	pH 5.6	pH 6.6	pH 7.3
γ-$Fe_2O_3 \cdot H_2O$	α-$Fe_2O_3 \cdot H_2O$	α-$Fe_2O_3 \cdot H_2O$	α-$Fe_2O_3 \cdot H_2O$
γ-Fe_2O_3	α-Fe_2O_3	α-Fe_2O_3	α-Fe_2O_3
Magnetic	Non-magnetic	Non-magnetic	Non-magnetic

It is hardly to be doubted that under the experimental conditions which lead to the quantitative formation of an intermediate bimolecular compound of magnetite structure, a quantitative yield of γ-$Fe_2O_3 \cdot H_2O$ is obtained. In case the ratio of the complex pyridine ferric chlorides to the pyridine ferrous chlorides in the mixture is different, then mixtures of α- and γ-hydrates will be obtained. By quantitative determination of the ferromagnetism of the oxides formed by dehydration of the hydrates produced from the yellow salt, it should be possible to control the autoxidation process and the composition of the intermediate products. Formerly it was only possible to do this by tedious determination of the atomic ratios of the iron, nitrogen, and halogen.

That under the conditions mentioned (pH from 5.6–7.3) α- and γ-hydrates and not the oxides, are formed, probably is to be traced to the fact that ferrous hydroxide, $Fe(OH)_2$ or $FeO \cdot H_2O$, remains as such in the intermediate ferrous–ferric compounds. In the course of the oxidation, the ferrous iron of the intermediate bimolecular complex is oxidized without

loss of the chemically combined water of hydration.   Therefore we obtain by autoxidation at a pH slightly on the acid side or near the neutral point on the basic side, *hydrates*, not oxides.

If the aforementioned experiments with the yellow salt are carried out in exactly the same manner in a strongly alkaline medium (e.g., with piperi-dine) we no longer obtain hydrates, but rather oxides, or mixtures of the two.   For example, if the yellow salt is put in piperidine and then diluted with water and shaken with air, there is intermediate formation of black magnetite, which on further treatment with air is completely oxidized to γ-iron oxide.   The γ-Fe_2O_3 formed possesses a beautiful velvety red color. On short heating to 260°C. the oxide loses its magnetism, and the color deepens.   The intermediate formation here of finely divided black mag-netite is in agreement with the supposed formation of the complex inter-mediates analogous to magnetite when the autoxidation of the yellow salt occurs in weakly alkaline solutions.

The oxidation of the yellow salt with hydrogen peroxide in a strongly alkaline solution does not lead to the formation of the α-hydrate, but instead to mixtures of considerable amounts of α-oxide with small amounts of γ-oxide.   Likewise, as we have already mentioned, the oxidation of aged $Fe(OH)_2$, in other words, of FeO, leads to the formation of α-Fe_2O_3, as we have proved by x-ray analysis.

From these experiments we may therefore draw the conclusion that the process of formation of the γ-*hydrate* is not fundamentally different from that of the formation of the γ-*oxide*.

When we work with Werner complexes we have from the start a spatial orientation, whether the solid salts or their solutions are used.   We have here an interesting case in which the spatial arrangement and the chemical composition of the intermediate complexes determine the type of lattice from which the hydrates and oxides that are formed are built up.

Besides differences in the lattice types of the iron oxides, we have to deal with differences in the surface properties, which are primarily governed by the "sensitive crystal properties" (empfindlich Krystalleigenschaften). In the following section we shall be principally concerned with the surface properties of iron oxides in their application to biochemistry.   In order to determine by physical measurement the sensitive crystal properties, we have applied the measurement of the hysteresis and coercive force of the magnetic oxides, as we shall report in the second part of this review.   We are therefore able to determine the fine structures of our iron oxides by x-ray measurement, and through the application of magnetic measure-ments, their hyperfine structures.

Since we may look upon the Werner complexes as a stage preceding the formation of submicroscopic crystals or colloidal particles, there is a logical

reason for treating the biological experiments with both types of iron compounds simultaneously in this review.

