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THE ROLE of CALCIUM in BIOLOGICAL SYSTEMS

Volume I

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FOREWORD

Calcium must certainly be the major bioelement of the times. Only a generation ago Ca^{2+} was known to physiologists and biochemists as a component of bone mineral and as a blood plasma constituent required in heart function and blood coagulation, but little more. Only a few, such as Baird Hastings and Walter Heilbrunn, saw more clearly into the future of Ca^{2+}, a future that was a long time coming. Then came the discovery of the role of Ca^{2+} in the contraction-relaxation cycle of skeletal muscle and the recognition that the free Ca^{2+} concentration of the resting sarcoplasm must normally be orders of magnitude lower than that in the blood plasma. Thus it was found that skeletal muscle must possess extremely efficient energy-dependent Ca^{2+} pumps. The discovery that mitochondria can accumulate Ca^{2+}, by my colleagues Vasington and Murphy, was at first regarded by many as an anomaly of in vitro conditions, since Ca^{2+} had earlier been found to uncouple oxidative phosphorylation. How could oxidative phosphorylation and Ca^{2+} transport be compatible? What possible role can mitochondria play in cellular Ca^{2+} distribution? And why does calcium phosphate form insoluble but noncrystalline granules in the mitochondrial matrix?

Answers to these and other questions came slowly at first, but in the 1970s a crescendo of Ca^{2+} research developed. Today we know dozens if not hundreds of different cellular and extracellular processes that are regulated by changes in the level of cytosolic or extracellular Ca^{2+}, in which at least three different membrane systems of the cell take part. Indeed, Ca^{2+} is now emerging as a most important and ubiquitous intracellular messenger, perhaps even broader in function than cyclic AMP, the original second messenger. What is even more remarkable is that cytosolic Ca^{2+} levels can regulate several different activities simultaneously in a single cell, raising fundamental questions regarding spatial and temporal regulatory fluctuations in cytosolic Ca^{2+} concentrations. Also remarkable are the biochemical mechanisms that keep calcium and phosphate, which occur in extracellular fluids and urine in supersaturating concentrations, from precipitating and turning us into stone. Central to all these questions is the chemistry of Ca^{2+}, its special features that endow it alone, of all the common cations, to participate in such a panoply of biological activities.

The papers in this volume address many aspects of these problems in the biochemistry and physiology of calcium and provide an important guide to recent progress.

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PREFACE

The purpose of this review is to summarize and correlate the recent advances in several fields of scientific research related to the involvement of calcium in the structure development and function of biological systems.

Considering the general interest in calcium, this publication which is a comprehensive collection of contributions on the biochemical properties of the ion, is aimed to be of interest to workers in many fields of biology and medicine whose investigations might be related, directly or indirectly to the role of this ion in biological systems. In addition to the benefit of presenting a concise review of the state-of-the-art on each subject, it will provide a useful reference source of the work done in a wide range of scientific disciplines such as biochemistry, analytical chemistry, cell biology, physiology, nutrition, pathology, pharmacology, toxicology, etc.

The text consists of six major divisions. The first deals with the chemistry of calcium and gives both the theoretical and practical basis to interpret the role of this element in the function of normal and pathological biological systems, as described by the other subsequent divisions.

It is not the aim of this publication to provide an exhaustive compilation of all the subjects concerning the biochemistry of calcium, but to give within the limits of the present work the most important and actual highlights related to this bioelement. In most instances the given information has been made as concise as possible to make feasible the coverage of all the different subjects, but without sacrificing the updated bibliographic references which constitute a quick access to the ultimate source of knowledge. To the contributors and publisher who have made possible this publication we are very much indebted.

Leopold J. Anghileri
Anne Marie Tuffet-Anghileri

THE EDITOR

Leopold J. Anghileri, Dr. Chem. is Senior Scientist and Research Group Leader at the Laboratory of Biophysics, Service of Nuclear Medicine and Diagnostic by Ultrasons and Thermography, Medicine Faculty B, University of Nancy, France. Dr. Anghileri graduated in 1951 from Buenos Aires University where he obtained his Dr. Chem. degree in 1957. Dr. Anghileri became a member of the American Chemical Society in 1961. Until 1964 he was Investigator at the Argentine Atomic Energy Commission. From 1964 to 1968 he was a fellow at the Johns Hopkins Medical Institutions, Division of Nuclear Medicine and Radiation Health, Institut de Radium, Laboratoire Curie, Paris, and Institut für Nuklearmedizin, Deutsches Krebsforschungzentrum, Heidelberg, West Germany. During the period 1970 to 1975 he was Investigator at the Tumorforschung, Ruhr Universität, Essen, West Germany.

