PRINCIPLES GOVERNING THE FORMATION OF METALLIC COMPLEXES OF POLYAMINO-POLYPHOSPHONIC ACIDS AND EXCRETION OF METALS FROM THE BODY

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Some principles governing the formation of metallic complexes with new chelating compounds, the polyaminopolyphosphonic acids, are described. These compounds are capable of forming stable complexes with Cu, Pb, Fe, and Be, but they practically do not react with Ca. The polyaminopolyphosphonic acids are shown to be effective agents for the elimination of Be, in agreement with physicochemical principles governing chelation of metals in vitro. However, in relation to elements such as Ni, Co, and V, chelation with phosphonate groups, just as with acetate groups (EDTA, DTPA, etc.) is ineffective. To explain these findings, facts are described indicating stability of the bond formed by these metals with amino acids and proteins. Competitive relationships of this type reduce excretion of the incorporated metal.

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The interest shown by biologists in chelating agents is due mainly to the ability of these compounds to form highly stable chelate complexes with most metals which are soluble in water, and also to their relatively low toxicity [1-8]. It is these properties of chelating agents which have helped to solve problems connected with the conservation of blood, and the excretion of radioactive isotopes and toxic metals (Pb, I, Zn, Mn, Cr, Po) from the body.

However, the universality of the action of these polydentate chelating compounds was in one respect an obstacle to elimination of highly toxic elements such as Be, Mg, Sr, and Ba, with a chelation stability constant somewhat lower than that of Ca, from the body [6, 9, 11]. Since the Ca concentration in blood and other biological media of the body is very high, this cation can compete for a place in the complex with any of these metals requiring excretion. The equilibrium point of chelation in the body depends on the ratio between the stability constants of the chelate of the metal to be excreted and of Ca. One of the principal characteristics determining the direction of chelation in the body is the displacement constant of Ca by the metal [6, 11, 15].

The very low value of this constant also explains the ineffectiveness of EDTA and other aminopolycarboxylic acids in eliminating elements such as Mg, Be, Sr, etc. from the body.

From this point of view chelating agents in whose molecule the carboxyl groups are replaced by alkylphosphonates are of considerable interest [2, 9, 10]. This modification of the molecule of the chelating compounds provided chemists and biologists with chelating agents with new and very valuable properties. These compounds can form stable complexes with Cu, Zn, Fe, rare-earth elements, and Be [1, 8, 9, 10], yet they practically do not react with Ca, a fact which served as the basis for the study of the usefulness of these compounds in biology and medicine.

The object of this investigation was to study the chelating properties of polyaminopolyphosphonic acids relative to Ca, Be, Mn, Fe, and Cu and their effect on the excretion of these elements from the body.

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TABLE 1. Excretion of Ca^{45} (mean data in percent of administered dose)

Compounds administered	Excretion of Ca^{45}		
	by the kidneys	by the intestine	total
Control ($Ca^{45}Cl_2$)	0.95±0.07	8.28±0.76	9.23±1.2
$Ca^{45}Cl_2$ + phosphicin (intraperitoneally)	1.75±0.35	6.14±0.52	7.89±0.71
$Ca^{45}Cl_2$ + phosphicin (by mouth)	0.88±0.10	9.02±1.1	9.90±2.0

TABLE 2. Effect of Polyaminophosphonic Acids on Content of Be^7 in Mouse Cadavers (mean data in percent of control)

Experimental conditions	γ-ray activity of cadavers after administration of Be^7	
	1 h	24 h
Be^7	100.0	100.0
Be^7 + I	62.5	52.4
Be^7 + II	77.8	48.4
Be^7 + III	75.0	48.4

Note: I) Ethylenediamino-bis-isopropylphosphonic, II) oxadiethyleneaminoisopropylphosphonic, III) diethylenetriamino-bis-isopropylphosphonic acids.

EXPERIMENTAL METHOD

The chelating properties of the polyaminopolyphosphonic and polyaminopolycarboxylic acids were investigated polarographically and by potentiometric titration. To investigate the effect of the chelating agents on excretion of the various elements, Ca^{45}, Be^7, Mn^{54}, and Cu^{64} were used, being injected intraperitoneally into albino rats in the form of $Ca^{45}Cl_2$, Be^7Cl_2, $Mn^{54}Cl_2$, and $Cu^{64}SO_4$ in indicator doses. The animals were kept in metabolism cages so that the excreta could be collected separately. The content of Be^7, Mn^{54}, and Cu^{64} in the animals' body and excreta, and also in their blood and organs was determined by measurement of the γ-emission by means of a special instrument (built by A. A. Petushkov and M. R. Zel'tser). In the case of animals receiving $Ca^{45}Cl_2$, the measurements were made with respect to β-particle activity.

