Biochemical Pharmacology, Vol. 23, pp. 2557–2565. Pergamon Press, 1974. Printed in Great Britain.

STUDIES WITH TRYPTOPHAN METABOLITES *IN VITRO*. EFFECT OF ZINC, MANGANESE, COPPER AND COBALT IONS ON KYNURENINE HYDROLASE AND KYNURENINE AMINOTRANSFERASE IN NORMAL MOUSE LIVER*

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(*Received* 27 *February* 1973; *accepted* 13 *February* 1974)

Abstract—The possible mechanisms by which increasing concentrations of Zn^{2+}, Mn^{2+}, Cu^{2+}, or Co^{2+} may affect the vitamin B_6-dependent enzymes kynurenine hydrolase and kynurenine aminotransferase, were studied in normal mouse liver homogenates. It was found that Zn^{2+} inhibited kynurenine hydrolase, whereas Mn^{2+} activated this enzyme, but both Zn^{2+} and Mn^{2+} activated kynurenine aminotransferase. Co^{2+} and Cu^{2+} inhibited both enzymes. This inhibition is attributed to the blocking and inactivation of the -SH groups of these enzymes and may be due to the adequate sequence of the -SH groups in both enzymes for Cu^{2+} or Co^{2+} action. This is in contrast to the inadequate sequence of these groups in kynurenine aminotransferase for Zn^{2+} action. The decreasing order by which these metal ions inhibit (a) kynurenine hydrolase is $Cu^{2+} > Co^{2+} > Zn^{2+}$, and (b) kynurenine aminotransferase is $Cu^{2+} > Co^{2+}$. The decreasing order of the per cent activation of the aminotransferase enzyme is $Mn^{2+} > Zn^{2+}$. These decreasing orders fall into a more reasonable order approximating the order of complex stability of these metal ions.

THE SITE of action of several members of the vitamin B complex on the metabolic pathway from the essential amino acid tryptophan to the vitamin niacin has been reviewed.[1,2] The role of B_1 and B_2 has not been clearly demonstrated, whereas the action of pyridoxine (B_6) in this pathway is well established.[2] Several enzymes along the tryptophan–niacin pathway (kynurenine pathway) e.g., kynurenine hydrolase, kynurenine aminotransferase and most probably quinolinic acid decarboxylase, require the participation of vitamin B_6 as coenzyme.[1–5]

It is known that pyridoxal chelates with cations[1] like Na^+, K^+, Li^+, Mg^{2+}, Ca^{2+} and Mn^{2+}, as well as with polyvalent cations.[1,6] It was also suggested that pyridoxal might function as a complex with a cation.[6] The metal ions which function in these reactions *in vivo* are unknown. The discovery that metal ions catalyze nonenzymatic reactions of pyridoxal with amino acids,[7,8] suggested that metal ions might participate in the action of pyridoxal phosphate-dependent enzymes.[9] However, little pertinent information is available on the effect of metal ions on the vitamin B_6-dependent enzymes kynurenine hydrolase and kynurenine aminotransferase.[10–15]

Since the polar side chains of proteins interact with metal ions, these ions often have a pronounced effect on enzyme function. The present studies were carried out

* This study was financially supported by the Alexandria Company for Pharmaceuticals and Chemical Industries.

to investigate the *in vitro* effects of some metal ions of biological interest e.g., Sb^{3+}, Pb^{2+}, Zn^{2+}, Mn^{2+}, Co^{2+} and Cu^{2+}, on the metabolism of kynurenine which is the central metabolite in the kynurenine pathway of tryptophan metabolism, and its conversion into anthranilic acid and kynurenic acid through the B_6-dependent enzymes, kynurenine hydrolase (EC 3.7.1.3) and kynurenine aminotransferase (EC 2.6.1.7), respectively.