VI. THE IMPORTANCE OF ACTIVE IRON (WERNER COMPLEXES AND IRON OXIDES OR HYDRATES) IN BIOLOGY

The reactions at the surfaces of solid substances must be similar to the reactions with similarly constructed Werner complex salts. On the surfaces of solid iron oxides there exist coördination centers in the form of loosely bound iron atoms which are able to draw in radicals or even whole molecules to form primary coördinative bonds. Then the exchange or displacement occurs by which the reacting substances become activated. In the active states, reactions which would otherwise proceed extremely slowly may be catalytically accelerated or may even first become at all possible.

It is therefore not only the number of atoms on the surface that determines the activity but there must exist, as we have indicated, a special property of individual active atoms which affects the adsorption and, because of that, the activity. The proportion of these atoms in a special condition to the total number present is to be regarded as the measure of activity.

The catalytic activity of iron oxide varies greatly with the method of preparation, and our first problem is to prepare it of the highest possible activity. Then we may proceed with the biological tests. Researches of this type are still in a preliminary stage and there is, no doubt, room for much development and improvement.

The first direct experiments to test the biological activity of iron oxides and complex iron compounds were those of Webster (78, 79). He established the fact that red corpuscles, which are necessary for the growth of *Bacterium lepisepticum* of virulent type D, may be replaced by sodium penta-cyano-aquo-ferroate ("aquo salt"). This typical Werner complex salt functions even when diluted to 0.002 mg. per cubic centimeter. Since these very dilute solutions gave benzidine reactions (blood test), the biological activities of both the iron compounds and the red corpuscles were measured as to their peroxidase properties, using the benzidine reaction as an indicator. As a result of these experiments of Webster, to whom we had supplied the so-called aquo salt, it appeared that *B. lepisepticum* is to be regarded as an organism so delicately adjusted to oxygen pressure that it can grow prolifically and possess the specific properties of the virulent type only when oxygen is mechanically removed, or when the effects of oxygen are suppressed by the addition of catalase (blood, aquo salt).

This important observation of Webster concerning the biological action of aquo salt led us to examine our iron oxides for peroxidase properties by

means of the benzidine test.   Since the oxides gave the typical blue colora-
tion in a very distinctive manner we assumed, at first, that their biological
activities could be characterized in a simple way by the color reaction.
Further investigation showed that the relations are not so simple.   Unfor-
tunately, however, our first statements regarding the benzidine activity of
iron oxides, which were made without a knowledge of the physical and
chemical relations to the fine structure, have been expanded far beyond
legitimate limits by other investigators, so that much confusion has arisen
regarding the meaning of "active iron" (18).

Since the term "active iron oxide" has been applied also, in inorganic
chemistry, to oxides having free energy and which approach a stable condi-
tion on aging, it is necessary to emphasize that when we speak of "active
iron" we have in mind only those oxides or those complex iron compounds
which have a favorable influence on the length of life, the growth, the
virulence, or, generally, on the biological activity of bacteria and tissue
cells.

Although we had previously carried the knowledge gained in our experi-
ments on nitrate and nitrite reduction over into the biological field, and had
shown that there is a connection between the iron content of highly virulent
cholera bacteria and the power to reduce nitrates, our systematic experi-
ments to obtain a deeper understanding of active iron compounds were
first begun after Webster's positive results with aquo salt became known.

In aquo salt we had at hand a biological catalyst of known constitution,
and we were justified in the hope that we could determine the nature of the
so-called X mechanism of hemophilic bacteria.   Avery (3) and his co-
workers had found that a so-called X factor is necessary for good growth
of hemophilic anaerobic bacteria and of pneumonia bacilli at atmospheric
pressure.   They found that red corpuscles do not lose their X factor
quality even after heating in steam at 120°C., and they were finally able to
replace the X factor by adding gum arabic–ferrous sulfate solution to the
culture medium.

The extensive experiments of Webster and Baudisch (79) showed that
aquo salt diluted to 0.01 mg. per cubic centimeter can replace the X factor
with hemophilic bacteria.   Influenza bacilli also grew in the presence of
aquo salt, as did *B. welchii* and *B. sporogenes.*   The experiments extended
over a period of a year, and could always be repeated with the same results.

