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The Biochemical Basis of Zinc Physiology

BERT L. VALLEE AND KENNETH H. FALCHUK

Center for Biochemical and Biophysical Sciences and Medicine, Department of Medicine, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts

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I. INTRODUCTION

Numerous enzymes and proteins utilize first transition and group IIB elements to carry out their biological functions. Zinc is the most widely used of these metals in biology, although not the most available, since it is only the 27th most abundant element in the earth's crust. In microorganisms, plants, and animals, over 300 enzymes have been identified representing more than 50 different types that are known to require zinc for their function. In contrast, there are many fewer different iron metalloproteins or enzymes, and even lesser numbers have been found to contain copper, molybdenum, selenium, nickel, manganese, or cobalt. Among the zinc enzymes there are oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases, i.e., examples from all six enzyme classes established by the International Union of Biochemistry. Indeed, zinc is the only metal encountered in each enzyme class. The notable selection and frequent utilization of zinc as the predominant functional metal of so many biological molecules have to be understood in terms of its chemical properties and how these are utilized in biochemical systems.

Two properties of zinc need to be highlighted. First, unlike other metals, including those of the IIB series, zinc is virtually nontoxic (37). The homeostatic mechanisms that regulate its entry into, distribution in, and excretion from cells and tissues are so efficient that no disorders are known to be associated with its excessive accumulation, in contrast to iron, copper, mercury, and other metals. Second, its physical and chemical properties, including its generally stable association with macromolecules and its coordination flexibility, make it highly adaptable to meeting the needs of proteins and enzymes that carry out diverse biological functions (522–524). These and yet other chemical properties form the basis for the extensive participation of zinc in protein, nucleic acid, carbohydrate, and lipid metabolism, as well as in the control of gene transcription and other fundamental biological processes.

The advances in knowledge of zinc chemistry and biochemistry in the past two decades have been striking and have now reached a level that provides predictive capacity for both the biology and pathology of zinc metabolism. We do not attempt an encyclopedic analysis of the astoundingly large body of observations now on record regarding the action of zinc ions in biology. Reviews of zinc metabolism have been written in the past decades addressing analysis of its content in biological samples (438), enzymology (529), and various aspects of its importance to biology, pathology, and medicine (139, 304, 419, 512, 520), to teratology (268), to chromatin

function (73, 145, 528, 569), and others too numerous to cite.

We offer, instead, a perspective on recent progress in the biochemistry of the metal in terms of the specific chemistry that characterizes its association with the many different zinc macromolecules now known. This should serve as a basis for understanding of the physiology of zinc in cell and molecular biology. We specifically examine its relationship to growth, development, differentiation, immune phenomena, receptor activity, and neurophysiology, among others. In some areas of physiology and pathology presented, the relevant zinc species are well-known metalloenzymes and metalloproteins. In others, they have not yet been identified and, therefore, are viewed as uncharacterized zinc proteins or zinc protein complexes. We believe that the hitherto inexplicable phenomena that have been reported in these other areas will become intelligible once the biochemistry of the pertinent biological zinc macromolecules has been established.

II. OVERVIEW

The last 30 years have established that zinc is an integral component of numerous functional proteins wherein it exerts specific properties over a wide range of physiological systems. Such knowledge has affected virtually all aspects of molecular and cell biology, resulting in new insights. This development owes much both to advances in the instrumental analysis of zinc that have generated extraordinarily low detection limits and to the advent of high-resolution methods for the isolation, purification, and characterization of macromolecules. Consequently, a large and diverse number of zinc-containing enzymes and proteins have been recognized to participate in the metabolism of proteins, nucleic acids, carbohydrates, and lipids. Identification of these zinc molecules and the elucidation of their biochemistry, together with information generated in the areas of nutrition, physiology, medicine, and pathology, have converged to establish a consolidated body of knowledge of zinc metabolism. The pertinent developments in these major disciplines are summarized briefly.

The induction of zinc deficiency in microorganisms, animals, and plants and the description in each case of the full spectrum of the resultant clinical manifestations have served as the basis for nutritional management of both animals and humans. One general conclusion from studies of zinc deficiency stands out. In all instances, zinc-deficient cells fail to divide and differentiate with consequent growth impairment in phytids and plants (425, 474), animals (168, 170, 171, 296, 319, 378, 382, 506, 508, 515), and humans (422).

