3. The lipid phosphorus changed little during the first 8 days and then decreased rapidly during the period 8–32 days after either nerve section or nerve crush. In the sectioned nerves the lipid phosphorus fell still further, whereas in the crushed nerves it had commenced to increase by 96 days and continued to increase throughout the period of regeneration.

4. The concentration of total nucleic acid increased after either nerve section or nerve crush, reaching a maximum in 16 days. Thereafter the concentration of nucleic acid fell in both the sectioned and crushed nerves. After each type of operation the PNA increased more rapidly than the DNA, so that there was an increase in the PNA/DNA ratio.

5. The PP fell throughout the whole course of the degeneration in the sectioned nerves. In the crushed nerves it had commenced to rise by 96 days, and by

250 days it approximated the concentration in the control nerves.

6. The 'inositide phosphorus' followed a course similar to that of the lipid phosphorus. After either type of operation there was little change during the first 8 days, followed by a rapid fall during the period 8–32 days. In the sectioned nerves the 'inositide phosphorus' fell still further, whereas in the crushed nerves it had commenced to increase by 96 days and continued to rise during the entire period of study.

7. The increase in the concentration of nucleic acid and the decrease in the concentration of lipid phosphorus and 'inositide phosphorus' still persisted 600 days after nerve crush.

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A Study of the Amino-acid Complexes Formed by Metals of Group II of the Periodic Classification

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Albert (1950) published a study of the formation of complexes between a number of metals and α -amino-acids containing only two ionizing groups. The mathematical treatment of the results from potentiometric titrations enabled the stabilities of the metal complexes to be calculated. Using his potentiometric method all the metals of Group II of the periodic classification were studied in their relation

to glycine, its derivatives and other α -amino-acids with only two ionizing groups. In this paper an attempt has been made to study the effect on complex formation of (a) the nature of the metal, and (b) substitution in the amino-acid. An attempt was also made to correlate the results obtained by potentiometry with the solubility studies of Monk (1951*a*, *b*).

EXPERIMENTAL

Materials. The following salts were used in 0.01M-solution: $\text{BaCl}_2 \cdot 6\text{H}_2\text{O}$; SrCl_2 ; CaCl_2 ; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; $\text{BeSO}_4 \cdot 4\text{H}_2\text{O}$; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; $3\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$; $\text{Hg}(\text{NO}_3)_2$; $\text{CoCl}_2 \cdot 5\text{H}_2\text{O}$; $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$; $\text{Pb}(\text{NO}_3)_2$. $\text{Hg}(\text{NO}_3)_2$ was made up in 0.025M- HNO_3 and excess acid back-titrated in the presence of the amino-acid investigated. AgNO_3 was used in 0.02M-solution. All salts were of A.R. quality.

All the amino-acids were vacuum-dried at 100° for at least 48 hr., weighed and made up in 0.02M-solution. Because of the low solubility of their complexes it was necessary to use 0.001M-concentrations of DL-norvaline and DL-norleucine. The purity of each amino-acid was assessed by two-dimensional chromatography and by potentiometric titration with 0.100M-KOH. All samples except DL- α -alanine gave titration curves which on calculation yielded constant pK values within ± 0.03 pK units over the entire curve. The pK values were calculated for each point on the curve using the method of Sørensen (1909). The values obtained were consistent with those in the literature. DL- α -Alanine of satisfactory purity was obtained after two recrystallizations from aqueous methanol.

Potentiometric titration. A Cambridge Instrument Company bench type pH meter was used with a glass electrode and saturated calomel half-cell. The titrations were carried out as outlined by Albert (1950) with minor modifications. The dissociation constant for the α -amino group was determined under the conditions subsequently used for the titrations in the presence of the metal. These titrations were made on solutions in which the ratio of acid to metal was 2:1 for bivalent and 1:1 for univalent metals. For most titrations equal volumes of stock metal and amino-acid solutions were mixed and then stirred with O_2 -free N_2 for 10 min. At the midpoint of the titration the volume was 50 ml.

Calculations. The two systems to be considered in calculating the stability of each complex are:

- $\text{M}^{++} + \text{amino-acid} = \text{complex I}^+ \text{ with dissociation constant} = K_1,$
- $\text{complex I}^+ + \text{amino-acid} = \text{complex II} \text{ with dissociation constant} = K_2,$

where M^{++} is a bivalent metal. The overall stability of the final complex $\log K_s$ is given by

$$\log K_s = \log K_1 + \log K_2.$$

The calculation of $\log K_s$ takes account of the proportion of amino-acid in the form available for chelation at a particular pH. This form of the amino-acid is that in which the carboxyl group is ionized and the amino group unionized. All values for $\log K_s$ were calculated from the point at which one molecule of the amino-acid was bound to the metal ion. At this point the following equation was used: $\log K_s = -2 \log [\text{Sc}]$, where $[\text{Sc}]$ is the concentration of free amino-acid available for chelation at this pH (Albert, 1950). The more critical method of calculating values for either $\log K_1$ or $\log K_2$ for each point on the titration curve was also used. These methods yielded values for $\log K_s$ agreeing within 0.1 unit for the amino-acids and 0.2 unit for glycylglycine.