It was but a short step to go from sodium penta-cyano-aquo-ferroate
(aquo salt) to the active iron oxide, γ-Fe_2O_3, since both compounds gave
positive benzidine reactions, showed definite catalase properties, and ab-
sorbed oxygen in aqueous solutions or suspensions.   If γ-Fe_2O_3 were heated
to 550°C. it changed its structure and lost these properties almost com-
pletely.   No observable chemical change occurred when the oxide was

heated, for there was no loss of weight and chemical analysis again gave Fe_2O_3 (inactive α-Fe_2O_3). The biological tests proved to be quite striking. While the γ-Fe_2O_3, similarly to the aquo salt, functioned as a very active X factor (biocatalyst, growth catalyst), the results with α-Fe_2O_3 were entirely negative.

It can be readily understood that we, at that time, attributed the surprising results primarily to the differences in crystal structure. We were then at the threshold of our oxide studies and as yet knew nothing of their great variability. Also, there was little that was definite about them to be found in the literature. This much, however, was definitely established by our experiments: the requirements of the X mechanism can be satisfied not only with red corpuscles but also with those iron compounds which show peroxidase properties and which absorb oxygen.

Our description of this biocatalytic action of certain iron oxides and of aquo salt excited the interest of other investigators and our experiments were variously repeated with both positive and negative results. When Miss Morris and Baudisch set about to repeat the Webster-Baudisch experiment half a year later, at the Rockefeller Institute, they at once met with difficulties, although both the media and the cultures were obtained from the laboratory of Webster.

In order to account for the discrepancies it seemed advisable to make a closer study of the aquo salt and its relation to the culture medium. The principal reason was Webster's proof that the ammino and nitro salts were far less active than aquo salt. In the ammino and nitro salts, NH_3 and NO_2 groups, respectively, replace the H_2O group in aquo salt. Dubos soon found a biological reaction with which to test the activity of aquo salt in the medium. This method could also be applied to iron oxide and proved to be far superior in sensitivity to the chemical tests for catalase.

The Dubos (15, 16, 22) test involves the longevity of pneumonia bacilli. Pneumococci die very rapidly in ordinary bouillon, but the addition of a small amount of blood will greatly extend their lives. There is, here, a certain analogy to the X mechanism. With pneumococci growing in flat dishes the presence of peroxides can be directly demonstrated with the benzidine reaction (benzidine + hydrogen peroxide). The peroxides shorten the lives of the cells and even kill them outright. The addition of catalase serves to keep them alive. We have here, then, a possible method of testing the catalase activity of aquo salt in bouillon and of comparing its activity with that of the ammino salt.

$$\left(Fe \begin{matrix} NH_3 \\ (CN)_5 \end{matrix} \right) Na_3$$
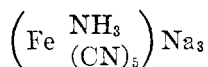
The method is as follows: 5 cc. of ordinary bouillon in a test tube is inoculated with a 12-hour old avirulent pneumococcus culture, strain RN derived from Type II, and the sterile salts are added. The tubes are kept at 37°C. for 24 hours and are then left to stand in the dark at room temperatures. From time to time, samples are removed and spread on blood agar.

The result was that aquo salt decidedly lengthened the lives of the pneumococci even in dilution to 10^{-3}, whereas there was no effect with ammino salt. This experimental procedure was applied to test the catalase action of iron oxides. Oxides prepared in different ways gave definitely different effects.

In the case of aquo salt, the mechanism of the reaction and its relation to the medium is clear in at least one respect. The inhibition reactions which we have previously described are color tests with which we can test the intensities and selectivities of the force fields of the sixth "free" coördination valence. However, they are not as sensitive as the biological reaction just described. Groups with strong affinity to divalent iron in a complex (NH, SH, CN, NO, CO, etc.) block the free valence on entry into the complex and inactivate it, either wholly or partly. We have already mentioned that potassium ferrocyanide is entirely inactive and becomes activated on irradiation. Activation by means of light can also be biocatalytically demonstrated, for it is only irradiated potassium ferrocyanide that acts as an X mechanism.

These considerations give us a simple explanation of the conflicting results in the Webster-Baudisch experiments. If the bouillon contains substances which "block" the coördination valences there is no X action because the catalytic (autoxidative, peroxidative) action of the complex is either greatly diminished or entirely destroyed. When aquo salt is to be used as an X factor in bouillon cultures one must, at the very least, test its catalase action in the medium, as can be done by the Dubos-Baudisch method.