Recent studies have shown that Sb^{3+}, as potassium antimonyl tartrate (tartar emetic), produces functional pyridoxine deficiency in the mouse liver.[16] The inability of pyridoxal hydrochloride, ATP, Ca^{2+} and Mg^{2+} to counteract the effects of tartar emetic, indicates that the phosphorylation of pyridoxal with ATP is impaired in the presence of Sb^{3+}. It is interesting to note that Mg^{2+} and Ca^{2+} in high concentrations are able, at least partially, to reverse the inhibitory effects of tartar emetic. Amer *et al.*[17] showed that four Sb^{3+}-containing drugs inhibit both kynurenine hydrolase and kynurenine aminotransferase in mouse liver homogenates. The inhibition was directly related to the antimonial contents of the drugs. Spectrophotometeric studies supported the possibility that an inactive chelate between antimony and pyridoxal phosphate is formed and offers an explanation for the mechanism of the observed inhibitions.[16,17] Kelada *et al.*[18] showed that tartar emetic inhibits both 3-hydroxykynurenine aminotransferase and quinolinic acid decarboxylase suggesting the existence of functional inactivation of vitamin B_6. However, vitamin B_6 was unable to overcome all tartar emetic induced abnormalities. It was striking that these abnormalities could be overcome completely by the use of 2,3-dimercaptopropanol (BAL) as an adjuvant to tartar emetic therapy.[18]

Recently, the effect of Pb^{2+} on the metabolism of kynurenine was studied.[19] It was shown that Pb^{2+} inhibits kynurenine hydrolase and it was suggested that the inhibitory effect of Pb^{2+} is brought about by inactivation of the sulphydryl groups of this enzyme.

The present study was planned, therefore, to investigate the effect of increasing concentrations of Zn^{2+}, Mn^{2+}, Cu^{2+} or Co^{2+} on the B_6-dependent enzymes kynurenine hydrolase and kynurenine aminotransferase, as indicated by the amounts of anthranilic acid and kynurenic acid produced, respectively.

MATERIALS AND METHODS

Materials. DL-Kynurenine sulphate was purchased from Schuchardt Co., Munchen, Germany. Kynurenic acid was supplied by Sigma Chemical Co., St. Louis, Mo., U.S.A. α-Ketoglutarate was supplied by L. Light & Co., Colnbrook, England. Anthranilic acid was purchased from Merck AG, Darmstadt, Germany. Manganese chloride was purchased from Riedel-De Haen AG, Seelze-Hannover, Germany, and zinc sulphate was purchased from Veb. Labor-Chemie, Apolda, Germany. Cobalt acetate was purchased from Johnson Malthey and Co. Ltd., London, England, and copper sulphate from Veb. Labor-Chemie, Apolda, Germany.

Preparation of the homogenates and incubations. Mouse liver homogenates were prepared and incubations were carried out as previously described.[20]

Quantitative determination of metabolites. The quantitative determinations of kynurenine, kynurenic acid and anthranilic acid were performed by a slight modification of the method used by Miller *et al.*[21]

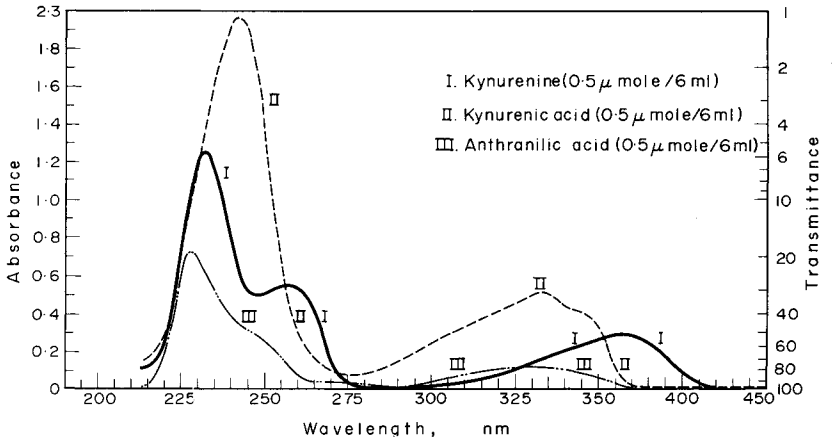

FIG. 1. Ultra-violet absorption spectrum of kynurenine (0·5 μmole/6 ml), kynurenic acid (0·5 μmole/6 ml) and anthranilic acid (0·5 μmole/6 ml) measured under the same experimental conditions.

