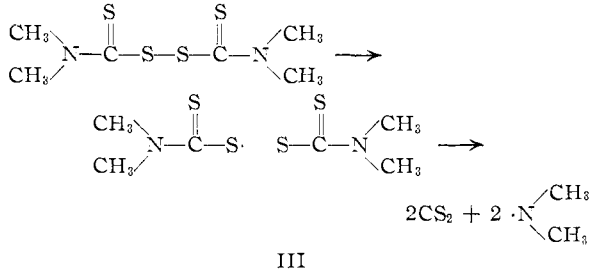
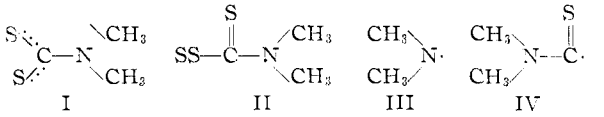
an over-all reaction for generation of radicals is as given below.



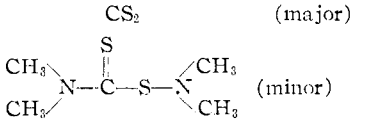
III

This picture has the advantage that it explains the pyrolysis products of TMTD. The radicals present due to the two possible modes of decomposition of TMTD are

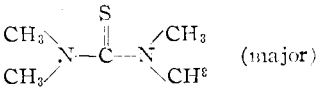


I II III IV

The observed pyrolysis products are[17]



and

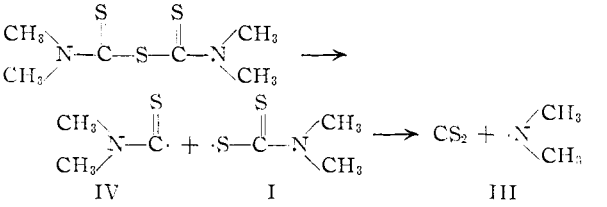


CS_2 is produced in the decomposition of intermediate I above.

The major product—tetramethylthiourea—is produced by the combination of radicals III and IV; the minor product by the combination of I + III or 2(III) + CS_2. The other possible combinations of these radicals would lead in general to regeneration of thiuram sulfides (mono, di, tri and tetra) and these would then go through the same sort of decomposition as described above. From the higher polysulfides one might expect to eventually get a trace of sulfur also, as has been observed.

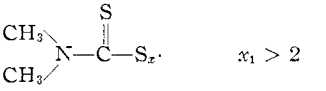
Koch[14] also has suggested a different complex mode of decomposition.

The behavior of TMTM also fits in the above picture. There is no possibility of forming radical II and this agrees with the fact that no inhibition is observed. Initiation can come about by a splitting at the C–S bond followed by splitting out of CS_2 in analogy with the above picture.



IV I III

The decomposition of the higher thiuram polysulfides would lead to the initiating radical but also to twice as many retarding radicals like II and a number of higher radicals which also would be expected to be retarders.[11] Thus in these compounds the initiating activity might be concealed by the large retarding power.



In the case of diphenyl disulfide cleavage at the S–S bond would produce the radicals $C_6H_5S\cdot$ which would have initiating activity, while induced cleavage at a C–S bond would produce the inhibiting radical $C_6H_5SS\cdot$.

This mechanism for the decomposition of thiuram compounds cannot be regarded as proved. However, it is in accord with all the facts which have been obtained in this polymerization study with this class of compounds.

PRINCETON, NEW JERSEY

(17) J. v. Braun and K. Weissbach, *Ber.*, **63**, 2836 (1930).

