Less direct methods also give no indication of interactions of albumin with cations. The cloud-point technique[2] indicates the absence of complexes with aliphatic amines, although interpretation of the data is complicated by factors other than binding. The *p*H displacement method[14] reveals a similarity in behavior of the quaternary amines tetrapropylammonium iodide and trimethylphenylammonium iodide, and sodium iodide. Since there is strong evidence[12] that sodium ion is not bound by albumin, it seems reasonable to interpret the *p*H behavior of the quaternary ammonium ions in a like fashion.

Thus it is clear that organic cations of a wide variety of structures do not form complexes with serum albumin with an affinity even approaching that of complexes with anions of similar structure.

Comparison of Proteins.—The binding behavior of albumin is markedly different from that of the fibrous protein wool. Steinhardt and Zaiser[15] have shown that quaternary ammonium ions affect the titration curves of wool with bases in a manner analogous to the effects of anions on acid titrations. Thus both anions and cations of about equal size seem to be bound by wool.

The behavior of albumin toward organic cations is as predicted by the postulate[3] that residues with –OH groups interact preferentially with $-COO^-$ side chains. Because of this internal bonding, an organic cation must have a much stronger interaction energy to be bound at a $-COO^-$ site than an organic anion requires to be bound at an $\equiv NH^+$ site. Among molecules of similar size and structure, the anions are therefore bound much more strongly than cations.

From the hypothesis of preferential hydroxyl–carboxyl internal bonding it also follows that the unique character of serum albumin in anion–protein interactions should disappear in cation–protein interactions. Among the proteins previously compared[3] in anion affinities, since all except albumin have an excess of –OH groups over carboxylic side chains all should have little affinity for organic cations. The globular proteins bovine γ-globulin and trypsin behave as predicted, neither binding the diethylcyanine ion (Table III). Similarly γ-globulin shows no interaction with streptomycin (Table IV).

Thus in interactions with organic cations albumin loses its unique position and acts as a typical corpuscular protein.

Acknowledgment.—This investigation was supported in part by research grants from the National Institutes of Health, Public Health Service, and from the Office of Naval Research (Project Number NR 121-054).

EVANSTON, ILL. RECEIVED APRIL 18, 1951

(14) G. Scatchard and E. S. Black, *J. Phys. Colloid Chem.*, **53**, 88 (1949).

(15) J. Steinhardt and E. M. Zaiser, *J. Biol. Chem.*, **183**, 789 (1950).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, STATE UNIVERSITY OF IOWA]

The Effect of *p*H on the Combination of Serum Albumin with Metals[1]

BY CHARLES TANFORD

A polarographic study has been made of the effect of *p*H upon the interaction between a number of metals and bovine serum albumin. It is concluded that the principal sites on the protein molecule responsible for metal binding are the imidazole groups. Approximate values of the logarithms of the intrinsic association constants with these groups are: Cu^{++} (in 0.15 *M* KNO_3) 3.7, Zn^{++} (in 0.15 *M* KCl) 2.9, Cd^{++} (in 0.15 *M* KCl), 2.8. Pb^{++} (in 0.15 *M* KNO_3) < 2.3, Tl^+ (in 0.15 *M* KCl) < 0. Weak binding also occurs at carboxyl groups. The extent of binding at these sites is determined largely by the competitive effect of other anions present in solution.

It is well-known that the limiting polarographic current due to the reduction of a metallic ion (or other reducible substance) may be considerably decreased by the presence of proteins.[2,3] Previous work in this Laboratory[4] has shown that this decrease is due to complex formation between the metallic ion and the protein; while a metal ion involved in such a complex may still be reduced, the resulting current will be smaller because of the smaller diffusion coefficient of a metal ion bound to a protein molecule, and also because of the fact that the reduction itself may proceed slowly.

Work is currently under way in this Laboratory to develop a method for obtaining thermodynamic constants for protein–metal interaction from this decrease in the polarographic current. The present paper, however, describes how semi-quantitative information on metal–protein complexes can be obtained with great rapidity from the polarographic current depression. The information obtained is similar to that which can be gained from the observation of shifts in absorption spectrum peaks. The work reported is a study of complex formation between serum albumin and cadmium, zinc, lead, copper and thallium, and of the effect of *p*H upon the extent of interaction.