Dr. Anghileri has presented invited papers at international meetings. He has published more than 210 research papers. His current major research interest includes hyperthermic treatment of cancer and the calcium-magnesium membrane relationship in tumors.

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The late **Anne Marie Tuffet-Anghileri, Dr. Sci.** was Investigator at the Centre National de la Recherche Scientifique, Paris. Dr. Tuffet-Anghileri graduated in 1969 from Paris University. She worked in carcinogenesis at the Laboratoire Curie, Paris and in Molecular Biology (DNA-metal ions interactions) at the Paris University.

To my wife and dedicated co-worker Anne Tuffet-Anghileri (1937-1981) whose life was sacrificed for and by the Science.

L. J. Anghileri

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THE CHEMISTRY OF CALCIUM ION AND ITS BIOLOGICAL RELEVANCE

B. A. Levine and R. J. P. Williams

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I. INTRODUCTION

The interaction of calcium ions with biological ligands is not easily understood. We shall start from a general equation which we have used previously:[1,2]

$$\text{The Effect of Calcium} \propto [Ca^{2+}] \cdot K_{aq} \cdot p \cdot \int [\text{Structure Factors}] \tag{1}$$

The equation states that in order to understand the action of calcium we must know the free ion concentration in a particular compartment at a given time and its time dependence. We must also know the equilibrium binding constants for calcium with the available liganding groups, L, when they are free in water, K_{aq}. As the binding in biology is not necessarily in an aqueous phase nor is it necessarily observable in the absence of fields, we correct K_{aq} to $K_{aq}p$ where p is a kind of partition coefficient which will account for the effect of local solvent changes and fields. Once the complex, CaL, is formed we need to understand its properties and they will depend upon its structure and the energy of other related structures. We need to look at the structure of complexes and at possible excited state energies and structures. Using the above equation we should be able to account for calcium activities in a qualitative manner. Since the whole of this paper will be based on the assumption that calcium interactions with ligands in complexes are electrostatic we start below with a description of electrostatic binding.

In all the solutions in which we find Ca^{2+} ions there are also H^+, Mg^{2+}, Na^+, and K^+ ions which can compete for L, following the pattern of Equation 1. We shall then have to make a comparison, especially with magnesium chemistry, in order to see why calcium behaves as it does. Biological systems have optimized the use of the different ion properties and they also use the competition between ions to control activities.

II. ELECTROSTATIC INTERACTION

It is usual to note that electrostatic interactions are simple. The energy of binding of a cation, e.g., the calcium ion to an anion in the gas phase, is given by

$$\Delta G = \int_1 \frac{z_1 z_2}{r_1 + r_2} \cdot \frac{1}{\epsilon} \tag{2}$$

where z_1 and z_2 are the ionic charges on the calcium ion (+2) and on the anion and r_1 and r_2 are their radii (Table 1). In the gas phase the dielectric constant, ε, is unity. A similar expression is readily derived for the binding of ions to neutral ligands — dipoles. The energy of interaction in a different medium is then reduced by the dielectric constant of the medium, i.e., 80 for water. If we then consider the energy of free separated ions in the dielectric to be simple functions of z/r we reach the energy of association of ions in a solvent as the difference between the bound and the free state

$$\Delta G_1 = \int_1 \left(\frac{z_1 z_2}{r_1 + r_2}\right)\Big/_{\epsilon} - \int_2 \left(\frac{z_1}{r_1} + \frac{z_2}{r_2}\right)\Big/_{\epsilon} \tag{3}$$

where f_1 and f_2 are simple functions. Analysis of this type of equation leads to a correct qualitative overall view of the variation of ion association with changes in ion charge and radius for cations of Group IA, IIA, and IIIA of interest in this article.[3]

Table 1
RADII OF IONS

Cation	Na^+	K^+	$H_9O_4^+$	NH_4^+ (H_3O^+)	$N(CH_3)_4^+$	Mg^{2+}	Ca^{2+}
Radius (Å)	0.95	1.35	2.0	~1.40	~2.0	0.65	0.95
Anion	F^-	Cl^-	Br^-	I^-	PO_4^{3-}	SO_4^{2-}	
Radius (Å)	1.4	1.8	1.95	2.2	2.1	2.1	

