Table II—Slopes and Intercepts of Graphs Plotted According to Eq. 4 for Methylparaben

Dielectric Constant, 25°[a]	R[b]	n[c]	Intercept	Slope	$P(1)$ at N_A = 1
17.6	0.985	8	98.8	27.7	121.4
18.9	1.000	8	94.8	26.8	121.5
21.3	1.000	8	88.4	32.6	121.0
23.5	0.998	9	106.1	16.8	122.8
21.3	1.000	7	78.6	42.7	121.3
28.5	1.000	5	51.7	69.7	121.4
23.5	0.986	8	117.6	3.33	120.9
13.3	0.994	7	109.2	15.3	124.5
23.9	1.000	9	101.7	21.0	122.7
20.8	0.998	9	108.4	13.8	122.2
29.6	0.999	9	100.5	22.7	123.2
24.9	0.999	6	73.6	48.3	122.0
26.1	1.000	5	71.4	49.8	121.2
27.5	1.000	5	69.4	51.5	120.7

[a] Solvents are listed in Table I. [b] Correlation coefficient. [c] Number of points from which the least-squares parameters were determined.

obeyed. The least-squares statistics for these lines are shown in Table II.

According to Eq. 4, if extrapolation is carried out to $N_A = 1$, a value is obtained for the polarization of the solute, *i.e.*, $P_A = P(1)$, where the latter is the extrapolated value. These values are listed in Table II; even though the extrapolation is long, there is good agreement. By inserting the average of these values (122.7), the density of methylparaben (1.09 g/cm^3), and its molecular weight (152) into Eq. 7*a*, one obtains:

$$Q = \frac{\epsilon - 1}{\epsilon + 2} = P_A\rho/M_A = 1.09(122.7/152) = 0.88 \qquad \text{(Eq. 8)}$$

This equation gives $\epsilon = 21.8$, which coincides with the previously quoted value $\gamma = 21.0 \pm 0.6$. This value may rationally be denoted ϵ_A^l, *i.e.*, the dielectric constant of the methylparaben in the dissolved state. This result differs significantly from values obtained by using a solids cell in the Q-meter [ϵ_A (solid) = 2.70] and suspension techniques ($\epsilon = 5.8$).

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Acid Dissociation and Metal Complex Formation Constants of Penicillamine, Cysteine, and Antiarthritic Gold Complexes at Simulated Biological Conditions

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Received April 13, 1978, from the *Department of Chemistry, State University of New York at Binghamton, Binghamton, NY 13901.* Accepted for publication September 11, 1978.

Abstract □ Ionization constants for acid functions of D-penicillamine, L-cysteine, thiomalic acid, and thioglucose were measured by pH titration at 37° and 0.15 *M* ionic strength. Chelate formation constants for these ligands with calcium(II), iron(III), and gold(I) were then determined under the same conditions chosen to approximate the *in vivo* situation. Only iron(III) formed both 1:1 and 1:2 chelates with D-penicillamine, L-cysteine, and thiomalate; calcium formed weak and gold strong 1:1 complexes with all ligands studied. Because of precipitate formation, the stability constants for the systems thioglucose-iron(III), D-penicillamine-gold(I), and L-cysteine-gold(I) had to be determined indirectly with thiomalic acid as the competing ligand. The *in vivo* fate of antiarthritic gold(I) compounds remained uncertain, but gold(I) chelates probably persist as such for extended periods.

Keyphrases □ Penicillamine—acid dissociation and metal complex formation constants with calcium(II), iron(III), and gold(I), simulated biological conditions □ Cysteine—acid dissociation and metal complex formation constants with calcium(II), iron(III), and gold(I), simulated biological conditions □ Thiomalic acid—acid dissociation and metal complex formation constants with calcium(II), iron(III), and gold(I), simulated biological conditions □ Thioglucose—acid dissociation and metal complex formation constants with calcium(II), iron(III), and gold(I), simulated biological conditions □ Gold complexes—stability constants of antiarthritic gold(I) complexes and penicillamine and cysteine, simulated biological conditions □ Iron(III) and calcium(II) complexes—with penicillamine, cysteine, thiomalic acid, and thioglucose, simulated biological conditions

D-Penicillamine (I), 3-mercapto-D-valine, is the accepted therapeutic agent for the treatment of Wilson's disease (1, 2) and cystinuria (3, 4). Compound I is also a well-known antidote in lead and mercury poisoning (5–7) and has been investigated as a protective agent against radiation (8). It has been approved for rheumatoid arthritis therapy (9) in several countries but not in the United States because of some potentially severe side reactions. However, I success rates in rheumatoid arthritis treatment are at least as high as those with the established drugs aurothioglucose (II) and aurothiomalate (III), and dangerous toxic side effects can largely be avoided by careful monitoring of the patient's blood (10).

The mode of action of I in cystinuria is well understood—the mixed disulfide (IV) formed with cysteine (V) is more soluble than cystine (VI) (11). The therapeutic value in Wilson's disease as well as in the treatment of heavy metal poisoning results from its strong *in vivo* metal

0022-3549/79/0400-0426$01.00/0

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chelating properties (1, 6, 7). The mechanism of action of I in rheumatic disease is not yet understood, although theories have been advanced (12). A striking similarity between side effects in rheumatoid arthritis patients caused by gold treatment (with II or III) and I therapy has been observed (13).