In applying this new information to the iron oxides, the methods were developed so that we could use synthetic media as well as bouillon. With these, also, we could demonstrate the selective action of certain oxides as X mechanisms. Previously it had been impossible to predict definitely biological activity by any physical or chemical tests.

These biological experiments have considerably clarified the complex X mechanism. The importance of catalase activity in iron oxides has come definitely to the fore and we must strive to prepare pure oxides that will be highly catalatic but not peroxidative. The previous emphasis on crystal structure retreats to the background. We now emphasize surface topography as it is determined by the pores of the clusters and by the degree of dispersion of the submicroscopic particles. It has become evident that

we are really only at the beginning of a knowledge of biologically active iron oxides. Our selection of γ- and α-oxides as the "active" and "inactive" ones, respectively, was, in fact, only the key with which we opened up a very wide field of investigation.

Many investigators are engaged on the problem of structure in oxides prepared in various ways, and new facts and relations are constantly being published. For example, the structures of simple oxides have been considered in terms analogous to those employed in the classical valence theory of organic chemistry (Krause) (54). Along with efforts such as this we have the catalytic tests, the x-ray analyses, and the magnetic measurements.

It would be highly advisable and worth while to select the most characteristic types of iron oxides and use them in such biological experiments as have been described in this paper. The iron cyanide complexes are also susceptible to further development, as far as biological activity is concerned, as has recently been shown by Margarethe Lwoff (58) at the Pasteur Institute.

REFERENCES FOR TABLE 1

(1) ANGELI-ARNDT: Sauerstoffhaltige Verbindungen des Stickstoffs. Samml. chem. chem.-tech. Vortr. 1908, XIII.

(2) BALY, EDWARD CHARLES CYRIL, HEILBRON, ISIDOR MORRIS, AND STERN, HAROLD JACOB: Trans. Chem. Soc. 123, 185 (1923).

(3) BAUDISCH, O.: Ber. 44, 1009 (1911). Über Nitrat- und Nitrit-Assimilation. I.

(4) BAUDISCH, O.: Schweiz. Naturforsch. Ges., 1911. Über Nitrat- und Nitrit-Assimilation. II.

(5) BAUDISCH, O.: Zentr. Bakt. Parasitenk. 32, No. 20/25 (1912). Über Nitrat- und Nitrit-Assimilation und über eine neue Hypothese der Bildung von Vorstufen der Eiweisskorper in den Pflanzen.

(6) BAUDISCH, O., AND MAYER, ERWIN: Ber. 45, 1771 (1912). Lichtchemische Vorlesungsversuche von pflanzenphysiologischen Interesse (III. lichtchemische Mitteilung).

(7) BAUDISCH, O.: Ber. 45, 2879 (1912). Über Nitrat- und Nitrit-Assimilation. IV. Eine Erwiderung an Herrn Oskar Loew.

(8) BAUDISCH, O., AND MAYER, ERWIN: Ber. 46, 115 (1913). Über Nitrat- und Nitrit-Assimilation.

(9) BAUDISCH, O.: Vierteljahrsschr. Naturforsch. Ges. Zürich 59, 1 (1913). Über das chemische Verhalten anorganischer stickstoffhaltiger Pflanzennahrungsstoffe gegenüber dem Sonnenlicht.

(10) BAUDISCH, O.: Z. angew. Chem. 26, 612 (1913). Über Nitrat- und Nitrit-Assimilation.

(11) BAUDISCH, O.: Jahrb. Photogr. Reprod. Technik (1913). Über das chemische Verhalten anorganischer stickstoffhaltiger Pflanzennahrungsstoffe gegenüber dem Sonnenlicht.

(12) BAUDISCH, O.: Naturwissenschaften 2, 199, 229 (1914). Zur Frage der Assimilation anorganischer stickstoffhaltiger Verbindungen in dem Pflanzen.

(13) BAUDISCH, O., AND MAYER, E.: Z. physiol. Chem. 89, 176 (1914). Photochemische Studien zur Nitrat- und Nitrit-Assimilation.

(14) BAUDISCH, O.: Jahrb. Photogr. Reprod. Technik (1914). Über chemische Lichtwirkung auf grossen Bergeshöhen.