RESULTS

Table 1 shows the $\log K_s$ values obtained for glycine and those of its derivatives in which the hydrogen atoms of the amino group were substituted. There are no values for Mg, Ca, Sr and Ba as these were too low to obtain by the present method. Fig. 1 shows the relative stability constants for glycine with Group IIb metals. Table 2 is a comparison of the stability constants obtained by different authors using mainly potentiometric methods.

Fig. 1. Typical distribution of stability constants of Group II metals with an amino-acid (in this case glycine). The figures for magnesium and calcium are quoted from Monk (1951c).

DISCUSSION

Potentiometric titrations of α -amino-acids with metals of Group II showed that the two sub-groups behaved differently. The Group IIa metals, barium, strontium and calcium either did not form complexes, or only did so with very low stability constants. In contrast, the Group IIb metals, zinc, cadmium and mercury formed complexes with stability constants which could be readily measured. The other elements of the group behaved in different ways. Beryllium appeared to form stable complexes and was therefore associated with Group IIb. Magnesium, however, formed complexes with low stabilities which could not be measured accurately and it behaved as though it were intermediate in properties between the two groups. Monk (1951c) quoted $\log K_s$ values for calcium which were well beyond the limit measurable by the method used. He also showed that magnesium had $\log K_s$ values of approximately 4.0. This is in agreement with the values of Albert (1950) and those found in the present investigation.

Throughout this investigation it was apparent that the metal was the dominating factor in determining the stability constants of the complexes. This confirmed the observation of Albert (1950) on a wide range of metals. Fig. 1 showing the relative stabilities of the metal complexes with glycine was typical of all the amino-acids studied. The order of stability was $\text{Hg} > \text{Be} > \text{Zn} > \text{Cd}$. So far no metal has

possible to assess complex formation on the alkaline side of this pH.

The effect of ligand structure on complex stability was examined. No appreciable effect was found when the hydrogen atom of the glycine amino group was substituted as in sarcosine, proline and hydroxyproline. The values in Table 1 show differences which are not temperature effects, but are slight

Table 1. *Stability constants for metal complexes of α -amino-acids with only two ionizing groups*

(The effects of substituting the hydrogen atoms of the amino group and of carbon chain length and isomerism are shown.)

Amino-acid	Temperature (°C)	pK	log K_s			
			Be	Zn	Cd	Hg
Glycine	22.0	9.73	13.3	9.2	7.9	18.2
Glycylglycine	21.0	8.21	9.8	6.4	5.4	12.4
Sarcosine	20.0	10.22	13.9	8.8	7.5	18.7
L-Proline	17.0	10.46	14.2	9.9	8.0	20.5
L-Hydroxyproline	17.0	9.70	12.7	9.6	8.2	17.7
DL- α -Alanine	21.0	9.90	13.1	9.1	7.6	18.4
DL- α -Amino- n -butyric acid	20.0	9.62	12.9	8.3	6.8	18.5
DL-Norvaline	20.0	9.87	12.6	8.1	6.6	17.7
DL-Norleucine	20.0	9.92	12.8	8.5	6.9	17.8
DL- α -Aminoisobutyric acid	19.0	10.24	12.4	8.8	7.2	18.3
DL-Valine (MBT)*	19.5	9.59	12.4	8.2	6.7	†
DL-Isoleucine	20.0	9.86	12.6	8.2	6.6	17.6
DL-Leucine	20.0	9.92	13.2	9.1	7.8	17.5

* Microbiologically tested.

† Insoluble complex.

