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Studies on the Sulfur-containing Chelating Agents. XXVI.[1] Formation of Mixed Ligand Chelates Containing Penicillamine and Sulfhydryl Compounds

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Formation of new mixed ligand chelates consisting of penicillamine, bivalent metals, such as mercury, lead and cadmium, and some of the secondary ligands, such as mercaptoacetic acid, N-acetylpenicillamine, glutathione, 2-mercaptoethylamine, α-mercaptopropionyl glycine and penicillamine methyl ester, in the ratio of 1:1:1 were recognized potentiometrically, but there was no indication for the existence of the similar mixed ligand chelate in the cases of ethylmercaptan, glycine and histidine. The mixed ligand chelate is formed in two steps; the simple penicillamine metal chelate is formed in the first step, and the secondary ligand becomes to coordinate to 1:1 penicillamine chelate to yield the 1:1:1 mixed ligand chelate in the second step. The order of the relative formation constants of mixed ligand chelates was $Cd^{2+}>Hg^{2+}>Pb^{2+}$. The formation constants of mixed ligand chelates are linearly correlated to the dissociation constants of sulfhydryl group in secondary ligands. Moreover, among these mixed ligand chelates, the decrease of binding affinity of the carboxyl group in penicillamine tends to increase the formation of the mixed ligand chelate.

In recent years the formation of the mixed ligand complexes, namely the complexes containing two different ligands, has become an interesting subject not only in coordination chemistry but also in biological chemistry as a model for the enzyme–metal–substrate complexes.[3] However, the formation of the mixed ligand complexes in which the sulfhydryl group is involved have been scarcely studied, although they are biologically of great importance.

We have investigated extensively the chelate formation of penicillamine in an attempt to discuss the relationship between the chelating ability and the antitoxic effect against heavy metal poisoning, and reported the correlation between the mode of the coordination and the antitoxic effect[4] against lead and mercury, and the mixed valence complex formed by the reaction of cupric ion.[1] In order to investigate the participation of the interaction between penicillamine and the metal ions, which are bound to protein, to the antitoxic mechanism against metal poisoning, the study of the mixed ligand complexes containing biologically important sulfhydryl ligands is considered to be of importance. The ternary complex of penicillamine with other ligands which contain sulfhydryl group should be considered as a model system in the antitoxic mechanism, because poisonous heavy metals such as lead and mercury are considered to bind with the sulfhydryl groups in protein. On the basis of these considerations, the mixed ligand chelates containing penicillamine and other sulfhydryl compounds were studied by the potentiometric method.

Experimental

Materials——DL-Penicillamine was purchased from the Sigma Company. Penicillamine methyl ester hydrochloride and N-acetylpenicillamine were obtained according to the method reported in the previous

1) Part XXV: Y. Sugiura and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **18**, 368 (1970).
2) Location: *Yoshida, Shimoadachi-cho, Sakyo-ku, Kyoto.*
3) *a*) R. Näsanen, P. Merilainen and S. Lukkari, *Acta. Chem. Scand.*, **16**, 2384 (1962); *b*) A.S. Mildvan and M. Cohn. *J. Biol. Chem.*, **241**, 1178 (1966); *c*) A.K. Babko, *Talanta*, **15**, 721 (1968); *d*) B. Sarkar and Y. Wigfield, *Can. J. Biochem.*, **46**, 601 (1968).
4) Y. Sugiura, A. Yokoyama and H. Tanaka, *Chem. Pharm. Bull.* (Tokyo), **18**, 693 (1970).

paper.[4] Mercaptoacetic acid, 2-mercaptoethylamine hydrochloride, glutathione, α-mercaptopropionyl glycine, ethylmercaptan, glycine and histidine as the second ligands were reagent grade materials, and were recrystallized whenever necessary. The solutions of nickel nitrate, zinc nitrate, cadmium nitrate, lead nitrate and mercuric chloride were prepared from reagent grade materials and were standardized complexometrically with EDTA. Carbonate-free potassium hydroxide was prepared by the procedure described by Armstrong[5] and was standardized by the titration with potassium hydrogen phthalate. Deionized water was used throughout the experiments.