Failure of cell division in response to zinc deprivation was first demonstrated by Raulin (430) in studies with *Aspergillus niger* and was later observed in bacteria, flagellates, and unicellular and multicellular organisms (27, 147, 168, 425, 508, 512, 513, 515, 542). Fetal and juvenile cells with high turnover, i.e., from skin, bowel, and gonads, are more sensitive to zinc deprivation than are those from tissues whose cells are more stationary. The proliferative arrest can be attributed to blockage at different stages in the cell cycle and may be characteristic of a given tissue or organism. Thus zinc-deficient rat brain cells are blocked at the G_0/G_1 transition (85), rat liver cells at G_1 (82), and *Euglena gracilis* cells at the late S/G_2 phase (147, 153, 542).

Correlation with molecular entities underlying these effects on cell growth began to emerge with the discovery of zinc metalloenzymes. The serendipitous finding of zinc in carbonic anhydrase first established its role in catalysis (271). Subsequent recognition of its presence in carboxypeptidases A and B, alcohol dehydrogenases, alkaline phosphatases, and thermolysin focused attention on the function of zinc in multiple types of enzymes that exhibit different specificities, while studies of its involvement in catalytic, regulatory, or structural roles provided a physical basis for zinc metalloenzymology (515, 529, 531).

The unambiguous identification of ligands specific for zinc in proteins and their modes of coordination in the active, coactive (or cocatalytic), and structural sites of some dozen or more metalloenzymes was the result of X-ray crystallographic analysis (53, 117, 121, 235, 261, 272, 307, 334, 335, 404, 434, 478, 487). The amino acids that serve as zinc ligands, the protein sequences that contain them, and the three-dimensional structures in which they are arranged have been summarized (522–526). The structures of enzymes examined by this method have also served as standards of reference essential to the comparison with other homologous enzymes belonging to the same class or subclass.

Inquiries into the metabolic functions of zinc have also been given new direction by studies of the metal's role in the control of gene expression (528) that have revealed its essentiality in cell division, development, and differentiation. It had been observed by Fujii (185) that cell nuclei contain zinc and that it binds to RNA (187, 543) and DNA (466). This ultimately focused on the discovery of enzymes involved in DNA replication and transcription and on the effects of zinc on the structure and composition of chromatin (145, 528). More recently, there has been a virtually explosive interest in zinc-dependent gene regulatory proteins.

These advances have had major implications for pathology and medicine. The wide metabolic spectrum governed by zinc proteins and the interrelationships between them implies the existence of processes, still largely unknown, that carefully regulate the distribution of the metal and its homeostasis. In fact, regulatory systems for zinc absorption, distribution, and excretion have evolved that are so effective that, under most conditions, they protect the organism from deficiency of the metal and almost always from its excess. These circumstances together with the chemistry of zinc (see below) likely account for the fact that ingestion of the element is essentially not toxic in humans (37). Adverse symptoms in humans are observed only on inhalation of zinc fumes, in exceedingly rare instances, after accidental

ingestion of unusually large amounts of zinc dissolved in acid fruit juices or infusion of fluids, both of which had been stored in galvanized containers, or as a consequence of a conditioned deficiency (141).

Deficiency occurs only after prolonged reduction of intake or excessive uncompensated losses. The clinical manifestations of zinc deficiency in animals (168, 170, 171, 296, 319, 378, 382, 425, 506, 508, 515) and humans (422) have been described and reproduced by experimentation in many species (30). It can also occur through chronic dietary inadequacy (418), conditioned deficiency (141, 144, 517), genetically determined factors (11, 289, 368, 412, 553), and iatrogenesis (264, 279).

Throughout the period of discovery of zinc enzymes, there has been a diligent search for alterations of their activities in organs and tissues of zinc-deficient animals. The results have been almost uniformly disappointing. This strongly suggests that whatever the distribution and control system may be, it first ensures functional adequacy of the zinc proteins (enzymes) that serve acute metabolic needs.