Anthranilic acid and kynurenine were determined by the method of Mason and Berg[22] and the concentrations of anthranilic acid and kynurenine were derived from the corresponding calibration curves prepared with aqueous solutions containing known concentrations of both metabolites. The amounts of kynurenine and anthranilic acid were corrected by subtracting the amounts present at the zero time.

Kynurenine and kynurenic acid were determined by the method of Miller *et al.*[21] These metabolites were estimated spectrophotometrically in presence of each other at the wavelengths 365 nm and 330 nm, corresponding to kynurenine and kynurenic acid, respectively. Figure 1 illustrates the possible interference at these wavelengths in the u.v. spectrum of acidified solutions (with TCA), of equimolar concentrations (0·5 μmole/6 ml) of these metabolites under the same experimental conditions. Since kynurenine and anthranilic acid have considerable absorption at 330 nm, the wavelength used for kynurenic acid analysis, it was possible to correct the absorption value of kynurenic acid by deducting the absorption values (at 330 nm) corresponding to the interfering quantities of kynurenine (recovered) and anthranilic acid (produced) from the absorption reading at 330 nm. Thus, calibration curves for kynurenine and anthranilic acid were prepared at 330 nm. The absorption at 330 nm over the concentration range employed, followed the Beer-Lambert law. Moreover, additive absorption readings at this wavelength were obtained when kynurenic acid was added to solutions containing kynurenine and anthranilic acid either alone or in mixture. These curves were used to determine the absorption at 330 nm corresponding to the amount of each of kynurenine and anthranilic acid present, and which were quantitatively determined by the method of Mason and Berg.[22] The absorption measured at 365 nm also represents the amounts of kynurenine recovered. The results obtained for kynurenine recovered either by the method of Mason and Berg[22] at 560 nm or by that of Miller *et al.*[21] at 365 nm were almost identical. Recovery experiments using known weights (in μmoles) of the individual metabolites or known amounts of their mixtures, were completely satisfactory by applying the above mentioned modification.

RESULTS

The effect of Zn^{2+}, Mn^{2+}, Co^{2+} and Cu^{2+} on the B_6-dependent enzymes kynurenine aminotransferase and kynurenine hydrolase is best illustrated by (a) the ratio of the amount of anthranilic acid (AA) to the amount of kynurenic acid (KA) produced (AA/KA), and (b) the percentage of activation of inhibition. This is calculated as the percentage difference between the amount of the metabolite produced in the control experiment and that produced at varying concentrations of each metal ion with reference to the control value.

The effect of increasing the concentration of each metal ion on kynurenine aminotransferase and kynurenine hydrolase, as indicated by the amounts of kynurenic acid and anthranilic acid (in μmoles/g liver) produced, respectively, is shown in Table 1. At a concentration of 3×10^{-5} M, Zn^{2+} activated kynurenine aminotransferase but had no demonstrable effect on kynurenine hydrolase (Table 1A; expt. 2). Increasing the concentration of Zn^{2+} to 3×10^{-4} M (expt. 3), 3×10^{-3} M (expt. 4), and to

TABLE 1. EFFECT OF INCREASING CONCENTRATIONS OF THE METAL IONS ON THE METABOLISM OF KYNURENINE BY NORMAL MOUSE LIVER HOMOGENATES*