Experimental

Polarographic currents were measured on a Sargent model XXI polarograph. The capillary used has a flow rate of 2.65 mg. of mercury per sec., with a drop time varying between 3.6 and 4.0 sec., depending on the solution used. The cell designed for this work and the technique for oxygen removal have been described elsewhere.[5] Polarographic currents were measured by the extrapolation method.

Armour crystalline bovine serum albumin was used, and its concentration in stock solutions was determined by means of ultraviolet light absorption at 280 mμ wave

(1) Presented at the XIIth International Congress of Pure and Applied Chemistry, New York, N. Y., September, 1951.

(2) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941, p. 121.

(3) J. K. Taylor and R. E. Smith, *Anal. Chem.*, **22**, 495 (1950).

(4) C. Tanford, THIS JOURNAL, **73**, 2066 (1951).

(5) C. Tanford and J. Epstein, *Anal. Chem.*, **23**, 802 (1951).

length.[6] Metal salts were C.P. reagents, used without further purification. All solutions were made up so that the total ionic strength was 0.15. The *p*H was adjusted by the careful addition of dilute KOH, and measured on a Beckman model G *p*H meter. All measurements were made at 25.0°.

Results

The effect of variation in *p*H upon the polarographic current of solutions containing 4×10^{-4} *M* cadmium in 0.15 *M* potassium chloride is shown in Table I. It can be seen that *in the absence of protein* the current remains unchanged between *p*H 2.5 and 7, after which there is a decrease, accompanied by the appearance of a precipitate of $Cd(OH)_2$. The constancy of the current is due to the fact that, in solutions containing chloride, cadmium exists largely in the form of various chloride complexes, the relative concentrations of which are unaffected by *p*H. When precipitation begins, of course, cadmium is removed from solution, and the current is reduced. Entirely different behavior occurs *in the presence of serum albumin*. There is first a small reduction in current as the *p*H is increased from 1.7 to 5.25. This indicates weak combination between cadmium and serum albumin, increasing with increasing *p*H. It can be assumed that this is due to attachment of cadmium ions to carboxyl groups, since these groups become ionized, and, hence, available for reaction, in this *p*H range. Above *p*H 5.25 a profound change takes place, and the limiting current drops rapidly to about 20% of the value it would have had in the absence of protein. Since the *p*H range in which this drop occurs is the range in which the imidazole groups of serum albumin lose their positive charge,[8] it can be concluded that very strong interaction occurs between cadmium and the neutral imidazole groups of albumin. It is probable that when the current has dropped to about 20% of its value in the absence of protein, virtually all of the metal is bound to the protein,[4] the current observed being due to the slow reduction of protein-bound cadmium. As the *p*H is increased above *p*H 8 the amino groups of the albumin molecule become available for reaction with cadmium. No further change in current takes place, however, because virtually all of the cadmium is already bound to imidazole groups at *p*H 8.[9]

TABLE I

LIMITING CURRENT FOR 4.0×10^{-4} *M* CADMIUM IN POTASSIUM CHLORIDE SOLUTIONS (μ 0.15)

	Without protein		In presence of 1.23 g. S.A./100 ml.		
*p*H	$(i_L)_0$, μa.		*p*H	i_L, μa[b]	$i_L/(i_L)_0$
2.5	3.34		1.69	3.25	0.97
3.1	3.34	Ave.	4.50	3.22	.96
6.5	3.41	3.35	5.25	3.07	.915
6.6	3.31		6.00	1.89	.565
7.2	3.24		6.89	1.00	.30
8.6	1.46[a]		7.68	0.72	.215
			8.95	.64	.19

[a] Precipitate of $Cd(OH)_2$ present. [b] These values were obtained by interpolation from experiments at a series of different protein concentrations.

The most convenient way to represent these data is by means of a plot of the ratio of the current observed in the presence of protein, i_L, to that observed for a similar solution in the absence of protein, $(i_L)_0$, against *p*H. For the experiments on cadmium this ratio is given in the last column of Table I, and is plotted against *p*H in Fig. 1. Above *p*H 7 the value of $(i_L)_0$ has been assumed the same as in the range acid to *p*H 7. This, of course, is very reasonable since that part of the cadmium still in solution in the free form will exist in the same chloride complexes as it does acid to *p*H 7. The only reason for the drop in current which takes place above *p*H 7 in the absence of protein is the fact that some of the cadmium is removed from solution by precipitation. No precipitation is observed in the presence of protein because the concentration of free cadmium above *p*H 7 is very small, *i.e.*, the solubility product constant for $Cd(OH)_2$ is not exceeded.

