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PROTEIN-METAL INTERACTIONS

Edited by
Mendel Friedman

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Preface

Metal ions and proteins are ubiquitous. Therefore, not surprisingly, new protein-metal interactions continue to be discovered, and their importance is increasingly recognized in both physical and life sciences. Because the subject matter is so broad and affects so many disciplines, in organizing this Symposium, I sought participation of speakers with the broadest possible range of interests. Twenty-two accepted my invitation. To supplement the verbal presentations, the Proceedings include five closely related invited contributions.

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This book encompasses many aspects of this multifaceted field. Topics covered represent biochemical, immunochemical, bioorganic, biophysical, metabolic, nutritional, medical, physiological, toxicological, environmental, textile, and analytical interests. The discoveries and developments in any of these areas inevitably illumine others. I feel that a main objective of this Symposium, bringing together scientists with widely varied experiences yet with common interests in protein-metal interactions, so that new understanding and new ideas would result has been realized. I hope that the reader enjoys and benefits from reading about the fascinating interactions of metal ions and proteins as much as I did.

Although an adequate summary of the Symposium is not possible in a brief preface, I wish to express particular interest in the ideas reported by Professor Frieden: that the relative occurrence and participation of the various metals as essential elements in enzyme action and other life processes is an adaptive relationship to their relative abundance in the ocean. Undoubtedly, this adaptation is a continuing process. A more immediate practical concern voiced by D. K. Darrow and H. A. Schroeder that has received widespread publicity and debate is that children are highly susceptible

to lead poisoning and that their exposure to lead nowadays comes mainly from automobile exhaust.

Of the invited contributions supplementing the Symposium, the paper by J. T. MacGregor and T. W. Clarkson deserves special mention. Dr. MacGregor collaborated with Dr. Clarkson, his former major professor, in this thorough review while the latter was out of the country dealing directly with an episode of mercury poisoning described in their paper. I believe their critical compilation of tissue distribution and toxicity of mercury compounds will greatly benefit the medical and other scientific communities in dealing with this useful but dangerous element.

I am confident that the Proceedings of the Symposium on "Protein-Metal Interactions" will be a valuable contribution to the literature. I am particularly grateful to Dr. I. A. Wolff, Chairman of the Protein Subdivision of the Division of Agricultural and Food Chemistry of the American Chemical Society, who invited me to organize this Symposium, to all contributors and participants for a well realized meeting of minds, and to R. N. Ubell, Editor-in-Chief, Plenum Publishing Company, for arranging publication as a volume in the series, ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY.

Mendel Friedman
Moraga, California
April 1974

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THE EVOLUTION OF METALS AS ESSENTIAL ELEMENTS [with special
reference to iron and copper]

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The past few years have witnessed exciting progress in our understanding of the elements required for the growth and survival of the higher animal. As anticipated, these new discoveries have been exclusively among the trace elements, those elements which in minute quantities are essential for growth and development. As shown in Table I, after the discovery of the requirement for cobalt in 1935, there was a hiatus of about two decades before the essentiality of molybdenum (1953), chromium (1957) and selenium (1959) was confirmed. This was the end of an era in trace element research. From here on a major change in the research technique had to be devised. The animals, their food and their entire environment had to undergo a complete trace element decontamination, using special plastic houses, highly purified diets and filtered air. After a decade of painstaking research, Dr. Klaus Schwarz of the Veterans Administration and others added three new elements to the essential list: fluorine, tin and vanadium. In 1972, Dr. Edith Carlisle, University of California, Los Angeles, proved that silicon also was required for the growth and development of chicks. We now know that at least twenty-five of the 96 elements found on earth are required for some form of life (Figure 1; see also Table III).

Two-thirds of the lightest elements, 22 out of the first 34, are essential. These 22 elements plus molybdenum (no. 42), tin (no. 40) and iodine (no. 53) complete the list of 25 essential elements. It is an impressive fact that despite our current sophistication in biochemistry and molecular biology, there remain many fundamental, unanswered questions concerning the elements required for animal or plant life. Moreover, there has been little effort to relate the evolution of the dependence on specific elements to the past and present status of living organisms.