[CONTRIBUTION FROM THE PHYSICAL CHEMISTRY DIVISION, NATIONAL CHEMICAL LABORATORY]

Metal Protein Interactions in Buffer Solutions. Part II. A Polarographic Study of the Interaction of Zn^{II} and Cd^{II} with Bovine Serum Albumin

BY M. S. NARSINGA RAO[1] AND HIRA LAL

RECEIVED DECEMBER 2, 1957

It is concluded that binding data obtained from polarographic current ratios are comparable in accuracy with those obtained from equilibrium dialysis. A polarograpic study of competition between Zn^{II} and Cd^{II} for interaction with bovine albumin has been made: it is concluded that bovine albumin contains two sites, more reactive than imidazole, which combine with Zn^{II} in preference to Cd^{II}. It has been shown that the intrinsic constant for the interaction of Cd^{II} with bovine albumin, the two most reactive sites of which are covered by Zn^{II} ions, agrees well with the expected value for a 1:1 interaction with imidazole sites.

In Paper I,[2] we outlined general concepts governing the interpretation of binding data obtained in buffer solutions. Binding data themselves have been obtained by equilibrium dialysis and polarographic measurements. Whereas the major data for the binding of metal ions by bovine serum albumins have been obtained by equilibrium dialysis, we thought it worth while to investigate a selected few metal–bovine albumin systems polarographically, and to see if these measurements yield binding data comparable in accuracy to those obtained

(1) Department of Chemistry, Clark University, Worcester 10, Mass.

(2) H. Lal and M. S. N. Rao, THIS JOURNAL, **79**, 3050 (1957).

from equilibrium dialysis. Saroff and Mark[3] have indeed shown that, in the lower binding region at least, the binding data obtained from diffusion currents agree well with those obtained from equilibrium dialysis.

It is well known that the limiting current due to the reduction of metal ions at the dropping mercury electrode may be considerably depressed by the presence of proteins. Tanford[4] has expressed the diffusion current in the presence of protein as a sum of two terms—one for the free and the other for the protein-bound metal ion; thus

$$(i_d)_p/i_d = \frac{A + \alpha(A_0 - A)}{A_0} = \frac{A + \alpha\bar{\nu}c_p}{A_0} = 1 - \frac{(1 - \alpha)\bar{\nu}c_p}{A_0} \quad (1)$$

In the above equation $(i_d)_p$ and i_d are the diffusion currents with and without the protein, respectively, A_0 the total concentration of metal ions and A that of free metal ions at equilibrium, $\bar{\nu}$ the average number of metal ions bound per protein molecule, c_p the concentration of the protein and α a small fraction indicative of the characteristics of the reduction of metal–protein complex ion at the dropping mercury electrode.

The binding data (*i.e.*, $\bar{\nu}$ and A) can be evaluated from current ratios provided the constant α is known. This constant has been determined by Tanford[4] for the systems: Cu^{II}–NBSA ($\alpha = 0$), Zn^{II}–NBSA ($\alpha = 0.10$), Cd^{II}–NBSA ($\alpha = 0.18$) and Pb^{II}–NBSA ($\alpha = 0.20$). With fixed protein and total metal concentrations (1.23% and 4×10^{-4} M, respectively), the current ratios were measured at various *p*H values, the limiting value of the current ratio being equated to α. From the polarographic data necessarily limited to a low binding region in view of the metal concentration used, Tanford[4] has concluded that the principal sites responsible for binding metal ions are the imidazole groups of the bovine serum albumin molecule. Saroff and Mark[3] have reached a similar conclusion from their study of the interaction of zinc ion with bovine albumin in an acetate buffer of *p*H 6.1 and ionic strength 0.05.

The present paper outlines a polarographic study of the interaction of Zn^{II} and Cd^{II} ions with bovine serum albumin and its derivatives in an acetate buffer of *p*H 6.50 and ionic strength 0.20.

Experimental

The native bovine serum albumin (NBSA) and its esterified (EBSA) and acetylated (ABSA) derivatives were as described previously.[2] One per cent. protein solutions were used. All measurements were made in a standard acetate buffer of *p*H 6.50 and ionic strength 0.20 at 30 ± 0.1°. The buffer solution as made from Analar grade sodium acetate gave a small polarographic wave at $E_{1/2} = -1.0$ v., thus interfering seriously with the studies involving zinc ion ($E_{1/2} = -1.02$ v.). In this and other cases, where the slight heavy metal impurity was likely to affect the results, buffer solution was made by adding Analar grade acetic acid to a solution of Merck grade sodium hydroxide and adjusting the *p*H to 6.50. This solution did not contain any detectable metallic impurity.