Expt. no.	Concn (M)	Metabolites determined (μmole/g liver)† Kynurenine utilized (KN)‡	Kynurenic acid (KA)	Anthranilic acid (AA)	Ratio (AA/KA)	% Activation KA	% Inhibition AA
			(A) Zn^{2+}				
1	0	7·44	5·10	0·80	0·16	0	0
2	3×10^{-5}	6·54	5·40	0·82	0·15	6	0
3	3×10^{-4}	6·64	6·00	0·60	0·10	18	23
4	3×10^{-3}	6·75	6·30	0·43	0·07	24	48
5	$7{\cdot}8 \times 10^{-3}$	6·75	6·30	0·39	0·06	24	51
			(B) Mn^{2+}			% Activation	% Activation
1	0	6·80	5·40	0·67	0·12	0	0
2	3×10^{-5}	7·32	6·30	0·78	0·12	17	16
3	3×10^{-4}	7·64	6·90	0·83	0·12	28	24
4	3×10^{-3}	8·64	7·50	0·90	0·12	39	34
5	$7{\cdot}8 \times 10^{-3}$	8·88	7·50	0·95	0·13	39	42
			(C) Co^{2+}			% Inhibition	% Inhibition
1	0	6·80	5·40	0·67	0·12	0	0
2	3×10^{-5}	5·22	4·17	0·51	0·12	23	24
3	3×10^{-4}	4·32	3·33	0·45	0·14	40	33
4	3×10^{-3}	3·32	2·94	0·35	0·12	46	48
5	$7{\cdot}8 \times 10^{-3}$	3·28	2·63	0·27	0·10	51	60
			(D) Cu^{2+}				
1	0	7·44	5·10	0·80	0·16	0	0
2	3×10^{-5}	5·64	4·92	0·41	0·08	4	49
3	3×10^{-4}	3·94	3·60	0·35	0·10	29	56
4	3×10^{-3}	2·35	2·10	0·14	0·07	59	83
5	$7{\cdot}8 \times 10^{-3}$	1·22	1·10	0·00	0·00	80	100

* The incubation medium (4 ml) contained 5 μmoles DL-kynurenine sulphate, 30 μmoles α-ketoglutarate, 40 μg pyridoxal phosphate, 0·005 M calcium chloride, 0·001 M magnesium sulphate and 10 per cent whole liver homogenate (2 ml) in 0·05 M potassium phosphate buffer pH 7·4.

† Average values of four experiments.

‡ These values represent the difference between the kynurenine recovered and that originally present in the medium.

$7{\cdot}8 \times 10^{-3}$ M (expt. 5), induced pronounced inhibition of kynurenine hydrolase and further activation of kynurenine aminotransferase (Table 1A). The ratio AA/KA was therefore, decreased from 0·16, 0·15, 0·10, 0·07 and 0·06 with increasing Zn^{2+} concentration (Table 1A). This decrease could be attributed to the inhibition of kynurenine hydrolase responsible for the production of anthranilic acid rather than to increased production of kynurenic acid. This interpretation could be further substantiated by comparing the per cent inhibition of anthranilic acid with the per cent activation of kynurenic acid production in the presence of increasing concentrations of Zn^{2+} in the incubation medium. The former percentage was increased from 0, 23, 48 and 51 per cent, whereas the latter percentage was increased from 6·0, 18, 24 and 24 per cent with increasing concentrations of Zn^{2+}, 3×10^{-5} M, 3×10^{-4} M, 3×10^{-3} M and $7{\cdot}8 \times 10^{-3}$ M, respectively (Table 1A).

On the other hand, increasing the concentration of Mn^{2+} in the incubation medium from 3×10^{-5} M, 3×10^{-4} M, 3×10^{-3} M, and $7{\cdot}8 \times 10^{-3}$ M, resulted in an activation of both enzyme systems to the same extent (Table 1B). Thus the activation of kynurenine hydrolase was increased from 16, 24, 34 and 42 per cent, and the activation of kynurenine aminotransferase was also increased from 17, 28 and 39 per cent with increasing concentrations of Mn^{2+} (Table 1B). The AA/KA ratio was constant (0·12), i.e. independent of the Mn^{2+} concentration in the medium (Table 1B).