(15) BAUDISCH, O.: Ber. **49**, 1148 (1916). Über Nitrat- und Nitrit-Assimilation. VIII. Über Cholera. I.

(16) BAUDISCH, O.: Ber. **49**, 1176 (1916). Über Nitrat- und Nitrit-Assimilation. IX.

(17) BAUDISCH, O., AND KLINGER, G.: Ber. **49**, 1167 (1916). Über Nitrat- und Nitrit-Assimilation. X.

(18) BAUDISCH, O.: Ber. **49**, 1176 (1916). Über Nitrat- und Nitrit-Assimilation. XI.

(19) BAUDISCH, O.: Ber. **50**, 652 (1917). Über Nitrat- und Nitrit-Assimilation. XII. O. Loew nochmal zur Erwiderung.

(20) BAUDISCH, O.: Ber. **51**, 793 (1918). Über Nitrat- und Nitrit-Assimilation. XIII. Eisen und Sauerstoff als notwendige Agenzien für die Reduktion von Alkalinitriten mit autoxydablen Verbindungen.

(21) BAUDISCH, O.: Ber. **52**, 40 (1919). Über Nitrat- und Nitrit-Assimilation. XIV.

(22) BAUDISCH, O.: Ber. **52**, 40 (1919). Über Nitrat- und Nitrit-Assimilation. XV.

(23) BAUDISCH, O., AND MAYER, P.: Biochem. Z. **107**, 1 (1920). Studien über die Reduktion der Nitrite und Nitrate. Eine neue Methode zur quantitativen Bestimmung dieser Salze.

(24) BAUDISCH, O.: J. Biol. Chem. **48**, 489 (1921). The Mechanism of Reduction of Nitrates and Nitrites in Processes of Assimilation.

(25) BHARGANO, L. N., AND DHAR, N. R.: J. Indian Chem. Soc. **10**, 453 (1933).

(26) HANTZSCH, A.: Ber. **66**, 1566 (1930).

(27) HARTEK, PAUL: Ber. **66**, 423 (1933).

(28) VON NAGEL, A.: Z. Elektrochem. **36**, 754 (1930).

(29) VORLÄNDER, D., AND GOHDES, WERNER: Ber. **64**, 1776 (1931). Die Allgegenwart von Stickoxyden.

(80) ZINTEL, E., AND HARDER, A.: Ber. **66**, 760 (1933).

REFERENCES

(1) ALBRECHT, W. H.: Ber. **62**, 1475 (1929).

(2) ANGELI-ARNDT: Samml. chem. chem.-tech. Vortr. 1908, XIII.

(3) AVERY, O. T.: J. Exptl. Med. **34**, 38–40 (1921).

(4) BAMBERGER, E.: Ber. **33**, 1781 (1900).

(5) BAMBERGER, E.: Ber. **33**, 1939 (1900).

(6) BAMBERGER, E., AND BAUDISCH, O.: Ber. **42**, 3568 (1909).

(7) BARKER: Z. Krist. **45**, 1 (1908).

(8) BAUDISCH, O.: Chem.-Ztg. **33**, 1298 (1909).

(9) BAUDISCH, O.: Chem.-Ztg. **35**, 913 (1911).

(10) BAUDISCH, O.: Ber. **49**, 172 (1916); **50**, 334 (1917).

(11) BAUDISCH, O.: Naturwissenschaften **5**, 289 (1917). Innere Komplexsalz in ihrer Beziehung zur physiologischen Chemie.

(12) BAUDISCH, O.: Ber. **53**, 413 (1920).

(13) BAUDISCH, O.: Ber. **54**, 413 (1921).

(14) BAUDISCH, O.: Ber. **62**, 2699 (1929). Die Bedeutung von Austausch oder Verdrängungsreaktionen bei katalytischen Vorgängen.

(15) BAUDISCH, O.: Biochem. Z. **232**, 35 (1931). Über den Einfluss koordinative gebundener Gruppen auf die Eigenschaften des zentralen Eisenatoms in Eisencyanverbindungen.

(16) BAUDISCH, O.: Biochem. Z. **245**, 265 (1932).