In vivo metal chelation by I and similar sulfur-containing amino acids has been studied (14–21), and stability constants for many biologically important metal ions were determined at standard conditions and varying ionic strengths. However, the formation constants of gold(I) and iron(III) complexes with I and V were never measured. Furthermore, the stability constants of II and III, the gold salts most widely used in rheumatoid arthritis treatment, were not known before this study. Acid dissociation constants (pKa) were measured previously for I, V, and thiomalic acid (VII), but not for thioglucose (VIII), at standard conditions (14, 18). So far, none has been determined at simulated biological conditions, *i.e.*, 37° and 0.15 ionic strength.

The technique chosen for this study was potentiometric titration of the acid protons (on the thiol, amino, and carboxylic functions), a method used previously with excellent success in a similar system (14, 16, 17). Titrations of the free ligands yield the pKa values, while titration after addition of the metal ions allows calculation of the metal complex formation constants from the respective curves. This method also permits the indirect determination of K_f values for inaccessible complexes by titration of the metal ion in the presence of two competing ligands (22, 23). A precipitation reaction with the systems thioglucose–iron(III) and cysteine–gold(I) and the unavailability of I and V complexes with gold(I) necessitated the use of this indirect technique.

EXPERIMENTAL

Materials—D-Penicillamine[1], L-cysteine[2], thioglucose[3], thiomalic acid[3], sodium aurothiomalate[4], and aurothioglucose[5] were standardized by potentiometric titration and characterized by optical rotatory dispersion, NMR, and IR spectra and melting points.

Metal-ion solutions were prepared from reagent grade nitrates[6] and were standardized according to routine procedures (24).

Potentiometric Measurements—The pH measurements were carried out in a 100-ml jacketed titration cell fitted with a magnetic stirrer and rubber stopper through which were inserted nitrogen and buret delivery tubes and glass and calomel electrodes. A research pH meter[7] was used; the system was calibrated at pH 4 and 9.22. The ionic strength was 0.15 in all cases (potassium nitrate). The temperature was held to ±0.05° of the desired value.

Acid Dissociation Constants—Each acid function was titrated with increments of 0.1000 *M* KOH, carbonate free[8].

Chelate Formation Constants—The stepwise chelate formation constants were determined according to the procedure of Doornbos and Faber (14). The calcium(II) or iron(III) solution was added to that of the ligand, except for the thioglucose–iron(III) system which was unstable. Solutions of aurothioglucose and sodium aurothiomalate were prepared from the salts and used as such. The thioglucose–iron(III), I–gold(I), and V–gold(I) formation constants were determined by ligand competition with the thiomalate complexes.

[1] Merck Sharp and Dohme, New Brunswick, N.J.
[2] Sigma Chemical, St. Louis, Mo.
[3] Eastman Organic, Rochester, N.Y.
[4] Aldrich, Milwaukee, Wis.
[5] Schering-Plough, Bloomfield, N.J.
[6] Baker Chemical Co., Phillipsburg, N.J.
[7] Radiometer M52-b.
[8] Calculations were performed with an APL program written by T. D. Zucconi based on the equations of Doornbos (19).

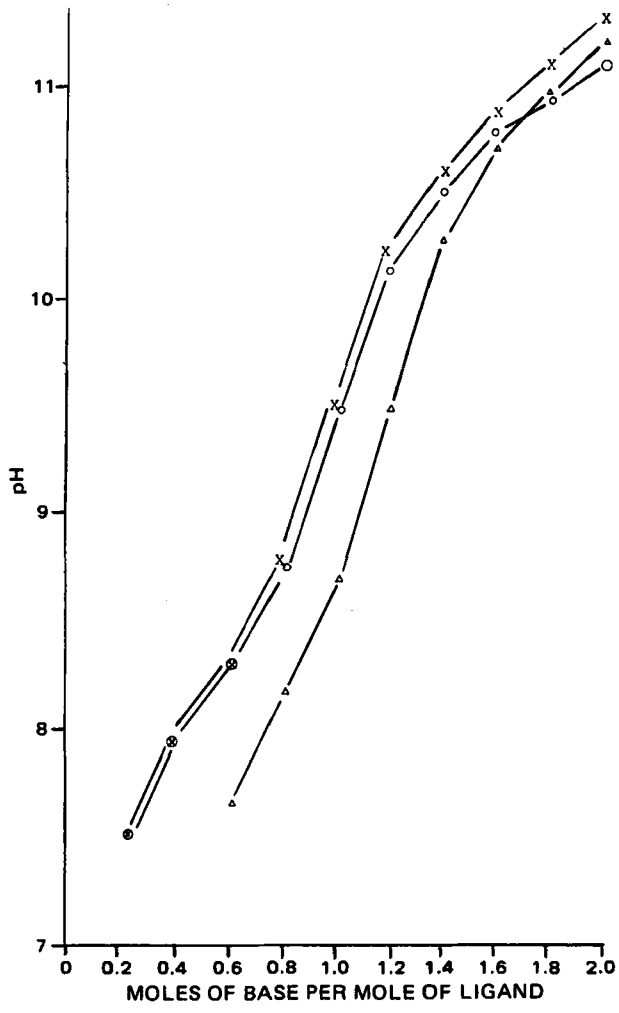

Figure 1—*Formation curves of penicillamine with calcium(II) (○), iron(III) (△), and free ligand (×) at 20°.*

Calculations—Calculations for K_1 and β_2 (*i.e.*, K_1K_2) were made with the FORTRAN IV program SCOGS[9] developed by Perrin and Sayce (25).