The results concerning the effect of increasing the concentration of Co^{2+} or Cu^{2+} on kynurenine aminotransferase and kynurenine hydrolase are given in Table 1C and 1D. Increasing the concentration of Co^{2+} or Cu^{2+} from 3×10^{-5} M, 3×10^{-4} M, 3×10^{-3} M and $7{\cdot}8 \times 10^{-3}$ M resulted in an inhibition of both enzymes. Co^{2+} inhibited both enzyme systems to the same extent. Thus the per cent inhibition of kynurenine hydrolase and kynurenine aminotransferase was increased to nearly the same extent by increasing the concentration of Co^{2+} in the incubation medium. The AA/KA ratio was, therefore, nearly constant and independent of the concentration of Co^{2+} (Table 1A). However, the inhibition induced by Cu^{2+}, was more pronounced on kynurenine hydrolase. The per cent inhibition of kynurenine hydrolase was increased from 49, 56, 83 and 100 per cent, whereas the per cent inhibition of kynurenine aminotranferase was increased from 4, 29, 59 and 80 per cent by increasing the concentration of Cu^{2+} from 3×10^{-5} M, 3×10^{-4} M, 3×10^{-3} M and $7{\cdot}8 \times 10^{-3}$ M. Furthermore, the AA/KA ratio was decreased from 0·16, 0·08, 0·10, 0·07 and 0, with the same increase in Cu^{2+} concentration in the medium. Thus, the per cent inhibition for the production of anthranilic acid was higher than that for kynurenic acid (Table 1D; expts 2, 3, 4 and 5).

The effect of Zn^{2+}, Mn^{2+}, Co^{2+} or Cu^{2+} on the B_6-independent enzyme kynurenine hydroxylase was also studied by determining the amounts of kynurenine utilized which reflect closely the amounts used in the synthesis of the two metabolites, as well as the amounts of kynurenine utilized in other pathways e.g., in the synthesis of 3-hydroxykynurenine. The amounts of kynurenine utilized in the absence of these metal ions were more than that converted to both metabolites (Table 1). The difference may reflect hydroxylation to 3-hydroxykynurenine or the further metabolism of either of the products. However, the amounts of kynurenine utilized were almost equivalent to that converted to both metabolites, kynurenic acid and anthranilic acid in the presence of these metal ions (Table 1). The latter finding may be taken as an

indication that the conversion of kynurenine to other metabolites e.g., 3-hydroxykynurenine, may be inhibited due to either the presence of these metal ions, or the presence of insufficient quantities of reduced triphosphopyridine nucleotide (NADPH) required for the conversion of kynurenine to 3-hydroxykynurenine through kynurenine hydroxylase in the homogenate; NADPH was not added to the incubation medium. This finding requires further investigation especially since the metal requirement for this enzyme is not known.[23]

DISCUSSION

It is evident from the present study that increasing concentrations of Zn^{2+} (Table 1A) inhibit the vitamin B_6-dependent enzyme kynurenine hydrolase which is responsible for the conversion of kynurenine to anthranilic acid, whereas Mn^{2+} activates this enzyme (Table 1B). Our previous studies have shown that the same enzyme system could be inhibited by increasing concentrations of Pb^{2+} in the incubation medium.[19] On the other hand, Zn^{2+} or Mn^{2+} activates the B_6-dependent enzyme kynurenine aminotransferase which is responsible for the conversion of kynurenine to kynurenic acid (Table 1A and 1B). However, Co^{2+} (Table 1C) and Cu^{2+} (Table 1D) inhibit both enzymes. Cu^{2+} has a more pronounced inhibitory effect than Co^{2+} at equimolar concentrations ($7{\cdot}8 \times 10^{-3}$ M) (expt. 5, Table 1C and 1D).

The present results are in accordance with the results of some studies carried out on the effect of these metal ions on kynurenine hydrolase. Saran[24] showed that *Neurospora* kynurenine hydrolase is inhibited by Zn^{2+} and Co^{2+} and various other metal salts at 10^{-5} M, but is activated by Mn^{2+}. The *in vivo* studies of Matsumura *et al.*[25] showed also an inhibitory effect of Co^{2+}, Cu^{2+} or Zn^{2+} on the enzyme activity when these metals were injected intramuscularly. Wiss and Weber[11] found no activation effects on a 150-fold purified enzyme from guinea-pig liver in the presence of either Co^{2+}, Zn^{2+} or Mn^{2+} in the incubation medium. However, Hagino found that various di- and mono-valent cations had no effect on the purified enzyme from rat liver.[15]