As an approximate generalization which can be readily tested, the equation says that anions and cations of similar size tend to associate more than ions of disparate size. It is the ratio of cation radius to anion radius which dominates association. This can be put in another way: large anions precipitate large cations; small anions precipitate small cations. Examples are the relative strengths of association and precipitation of calcium but not magnesium ions with sulfate (Ca > Mg) and the relative strengths of association of the same ions with hydroxide (Mg > Ca). In effect, of course, we are discussing the sizes of ligand donors relative to the amount of displaced water (Equation 3). A large donor will displace proportionately more water from around a small cation and this will effectively destabilize the complex. The proton is a very small ion, hence the anion association of small cations parallels the proton association — small cations bind best to weak acid anions, e.g., OH^-, F^- but not $ClO^{2-}{}_4$, $SO^{2-}{}_4$ which are large strong-acid anions. The behavioral differences between Na^+ and K^+, Mg^{2+} and Ca^{2+}, or Al^{3+} and Ln^{3+} all conform to these radius-ratio patterns. However as we shall see, the deviations from expectations from donor atom size differences or from weak-acid/strong-acid donor classifications are very marked for ligands which are multidentate, i.e., complex ligand structures and size factors confuse the above simple expectations. Thus, although the qualitative description of behavior of simple salts is correctly given by the simple theory outlined above, it does not give a satisfactory quantitative treatment of association energies nor does it describe the stereochemistry or kinetics of complexes at all. It is usual, though, to say that the ionic model is correct in principle and no covalent forces need to be inferred in the compounds of groups IA, IIA, and IIIA of the Periodic Table, but it is not useful in practice in its predictions of chemical equilibrium. We then resort to a more chemically qualitative description, finding some empirical rules about binding from measurement of solubilities and complex ion formation and making generalizations.

Before doing so it is wise to note why the theory fails. The above picture of ionic bonding is not a molecular picture, and only a bulk dielectric constant represents the solvent. In the real world this concept must be replaced by a molecular model for water. In itself this is technically very difficult,[4] but an even harder problem is the description of the solvent, ligand, and cation (both free and in a complex) in molecular terms. At the center of the problem is the conformation and conformational dynamics of water and the ligands, especially in the complex formed. In particular, the stability of lattices and complexes will be affected by the exclusion volumes of the ions and solvent, especially since in the equilibria the ligands displace different numbers of water molecules. The multidimensional terms involving ligand-ligand, ligand-water as well as metal-ligand and metal-water distances and the uncertain numbers of water molecules involved in the equilibria as opposed to the simple descriptions using charges, radii, and dielectric constants, make numerical analysis exceedingly complicated. No detailed calculations are available. Even the form of equation to be used for the repulsions is not known since exclusion volumes do not depend on ionic interactions alone. In such circumstances an empirical approach is the only one possible although the ions behave as charged spheres. We must look first at the structures of calcium salts.

Table 2
SOME Ca^{2+} STRUCTURES OF SMALL MOLECULES OF BIOLOGICAL INTEREST

	Coordination no.	Ca—O distances (nm)		Ref.
		Min.	Max.	
Ca^{2+} thymidylate	7	0.230	0.265	1
Ca^{2+} diphosphonate	8	0.240	0.260	2
Ca^{2+} galactose	8	0.235	0.255	3
Ca^{2+} blephavismin	7	0.235	0.245	4
Ca^{2+} trehalose	7	0.235	0.255	5
Ca^{2+} arabonate	8	0.245	0.250	6

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III. CRYSTAL STRUCTURES OF CALCIUM SALTS

The first step in this approach comes from an examination of the crystal structures of calcium and other cation salts.[1] Table 2 gives some data which show that:

1. The calcium ion overwhelmingly prefers oxygen (O) — atom donor groups of ligands.
2. The calcium ion has no simple apparent radius, i.e., there is not a constant distance from Ca^{2+} to its nearest oxygen neighbors in the salts of Table 2. The problem is not only that the coordination number is variable from salt to salt, but that even within any one crystal the Ca^{2+} ion does not form the center of a regular geometric figure, e.g., an octahedron. In fact the coordination geometry is invariably extremely irregular and the Ca^{2+} — oxygen distance varies from 2.4 to 3.2Å.
3. The calcium ion has no fixed coordination number — values from 6 to 12 have been observed — insofar as coordination number has a meaning in the irregular geometries observed. The radius commonly given to the calcium ion (0.95 Å) is for a coordination number of 8 observed in simple *anhydrous* halide lattices.
4. The hydration of a salt in a crystal is not predictable.

We must discover why structural simplicity is lost especially in hydrated salts.

A first step is taken by a comparison with other metal ion crystals. Table 3 shows that the Mg^{2+} ion has a very different behavior.[1] First, the degree of hydration of equivalent salts is very different. Second, the magnesium ion nearly always occupies the center of an *octahedron* of oxygen atoms with a *fixed* ionic bond distance of 2.05