The validity of the experimental procedures and apparatus was tested as a whole by determination of the pKa of benzoic acid at 25°. The determined value of 4.25 ± 0.02 compared exactly with the literature value of 4.25.

RESULTS

Acid Dissociation Constants—Table I shows the values obtained for the ligands studied and available literature values. These values were used in calculating the stability constants because both sets of determinations were done under the same conditions. Some well-known pKa values were also redetermined to evaluate the precision and accuracy of the experimental technique and procedures.

Metal Complex Formation Constants—The values of the formation constants K_1 and β_2 are listed in Table II. These constants were calculated from the curves of the number of equivalents of base added *versus* pH (Figs. 1–8). The constants for some systems could not be determined directly. The VII–iron(III) system gave a precipitate under all conditions studied. It was evaluated as VII–VIII–iron(III) indirectly.

Compounds I, V, and VII gave precipitates at high values (≥9), but sufficient data points were available at lower pH values to permit calculation of the constants. No point where a precipitate existed was used in finding the reported values in Table II. The I- and V-gold(I) systems were determined by ligand competition with aurothiomalate because of the unavailability of the I- and V-gold(I) salts.

[9] Modified for use on the campus computer, IBM 370-158.

Table I—Acid Dissociation Constants

	$\overset{O}{\overset{\|}{C}}OH$	NH_3^+	SH
	pKa at 20°, μ = 0.15 (Literature Values)		
Penicillamine	(2)[a]	8.11 ± 0.02 (8.03)[a]	10.82 ± 0.04 (10.83)[a]
Cysteine	(1.88)[a]	8.38 ± 0.02 (8.32)[a]	10.60 ± 0.03 (10.48)[a]
Thiomalic acid	4.68 ± 0.03 (4.68)[b]	—	10.51 ± 0.03 (10.55)[b]
Thioglucose	—	—	11.67 ± 0.05
	pKa at 37°, μ = 0.15		
Penicillamine	—	7.83 ± 0.01	10.34 ± 0.04
Cysteine	—	8.04 ± 0.02	10.21 ± 0.06
Thiomalic acid	4.68 ± 0.04	—	10.24 ± 0.02
Thioglucose	—	—	11.51 ± 0.03
	pKa at 25°		
Benzoic acid	—	4.25 ± 0.02	(4.25)[c]

[a] According to D. A. Doornbos, *Pharm. Weekbl.*, **102**, 269 (1967). [b] According to J. Bjerrum, G. Schwarzenbach, and L. G. Sillén, "Stability Constants of Complex Compounds," Chemical Society, London, England, Special Publication 6, 1957. [c] Standard material used for evaluating equipment performance.

DISCUSSION

Values obtained for acid dissociation constants of I, V, and VII at 20° (Table I) were in excellent agreement with previously published values (14–18), allowing for differences in ionic strength. The precision of all pKa values determined ranged from ±0.01 to ±0.06 unit, well within acceptable limits. The pKa found for VIII was significantly higher than the pKa values of both I and V. It was of a magnitude typical for isolated thiol groups.

The increase in temperature from 20 to 37° decreased most pKa values. However, the magnitude of the change was quite different for different functions and different compounds. The carboxyl group of thiomalic acid was unaffected while the pKa values of the amino groups of all four ligands were reduced by 0.3 unit. The thiol group showed the greatest variation in its decrease of pKa: 0.16 unit for thioglucose, 0.27 for thiomalic acid, 0.29 for V, and 0.48 for I. Because the pKa values for the functional groups of interest were far enough apart, the groups could be treated as monoprotic acids.

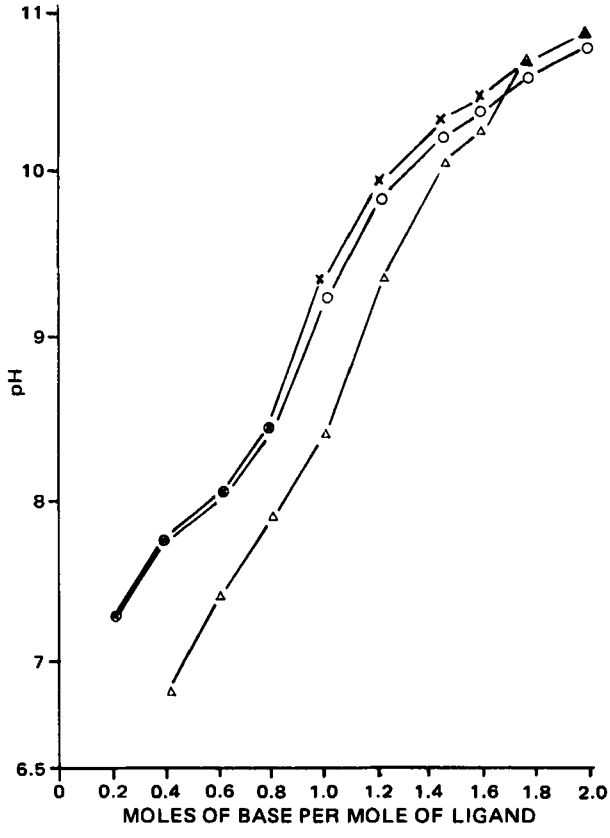

Figure 2—*Formation curves of penicillamine with calcium(II) (○), iron(III) (△), and free ligand (×) at 37°.*