(17) BAUDISCH, O.: Biochem. Z. **258**, 69 (1933).
(18) BAUDISCH, O.: Aktives Eisen. Carl Oppenheimer Handbuch der Biochemie des Menschen und der Tiere, 2te Auflage, Ergänzung zu Bd. I–III des Hauptwerks, p. 749. Gustav Fischer, Jena (1933).
(19) BAUDISCH, O., AND ALBRECHT, W. H.: Naturwissenschaften **20**, 639 (1932).
(20) BAUDISCH, O., AND ALBRECHT, W. H.: J. Am. Chem. Soc. **54**, 943 (1932).
(21) BAUDISCH, O., AND DAVIDSON, DAVID: J. Biol. Chem. **71**, 501 (1927).
(22) BAUDISCH, O., AND DUBOS, R.: Biochem. Z. **245**, 278 (1932).
(23) BAUDISCH, O., AND FÜRST, ROSE: Ber. **48**, 1665 (1915).
(24) BAUDISCH, O., GUREWITSCH, H., AND ROTHSCHILD, S.: Ber. **49**, 180 (1916).
(25) BAUDISCH, O., AND KARZEFF, N.: Ber. **45**, 1164 (1912).
(26) BAUDISCH, O., AND KING, VICTOR L.: Ind. Eng. Chem. **3**, 629 (1911).
(27) BAUDISCH, O., PISTOR, A. E., AND SILBERBLATT, B.: Ber. **49**, 191 (1916).
(28) BAUDISCH, O., AND ROM, HERMANN: Ber. **49**, 203 (1916).
(29) BAUDISCH, O., AND ROTHSCHILD, S.: Ber. **48**, 1660 (1915).
(30) BAUDISCH, O., AND WELO, L. A.: J. Biol. Chem. **61**, 261 (1924).
(31) BAUDISCH, O., AND WELO, L. A.: Naturwissenschaften **21**, 659 (1933).
(32) BILTZ, W.: Z. anorg. allgem. Chem. **145**, 63 (1925). Résumé of references.
(33) BILTZ, W.: Z. anorg. allgem. Chem. **164**, 245 (1927).
(34) BILTZ, W.: Z. anorg. allgem. chem. **164**, 245 (1927); **166**, 351 (1927).
(35) BILTZ, W., AND HÜTTIG, G.: Z. anorg. allgem. Chem. **114**, 241 (1920).
(36) BÖHM, I.: Z. Krist. **68**, 567 (1928).
(37) CAMBI, L.: Chem. Zentr. **1914**, II, 1100.
(38) CAMBI, L., AND SZEGÖ, L.: Ber. **66**, 656 (1933).
(39) ÇOSTACHESCU, N., AND SPACU, G.: Chem. Zentr. **1912**, II, 1972.
(40) DAVIDSON, D.: J. Am. Chem. Soc. **50**, 2622 (1928).
(41) EICHHOLTZ, F., AND BERG: Biochem. J. **225**, 353 (1930).
(42) EPHRAIM, F.: Ber. **50**, 1069 (1917); **51**, 644 (1918).
(43) FEIGL, F., AND BONDI, A.: Ber. **64**, 2819 (1931).
(44) GRIMM, H.: Naturwissenschaften **17**, 536 (1929). Isostere Molekeln.
(45) GROSSMANN AND HÜNSELER: Z. anorg. allgem. Chem. **46**, 370 (1905).
(46) HAUROWITZ, F., AND KITTEL, H.: Ber. **66**, 1046 (1933).
(47) HENSCHEL, H., AND RINNE, FR.: Ber. Sachs. Ges. Wiss. math.-phys. Klasse **79**, 17, 1 (1927).
(48) HIEBER, S.: Z. Elektrochem. **39**, 27 (1933); Ber. **60**, 2300 (1927); Z. anorg. allgem. Chem. **186**, 102 (1930); **190**, 197 (1930).
(49) HOFMANN, K. A.: Ann. **312**, 1 (1900).
(50) JOHNSON, T. B., AND BAUDISCH, O.: J. Am. Chem. Soc. **43**, 2670 (1921).
(51) KLAUS, F., AND BAUDISCH, O.: Ber. **51**, 1036 (1918).
(52) KLEMM, W., JACOBI, H., AND TILK, W.: Z. anorg. allgem. Chem. **201**, 1 (1931).
(53) KLEMM, W.: Z. anorg. allgem. Chem. **201**, 1 (1931); Z. angew. Chem. **190**, 193 (1930).
(54) KRAUSE, A.: Z. anorg. allgem. Chem. **208**, 282 (1932).
(55) LANGMUIR, I.: J. Am. Chem. Soc. **41**, 1573 (1919).
(56) LANGMUIR, I., AND HÜCKEL, W.: Z. Elektrochem. **27**, 305 (1921).
(57) LEY, H.: Die Beziehungen zwischen Farbe und Konstitution. S. Hirzel, Leipzig (1911).
(58) LWOFF, M.: Compt. rend. soc. biol. **107**, 1438 (1931); Ann. inst. Pasteur **50**, 1, 55, 707 (1933).
(59) MAGNUS, A.: Z. anorg. allgem. Chem. **124**, 293 (1922).