Data similar to those given in Table I have been obtained for the other metals studied. The results are all summarized in Fig. 1, and are discussed in the following paragraphs.

In the case of thallium there is no change in diffusion current with *p*H in the absence of protein. What is much more remarkable is that there is also virtually no change in the presence of serum albumin. Even what little change does occur, a decrease of 1 to 2%, can be accounted for in part by the fact that there is a small reduction in the drop time of the capillary when serum albumin is present. It can therefore be concluded that there is virtually no interaction between serum albumin and thallous ion up to *p*H 10.

The results obtained with zinc are very similar to those obtained with cadmium, and no special discussion of them is required. No measurements can be made with zinc at acid *p*H values because the zinc wave there overlaps with the wave due to reduction of hydrogen ion.

The determinations with lead and copper were made using potassium nitrate as supporting electrolyte in place of potassium chloride. In this case it was found that some change in current with *p*H occurs even in the absence of protein. The reason for this is not quite clear. The formation of $M(OH)^+$ ions with increasing *p*H may be partly responsible. Precipitation of lead and copper hydroxides takes place at a *p*H about 0.5 lower than was found for the precipitation of cadmium hydroxide. In computing $i_L/(i_L)_0$ for these metals the change in $(i_L)_0$ with *p*H has been taken into consideration. Alkaline to the precipitation *p*H value of $(i_L)_0$ has again been assumed constant.

Figure 1 shows that, like zinc and cadmium, lead and copper are apparently bound principally by the imidazole groups of serum albumin. However, lead is bound much less strongly than cadmium or zinc, while copper appears to be bound much more strongly. Some copper binding also

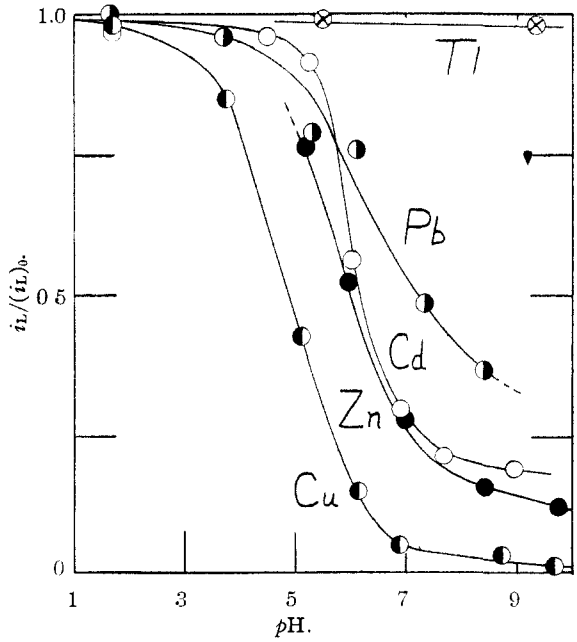


Fig. 1.—The ratio $i_L/(i_L)_0$ as a function of *p*H. The protein concentration is 1.23 g./100 ml. of solution.

(6) The method was standardized by measurements on solutions whose concentration was determined independently by drying at 105°. The value of $E_{1\%}^{1\ cm.}$ was found to be 6.60, in agreement with a value reported by Cohn, Hughes and Weare (ref. 7).

(7) E. J. Cohn, W. L. Hughes, Jr., and J. H. Weare, THIS JOURNAL, 69, 1753 (1947).

(8) C. Tanford, *ibid.*, **72**, 441 (1950).

(9) It is interesting to compare these data with those obtained in a preliminary study using pepsin instead of serum albumin. Pepsin has only two imidazole groups per molecule (ref. 10), and it is found that the current depression of a 4×10^{-4} *M* cadmium solution in the presence of pepsin is much less sharp from *p*H 5 to 7, and continues until a *p*H of 10 is reached, *i.e.*, in this case amino, tyrosyl or sulfhydryl groups must be utilized to produce complete binding.

(10) G. R. Tristram, *Advances in Protein Chemistry*, **5**, 145 (1949).

takes place over the acid range, presumably on carboxyl groups.[11]

pH for 50% Reaction.—It has been seen above that most of the change in the polarographic current occurs roughly in the pH region in which the albumin imidazole groups lose their protons. Actually, of course, the region of combination is displaced somewhat to the acid side of the normal imidazole ionization range, because the very fact of combination stabilizes the neutral form of this group, and, therefore, decreases the apparent ionization constant. The shift to the acid side is a good measure of the strength of combination. From this fact, too, therefore, it can be concluded from Fig. 1 that the strongest complexes with the albumin imidazole groups are formed by copper, followed by zinc, cadmium and lead.