The ionic strength, μ, was 0.15 (potassium nitrate) in all experiments to approximate *in vivo* conditions, although this value was not high enough to guarantee a completely constant ionic strength medium. Changes in K values with changing ionic strength can sometimes be appreciable for sulfur-containing amino acids (26). However, no attempt was made to correct for changes in ionic strength due to changes in species distribution as a function of pH or different concentrations of metal ions and ligands used. The maximal change was estimated as 0.024. Like most other authors (14–21), we neglected these small changes since the main emphasis of this work was not on their effect.

The formation constant for the I chelate with calcium was determined at 20° to permit comparison and correlation of results with those of previous investigators. The 3.15 value for log K_f compared well with the 2.7 obtained by Doornbos and Faber (19), considering the difference in ionic strength and the usual variance among laboratories. The stabilities of the iron(III) complexes were also determined at 20° with I, V, and VII (Table II) because no literature data were available although the iron(II) complexes had been studied earlier (14). The stability constants for both calcium(II) and iron(III) with all four ligands were determined at 37° (Table II).

The formation curves (Figs. 1 and 2) do not reveal anything unexpected for the interaction of I with calcium, which forms a rather weak complex. Actually, there was no deviation of the curves with calcium present until after addition of 1 mole equivalent of base. This finding may indicate that bonding occurs at the thiol group. The complex may be with the thiol group alone or with it and one of the other two functions. The carboxyl site seems less likely than the amino group, however, because of its greater distance from the thiol group.

Table II—Formation Constants Determined

System	log K_1	log β_2[a]
	20°, μ = 0.15	
I-Calcium(II)	3.15	—
VII-Calcium(II)	2.84	—
I-Iron(III)	—	—
I-Iron(III)	11.27	16.25
V-Iron(III)	10.85	14.49
VII-Iron(III)	9.18	11.98
I-Gold(I)	12.50[b]	—
V-Gold(I)	11.11[b]	—
VII-Gold(I)	10.27	—
VIII-Gold(I)	8.51	—
	37°, μ = 0.15	
I-Calcium(II)	3.01	—
V-Calcium(II)	2.98	—
VII-Calcium(II)	2.79	—
VIII-Calcium	1.58	—
I-Iron(III)	11.02	15.79
V-Iron(III)	10.63	14.01
VII-Iron(III)	9.01	12.52
VIII-Iron(III)	—	7.25[b]
I-Gold(I)	13.50[b]	—
V-Gold(I)	12.04[b]	—
VII-Gold(I)	11.23	—
VIII-Gold(I)	8.87	—

[a] $\beta_2 = K_1K_2$. [b] Determined by ligand competition.

Comparison of the iron(III) curves for I and V (Figs. 5–8) revealed a larger deviation from the free ligand curve for I–iron(III). Above pH 10.5, the curve of V–iron(III) actually was higher than that for the free ligand. Moreover, a precipitate began to form above pH 9 with V–iron(III). The fact that this phenomenon was not seen with I may be taken as evidence for a stronger complex. Values of pH in regions where a precipitate formed were not used to calculate K_f.

The rather large deviation at pH 6 (I, 37°) may not have been due to chelate formation but, perhaps, to a weaker association with the ionized acid group and was definitely in part due to the formation of some hydroxide compounds. The presence of an iron hydroxide–V complex was investigated by Tanaka (27), although Gruenwedel and Hsien-Chung (28) concluded that the onset of precipitation would not allow its investigation.

The VII–iron system and the I–iron system both turned purple as soon as iron was added, but the color disappeared upon mixing. When base was added to VII–iron, the color formed at pH 5, much lower than with I–iron. This result is expected because of the two comparatively strong acid groups in VII. The color at a pH well below the pKa of the thiol group indicated a chelation through the two carboxyl groups and no involvement of the thiol. The meter reading became unstable at about pH 7 and, again, a precipitate formed at pH 9. The thiol group could not play an important role in this complex unless its acidity were greatly enhanced by complex formation. The VIII–iron system was not stable at any ratio of ligand to metal. Therefore, the K_f values were determined by titrating VIII together with VII and iron(III), followed subsequently by resolution of the data by a computer. Thus, no formation curve is shown.

Comparison of the K_f values at 20 *versus* 37° revealed a decreasing tendency to complex at the higher temperature for calcium(II) and iron(III) while the opposite was true for gold(I). The difference was large enough to be considered real. The same value of gold hydroxide formation (29) was used for calculation of K_f at both temperatures. This procedure probably introduced only a small error between the K_f values obtained at different temperatures and may not account for the total observed difference.

A larger error at any given temperature could be caused by the use of a "wrong" value for the formation of gold hydroxide; literature values for equilibrium constants of metal-ion protolysis equilibria often range over many orders of magnitude as obtained by research workers using different techniques in different laboratories. Quantitative information concerning gold(I) hydrolysis is particularly scarce (30). Of course, certain hydrolysis equilibria might themselves be strongly affected by temperature changes. Earlier work on thiomalic acid chelation of silver(I) as a function of temperature (31) showed an increase of K_f with increasing temperature in that system.