TABLE I

DISCOVERY OF TRACE ELEMENTS REQUIREMENTS OF ANIMALS

Iron17th century	
Iodine1850	Chatin, A.
Copper1928	Hart, E.B., H. Steenbock, J. Waddell, and C.A. Elvehjem
Manganese . .	.1931	Kemmerer, A.R., and W.R. Todd
Zinc1934	Todd, W.R., C.A. Elvehjem, and E.B. Hart
Cobalt1935	Underwood, E.J., and J.F. Filmer Marston, H.R. Lines, E.W.
Molybdenum . .	.1953	deRenzo, E.C., E. Kaleita, P. Heytler, J.J. Oleson, B.L. Hutchings, and J.H. Williams Richert, D.A., and W.W. Westerfield
Selenium1957	Schwarz, K., and C.M. Foltz
Chromium1959	Schwarz, K., and W. Martz
Tin1970	Schwarz, K., and D.B. Milne, and E. Vinyard
Vanadium1971	Hopkins, L.L., and H.E. Mohr Schwarz, K., and D.B. Milne
Fluorine1971	Schwarz, K., and D.B. Milne
Silicon1972	Carlisle, E.M. Schwarz, K., and D.B. Milne

Adopted from K. Schwarz in "Trace Element Metabolism in Animals", C. F. Mills, Ed., E85 Livingstone, Edinburgh, p. 25 (1970). See this article for appropriate references.

In his classic text, The Fitness of the Environment, published in 1913, Lawrence J. Henderson concluded:

The properties of matter and the course of cosmic evolution are now seen to be intimately related to the structure of the living being to its activities; they become, therefore, far more important in biology than has been previously suspected. For the whole evolutionary process, both

cosmic and organic, is one, and the biologist may now rightly regard the universe in its very essence.

Despite the title of this far reaching work, Henderson put as much emphasis on the organism fitting to the environment through Darwinian evolution as on the milieu fitting the organism. Henderson wrote that the fitness of the environment was just one part of a reciprocal relationship of which the fitness of the organism is the other. This condition of life was expressed in the characteristics of water, carbonic acid and the compounds of carbon, hydrogen, and oxygen. These combined to provide "the best of all possible" environments for life. Henderson's basic approach is none the less applicable today and provides us with the starting point for our consideration of the basis for the selective dependence of life on the 25 natural elements. To fit their environment, living systems were involved in two major adaptations--first to the origin of life in the oceans and second to the utilization of oxygen in the atmosphere. These two adaptations have established the major guidelines for the requirement of the elements and the determination of the chemical structures accompanying the development of life on earth.

EVOLUTION OF LIFE IN THE SEA

It is widely accepted that life on earth first evolved in the sea or in saline tidal pools. This has led to an indelible imprint of the composition of the oceans in the chemistry of all cells and organisms. The oceans provided a convenient medium in which the primitive organism could float freely in a solution of the basic chemicals required to maintain life. For these primitive organisms the fluidity of a medium like water was indispensable. A denser matrix (e.g., solids) would have been too restrictive on the mobility of dissolved ions or metabolites. Finally, after millions of years, the metamorphic transition from aquatic to terrestrial existence began. This development was successful only if the transforming organisms were able to carry part of their marine environment with them. The distribution of salts in the blood and other body fluids reflects the oceanic nature of their evolutionary antecedents. The choice of sodium, potassium, calcium, magnesium and chloride ions for the major ionic environment of the cell, both inside and outside, stems from their availability in the primeval seas.

All this has been a widely recognized facet of comparative biochemistry (Prosser and Brown, 1961). What has not been fully appreciated is that the nature of the trace element requirement, particularly the metal ions, appears to have been limited by their availability in the early oceans. This is evident upon an examination of the concentrations in the oceans of the five bulk ions and the fourteen trace elements, (Table II; compare with Table III).