The finding that both Co^{2+} and Cu^{2+} inhibit, whereas, Zn^{2+} and Mn^{2+} activate the kynurenine aminotransferase enzyme system, coincides with that of Ogasawara[26] who found that a 200-fold purified kynurenine aminotransferase was inhibited by Co^{2+}, Hg^{2+}, Sn^{2+} or Ni^{2+} at 10^{-3} M. Moreover, the *in vitro* studies of Matsumura *et al.*[25] revealed that Zn^{2+} (10^{-2} M to 10^{-4} M) activated kynurenine aminotransferase, but, no effect was found *in vivo*. Shibata *et al.*[27] found that when $ZnSO_4$ was administered to rats the activity of kynurenine aminotransferase was not changed, whereas kynurenine hydrolase was inhibited. This latter finding coincides with that found in the present study concerning Zn^{2+}, although Mason demonstrated no metal effect on kynurenine aminotransferase.[28]

Such reports of partial activation of enzymes in impure preparations must be interpreted with caution, since many unexpected reactions can occur. For example, a metal might act as a protective agent by complexing with an inhibitory compound, or it might activate another enzyme whose activity would benefit the system under study. Zn^{2+} and other divalent ions activate pyridoxal kinase[29] and it is conceivable that under some experimental conditions this could lead to apparent metal activation of a pyridoxal phosphate requiring enzyme.

A direct comparison of results is not possible since experimental conditions differ. In some studies the activity of kynurenine hydrolase[11,15,24] and kynurenine aminotransferase[26,28] at different degrees of purity was assayed. Moreover, different techniques were used to study the *in vivo* effects of metal ions on both kynurenine hydrolase and kynurenine aminotransferase enzyme systems e.g., administration of metal ions either orally or by injection and *in vitro* by the addition of metal ions to the medium. The sources of kynurenine hydrolase and kynurenine aminotransferase in the *in vitro* studies also differ e.g., from *Neurospora*, guinea pig liver and rat liver, and different animal species were used for the *in vivo* studies. Different sensitivities of pyridoxal phosphate-requiring enzymes have been reported in various species.[1] Nevertheless there does not seem to be an effective means of determining to what degree these B_6-dependent enzymes are saturated with the coenzyme in the tissues (*in vivo* techniques[30]). Under certain conditions, these values decline rapidly following homogenization.[31] Rosen has raised the possibility that *in vitro* the B_6-dependent enzymes may undergo a conformational change that allows for greater binding of pyridoxal phosphate, and thus increases enzyme activity in the *in vitro* assay system.[32] It seems, therefore, that the per cent saturation[30] cannot be taken as an accurate representation of *in vivo* binding.

The amounts of kynurenine utilized, in the absence of these metal ions, were more than those converted to both metabolites. The difference might reflect hydroxylation of kynurenine to 3-hydroxykynurenine or the further metabolism of either of the products. However, there is evidence that the conversion of kynurenine to other metabolites *viz.*, 3-hydroxykynurenine, may be inhibited in the presence of increasing concentrations of Zn^{2+} (Table 1A), Co^{2+} (Table 1C), Cu^{2+} (Table 1D) or Mn^{2+} (Table 1B), since the amounts of kynurenine utilized were equal to those convered to both metabolites (kynurenic acid and anthranilic acid). It seems, therefore, that increasing concentrations of Zn^{2+}, Co^{2+}, Cu^{2+} or Mn^{2+} further inhibit the vitamin B_6-independent enzyme kynurenine hydroxylase, which is responsible for the conversion of kynurenine to 3-hydroxykynurenine. No specific inhibitors of kynurenine hydroxylase are known, but being an FAD-containing enzyme, it might be inhibited by anything that would inhibit any flavoprotein enzyme such as Cu^{2+}, Ag^{+} and others.[23] Further investigations are urgently needed to investigate the possible role of these metal ions on the other vitamin B_6-independent enzymatic reactions along the kynurenine pathway.