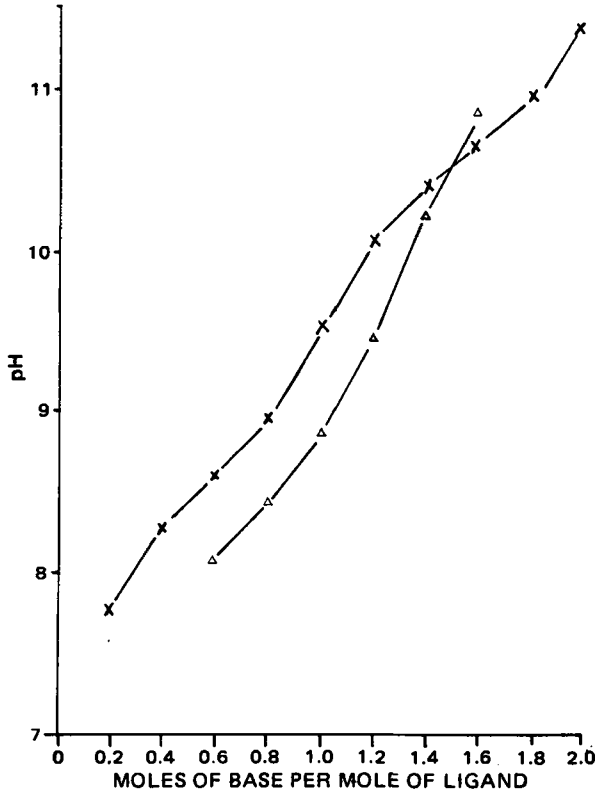

Figure 3—*Formation curves of cysteine with iron(III) (△) and free ligand (×) at 20°.*

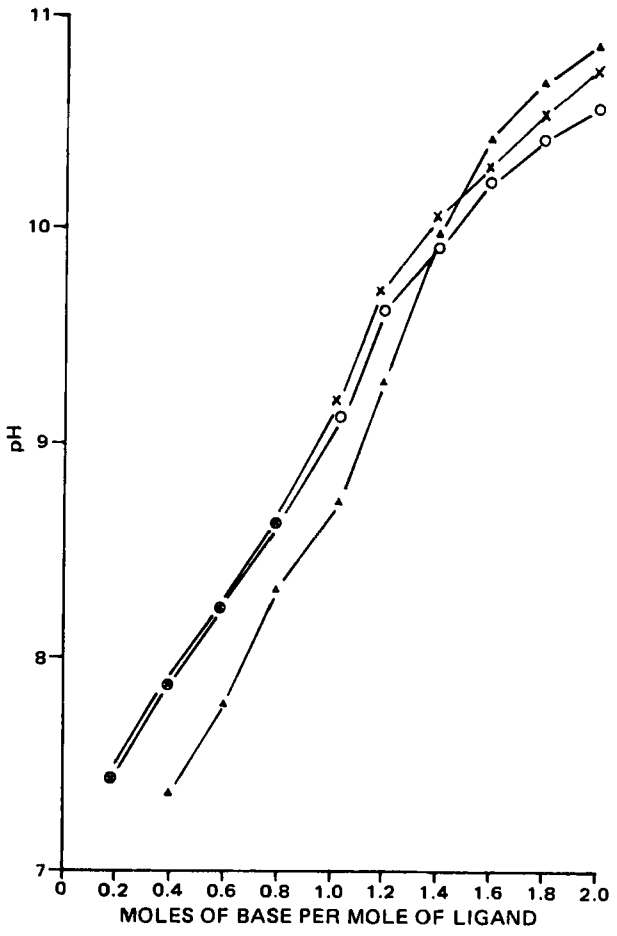

Figure 4—*Formation curves of cysteine with calcium(II) (○), iron(III) (△), and free ligand (×) at 37°.*

The ligand effect on the order of stabilities was the same with all three metal ions studied: I > V > VII > VIII. This order was also found in the literature for complexes of three of these ligands with other metals (14–17). No data were available for VIII.

In all cases, I formed a more stable complex than did V. Obviously, steric hindrance by the two added methyl groups in I is not a destabilizing factor but may have a small stabilizing effect on the chelate ring formed, thus giving rise to a higher K_f value of the I complex relative to the corresponding V complex. The lower stability of complexes with VII was probably due to the weaker interaction of the metal ions with the carboxyl group. Compound VIII was the weakest ligand. This finding is easily explained by the presence of only one strongly interacting group and the geometry that precludes the formation of a stable ring structure. The effects of structure and various substituents on the biochemical properties and the activity of I derivatives were studied previously (32–34), and chelate formation tendency was only one of a number of factors investigated.

Major differences between the V and I complexes existed that are not evident from the data shown. It was reported (17, 18, 26) that V tends to form precipitates with some metal ions, especially copper. This tendency was found with gold(I) as well. When V was added to II, a white precipitate formed immediately. The insoluble matter became slowly soluble after addition of some base [this same phenomenon was observed by Perrin and Sayce (15) for the V–zinc system]. The precipitate formed even when a large excess of V was added. Compound I also gave a precipitate with II in a 1:1 ratio but did not precipitate in a 4:1 ratio mixture.