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	197	198	199	200	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681	682	683	684	685	686	687	688	689	690	691	692	693	694	695	696	697	698	699	700	701	702	703	704	705	706	707	708	709	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755	756	757	758	759	760	761	762	763	764	765	766	767	768	769	770	771	772	773	774	775	776	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791	792	793	794	795	796	797	798	799	800	801	802	803	804	805	806	807	808	809	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	858	859	860	861	862	863	864	865	866	867	868	869	870	871	872	873	874	875	876	877	878	879	880	881	882	883	884	885	886	887	888	889	890	891	892	893	894	895	896	897	898	899	900	901	902	903	904	905	906	907	908	909	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926	927	928	929	930	931	932	933	934	935	936	937	938	939	940	941	942	943	944	945	946	947	948	949	950	951	952	953	954	955	956	957	958	959	960	961	962	963	964	965	966	967	968	969	970	971	972	973	974	975	976	977	978	979	980	981	982	983	984	985	986	987	988	989	990	991	992	993	994	995	996	997	998	999	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021	1022	1023	1024	1025	1026	1027	1028	1029	1030	1031	1032	1033	1034	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061	1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088	1089	1090	1091	1092	1093	1094	1095	1096	1097	1098	1099	1100	1101	1102	1103	1104	1105	1106	1107	1108	1109	1110	1111	1112	1113	1114	1115	1116	1117	1118	1119	1120	1121	1122	1123	1124	1125	1126	1127	1128	1129	1130	1131	1132	1133	1134	1135	1136	1137	1138	1139	1140	1141	1142	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165	1166	1167	1168	1169	1170	1171	1172	1173	1174	1175	1176	1177	1178	1179	1180	1181	1182	1183	1184	1185	1186	1187	1188	1189	1190	1191	1192	1193	1194	1195	1196	1197	1198	1199	1200	1201	1202	1203	1204	1205	1206	1207	1208	1209	1210	1211	1212	1213	1214	1215	1216	1217	1218	1219	1220	1221	1222	1223	1224	1225	1226	1227	1228	1229	1230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Table II
Concentration of Some Crucial Elements in Sea Water

Element	Parts per Million	Element	Parts per Billion
K	380	Fe	10
Mg	1,350	Cu	3
Ca	400	Zn	10
Cl	19,000	Mn	2
Na	10,500	Mo	10
B	4.6	Co	.27
F	1.3	Se	.09
Si	3.0	Cr	.05
I	.06	V	2
		Sn	3

Taken from: E. D. Goldberg in The Sea (ed. by M. N. Hill), pp. 4-5, (1963), J. Wiley, New York.

The macro ions are present in concentrations $10^5 - 10^6$ times those of the trace metals. The ten essential trace metals are present in 10 parts per billion or less. Three required non-metals, boron, fluorine, and silicon are present in 1-5 parts per million, but are still only trace elements in most systems, possibly due to the lack of the unique binding properties exhibited by these non-metals. Finally iodine proves to be an essential trace element to mainly non-marine forms. The special properties of these trace elements in both simple and complex organisms will be discussed later.

AEROBIC CELLS

Another major evolutionary adaptation met by living systems arises from changes in the atmosphere leading to an accumulation of and a dependence on oxygen. My colleague, Hans Gaffron (1960), of Florida State University, has summarized the major stages of biogenesis in terms of the transition from a highly reducing atmosphere to our present oxygen rich world. There was first an anaerobic era of excess hydrogen with ammonia, methane and water vapor and an accumulation of highly reduced organic substances. This was followed by a hydrogen poor period with the beginnings of inorganic or organic catalysis and traces of oxygen. In the third period, the anaerobic organisms deplete their useful reservoir of organic substances dooming the earth to essential dependence on photoreduction as the major initial source of energy. This leads to today's era of photosynthesis and ascendancy of cells dependent on oxidative energy, eliminating or driving the obligate anerobes "underground". Oxygen

itself then becomes a major evolutionary force. As we shall see later the essentiality of numerous metals arises from their ability to promote the effective utilization of oxygen.