Potentiometric Titrations——Solutions containing the metal ion (2.0×10^{-3}M), penicillamine (2.0×10^{-3}M) and the second ligand (2.0×10^{-3}M) in the molar ratio of 1:1:1 were prepared and the ionic strength was made 0.1 with a 1.00M potassium nitrate solution. After complete attainment to equilibrium at 22°±0.1, the solution was titrated potentiometrically as previously described.[4]

Calculations——Formation constants of the mixed ligand chelates were determined in favorable cases for the combination of the 1:1 penicillamine chelates (ML) with a second ligand (A) by the methods similar to those employed by Thompson and Loraas.[6]

In the formation of the mixed ligand chelate (LMA) which is formed by the reaction of the primary chelate (ML) with the second ligand (A), the total concentration of metal ion (C_M) and the total concentration of the second ligand (C_A) are expressed by the equations (2) and (3). Moles of base added per mole of A (a^*) is expressed by the equation (4), because two moles of base is needed to neutralize free primary ligand (H_2L). Combining the equations (3) and (4), the concentration of the second ligand ([A]) can be expressed by the equation 5. In the equation 5, K_1 and K_2 are the acid dissociation constants of A. The formation constant (k) for the equation (1) is calculated by the equation (6).

$$ML+A \rightleftharpoons LMA \tag{1}$$

$$C_M=[ML]+[LMA] \tag{2}$$

$$C_A=[H_2A]+[HA]+[A]+[LMA] \tag{3}$$

$$a^*C_A+[H]-[OH]=[HA]+2[A]+2[LMA] \tag{4}$$

$$[A]=\frac{(2-a^*)C_A-[H]+[OH]}{2-\dfrac{[H]^2}{K_1K_2}+\dfrac{[H]}{K_2}} \tag{5}$$

$$k=\frac{C_A-[A]\left\{\dfrac{[H]^2}{K_1K_2}+\dfrac{[H]}{K_2}+1\right\}}{[A]\left\{C_M-C_A+[A]\left(\dfrac{[H]^2}{K_1K_2}+\dfrac{[H]}{K_2}+1\right)\right\}} \tag{6}$$

Results and Discussion

The complex formation which involves two different ligands can generally be classified into the following four types.[7]

a $\quad L + M + A \rightleftharpoons LMA$

b $\quad L + M + A \rightleftharpoons ML + A \rightleftharpoons LMA$

c $\quad xL + yA + 2M \rightleftharpoons MLx + MAy$

d $\quad L + M + A \rightleftharpoons ML + A$

(In these equations, L, A and M represent a primary ligand, a secondary ligand and a metal ion whose ionic charges are omitted respectively.)

In the titration curve of 1:1:1 penicillamine-Hg^{2+}-N-acetylpenicillamine system, the formation of the mixed ligand chelate can be traced in the following way. The titration curve of 1:1:1 penicillamine-Hg^{2+}-N-acetylpenicillamine exhibits two inflections at $a=3.0$ and $a=4.0$ as shown in Fig. 1 (C).

5) D.M.G. Armstrong, *Chem. Ind.* (London), **1955**, 1405.
6) L.C. Thompson and J.A. Loraas, *Inorg. Chem.*, **2**, 89 (1963).
7) G.H. Carey, R.F. Boguchi and A.E. Martell, *Inorg. Chem.*, **3**, 1288 (1964).

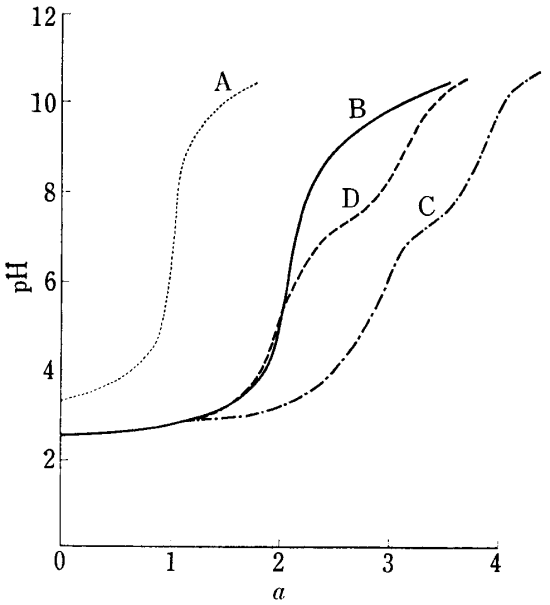

Fig. 1. Potentiometric Titrations of Mixed Ligand Systems of Penicillamine, Hg^{2+} and N-Acetylpenicillamine with Potassium Hydroxide

a=moles of base added per mole of metal ion
A: N-acetylpenicillamine
B: 1:1 Hg^{2+}-penicillamine
C: 1:1:1 penicillamine-Hg^{2+}-N-acetylpenicillamine
D: 1:1:1:1 penicillamine-Hg^{2+}-N-acetylpenicillamine-alkali