No precipitate developed with V or I when added in excess to aurothiomalate.

Both ligands formed a dark precipitate with iron(III). The V system,

however, did precipitate at lower pH values (10 *versus* 11). At the same concentration, the solution of I–iron was a darker purple, indicating a stronger complex.

The V–gold(I) system was the most troublesome. Additions of the ligand to aurothioglucose resulted in immediate formation of a white precipitate. The precipitate very gradually became soluble with additions of base. However, this compound was not used to calculate K_f because of its unknown fate after the solid formation. The reaction may be analogous to the well-known copper–V oxidation–reduction reaction (26).

The iron(III)-V system was more stable, especially when some base was added to the solution before V. This system was used to calculate the K_f value of the V–gold complex.

The bonding in silver amino acid complexes was previously (17) postulated to occur through the amino nitrogen with very little, if any, contribution from the sulfur. These gold complexes were linear, as can be expected for all gold complexes studied. However, in the case of gold(I) complexes, the main bonding site is the thiol group. The K_f value for VIII–gold is about 75% of that with I or V. This value is quite high considering the absence of the amine group, since increased stability of the I and V complexes is due to the formation of a chelate through the nitrogen. The lower value obtained with thiomalic acid is attributed to a smaller affinity of gold for the carboxyl group, reaffirming the role of the sulfur.

The results of this study have some limited implications for the possible fate of I *in vivo*. Thus, the lower pKa values at physiological temperature will change somewhat the fraction of protonated, neutral species. While I sorption from the stomach will remain virtually unaffected (because the pH of the gastric solution is below the pKa of all acid groups present), the bioavailability of I *via* colonic absorption will be even smaller than would be predicted from pKa values at lower temperatures (20–25°). Similarly, the fraction of ionized I species in blood will be increased. As is generally recognized, oral administration is the only logical route for I, even though I has been given by injection (35, 36). Different therapeutic results with oral administration *versus* injection should have been expected.

While I has significantly greater *in vitro* complex formation constants with all metal ions than V, as exemplified again in this work, the *in vivo* *stability* of I, especially against L-amino acid hydrase, is the overriding factor (37). Enhanced excretion of metal ions induced by I (but not by V) may be much more a consequence of the relative biological stability of the thiol group in I than of higher *in vitro* K_f values. Even so, a correlation exists between the actual values of K_f (largest for the most toxic heavy metals mercury and lead) and metal-ion removal from the body, the excretion of copper having been studied most thoroughly.

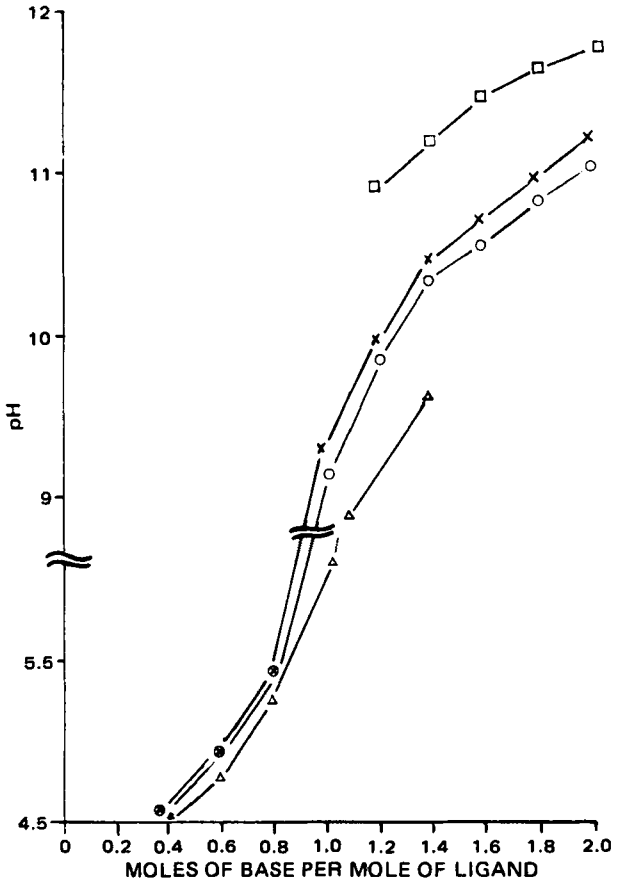

Figure 5—*Formation curves of thiomalic acid with calcium(II) (○), iron(III) (△), gold(I) (□), and free ligand (×) at 20°.*