III. CHEMISTRY AND BIOCHEMISTRY

A. Chemistry

In biological systems, very little, if any, zinc is free in solution. Zinc carries out its biochemical functions as a divalent cation primarily when bound to enzymes and other proteins. Its chemical properties are particularly favorable to a multiplicity of functionally significant interactions, since under physiological conditions, it does not undergo reduction or oxidation. This lack of redox change renders it stable in a biological medium whose oxidoreductive potential is subject to continual flux. Other physicochemical qualities that provide important advantages to biological systems include the fact that it is amphoteric, existing both as the aquo and hydroxo metal complex at pH values near neutrality. It has a variable coordination sphere and the stereochemical adaptability to assume multiple coordination geometries, unusual features that contribute to its biochemical versatility. These different geometries result in coordination numbers varying from two to eight (88). However, four, five, and six coordinate complexes are the ones most frequently encountered in enzymatic, and other, biological functions of zinc, with geometries ranging from regular or distorted tetrahedral to trigonal bipyramidal, square pyramidal, and octahedral. This plethora of coordination numbers and geometries reflects the ability of zinc to cooperate with the demands of its ligands, allowing them to alter its intrinsic reactivity. Importantly then, protein structure affects the chemistry of bound zinc as much as zinc, in turn, affects the conformation and adaptability of these macromolecules. Collectively, the physicochemical features of zinc are important avenues for translating chemical structure into multiple biological functions. Zinc thereby becomes a versatile interactant for different donor groups of varying ligand types, resulting in a broad range of stability constants, mobilities, reactivities, and function.

B. Spectroscopy

Recognition of zinc in all biological matter, its role in life processes, and association with specific function were delayed unduly as compared with that of other essential metabolites. This circumstance is attributable to the lack of suitable analytical methods in the past. Measurements of zinc that are sufficiently facile, sensitive, precise, and quantitative to define the central role of zinc in biology have been developed only during the last two decades. Early methods with limits of zinc detection of 1 μg/g were chemical (Table 1), based on the formation of colored zinc chelate complexes that proved inherently insensitive and unreliable for the ultimate purpose (530).

Present ultrasensitive methods for the routine detection and measurement of zinc in any biological matter are based on atomic spectroscopy and the interaction of its atoms with electromagnetic radiation. The detection limits of such procedures are one million to one billion times more sensitive than are chemical methods when comparing their absolute detection limits and the amounts of analyte required for each (Tables 1 and 2). The ultrasensitive methods employed most widely now for the determinations of metals in general and zinc in particular are based on atomic spectroscopy. Broadly, these procedures fall into three categories: atomic emission, atomic absorption, and atomic fluorescence spectroscopy. Instrumentally, all three techniques share many similar features, but each of them has peculiar advantages and disadvantages depending on the appli-

TABLE 1. *Detection limits for zinc in biological matter of various analytic techniques*

Technique	Absolute Detection Limit, g
Atomic emission spectroscopy	
Direct-current arc	1×10^{-7}
Copper spark	1×10^{-8}
Graphite spark	1×10^{-8}
Microwave excitation and other plasmas	2×10^{-12}
Atomic absorption spectroscopy	
Air acetylene flame	6×10^{-10}
Boat technique	3×10^{-11}
Delves technique	5×10^{-12}
Graphite furnace (flameless)	5×10^{-14}
Atomic fluorescence	2×10^{-10}
Microchemistry	1×10^{-6}
Polarography	1×10^{-7}
Anodic stripping voltametry	4×10^{-12}
Neutron activation analysis	1×10^{-8}
Spark source mass spectrometry	1×10^{-10}
Chemical ionization mass spectroscopy	1×10^{-11}
X-ray fluorescence	4×10^{-11}
Photon induced X-ray fluorescence	1×10^{-11}

From Vallee (518).

TABLE 2. *Spectroscopic limits for zinc and amounts of biological matter required for analysis*

Technique	Detection Limit, μg	Amount of Protein Required
1955		
Microchemistry	1.0	mg
Direct-current arc emission	1.0	mg
Graphite spark emission	1.0	mg
1970		
Neutron activation	0.1	mg
Flame atomic absorption	0.001	μg
Long-tube atomic absorption	0.00002	μg
1990		
Microwave plasma emission	0.000002	ng
Flameless atomic absorption	0.00000005	ng

cation. These three instrumental categories, their virtues, and their limitations have been detailed (539).

Among them, atomic absorption spectrometry has proven to be the approach that meets most, if not all, of the requirements of biology-oriented work and is, in effect, the present approach of choice. Its development has received by far the greatest attention by instrument manufacturers and has therefore become the most realistic methodological route. Much of the current knowledge of and interest in zinc in biological systems, including zinc enzymes and DNA binding zinc proteins, is due to the development of these methods. The features of the most readily available of the techniques, i.e., flame and electrothermic atomic absorption spectrometry, their advantages, and their applications to the analysis of biological samples have been described. Furthermore, methods for preparing zinc-free buffers and reagents, standards, and preparation for analysis of biological fluids and tissues have been detailed (151).