The region from $a=0$ to $a=3$ of the titration curve (C) coincided approximately with the composite curve formed by the curve of 1:1 Hg^{2+}-penicillamine (B) and that of N-acetylpenicillamine (A). This can be verified by the fact that the titration curve of 1:1 Hg^{2+}-penicillamine system coincided up to $a=2.0$ with that of the mixed ligand system (D) to which one molar alkali was previously added. However, the pH of the region from $a=3.0$ to $a=4.0$ in the mixed ligand system is evidently lower than that in the titration of N-acetylpenicillamine itself. Therefore, the formation of the mixed ligand chelate may be explained in two steps as shown in equation (b). Namely, in the region from $a=0$ to $a=3.0$, simple 1:1 penicillamine-Hg^{2+} chelate is formed, while in the region from $a=3.0$ to $a=4.0$, N-acetylpenicillamine coordinates to 1:1 penicillamine chelate to yield the 1:1:1 mixed ligand chelate. In the cases of 1:1:1 penicillamine-M^{2+}-mercaptoacetic acid, glutathione, 2-mercaptoethylamine, α-mercap-

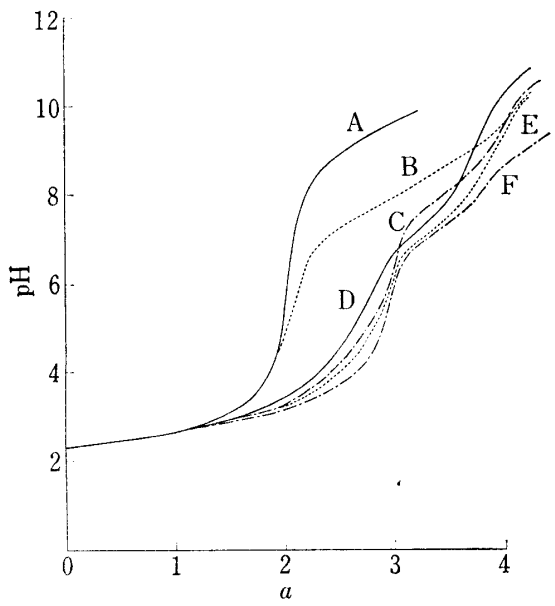

Fig. 2. Potentiometric Titrations of Mixed Ligand Chelate Systems of Hg^{2+} and Penicillamine with Potassium Hydroxide

a=moles of base added per mole of metal ion
A: 1:1 Hg^{2+}-penicillamine
B: 1:1:1 penicillamine-Hg^{2+}-2-mercaptoethylamine
C: 1:1:1 penicillamine-Hg^{2+}-mercaptoacetic acid
D: 1:1:1 penicillamine-Hg^{2+}-penicillamine methyl ester
E: 1:1:1 penicillamine-Hg^{2+}-α-mercaptopropionyl-glycine
F: 1:1:1 penicillamine-Hg^{2+}-glutathione

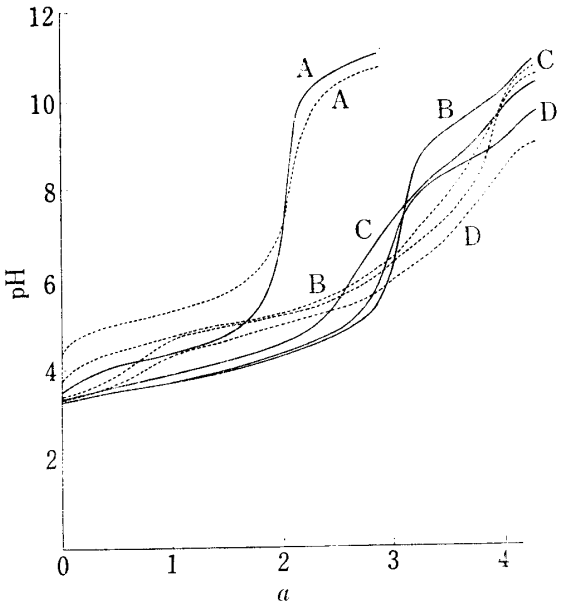

Fig. 3. Potentiometric Titrations of Mixed Ligand Chelate Systems of M^{2+} and Penicillamine with Potassium Hydroxide (where $M^{2+}=Pb^{2+}$ and Cd^{2+})