EXPERIMENTAL RESULTS

The experimental results showed a full correlation between instability of the Ca complex with ethylenediamino-bis-isopropylphonic acid (phosphicin) and absence of ability of this compound to increase the excretion of Ca from the body (Table 1). In contrast to phosphicin, under analogous conditions Na_2EDTA increases Ca excretion by 2.4-4 times [15]. For this reason, despite the lower value of the stability constant of complexes formed by different metals with organophosphorus chelating agents compared with those containing carboxyl groups, the displacement constants were higher in the first case. The absence of effect of Ca on binding of metals in the body by organophosphorus chelating agents may in fact explain the efficacy of phosphicin in lead poisoning [1]. The same principle is also seen in relation to Cu and Fe. The disodium salt of phosphicin was found to produce rapid excretion of Cu and Fe from the body, in full agreement with the high values of the stability constants of their complexes.

Particular attention should be given to the possibility of using organophosphorus derivatives of polyamines for the elimination of Be from the body. The formation of complexes of Be with these chelating agents possesses a number of characteristic features making these compounds specific reagents for Be. Among these properties are the absence of Be – N bonds in the complex, the formation of polycyclic Be complexes, high stability constants compared with the hydrogen complexes of other metals, and also the formation of polymetallic complexes. The structure of these complexes includes one or two Be ions and an ion of another metal possessing high affinity for N (for example, bivalent ions of the first transition period). The high stability of homo- and heteronuclear Be complexes with chelating agents in whose molecule the carboxyl groups are completely replaced by phosphonate groups is the basis on which effective elimination of this highly toxic element from the body can be expected to take place (Table 2).

Polymetallic complexes incorporating Be in the case of completely phosphorylated analogs have high constants relative to Ca. However, in the case of dicarboxymethylethylenediamino-bis-methylphosphonic acid, because of the presence of acetate groups forming bonds with Ca, the polynuclear complex has very high stability constants relative to Ca, leading ultimately to a low rate of Be elimination from the body.

The facts described thus indicate correlation between the physicochemical principles governing chelation of metals in vitro and the formation of complexes in vivo. Chelating agents with phosphonate groups, such as EDTA and DTPA, were found to be practically ineffective in relation to elements such as Ni, Co, and Cd. The displacement of Ca by the metal are high for Ni^{++}, Co^{++}, and Cd^{++}, whether for aminopolyacetic or aminopolyphosphonic acids, and they sometimes exceed those for Pb [10, 16, 17]. Consequently,

the lower effectiveness of chelating compounds of both series in these cases cannot be explained by competitive relationships with Ca. To explain this phenomenon, data for the stability of bonds between the incorporated metal and biocomplexes of the body must be invoked. The commonest and most widespread mechanism of metal binding in the body is the formation of complexes with amino acids and proteins, and the reaction of the metals with precipitating anions (PO_4, CO_3, etc.). It has been shown [19] that mono- and polynuclear complexes of metals with amino acids and proteins formed in the body have a substantial influence on complex formation with low-molecular weight aminopolyacetic acids administered from outside. Many investigations have been made of the stability of bonds formed by amino acids with metallic ions, from which the differences in stability of the complexes can be judged. Stability of complexes with amino acids increases from Mn^{++} to Cu^{++} roughly in the following order: $Mn^{++} < Fe^{++} < Pb^{++} < Co^{++} < Zn^{++} < Ni^{++} < Cu^{++}$. The binding of Co^{++}, Ni^{++}, Zn^{++}, and Cu^{++} with biocomplexes of the body is most stable. The stability constants of their complexes with amino acids are higher in value than the corresponding constants of displacement of Ca by the metal. For example, log K (the stability constant) of cysteine with Zn is 18.7, of asparagine with Ni it is 12.39, with Co 10.18, and so on. This comparison explains the low level of excretion of Ni, Co, and Zn by both aminopolyacetic and aminopolyphosphonic acids. The values of the stability constant for complexes of metals with amino acids indicate that competitive relationships of this type must have a greater, and not a lesser influence on binding of the metal by aminopolyphosphonic and polyacetic acids than that of Ca. Consequently, competitive relationships are created in the body not only between cations, but also between the organic anions binding the cations with different degrees of stability. Competitive relationships of this type may reduce the degree of elimination of the incorporated metal.

Competitive relationships between aminopolyphosphonic and polyacetic acids and amino acids are only one example of possible interactions in the body. The processes of interaction between exogenous chelating agents and high-molecular weight biocomplexes, and especially proteins forming polynuclear complexes with metals, are of a much more intricate nature.

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