Of particular interest is the finding, in the present and in our previous work,[19] that Zn^{2+} or Pb^{2+} are two metal ions which inhibit only the kynurenine hydrolase enzyme. Pb^{2+} and Zn^{2+} are known sulphydryl inhibitors.[19,33–36] Therefore, the inhibition induced by Zn^{2+} or Pb^{2+} may be brought about by inactivation of the -SH groups of kynurenine hydrolase. The finding that Zn^{2+} or Pb^{2+} does not inhibit kynurenine aminotransferase may be attributed to the inadequate sequence of the -SH groups of this enzyme for their action. It was previously reported that kynurenine hydrolase contains functional -SH groups.[24] It seems that Pb^{2+} has a higher affinity than Zn^{2+} for the -SH groups of kynurenine hydrolase, since the percentage inhibition of anthranilic acid production in the presence of Pb^{2+} (73%)[19] was found to be higher than that induced by Zn^{2+} (51%, Table 1A) on the same enzyme system.

The -SH groups of enzyme protein have a natural chelating ability for arsenic, zinc, copper, cobalt and lead.[19,33–38] However, since it is known that kynurenine hydro-

lase contains functional -SH groups,[24] the inhibitory effect of Zn^{2+}, Co^{2+}, Cu^{2+} or Pb^{2+} on kynurenine hydrolase may be brought about by blocking and inactivating the -SH groups of the enzyme. The finding that Co^{2+} or Cu^{2+} inhibits both kynurenine hydrolase and kynurenine aminotransferase (Table 1C and 1D) may be cautiously attributed to the adequate sequency of the -SH groups on both enzyme systems for Co^{2+} or Cu^{2+} action. This is in contrast to the inadequate sequence of the -SH groups on kynurenine aminotransferase for Zn^{2+} or Pb^{2+} action. However, this interpretation requires further investigation using another sulphydryl inhibitor e.g., *p*-chloromercuribenzoate. It may be that active sites other than the -SH groups, are functioning in the case of kynurenine aminotransferase e.g., carboxyl or amino groups, since these metal ions are known to have different affinities for these groups.[39]

The decreasing order by which the metal ions tested in these and previous experiments[19] inhibit kynurenine hydrolase is in the following order, Cu^{2+} (100%) > Pb^{2+} (73%) > Co^{2+} (60%) > Zn^{2+} (51%), and the decreasing order by which these metal ions inhibit kynurenine aminotransferase is Cu^{2+} (80%) > Co^{2+} (51%). However, Mn^{2+} activates both enzyme systems, whereas Zn^{2+} or Pb^{2+} activates only kynurenine aminotransferase. The decreasing order of the per cent activation is Mn^{2+} (39%) > Pb^{2+} (34%) > Zn^{2+} (24%). It seems that the decreasing order, either of inhibition or activation, falls into a more reasonable order approximating the order of the complex stability of these metal ions i.e., $Cu^{2+} > Ni^{2+} > Co^{2+} > Pb^{2+} > Mn^{2+} > Ca^{2+} > Mg^{2+}$. The position of zinc in the latter series varies, but it always forms less stable complexes than copper.[13,14] On the other hand Zn^{2+}, Mn^{2+}, Co^{2+} and Cu^{2+} inhibit the B_6- independent enzyme kynurenine hydroxylase. Pb^{2+} has no inhibitory effect on this enzyme.[19]

Schwarz and Mertz[40] reported that it is necessary to supply the metal ion in a complex of intermediate stability since very stable complexes are inactive. It is also reported that only those metal ions which can make and break bonds rapidly (the very labile ones) will enhance the reaction rate.[41] Thus, comparing the activation per cent of kynurenine aminotransferase in the presence of equimolar concentrations ($7{\cdot}8 \times 10^{-3}$ M) of Zn^{2+} or Mn^{2+}, it could be concluded that the activation per cent is decreased in the order Mn^{2+} (39%) > Zn^{2+} (24%) (Table 1B and Table 1A), which falls into a more reasonable order approximating the order of their complex stability.[13,14]

The exact explanation of metal toxicity effects on tryptophan metabolism may be, therefore, a direct result of metal ion imbalance. Decreased enzyme activity may result directly from inhibition by some metal ions through the formation of a strong stable inactive Shiff's base complex of vitamin B_6.

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