a=moles of base added per mole of metal ion
A: 1:1 M^{2+}-penicillamine
B: 1:1:1 penicillamine-M^{2+}-N-acetylpenicillamine
C: 1:1:1 penicillamine-M^{2+}-penicillamine methyl ester
D: 1:1:1 penicillamine-M^{2+}-glutathione
———: Pb^{2+} .....: Cd^{2+}

topropionyl glycine and penicillamine methyl ester (where $M^{2+}=Hg^{2+}$, Pb^{2+} and Cd^{2+}), the curves also exhibit the inflections at $a=3.0$ or $a=4.0$ as presented in Figs. 2 and 3. Therefore, it is evident that these systems undergo the similar reactions to that of the N-acetylpenicillamine system, namely simple 1:1 penicillamine metal chelate is formed in the first step, and then the secondary ligand becomes to coordinate to penicillamine chelate to yield the 1:1:1 mixed ligand chelate in the second step. While, in the 1:1:1 mixture of penicillamine-M^{2+}-ethylmercaptan, glycine and histidine (where $M^{2+}=Hg^{2+}$, Pb^{2+} and Cd^{2+}), the titration curve is identical with that of 1:1 penicillamine-M^{2+} in the range from $a=0$ to $a=2.0$, and in the region from $a=2.0$ to $a=4.0$ the titration curve is identical within experimental error with that of secondary ligand itself, and hence the presence of any mixed ligand chelate cannot be recognized.

Table I. Formation Constants of Mixed Ligand Chelates from 1:1 Chelates of Cd^{2+}, Hg^{2+} and Pb^{2+} with Penicillamine (H_2L) and 1 Mole of Secondary Ligand (H_2A)

Secondary ligand	Acid dissociation constant pK_a	Equilibrium quotient	Formation constant, log k		
			Cd^{2+}	Hg^{2+}	Pb^{2+}
None	—	[ML]/[M][L]	10.92[4]	16.4[4]	12.88[4]
2-Mercapto-ethylamine	8.35 10.81	[LMA]/[ML][A]	6.85±0.02	6.64±0.02	4.68±0.03
N-Acetylpenicillamine	3.28 10.26	[LMA]/[ML][A]	5.71±0.02	5.65±0.02	4.06±0.04
Mercaptoacetic acid	3.42 10.20	[LMA]/[ML][A]	5.19±0.03	5.09±0.01	4.16±0.01
Glutathione	8.75 9.65	[LMA]/[ML][A]	5.73±0.03	5.33±0.03	4.47±0.02
Penicillamine methyl ester	6.30 9.17	[LMA]/[ML][A]	5.02±0.02	4.90±0.02	3.76±0.02
α-Mercaptopropionyl glycine	3.60 8.74	[LMA]/[ML][A]	4.46±0.03	4.38±0.01	3.30±0.03

In 1:1:1 penicillamine-M^{2+}-sulfhydryl compound systems, the formation constants of the mixed ligand chelates by the reaction as shown in equation (b) were listed in Table I. On the basis of these results, the following discussions on the formation of the mixed ligand chelate will be possible.

The formation constants of the mixed ligand chelates (log k) are directly correlated to the dissociation constants of sulfhydryl group (pK_{-SH}) in secondary ligands as shown in Fig. 4. As previously reported,[4] the binding affinity of the carboxyl group in penicillamine to the metal ion decreases in order $Pb^{2+}>Hg^{2+}>Cd^{2+}$. The order of the relative formation constants of the mixed ligand chelates determined in the present investigation, however, was $Cd^{2+}>Hg^{2+}>Pb^{2+}$. This fact suggestes that decrease of the binding affinity of the carboxyl group in penicillamine tends to increase the formation of the mixed ligand chelate. In fact, the plots of the formation constants of the