The polarographic constant, K, relating diffusion current with metal ion concentration, was measured from polarographic analyses of standard solutions of zinc sulfate and cadmium acetate in the acetate buffer medium. The diffusion current was found to be proportional to the concentration of metal ion. The constant K had a value of 8.99 μa. per millimole of zinc ion per liter (flow rate of mercury, $m = 3.26$ mg. per sec., drop time, $t = 2.3$ sec. per drop at $E_{1/2} = -1.02$ v.) and 7.99 μa. per millimole per liter of cadmium ion ($m = 2.9$ mg. per sec., $t = 2.67$ sec. per drop at $E_{1/2} = 0.60$ v.). The corresponding values calculated from the Ilkovic equation were 8.59 and 8.11 for the zinc and cadmium ions, respectively.

Diffusion current measurements were made with a Tinsley Polarograph using a conventional H-type cell. One limb of the cell formed the saturated calomel electrode and was connected to the other limb containing the experimental solution (about 7 ml.) through a sintered glass disc and an agar bridge. The limbs were joined through a ground glass joint. The experimental limb could thus be detached and cleaned satisfactorily. Standard procedure for polarographic measurements was followed. The buffer medium acted as the supporting electrolyte. Dissolved oxygen was removed by passing O_2-free nitrogen through the solutions for 30–45 minutes.

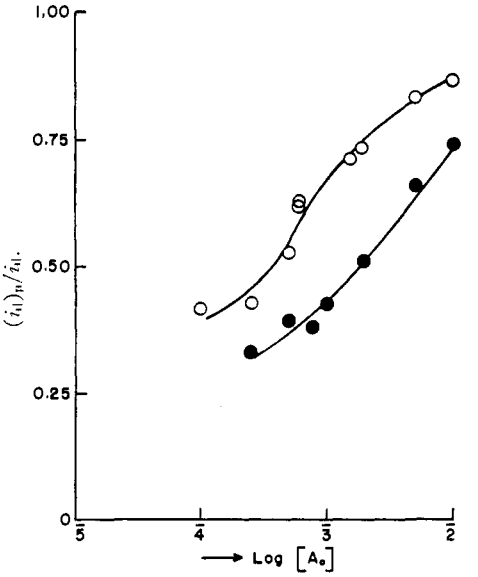


Fig. 1.—Zn^{II}–NBSA, ⊙; Zn^{II}–ABSA, ●.

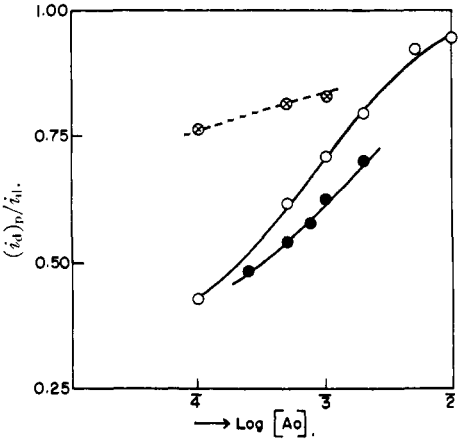


Fig. 2.—Cd^{II}–NBSA, ⊙; Cd^{II}–ABSA, ●; Cd^{II}–EBSA, ⊗.

Results and Discussion

The values for the current ratio, $(i_d)_p/i_d$, are represented in Fig. 1 as a function of the logarithm of the total metal ion concentration, A_0, for the systems Zn^{II}–NBSA and Zn^{II}–ABSA. Similar plots for cadmium–serum albumin systems are shown in

(3) H. A. Saroff and H. J. Mark, THIS JOURNAL, **75**, 1420 (1953).