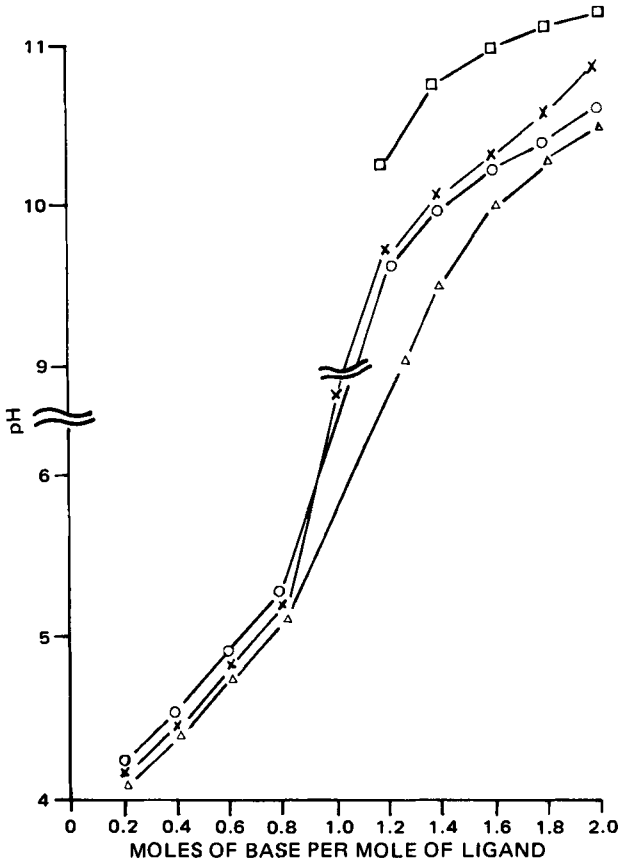

Figure 6—*Formation curves of thiomalic acid with calcium(II) (○), iron(III) (△), gold(I) (□), and free ligand (×) at 37°.*

The complex formation constants determined for gold(I) complexes in the present study are relatively high. While these values might be taken as evidence that I can remove previously deposited gold from the body of rheumatoid arthritis patients, they are not sufficient proof that that effect is important *in vivo*. In fact, some experts on I treatment of rheumatoid arthritis do not believe that I is beneficial in cases of gold poisoning[10].

For gold removal to occur, some, if not most, of the gold would have to be present in ionic form since I could obviously not complex directly with the free metal. It seems that an equilibrium between gold metal and gold ions is very unlikely (kinetically) *in vivo*. Conclusions concerning the oxidation state of gold species after injection in the body are hard to draw. In aqueous solutions, gold(I) has a tendency to disproportionate into gold metal and gold(III) (30), which is enhanced by increasing the temperature.

Furthermore, I would have to remain in a form that complexes the gold. Gold is supposed to be removed from areas where it is deposited. It is not circulating in the blood. Therefore, the I may have to circulate through the entire body before it reaches eventually the sites of gold deposition. During this time, it would be subject to many other reactions that can reduce the amount of I available for complex formation with gold. Only with extended administration of I could this condition be expected to occur. A one-time I dose for gold intoxication seems very unlikely to be effective.

Yet another consideration concerns the fact that the very strong 2:1 chelates formed with mercury, lead, nickel, copper, zinc, *etc.*, have significantly greater overall stabilities than the gold(I)–I complex, which has a 1:1 stoichiometry. The efficient removal of such heavy metal ions

[10] I. A. Jaffe, New York Medical College, New York, NY 10029, personal communication.

from the human body possibly is aided by the formation of "mixed chelates" *in vivo* (*e.g.*, I–metal ion–V). Mixed ligand chelates are believed to play an important role in biological systems (38) and are often significantly stronger than corresponding homoligand chelates. Gold(I) cannot enter into mixed ligand chelates and, therefore, would not be complexed as effectively *in vivo*.

Gold therapy is useful in a significant fraction of rheumatoid arthritis patients. The assumption is that the metal is the active agent of the gold compounds administered. The following observations, when considered together, may encourage speculation that this assumption need not necessarily be true:

1. Patients respond quite differently to different gold preparations (39).
2. Colloidal gold and gold compounds other than VII and VIII have been discontinued as ineffective or too toxic (40).
3. Ligands now used in rheumatoid arthritis therapy [with gold(I)] have a thiol group.
4. The biochemical activity of the only alternative drug to gold, I, is attributed to its thiol function (41).
5. Toxic side effects with I resemble those seen with patients receiving gold therapy (13).

The therapeutic effect of gold(I) compounds currently in use may be partly or entirely due to the thiol-containing ligand, *i.e.*, thioglucose or thiomalate. Apparently, this possibility has not been studied (42).

The relatively high K_f values for I with many metals also raise questions about the effect of I therapy on the balance of essential metals in the body. It will be remembered that the removal of metals is the basis of major accepted therapeutic uses of I.

The loss of taste due to a reduced zinc level has been reported by subjects during I therapy (43). Zinc supplements were given and normal taste sensitivity was restored, but normal taste returned by itself as well (44). The effect of I on body calcium levels is probably very small because of the small K_f and the large amount of the metal ion present in the body (10 mg/100 ml of blood). The amount of I complexed with iron might be expected to be quite high due to the relatively large K_f values determined in this study. However, most iron in the body is strongly tied up in hemoglobin. The actual ligand that chelates this iron *in vivo* is porphyrin, which is specific for iron; the complex has the very large K_f of $\sim 10^{30}$. Still, the fact that iron deposits are removed from synovial tissue by I (45) is in accord with iron complexing by I *in vivo*.

Similar considerations for some other trace metals in the body have not revealed extensive depletion of essential metals, although there have been no systematic studies except with copper and zinc. Certainly, the possibility of depleting metal ions such as cobalt(II) or chrome(III) needs further study.

Such studies as well as investigations into the pharmacokinetics of I and just patient monitoring would be considerably facilitated by reliable and rapid separation/analysis procedures for I and its complexes, metabolites, and derivatives. Current efforts are directed toward developing such methodology[11].