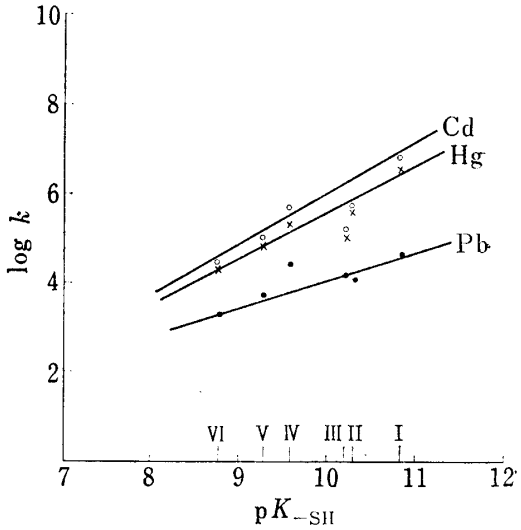

Fig. 4. Correlation of Formation Constants of Mixed Ligand Chelates and Dissociation Constant of Sulfhydryl Group in Secondary Ligands

I: 2-mercaptoethylamine
II: N-acetylpenicillamine
III: mercaptoacetic acid
IV: glutathione
V: penicillamine methyl ester
VI: α-mercaptopropionyl glycine

mixed ligand chelates against the differences of the stability constants (Δ log k') between penicillamine chelates and penicillamine methyl ester chelates[4] were found to be linear as shown in Fig. 5. In addition, as shown in Fig. 6, the relationship between the formation constants of the mixed ligand chelates and differences of chemical shift of the methine proton at pH 2—3 between penicillamine and penicillamine metal chelates (Δ cps) give a linear relation as to each ligand. It was previously reported[4] that the change of the chemical shift of the methine proton at pH 2—3, where the carboxyl group dissociates, is based on the ability of coordination of the carboxyl group to penicillamine.

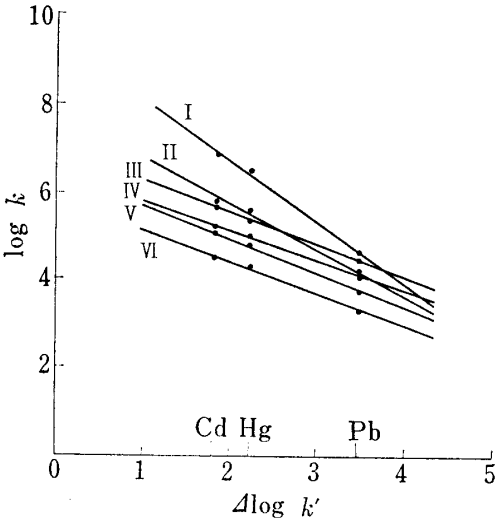

Fig. 5. Correlation of Formation Constants of Mixed Ligand Chelates with Differences of Stability Constants between Penicillamine Metal Chelates and Penicillamine Methyl Ester Chelates

I: 2-mercaptoethylamine
II: N-acetylpenicillamine
III: mercaptoacetic acid
IV: glutathione
V: penicillamine methyl ester
VI: α-mercaptopropionyl glycine

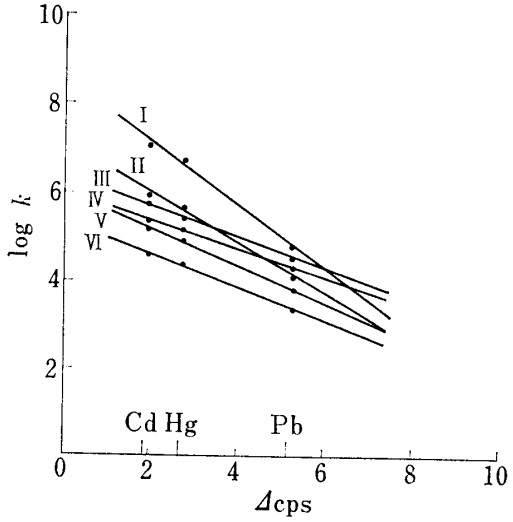

Fig. 6. Correlation of Formation Constants of Mixed Ligand Chelates with Differences of Chemical Schift of Methine Proton between Penicillamine Metal Chelates and Penicillamine