How environmental circumstances may determine whether an organism will survive such drastic changes as the step from anaerobic to aerobic conditions was shown in an actual laboratory model. In 1954, Jensen and Thofern found a micrococcus strain which grew well heterotrophically under anaerobic conditions. On contact with oxygen the cells die because they have neither an efficient mechanism for destroying the hydrogen peroxide which might be formed nor a respiratory system. But, when the nutrient medium contained a simple iron porphyrin, ordinary protohemin, these bacteria survived in air. The hemin was taken up into the cells and there combined with the right kind of protein to provide the bacteria with catalase and respiratory enzymes. The presence or absence of hemin in the surroundings at the time oxygen reaches the cells determines their fate.

THE THREE GROUPS OF ESSENTIAL ELEMENTS

The current state of knowledge about the 25 elements now recognized to be essential to the various types of organisms is summarized in Table III. In this table the elements are classified in three categories: (++) is used to describe the elements which have been shown to be essential to numerous representative animals in that group and are presumed to be required for life and survival by virtually every member of that group, (+) designates those elements for which a requirement has been demonstrated but the number of examples or evidence is so sparse as to limit any generalization of requirement (although the "unity" of nature strongly suggests a group requirement), and (-) indicates the lack of any definitive data supporting the essentiality of that element for the particular class of organism. This may be due to the absence of any experiments or to the failure to show a requirement in those experiments reported.

Each of the first group of six elements is required because they provide most of the atoms for the primary molecular building blocks for all forms of living matter: amino acids, sugars, fatty acids, purines, pyrimidines and their nucleotides. These molecules not only have independent biochemical roles but also are the respective constituents of the major categories of large molecules: proteins, cellulose, glycogen, starch, lipids and nucleic acids. Several of the 20 amino acids contain sulfur in addition to carbon, hydrogen, and oxygen. Phosphorus plays an important role in the nucleotides, such as adenosine triphosphate (ATP), which is central to the energetics of the cell. Adenosine triphosphate includes components that are also one of the four nucleotides needed to form

TABLE III

ESSENTIALITY OF THE ELEMENTS FOR LIVING ORGANISMS

Element	Bacteria	Algae	Fungi	Higher Plants	Inverte- brates	Verte- brates
H,C,N, O,P,S	++	++	++	++	++	++
K	++	++	++	++	++	++
Mg	++	++	++	++	++	++
Ca	+	++	+	++	++	++
Cl	++	++	-	++	++	++
Na	+	+	-	+	++	++
Fe	++	++	++	++	++	++
Cu	++	++	++	++	++	++
Zn	++	++	++	++	++	++
Mn	++	++	++	++	++	++
Mo	++	++	++	++	++	++
Co	++	+	+	+	++	++
Se	+	-	-	+	-	++
Cr	+	-	-	-	-	+
V	+	+	-	-	+	+
Sn	-	-	-	-	-	+
Ni	-	-	-	-	-	+
B	-	+	-	++	-	-
F	-	-	-	-	-	+
Si	-	+	-	+	-	+
I	-	+	-	+	+	++

KEY: (++) - Essential for several and, probably, all species in the group.
 (+) - Essential for one or a few species in the group.
 (-) - Not known to be essential for any species.

the double helix of deoxyribonucleic acid. Polysaccharides, such as cellulose, form the major structural component of plants and trees. Both sulphur and phosphorus are present in many of the small indispensable molecules called coenzymes. In bony animals phosphorus and calcium help to create strong supporting structures.

The second group of essential elements play a critical role in the electrochemical properties of cells from all forms of life. The principal cations are potassium and magnesium with a somewhat

less versatile role for calcium and sodium. The principal anions or negative charges are provided by chloride, sulfate and phosphate and are widely distributed with an anomalous feature of fungi in not requiring chloride. These seven ions maintain the electrical neutrality of body fluids and cells and also play a part in maintaining the proper liquid volume of the blood and other fluid systems. Whereas the cell membrane serves as a physical barrier to the exchange of large molecules, it allows small molecules to pass relatively freely. The electrochemical functions of the anions and cations serve to maintain the appropriate relation of osmotic pressure and charge distribution on the two sides of the cell membrane. One of the striking features of the ion distribution is the specificity of these different ions. Cells are rich in potassium and magnesium, and the surrounding plasma is rich in sodium and calcium. It seems likely that the distribution of ions in the plasma of higher animals reflects the oceanic origin of their evolutionary antecedents.