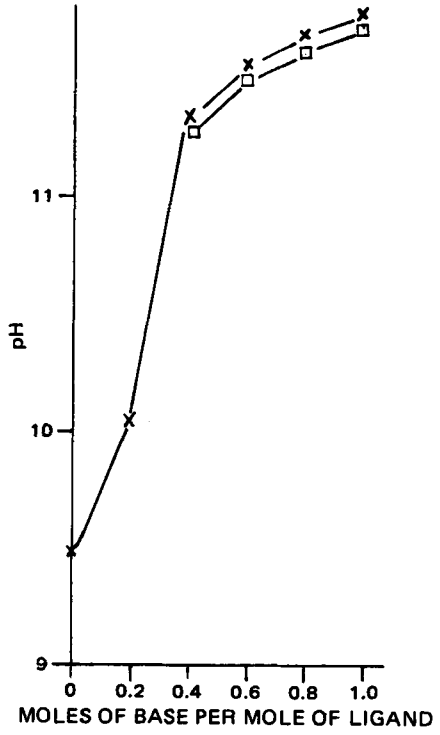

Figure 7—*Formation curves of thioglucose with gold(I) (□) and free ligand (×) at 20°.*

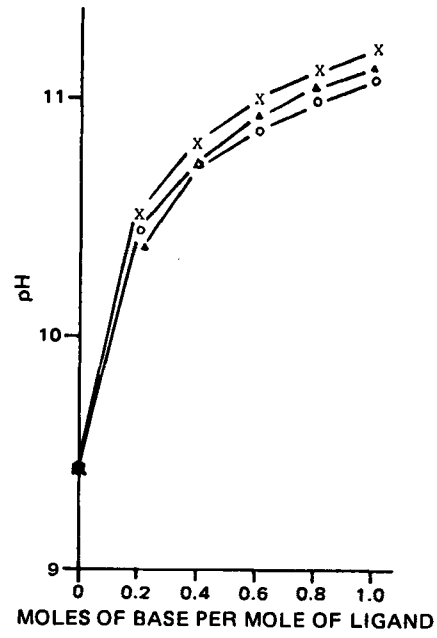

Figure 8—*Formation curves of thioglucose with calcium(II) (○), gold(I) (△), and free ligand (×) at 37°.*

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ACKNOWLEDGMENTS

Presented in part at the Division of Medicinal Chemistry, American Chemical Society, Chicago meeting, August 28–September 2, 1977.

The authors thank Dr. Vincent Maddi and Dr. John Baum for helpful advice and Dr. Israeli Jaffe for informative discussions as well as important literature references. A grant from the National Institutes of Health-Biomedical Research Support Grant fund of the State University of New York at Binghamton has enabled G. E. Janauer to continue this project.

Predictability of Warfarin Dose Requirements: Theoretical Considerations

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Abstract □ The theoretical basis of the predictability of warfarin maintenance dose requirements was evaluated using computer-generated hypothetical patient responses to a 10-mg/day "loading" dose regimen. Correlations between these responses and projected maintenance dose requirements were evaluated statistically, and a significant relationship was identified.

Keyphrases □ Warfarin—predictability of dose requirements, theoretical considerations, correlations between hypothetical patient responses and projected maintenance dose requirements □ Doses—predictability of warfarin requirements, theoretical considerations □ Maintenance dose requirements—predicting warfarin levels, theoretical considerations

Problems encountered in selecting an appropriate warfarin dosing regimen continue to represent a major obstacle to improvements in the quality and efficiency of oral anticoagulant therapy (1). Although the daily maintenance warfarin dose usually falls between 5.0 and 7.5 mg in large patient populations studied (2), wider variations in daily dose requirements are seen clinically, and the dose titration process used to determine this maintenance dose may be time consuming and relatively inefficient. The cost of added days of patient hospitalization required to select the maintenance dose may be significant.

Efforts to improve the efficiency of warfarin therapy have proceeded in two areas: (*a*) mathematical treatments describing the time course of warfarin's anticoagulant effects (3–5), and (*b*) clinical studies evaluating the predictability of steady-state warfarin dose requirements based on initial patient drug response (6–8). Two clinical investigations suggested a degree of predictability of anticoagulant maintenance dose requirements. A correlation was observed between the time required to achieve therapeutic anticoagulation (using a 15-mg/day "loading" dose regimen) and the maintenance warfarin dose finally required (6). Recently, a significant correlation between patient response to a specific loading dose regimen (10 mg/day for 3 days) and the maintenance dose required to achieve a desired steady-state degree of anticoagulation was demonstrated (7).

To evaluate the potential of these findings, the theoretical basis of observed correlations between loading and maintenance warfarin doses was investigated, using a previously described mathematical model for warfarin effect. The results of this evaluation and their clinical implications are described here.

BACKGROUND

Warfarin pharmacokinetics have been extensively investigated and are considered to be first order (3). They are described by:

$$C_p(t) = C_p^0 e^{-k_r t} \qquad \text{(Eq. 1)}$$

where $C_p(t)$ is the plasma drug concentration at any time t during a dosing interval, C_p^0 is the initial plasma concentration, and k_r is the first-order warfarin degradation constant.

0022-3549/79/0400-0432$01.00/0
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