I: 2-mercaptoethylamine
II: N-acetylpenicillamine
III: mercaptoacetic acid
IV: glutathione
V: penicillamine methyl ester
VI: α-mercaptopropionyl glycine

In consideration of the results the mechanism presented in Chart 1 seems most reasonable to explain the formation of the mixed ligand chelates containing penicillamine and other sulfhydryl compounds. In these systems, stable penicillamine chelate is formed primarily. When the sulfhydryl group of the secondary ligand is able to dissociate to its anion in mild alkaline medium, its attack to the central metal may occur, because these metals have extremely strong affinity to the sulfide anion. Finally, the mixed ligand chelate is formed by the coordination of the secondary ligand which acts as the bidentate ligand.

The sulfhydryl compounds which act as the bidentate ligand to Hg^{2+}, Pb^{2+} and Cd^{2+} form the mixed ligand chelates containing penicillamine, but there is no indication for the formation of the chelates of the similar type in the cases of ethylmercaptan, which act as the monodentate ligand to these metal ions. On the basis of the above considerations it seems reasonable to assume the structure of the mixed ligand chelate as shown in Chart 1. However, no mixed ligand species was detectable as to nickel and zinc by means of the present method. In these cases, the formation of a simple mixture of the respective chelate is recognized. This fact will be attributable to the property of nickel and zinc to form the chelate of the ligand–to–metal ratio 2:1. It seems particularly interesting that the metal ions, such as Hg^{2+}, Pb^{2+} and Cd^{2+} to which penicillamine is an effective antidote against their poisoning,[8] can form 1:1 chelate

8) M.B. Chenoweth, *Clin. Pharmacol. Therap.*, 9, 365 (1968).

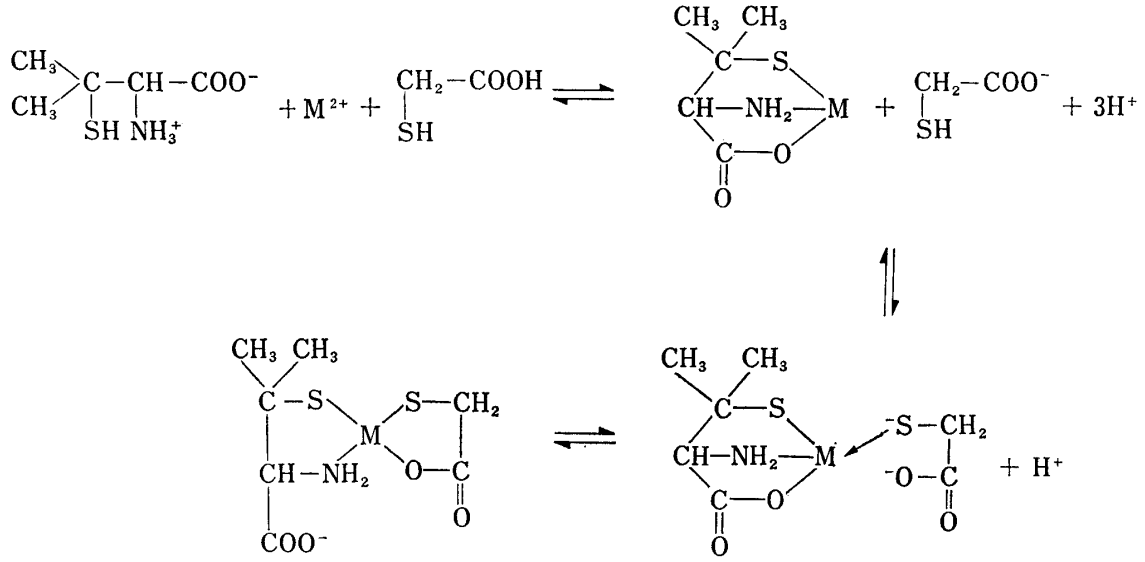

Chart 1

and also the mixed ligand chelate with penicillamine. It is presumed that the formation of the mixed ligand chelate have close connection to the interaction of penicillamine to the metal bound to protein *in vivo*, and hence to the removal of the poisonous metal. From this point of view the interaction of penicillamine to the metal bound to protein has been investigated by the use of radioisotopes in our laboratory.[9]

9) Y. Sugiura and H. Tanaka, *Radioisotopes* (Tokyo), **19**, 7 (1970).