(4) C. Tanford, *ibid.*, **74**, 211 (1952); see also C. Tanford, *ibid.*, **73**, 2066 (1951).

Fig. 2. It may be noticed that the acetylated derivative causes a much larger reduction in the diffusion current than the native protein due, no doubt, to a greater binding of metal ions by the former. . The esterified derivative does not seem to have much ability to bind the cadmium ion—a conclusion supported by equilibrium dialysis studies.[5] Similar experiments for the system Zn^{II}–EBSA, however, were not successful as the ester shifted the potential for hydrogen ion discharge to the vicinity of that for the zinc ion, thus rendering the evaluation of the requisite diffusion current impossible.

An evaluation of the binding data from the current ratio entails a knowledge of α (eq. 1). For reasons which will become obvious presently, we have preferred to estimate α from the diffusion currents given by equilibrium-dialyzed solutions together with the corresponding binding data. Thus

$$\alpha = \frac{(i_d)_{\text{internal}} - (i_d)_{\text{external}}}{Kc_p\bar{\nu}} \qquad (2)$$

where $(i_d)_{\text{internal}}$ and $(i_d)_{\text{external}}$ are the diffusion currents given by the protein-containing and protein-free solutions at equilibrium, and the other terms are as defined earlier. The constant α thus determined was found to be independent of metal ion concentration (and hence of $\bar{\nu}$) up to 2.0×10^{-3} M. Measurements at larger concentrations of metal ion were, however, less reliable. The relevant values of α are given in Table I.

TABLE I

VALUES OF α IN ACETATE BUFFER

(pH 6.50, ionic strength 0.20, 30°)

	Zn^{II}	Cd^{II}
NBSA	0.22 ± 0.03	0.30 ± 0.02
ABSA	0.20 ± 0.03	0.28 ± 0.01

It may be concluded therefore that, for a given system, the quantity α is constant and independent of $\bar{\nu}$. It may be expected, therefore, that the binding data evaluated from the current ratios by the use of eq. 1 would agree with the equilibrium dialysis data. A comparison of equilibrium dialysis data with those derived from polarographic measurements reveals that this is in fact the case.[5]

The values of α given in Table I differ markedly from those reported by Tanford. A few experiments were conducted to study the effect of pH on the constant α. Studies were made at pH 5.60, 5.93 and 6.50 in the acetate buffer of ionic strength 0.20. Keeping the protein and Zn^{II} ion concentrations fixed at 1.0% and 1.0×10^{-3} M, respectively, the extent of binding and the value of α were determined by equilibrium dialysis supplemented by polarographic measurements. The relevant values are given in Table II.

TABLE II

THE EFFECT OF pH ON THE VALUE OF CONSTANT α FOR SYSTEMS Zn^{II}–NBSA AND Cd^{II}–NBSA

pH	Zn^{II}	Cd^{II}
5.60	0.32	..
5.93	.30	..
6.50	.22	0.30
Limiting value (Tanford)	.10	0.18

(5) M. S. N. Rao and H. Lal, THIS JOURNAL, **80**, 3226 (1958).

It may therefore be concluded that the value of α decreases with pH. This conclusion is further supported by the fact that the values reported by Tanford are appreciably less—a result which may be expected from an extension of our data to pH values larger than 6.50. It would thus appear that the suitability of the limiting method of Tanford for the evaluation of α needs to be reconsidered for the simple reason that this constant is pH-dependent.