(60) MEISEL, K., AND TIEDJE, W.: Z. anorg. allgem. Chem. **164**, 223 (1927).

(61) MICHAELIS, L., AND SMYTHE, C. V.: J. Biol. Chem. **94**, 329 (1931).

(62) MÜLLER, E.: Z. Elektrochem. **31**, 43 (1925).

(63) PASCAL: Rev. gén. sci., July, 1923. Diamagnetism et Constitution Chimique.

(64) PFALTZ, M. H.: J. Am. Chem. Soc. **45**, 2980 (1923).

(65) PFALTZ, M. H., AND BAUDISCH, O.: J. Am. Chem. Soc. **45**, 2972 (1923).

(66) PFEIFFER, P.: Ber. **63**, 1811 (1930).

(67) RABINOWITSCH, E.: Z. Elektrochem. **39**, 702 (1933).

(68) RAIKOWA, THEODORA P.: Ber. **62**, 1626, 2142 (1929).

(69) RAY, R.: Z. anorg. allgem. Chem. **174**, 189 (1928).

(70) ROSENHEIM, A.: Z. anorg. allgem. Chem. **216**, 173 (1934).

(71) ROSENHEIM, A., ROEHRICH, E., AND TREWENDT, L.: Z. anorg. allgem. Chem. **207**, 107 (1932).

(72) SCHWARZ, ROBERT, AND BASS, LAWRENCE W.: The Chemistry of the Inorganic Complex Compounds. John Wiley and Sons, Inc., New York (1923).

(73) SPACU, G.: Z. anorg. allgem. Chem. **216**, 165 (1934).

(74) STELLING, O.: Z. physik. Chem. **16B**, 317 (1932).

(75) THILO, ERICH: Die Valenz der Metalle Fe, Co, Ni, Cu und ihre Verbindungen mit Dioximen. Samml. chem. chem.-tech. Vortr., neue Folge, Heft 13. Stuttgart (1932).

(76) TRAUBE, W., AND WOLFF, W.: Ber. **60**, 43 (1927).

(77) VAN ARKEL AND DE BOER: Chemische Bindung als elektrostatische Erscheinung, p. 161. Translated by Li Klemm and Wilhelm Klemm. S. Hirzel, Leipzig (1931).

(78) WEBSTER, L. T.: Proc. Soc. Exptl. Biol. **22**, 139 (1924).

(79) WEBSTER, L. T., AND BAUDISCH, O.: J. Exptl. Med. **42**, 473 (1925).

(80) WEINLAND, R.: Einführung in die Chemie der Komplexverbindungen, 2te Auflage. Ferdinand Enke, Stuttgart (1924).

(81) WELO, LARS A.: Phil. Mag. **6**, 481 (1928).

(82) WELO, L. A., AND BAUDISCH, O.: Nature **116**, 606 (1925).

(83) WELO, L. A., AND BAUDISCH, O.: Phil. Mag. **6**, 50, 399 (1925).

(84) WERNER, A.: Neuere Anschauungen auf dem Gebiete der anorganischen Chemie, 5te Auflage, neu bearbeitet von P. Pfeiffer. Braunschweig (1923).

(85) WYCKOFF, R. W. G., AND McCUTCHEON, TH. P.: Am. J. Sci. **13**, 223 (1927).