The third and most numerous group of essential elements is those required only in very small, trace amounts. This latter fact in no way diminishes their great importance to the survival and proper functioning of all organisms. For the purpose of this discussion we are going to make a distinction within this group between the metals and the non-metals.

The non-metals, boron, fluorine, silicon and iodine; will be mentioned briefly for several reasons. The first is that we don't know much about their requirement or biochemical role except for iodine. The indispensability of fluorine and silicon in the mammal has been reported in the last 18 months although the role of fluorine in preventing tooth decay was well known. Boron is required only in some plants and algae with no positive role in animals. Iodine, the heaviest required element presents a more interesting case. It is, of course, best known in the vertebrates as an essential part of the molecules which comprise the thyroid hormones, iodothyronine. These hormones play two probably related roles in the vertebrates (Frieden and Kent, 1974): (1) the initiation and control of metamorphosis and development in amphibia and in the higher vertebrates and (2) the adaptation of animals to homeothermy, which utilizes the calorigenic properties of these hormones. Yet the precise function of iodine in hormonal activity is still not understood. The role of iodine in invertebrates and plants is spotty and uncertain--there is no certainty of a group requirement for this element. The trace non-metals may be a more interesting group than imagined, but we will need much more information than is currently available.

* Boron and silicon are sometimes considered as metalloids, since they are at the borderline in the delineation of metals and non-metal properties.

The indispensable elements which most excite the bio-chemists are the trace metal ions, the most numerous category listed in Table III. They are especially interesting because of their widespread distribution, importance, and unique chemical properties. Five of these metal ions, Fe, Cu, Zn, Mn, Mo, appear to be generally required by all organisms. Cobalt will undoubtedly end up in this category. The other metals are required in such small amounts and the chances of contamination at this level are so great that highly refined experimental techniques were required to show their indispensibility. These metals include selenium, vanadium, chromium, and tin ions. Recently selenium has been identified as an essential component of an enzyme in the red blood cell, glutathione peroxidase, which is involved in the protection of the erythrocyte against the toxic, oxidative effects of hydrogen peroxide. There is growing evidence that nickel is also a required trace element. When we learn how to cope with the experimental conditions in the various media required for proper testing of different kinds of organisms, these four metals may prove to be needed by all cells. This experimental approach may also reveal additional new essential metals, the most likely being aluminum or germanium.

DEVELOPMENT OF CATALYSIS

What more can be said about the development of the need of cells for these 25 elements? The literature is replete with discussions of proteins, nucleic acids and informational macromolecules. Accordingly let us focus our attention on the development of the need for certain elements, in particular the metal ions. The six metals, iron, copper, zinc, manganese, molybdenum and cobalt, which are required by every major group of organisms have a reasonably well defined biological function. These six metals belong to a group known as the transition elements. The transition metals serve as essential constituents of a wide range of metalloproteins, usually enzymes, which participate in important oxidative, hydrolytic or transfer processes. Most of them undergo reversible changes in oxidation state which make them especially useful as intermediates in electron transfer or oxidation reactions. They also tend to form strong complexes with a variety of ligands of the type present in the side chains of proteins, and which gave extra stability to metalloprotein complexes.

How a typical catalytic activity of a transition metal enzyme might have evolved has been described by Melvin Calvin (1969) of the University of California (Berkeley). He pointed out the similarity between the enzymic reactions and the reactions known to the inorganic chemist. The model which he chose centers around the element iron, particularly in catalase, peroxidase and the cytochromes. Here a quantitative comparison was made between the ability of the simplest iron compounds to perform the same catalytic function as it has evolved in biological systems. Thus the accompanying (Figure 2)