Although the experiments here described were not originally intended for precise analysis, approximate values of the complexing constants can be computed from the data on the pH shift. As has been previously mentioned,[4] the total polarographic current can be expressed as the sum of two terms, one for free metal (concentration C_F) and one for protein-bound metal (concentration C_B)

$$i_L = A(C_F + \alpha C_B) \qquad (1)$$

where A is the normal constant relating current and concentration in the absence of protein, and α is a small fraction. Where all experiments are conducted at the same total metal concentration, C_O, therefore

$$i_L/(i_L)_0 = (C_F + \alpha C_B)/C_O \qquad (2)$$

If it is assumed that α is a constant, then α can be taken to be the value of $i_L(i_L)_0$ when all the metal is protein-bound, *i.e.*, when $C_B = C_O$, and can be obtained by extrapolation of the curves of Fig. 1. Values of α so obtained are given in Table II.[12]

TABLE II

APPROXIMATE INTERACTION CONSTANTS

	α	$i_L/(i_L)_0$ at 50% Rxn.	pH at 50% Rxn.	log $K°$	log $K°$ 25% Rxn.	log $K°$ 75% Rxn.
Cu	0	0.50	4.96	3.7	3.9	3.3
Zn	0.10	.55	5.82	2.9	3.0	2.8
Cd	.18	.59	5.93	2.8	2.6	2.9
Pb	.20	.60	7.35	<2.3	<2.6	<2.4
Tl	..	..	..	<0	...	...

Using these values, it is possible to calculate the current ratios corresponding to 50% combination from equation (2), and, hence, to read off the corresponding pH values from Fig. 1. These values are also listed in Table II. For equilibrium between metal and the imidazole sites we now have the equation

$$\frac{\bar{\nu}}{(n - \bar{\nu} - \bar{\nu}_H)C_F} = K° e^{-2Z_M Z_P w} \qquad (3)$$

where n is the total number of such sites, in this case 17,[13] $\bar{\nu}$ the number of these sites covered by metal ions, and $\bar{\nu}_H$ the number which are protonated, each number referring to one albumin molecule. $K°$ is the intrinsic association constant between the metal and the imidazole group, and the exponential factor is the usual electrostatic correction factor.[14] In this factor, Z_M represents the charge on the metal ion, Z_P the charge on the protein molecule, and w is a constant which has the value 0.0303 for human serum albumin at 25° and ionic strength 0.15,[8] and, since the molecules have the same size, presumably the same value for bovine albumin. A similar equation can be written for the association of imidazole groups with hydrogen ion

$$\frac{\bar{\nu}_H}{(n - \bar{\nu} - \bar{\nu}_H)a_{H^+}} = K_H^\circ e^{-2Z_P w} \qquad (4)$$

where the value of intrinsic constant is known from the author's work on human albumin (log $K_H^\circ = 6.10$).[8] The charge on the metal ion, Z_M, has the value +2 for Cu^{++}. For cadmium in 0.15 M KCl, however, the major component is $CdCl^+$.[15] There appears to be no reason why the chloride ion should be lost on protein binding, so that the effective change on the metal ion is +1 rather than +2. For zinc in KCl the tendency for association with chloride ion is less than for cadmium,[16] but the average composition is still probably closer to $ZnCl^+$ than to Zn^{++}. For this metal, therefore, Z_M has also been placed equal to unity. The extent of association of lead with nitrate is also considerable.[16] A charge of +1 has been assigned here, but this unquestionably overcorrects for the association, and the constants computed for lead are therefore certainly too high. To compute Z_P, we need to know the charge on the albumin molecule due to hydrogen ion dissociation, that contributed by bound anions, and that contributed by bound metal. The first of these, at any pH, is taken to be the same as for human serum albumin at that pH and at the same ionic strength and temperature[8,17]; data on chloride binding by serum albumin are taken from the work of Scatchard and co-workers[18]; nitrate ion is assumed to be bound to about the same extent.[19] The contribution of the bound metal to the charge, of course, is obtained by multiplying $\bar{\nu}$ by Z_M.