It may be of interest to speculate on the nature of the constant α. Saroff and Mark[3] have equated this constant to the ratio of the square roots of the diffusion coefficients of the protein and the metal ion; thus

$$\alpha = \left[\frac{D_{\text{albumin}}}{D_{\text{metal ion}}}\right]^{1/2} \qquad (3)$$

From the data of Champagne,[6] the diffusion coefficient of NBSA at pH 6.5 may be expected to be 7.2×10^{-7} cm.²/sec. at 30°. The polarographic diffusion coefficient of the zinc and the cadmium ion[7] may be expected to be 0.78×10^{-5} cm.²/sec. at 30°. Thus, the value of α should be of the order of 0.30 as compared to the experimental values of 0.22 and 0.30 for Zn^{II}–NBSA and Cd^{II}–NBSA systems, respectively.

If diffusion were to play a predominant role in determining the value of α, it is obvious that the observed decrease in α with pH should be attributable to changes in the diffusion coefficient of serum albumin and/or of the metal ion. Champagne[6] has reported that, in the pH range 3.95 to 7.40, the diffusion coefficient of NBSA increases slightly with pH. This should result in a slight increase in α with an increase in pH—a conclusion which is contrary to our observations. The probability that the observed effect of pH on α may be due to an increase in the diffusion coefficient of the metal ion with pH was explored. It was observed that the polarographic constant K corrected to the same value of $m^{2/3}t^{1/6}$ decreases with increased pH. The relevant data for zinc ion, obtained from an independent set of experiments, are given in Table III.

TABLE III

THE POLAROGRAPHIC CONSTANT FOR ZINC ION AS A FUNCTION OF pH

Acetate buffer, ionic strength 0.20, 26°

pH	m (mg./sec.)	t (sec.)	K (obsd.)	K (Ilkovic eq.)
6.58	1.43	4.3	5.68 ± 0.10	5.33
6.02	1.41	4.3	5.77 ± .10	5.26
5.10	1.45	4.2	6.22 ± .10	5.36

If the effect of pH on the polarographic constant, K, be attributed solely to changes in the diffusion coefficient of zinc ion, it is obvious from eq. 3 that α should increase with an increase in pH. The observed trend is, however, in the opposite direction.

It may be concluded, therefore, that whereas diffusion may play a predominant role in determining the value of α, there may be other modifying factors. It is of interest to note, however, that

(6) M. Champagne, *Compt. rend. acad. sci.*, **237**, 521 (1953).

(7) I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Pub. Inc., New York, N. Y., 1952, p. 52.

TABLE IV

COMPETITION BETWEEN Zn^{II} AND Cd^{II} IONS

(1.0% NBSA; acetate buffer *p*H 6.50, ionic strength 0.20; 30°)

$A_0 \times 10^4$, *M*		$(i_d)_p/i_d$		$\bar{\nu}$		$(A) \times 10^4$, *M*	
Zn^{II}	Cd^{II}	Zn^{II}	Cd^{II}	Zn^{II}	Cd^{II}	Zn^{II}	Cd^{II}
a							
1.00	0.00	0.41	..	0.52	..	0.22	..
1.00	1.00	.39	0.54	.51	0.42	.22	0.36
1.00	5.00	.40	0.67	.50	1.52	.23	2.66
b							
0.00	1.00	..	0.42	..	0.53	...	0.18
1.00	1.00	0.39	.54	0.51	.42	0.22	.36
5.00	1.00	0.58	.73	1.75	.27	2.31	.58
c							
5.00	0.00	0.53	..	2.05	..	1.85	...
5.00	1.00	.58	0.73	1.75	0.27	2.31	0.58
5.00	5.00	.58	.80	1.75	0.93	2.31	3.57
5.00	10.00	.58	.85	1.75	1.37	2.31	7.89
5.00	20.00	.58	.88	1.75	2.20	2.31	16.60
d							
0.00	5.00	..	0.61	..	1.79	...	2.25
1.00	5.00	0.40	.67	0.50	1.52	0.23	2.66
5.00	5.00	.61	.81	1.62	0.86	2.51	3.68
10.00	5.00	.66	.87	2.87	.60	5.59	4.08
20.00	5.00	.78	.93	3.70	.33	14.30	4.49