Table 2. *Comparison of stability constants in the literature of complexes formed with glycine, α -alanine and glycylglycine with various metals*

Amino-acid or peptide	Metal									
	Cu	Ni	Zn	Pb	Co	Mg	Ca	Ag†	Cd	Hg
Glycine	(1) 15.4	11.0	9.3	—	8.9	4.0	—	—	8.1	—
	(2) 15.6*	11.1	10.0	8.9	9.3	3.4†	1.4†	3.5*	—	—
	(3) —	11.0	9.2	9.3	8.8	—	—	3.7	7.9	18.2
	(4) 15.2	10.6	8.9	—	8.4	—	—	3.7	7.1	19.2
	(5) 15.4	—	9.7	—	8.9	—	—	—	—	—
α -Alanine	(1) 15.1	—	—	—	8.4	—	—	—	—	—
	(2) 15.4*	10.7	9.5	8.2	8.5	2.0†	1.2†	3.6*	—	—
	(3) —	—	9.4	—	—	—	—	—	7.2	—
Glycylglycine	(2) 11.7*	7.9	6.6	6.0	5.9	1.1†	1.2†	2.7*	—	—
	(3) —	7.6	6.4	5.8	5.8	—	—	3.1	5.4	12.4

(1) Albert (1950). (2) Monk (1951*a, b, c*). (3) Present work. (4) Flood & Loras (1945). (5) Maley & Mellor (1950).

* Solubility studies.

† First constant only.

been quoted in the literature as showing greater complex stability than mercury. The anomalous increase in stability when passing from cadmium to mercury was in accord with the work of Bjerrum (1941) on metal ammine formation. Experiments using mercuric chloride gave fallacious low results due to the mainly covalent nature of this salt. The ionized mercuric nitrate, however, gave the high values recorded here. The values for beryllium must be treated with caution as both its salts and complexes hydrolyse above pH 6. This made it im-

compared with the metal effect. The substitution of the α -amino hydrogen atom by another amino-acid as in glycylglycine, however, produced a decrease in stability comparable to the metal effect.

In Table 2 a comparison is made between the results of a number of workers. In general, good agreement was obtained for glycine and α -alanine. The purpose of this part of the work was to check the results from glass-electrode measurements with those of other authors and with the solubility measurements of Monk (1951*c*). Monk (1951*a*)

had already shown that solubility and titration measurement gave comparable results.

The results for glycylglycine agreed well with those of Monk (1951c). They were all measured at pH more acid than 8.0. At more alkaline pH values Dobbie, Kermack & Lees (1951) have shown that with cupric ions the peptide nitrogen also takes part in chelation. Evidence of this further chelation was obtained with mercury in this study. The titration curve obtained was symmetrical and yielded satisfactory results for $\log K_1$ and $\log K_2$.

The marked decrease in stabilities from glycine to glycylglycine is of interest, as according to Albert (1950) the metal bridges the carboxyl and amino groups of glycine. In the case of glycylglycine such ring formation is unlikely in view of the size of such a ring. As, however, there is evidence for the peptide losing a second proton per molecule (Dobbie *et al.* 1951) it is more probable that the metal chelates first with the terminal amino groups and then with the peptide nitrogen. Further work on metal peptide complexes is in progress.

The effect of lengthening the chain length on stability constants was small, but produced a still detectable decrease in stability from glycine to norvaline (Table 1). Norleucine, however, gave an unexpected increase in stability. The effect of isomerism in the chain was also studied. From Table 1

it will be seen that the isomers of norleucine gave different stability constants but the isomers of norvaline gave identical values.

SUMMARY

1. The stability constants of some complexes of α -amino-acids with metals of Group II of the periodic classification were measured by a potentiometric method.

2. The predominant part played by the metal in determining the stability constant was confirmed.

3. Metals of Group IIa formed complexes with stability constants too low to be measured. Group IIb metal complexes yielded measurable constants in the order of magnitude $\text{Hg} > \text{Be} > \text{Zn} > \text{Cd}$. The results of a number of investigators using a range of metals with glycine, α -alanine and glycylglycine were compared.

4. The effect of substituting the hydrogen atoms of the amino group of glycine on stability constants was studied. The only appreciable structural effect observed was the decrease in stability produced by peptide formation. Increase of chain length resulted in decreased stabilities.

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The Crystallization of Fumarase

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Fumarase, the enzyme catalysing the reversible hydration of fumaric acid to L-malic acid, was discovered in 1911 by Batelli & Stern. Since then it has been shown to occur in a large number of plant and animal tissues, and in lower organisms.

In 1941 Laki & Laki claimed to have isolated crystalline fumarase from pig-heart muscle, but Scott (1948), Scott & Powell (1948) and Racker (1950) have shown this preparation to contain considerable quantities of aconitase and lactic de-

hydrogenase. Scott (1948) has also obtained amorphous fractions with over three times the specific activity of the preparation of Laki & Laki. In this communication is reported the isolation from pig-heart muscle of crystalline fumarase, homogeneous by sedimentation and electrophoretic criteria, which has a specific activity almost ten times that reported by Laki & Laki. A preliminary account of this work has been published (Massey, 1951).