It remains only to calculate $\bar{\nu}$ and C_F at the point of 50% combination. Since in all of the solutions used in this study the metal concentration is 4×10^{-4} M, the concentration of free metal (in all of its forms) at the point of 50% combination must be 2×10^{-4} M. The concentration of metal bound to the imidazole groups must have the same value (ignoring the small amount bound to carboxyl groups). Since the protein concentration in each case is 1.78×10^{-4} M, the value of $\bar{\nu}$ must be 1.12. Using equation (4), it is now possible to calculate $\bar{\nu}_H$ at the measured pH for 50% reaction, and, substituting this value in equation (3) immediately leads to a value for the intrinsic binding constant, $K°$. The values so calculated are shown in Table II. In a similar way $K°$ values can be obtained from the pH at 25 or 75% combination. These values are also listed in Table II. They are in satisfactory agreement with the values obtained from the point of 50% combination, except in the case of copper (see below).

A constant has also been calculated for thallium, in a different manner. It is that which would result in a 2% reduction of the polarographic current at

(11) It might be mentioned here that complications arise in the measurement of polarographic currents of lead and copper in KNO_3 in the presence of albumin acid to pH 4. The values can still be determined with an accuracy of a few per cent., however. The complications which arise are not in any way connected with metal–protein complex formation and will be discussed elsewhere when they have been fully interpreted.

(12) In view of the steepness of the curves of Fig. 1, there is actually little error involved in the choice of α. For cadmium, for example, if α were zero rather than 0.18, the pH at the point of 50% combination, corresponding to $i_L/(i_L)_\infty = 0.50$ rather than 0.59, would be 6.17 instead of 5.93. The resulting value of log $K°$ would be 2.6, while the values for 25 and 75% combination would be 2.5 and 2.6, respectively. These values do not differ significantly from those obtained with α equal to 0.18. For zinc and copper the possible range of α is smaller than for cadmium, so that the error that can result from the choice of α is even less. It might be mentioned that it is theoretically not impossible that α may vary with pH. Studies currently in progress, however, indicate that such variation is likely to be quite small.

(13) B. Brand, *Ann. N. Y. Acad. Sci.*, **47**, 187 (1946).

(14) G. Scatchard, *ibid.*, **51**, 660 (1949).

(15) I. Leden, *Z. physik. Chem.*, **A188**, 160 (1941).

(16) J. Bjerrum, *Chem. Revs.*, **41**, 381 (1950).

(17) The binding of metal at the imidazole sites, of course, disturbs the hydrogen ion equilibrium. As long as $\bar{\nu}$ is only about 1, as is true here, the error involved is only of the order of a few hundredths in log $K°$.

(18) G. Scatchard, A. C. Batchelder and A. Brown, THIS JOURNAL, **68**, 2320 (1946); G. Scatchard, I. H. Scheinberg and S. H. Armstrong, Jr., *ibid.*, **72**, 535 (1950).

(19) Actually nitrate is probably somewhat more tightly bound. Once again, however, the effect on log $K°$ would be in the second decimal place only.

pH 9, as actually observed. Since this reduction can be accounted for in part on the basis of drop time reduction, the value given represents a maximum value for thallium–albumin interaction.

It is important to emphasize that these constants are merely approximate. It is not possible to obtain a thermodynamically sound association constant where the metal ion exists in a number of different complexes with the anions of the supporting electrolyte, as is true in every case here studied, except copper and thallium. In the case of copper another error is brought in: there is undoubtedly considerable copper binding by the carboxyl groups of the albumin molecule. This would lead to relatively high values of K° at lower pH's. The values themselves, however, may well be too low rather than too high.

Finally, while in the present case a considerable error in the choice of α is apparently permissible (footnote 12), precise values of α must be known if precise values of the association constants are to be computed from polarographic currents.

In spite of their relative inaccuracy, the constants here found serve a useful purpose in that they confirm the supposition that it is the imidazole groups which are primarily concerned with metal binding. If it were not so, the constants obtained from the points of 25, 50 and 75% combination would not agree so well. If, for example, it is supposed that the binding takes place at the ϵ-amino groups instead, only poor agreement can be obtained. For zinc, for example, using 9.40 for $\log K^{\circ}_{H}$ of the amino groups,[8] one would obtain $\log K^\circ$ values of 5.8, 5.6, and 5.2, from the points at 25, 50 and 75% reaction, respectively. These values not only show systematic variation, but are also much higher than one would expect for combination with amino groups ($\log K$ for the association of zinc with one ammonia molecule is only 2.37[16]).