the observed deviations in the values of α from those to be expected from a diffusion controlled discharge are related to the affinity of bovine albumin for metal ions. Thus, the affinity of bovine albumin for metal ions follows the increasing order: $Cd^{II} \rightarrow Zn^{II} \rightarrow Cu^{II}$, and is accompanied by a correspondingly decreasing order of α. Similarly, α decreases with acetylation of bovine albumin (Table I), and with an increase in *p*H (Table II), and is accompanied by a relatively large increase in the affinity of the protein for metal ions. It is obvious, therefore, that intrinsic and electrostatic factors may have some influence on the value of α. It may thus be expected that, for metal–albumin systems under study, α should increase somewhat with $\bar{\nu}$. The present investigations are, however, limited to a low binding region, and it is probable that the error in the experimental values may be larger than the expected changes in α. This does not however vitiate the suitability of the polarographic method for investigating metal–protein interactions at any given *p*H at which α is, to within experimental error, a constant quantity, and can often be determined through a single equilibrium dialysis experiment. Furthermore, the binding data calculated from current ratios are relatively insensitive to appreciable errors in the value of α. With the added advantage of rapid measurements, the polarographic method has much to recommend itself whenever it can be used.

A particularly useful aspect of polarographic measurements pertains to a study of competition between metal ions, say, Zn^{II} and Cd^{II} for combining with bovine albumin. These studies have helped substantially in elucidating the nature of the interactions. In Table IV, we have listed the current ratios, together with the evaluated binding data, for 1.0% bovine albumin solutions containing varying concentrations of Zn^{II} and Cd^{II} ions. Four sets of experiments were tried. In the first set, the concentration of Zn^{II} was kept fixed at 1.0×10^{-4} *M* and that of Cd^{II} was varied from 0 to 5.0×10^{-4} *M* (Table IVa); the binding of Zn^{II} ions remained unaffected indicating that 0.5 mole of Zn^{II} was firmly bound to NBSA. In the second set, the concentration of Cd^{II} was kept constant at 1.0×10^{-4} *M* and that of Zn^{II} varied from 0 to 5.0×10^{-4} *M*; Zn^{II} progressively replaced Cd^{II} (Table IVb). In the third set of experiments, the concentration of Zn^{II} was kept fixed at 5.0×10^{-4} and that of Cd^{II} varied from 0 to 2×10^{-3} *M*; apart from an initial small displacement of zinc, the binding of Zn^{II} remained unaffected indicating that approximately two zinc ions are bound firmly to bovine albumin (Table IVc). In the corresponding set of experiments in which Cd^{II} was kept constant at 5×10^{-4} *M* and Zn^{II} varied from 0 to 2×10^{-3} *M*, the zinc ions were able to replace cadmium ions practically completely[8,9] (Table IVd).

The data of Table IVc are particularly illuminating in that in this set of experiments we are studying the binding of Cd^{II} to bovine albumin the two most reactive sites of which have been blocked by combination with Zn^{II} ions. The first association constant of Cd^{II} to this "modified" albumin has a log k_1 value of 3.78 as against the log $k_1 = 4.6$[5] obtained from data in the absence of Zn^{II} (Fig. 3A). The corresponding data for the binding of Zn^{II} in the presence of 5.00×10^{-4} *M* Cd^{II} (Table IVd) reveal a log k_1 value of 4.48 which is close to log $k_1 = 4.60$[5] obtained in the absence of Cd^{II} (Fig. 3B). The dashed curve in Fig. 3 shows the progressive displacement of Cd^{II} by Zn^{II}.

The significance of the results outlined above may be illustrated by anticipating the discussion of binding data to be presented in the succeeding paper.[5] The intrinsic constants for the interaction of Zn^{II} and Cd^{II} with bovine albumin, assuming interaction at imidazole sites, have log k^0 values of 3.87 and 3.84, respectively. The large values of log k^0 as compared to the corresponding metal–imidazole systems,[10,11] together with the fact that log k^0 decreases with $\bar{\nu}$, point to the possibility that the first one or two metal ions are bound to

(8) These results appear to contradict those reported by Gurd.[9] Gurd has studied the competition between Zn^{II} and Cd^{II} to interact with human serum albumin in the higher binding region ($\bar{\nu}$ = 8–15) and shown that Zn^{II} and Cd^{II} compete with each other to combine with the same sites on the protein molecule. Whereas our data suggest that bovine albumin contains two sites which react with Zn^{II} in preference to Cd^{II}, once these sites are occupied Cd^{II} may compete successfully with Zn^{II}. This is indeed indicated from our data. Thus, at a total Zn^{II} concentration of 2.0×10^{-3} *M*, the number of Zn^{II} bound to NBSA is 3.7 in the presence of 5.0×10^{-4} *M* Cd^{II} (Table IVd) as against 4.7 in the absence of Cd^{II}. Similarly, at a total Cd^{II} concentration of 2.0×10^{-3} *M*, the number of bound Cd^{II} ions is 2.2 in the presence 5.0×10^{-4} *M* Zn^{II} (Table IVc) as against 3.8 in the absence of Zn^{II}. It may thus be seen that our data in the lower binding region, which is a distinctive feature of our investigations, supplement rather than contradict those reported by Gurd.

(9) F. R. N. Gurd, "Ion Transport Across Membranes," ed. H. T. Clarke, Academic Press, New York, N. Y., 1954, p. 146.

(10) J. T. Edsall, G. Felsenfeld, D. S. Goodman and F. R. N. Gurd, THIS JOURNAL, **76**, 3054 (1954).

(11) C. Tanford and W. L. Wagner, *ibid.*, **75**, 434 (1953).

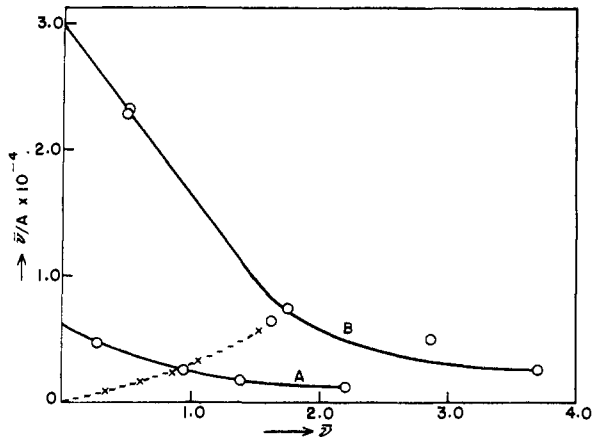


Fig. 3.—The interaction of Zn^{II} and Cd^{II} with bovine albumin: (A) the binding of Cd^{II} in the presence of 5.0×10^{-4} *M* Zn^{II}; (B) the binding of Zn^{II} in the presence of 5.0×10^{-4} *M* Cd^{II}. (The broken curve shows the progressive displacement of Cd^{II} by Zn^{II}).

sites more reactive than imidazole: either the 0.7 sulfhydryl group of total albumin or a chelate site formed by an imidazole group and a neighboring carboxyl group. Klotz and co-workers[12] have shown that the 375 mμ absorption peak characteristic of the interaction of Cu^{II} with the sulfhydryl group of bovine albumin is depressed by Zn^{II} and Cd^{II}, more so by Cd^{II} than by Zn^{II} and, consequently, we may assume that the ability of Cd^{II} to react with the sulfhydryl group of bovine albumin is somewhat more than, or at least equal to, that of Zn^{II}. We should expect therefore that Cd^{II} would be able to compete successfully or even displace Zn^{II} from combination with bovine albumin. We have, however, shown above that even when (approximately) two Zn^{II} ions are bound to bovine albumin, Cd^{II} ions are not able to displace them from combination, and that, in fact, log k_1 for the interaction of Cd^{II} with bovine albumin is reduced to 3.78: assuming a 1:1 interaction with imidazole sites, we thus have log $k^0_{CdI_m} \simeq 3.0$,[13] a value which is in good agreement with the first association constant of Cd^{II} with free imidazole.[12]