Half-wave Potentials.—Quantitative information on complex stability can be obtained from polarographic half-wave potentials only if special conditions are met.[20] These conditions are not satisfied by protein–metal systems even if the polarographic reduction proceeds reversibly.[21] However, observed changes in half-wave potential do serve as qualitative confirmation of the conclusions reached above. Thus the half-wave potentials of cadmium in acid solutions and of thallium at all pH values show a change of less than 0.01 volt upon the addition of protein. Alkaline to pH 6, however, shifts of several hundredth of a volt are observed for lead, cadmium and zinc, while a shift of about 0.2 volt occurs with copper. These observations substantiate the conclusion that the copper complex with the imidazole groups of serum albumin is stronger than that formed by any of the other metals.

Solubility of Metals Above pH 7.—As has already been mentioned, solutions of lead, cadmium, zinc or copper normally precipitate metal hydroxides; for 4×10^{-4} M solutions such a precipitate begins to appear near pH 7. No such precipitate appears if a complexing agent is present which removes free metal ions from solution. As expected, therefore, no precipitation occurs at pH 7 in the presence of serum albumin. With lead, and lead alone, a precipitate of lead hydroxide *does* appear above pH 9. This is further proof that at that pH all of the metal has entered into combination with albumin under the conditions of our experiments in the case of copper, zinc and cadmium, but not in the case of lead.

Absorption Spectra.—The studies of Klotz and co-workers[22] on the effect of pH on the absorption spectra of copper–serum albumin solutions lead to precisely the same results as are reported here. There is an increase in absorbance, but little change in frequency, upon the addition of albumin to copper sulfate near pH 4. A very pronounced shift takes place at pH 7, however, which is due to the strong copper complex with imidazole groups.

Discussion

One of the most interesting questions on the combination of metals with proteins is: can the affinity be explained merely on the basis of the combining power of the protein's constituent amino acid residues? To answer this question for the results reported in this paper we should need data on the combination of imidazole with metals. Unfortunately, such data are not available.[23] It is interesting to observe, however, that the order of complexing strength found in this investigation, namely, $Cu^{++} > Zn^{++} > Cd^{++} > Pb^{++}$, is exactly the same as has been observed by Irving and Williams for these metals in complexes with 8-hydroxyquinoline and dithizone, both of which also contain, as does the imidazole group, secondary or tertiary amino nitrogen.[24]

An interesting analysis also can be made of the extent of combination with the albumin carboxyl groups. Below pH 4 the imidazole groups of serum albumin are all positively charged, as mentioned above, and all the combination with metals presumably occurs at carboxyl groups. This combination is evidently quite weak, so much so that the extent of combination may be largely determined by the competitive effect of the anions of the supporting electrolyte. At pH 4, at an ionic strength of 0.15, about 65 of the carboxyl groups of serum albumin are ionized and available for reaction.[8] In solutions containing 1.23 g. of albumin per 100 ml. the albumin concentration, using 69,000 as its molecular weight,[7] is 1.78×10^{-4} M, *i.e.*, the effective carboxyl concentration is 0.012 M, compared to the available anion concentration of 0.15 M. If the reactivity of the carboxyl groups is taken to be roughly equivalent to that of acetate ions, we can use the recent compilation by Bjerrum[16] on the tendency for complex formation by metals with various anions to determine the relative extent of protein-binding expected. Using the figures of Table III we see that copper, lead, and cadmium all show considerable tendency for formation of acetate complexes. In the case of copper there is no tendency for formation of nitrate complexes, *i.e.*,

(20) Ref. 1, p. 161.

(21) Ref. 8, footnote 8.

(22) I. M. Klotz, I. L. Faller and J. M. Urquhart, *J. Phys. Colloid Chem.*, **54**, 18 (1950).

(23) Data on the combination of histidine with metals are not valid because the carboxyl and amino groups present in histidine are not available when the histidine is part of a peptide chain.

(24) H. Irving and R. J. P. Williams, *Nature*, **162**, 746 (1948).