The evidence outlined above suggests that bovine albumin molecule contains approximately two especially reactive sites—presumably compound sites involving an imidazole and a neighboring carboxyl group—to which Zn^{II} are bound in preference to Cd^{II} and, furthermore, that if these two sites are occupied by Zn^{II} ions, the intrinsic association of Cd^{II} to bovine albumin corresponds to that expected for a 1:1 combination with imidazole sites. A fuller discussion of the interaction of metal ions with bovine serum albumin as evidenced from electrophoretic, polarographic and equilibrium dialysis studies is presented in the succeeding communication.[5]

Acknowledgment.—The authors are grateful to Dr. A. B. Biswas for helpful suggestions.

(12) I. M. Klotz, J. M. Urquhart and H. A. Fiess, THIS JOURNAL, **74**, 5537 (1952); see also: I. M. Klotz, J. M. Urquhart, T. A. Klotz and J. Ayers, *ibid.*, **77**, 1919 (1955).

(13) For the evaluation of intrinsic constant, k^0, *see* ref. 2.

POONA 8, INDIA

[CONTRIBUTION FROM THE PHYSICAL CHEMISTRY DIVISION, NATIONAL CHEMICAL LABORATORY]

Metal Protein Interactions in Buffer Solutions. Part III. Interaction of Cu^{II}, Zn^{II}, Cd^{II}, Co^{II} (and Ni^{II}) with Native and Modified Bovine Serum Albumins

BY M. S. NARSINGA RAO[1] AND HIRA LAL

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Quantitative binding data for the interaction of metallic ions with bovine serum albumin have been interpreted in the light of electrophoretic data. It is concluded that the interaction of Cu^{II}, Zn^{II} and Cd^{II} with bovine albumin, in the initial stages, occurs through 2–3 compound sites involving, presumably, an imidazole site and a neighboring peptide N or peptide O. Peptide N appears to be involved in interactions with Cu^{II} and peptide O with Zn^{II} and Cd^{II}. Co^{II} (and presumably Ni^{II}) are, however, bound to free carboxyl sites of bovine albumin. A probable explanation of the distinctive feature of these interactions, as also the effect of acetylation and esterification of bovine albumin, has been put forward. It is further concluded that interactions involving Cu^{II}, Zn^{II} and Cd^{II}, in the higher binding region, are governed by a competition between imidazole and carboxyl sites to interact 1:1 with the metal ions and may be accompanied by configurational changes in the protein molecule.

Gurd and Goodman[2] have concluded from their studies on the interaction of Zn^{II} with human serum albumin (HSA) in the neutral *p*H region that the metal ion is bound 1:1 with the imidazole sites of the protein molecule; the evaluated intrinsic association constant (log $k^0 = 2.82$) agreed well with the first association constant for the Zn^{II}–imidazole system.[3] This agreement is, however, fortuitous in that the intrinsic association constant, as pointed out earlier,[4] must be upgraded to give log $k^0 = 3.62$. If we further note that the affinity of Zn^{II} for 4-methylimidazole[5] (in which the methyl group is substituted at the same position as the histidyl side chain) is somewhat lower than that for unsubstituted imidazole, we are led to conclude that Zn^{II} is bound to serum albumin much more firmly than suggested from a combination with the imidazole sites of the protein molecule. A reconsideration of the polarographic data of Tanford[6] for

(1) Chemistry Department, Clark University, Worcester 10, Mass.

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(5) Y. Nozaki, F. R. N. Gurd, R. F. Chen and J. T. Edsall, *ibid.*, **79**, 2123 (1957).

(6) C. Tanford, *ibid.*, **74**, 211 (1952).