TABLE III

TENDENCY FOR COMPLEX FORMATION (BJERRUM)

	Supporting electrolyte anions		
	Ac^-	Cl^-	NO_3^-
Cu^{++}	2.6	...	<0
Pb^{++}	2.7	...	(1.1)
Cd^{++}	2.2	2.3	...
Tl^+	(0)	(0.7)	...

no competition from the anions of the supporting electrolyte. Such competition does exist in the case of lead, however, and is very strong in the case of cadmium. Hence it would be expected that the strongest reaction with the carboxyl groups of serum albumin, in the presence of the particular supporting electrolytes used in this investigation, will occur with copper. Lead should be next strongest, with cadmium showing little interaction. This is precisely what is observed acid to *p*H 4, as can be seen from an examination of Fig. 1. No combination at all is to be expected for thallium.

Note.—After this paper was completed, it was learned that a precise study of the reaction between zinc and human serum albumin by the equilibrium dialysis method has been made by F. R. N. Gurd and D. S. Goodman, of Harvard University. Their results, too, show that binding occurs primarily at the imidazole groups. The value of log K_0 at 0° in 0.15 *M* $NaNO_3$ was found to be 2.82; the value of 2.9 given in this paper (Table II) is in good agreement with that. Gurd and Goodman have also studied the reaction between zinc and imidazole itself, and have found for the logarithm of the first association constant the value 2.76. At the same time, G. Felsenfeld and J. T. Edsall determined the association constants between copper and imidazole: for the logarithm of the first constant in 0.15 *M* $NaNO_3$ at 22.5° they obtain the value 4.40. This value is higher than that reported in this paper for serum albumin, but not too much so, considering that carboxyl group binding, which is very pronounced in the case of copper, has been entirely ignored in our analysis. In any event, these values indicate that the properties of the imidazole groups of serum albumin, with respect to metal binding, are certainly not very different from the properties of imidazole itself.

IOWA CITY, IOWA RECEIVED MAY 14, 1951

[CONTRIBUTION FROM THE CHEMISTRY LABORATORY OF THE UNIVERSITY OF MICHIGAN]

Preparation and Properties of N-Monosubstituted Ethylenediamine Complexes of Cobalt and Nickel[1,2]

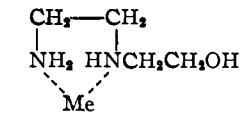
BY R. N. KELLER[3a] AND L. J. EDWARDS[3b]

The tris-cobalt(III) complex of $NH_2CH_2CH_2NHCH_2CH_2OH$ was prepared with the expectation that coördination through the nitrogen atoms would allow the hydroxyl group to remain free for further reaction. Not only did these hydroxyl groups appear to be unresponsive chemically but the resulting compound showed a remarkable inertness toward most common reagents. Other cobalt(III) and nickel(II) compounds of this hydroxy-diamine and other N-monosubstituted ethylenediamines were prepared but none possessed the inert character of the above complex. Of the hydroxy derivatives investigated, the greatest stability of the coördination compounds is attained when the hydroxyl group is two carbon atoms removed from the nitrogen.

Introduction

The change in properties and reactivity of the central metal ion accompanying coördination has been quite thoroughly investigated, but little attention has been given to the effect of coördination upon the chemical nature of the ligands in a complex compound. Sparsely scattered throughout the literature are references to a number of reactions which have been carried out on coordinated molecules and ions but most of these reactions are merely incidental to other lines of investigation.[4-8]

In the present work the first substance studied as a coördinating molecule was the diamine N-hydroxyethylethylenediamine, $NH_2CH_2CH_2NHCH_2CH_2OH$. Coördination to a metal ion would be expected to occur through the nitrogen atoms



The hydroxyl group consequently should be left free for further reaction. If this group retains typical organic characteristics in the resulting coordination compound, esterification of the group or replacement with other groups might be anticipated. The unexpected extreme stability encountered in the tris-hydroxyethylethylenediamine cobalt(III) salts and the unreactivity of the hydroxyl group in these complexes prompted an investigation of other N-monosubstituted ethylenediamine complexes. The diamines employed and the symbolization used in this paper are indicated below.

Diamine	Symbol
Ethylenediamine, $NH_2CH_2CH_2NH_2$	en
N-Methylethylenediamine, $NH_2CH_2CH_2NHCH_3$	me-en
N-Ethylethylenediamine, $NH_2CH_2CH_2NHCH_2CH_3$	et-en
N-(*n*-Propyl)-ethylenediamine, $NH_2CH_2CH_2NHCH_2CH_2CH_3$	pr-en
N-Hydroxyethylethylenediamine, $NH_2CH_2CH_2NHCH_2CH_2OH$	etol-en

(1) From the Ph.D. Thesis of L. J. Edwards, University of Michigan, June, 1950.

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