It is instructive to illustrate these remarks by the presentation of the absolute detection limits of the analytical techniques for the determination of zinc in use by 1985 (518) (Table 1). Most of the methods, other than spectroscopic ones, proved very cumbersome and unreliable, quite apart from their limits of detection. Moreover, most of them were time consuming, minimally requiring a day's work for a dozen samples.

Table 2 is more explicit and lists pertinent techniques, their detection limits, and amounts of material required for analyses between 1955 and 1990. Neglecting details in the evolution of the analytical procedures shown in Table 1, analyses can now be performed on ~30 samples/h, each containing picogram amounts of zinc in nanogram quantities of material, with a precision of 1–2%. Thus the detection and quantitative measurement no longer limit the study of zinc in biology. These facts should be kept in mind when reflecting on the history of the field and the rate of progress in the delineation of zinc chemistry, physiology, and pathology during the last part of this century.

C. Zinc Enzymes

The magnitude of the stability constants of metal binding proteins varies quite widely and has served to differentiate operationally between two classes, metalloproteins and metal-protein complexes (514). The metal is bound sufficiently firmly to metalloproteins so that it is not removed during isolation procedures. Zinc metalloenzymes, which are included in this group, can be characterized chemically and their activities related directly to their metal content (Table 3). X-ray analysis of homogeneous crystals has allowed the elucidation of the specific metal binding sites in a number of these enzymes, and this has led to an emerging understanding of the relationship between the structure of the zinc binding site and its function.

In contrast, in metal-protein complexes, the metal is bound loosely. In most instances, chemical characterization of these molecules has not been achieved. Therefore, the specificity and mode of interaction of the metal with the proteins, or other molecules, as well as the ligands to which the metal binds are ill defined (531). In this group are found many uncharacterized molecules of heterogeneous functions including some that possess zinc binding sites and thus are manifest as zinc-protein complexes. Many of these as yet undefined species have been detected in neural, endocrine, ocular, and hematological tissues, as described in section V.

The number and diversity of zinc enzymes and the known or postulated roles of the metal in their function can be appreciated by examination of Table 3. The isolation of many of these enzymes, their spectroscopic and metal binding properties, their metal stoichiometries, and the effects of chelating agents on their activities have been reviewed in detail, and many other aspects of their biochemistry have been summarized (529) and will not be repeated here. Instead, the types of functions zinc subserves in these enzymes and the way it interacts with amino acid ligands are emphasized, since these are essential to an understanding of its biological roles in zinc metalloenzymes.

Zinc has three functions in zinc enzymes: catalytic, coactive (or cocatalytic), and structural (525). A catalytic role specifies that the metal participates directly in enzyme catalysis. If the metal is removed by chelating or other agents, the enzyme becomes inactive. This abolition of activity is attributed primarily to the fact that zinc itself participates directly in the catalytic process; this does not exclude the possibility that there may also be a concomitant structural change (e.g., in local conformation and/or that of the ligands). A coactive (or cocatalytic) zinc atom enhances or diminishes catalytic function in conjunction with another active site zinc atom in the same enzyme, but is not indispensable of itself for either enzyme activity or stability (525). Structural zinc atoms are required solely for structural stability of the protein and can help stabilize the quaternary structure of oligomeric holoenzymes. Alcohol dehydrogenases of vertebrates are the only enzymes known thus far to contain both a catalytic and a structural zinc atom.

The amino acid ligands to which zinc binds and the resultant structure generated about the metal in catalytic, coactive, and structural sites have only been identified in relatively few enzymes, thus far, by X-ray crystallographic analysis (522–526). However, enough have

TABLE 3. *Zinc enzymes*

Name	Source	Role
Class I, Oxidoreductases		
Alcohol dehydrogenase	Yeast	c, s
Alcohol dehydrogenase	Vertebrates, plants	c, s
Sorbitol dehydrogenase	Vertebrates	c
D-Lactate dehydrogenase	Barnacle, bacteria	?
D-Lactate cytochrome reductase	Yeast	?
Superoxide dismutase	Vertebrates, plants, fungi, bacteria	ca
Class II, Transferases		
Transcarboxylase	*P. shermanii*	?
Aspartate transcarbamylase	*E. coli*	s
Phosphoglucomutase	Yeast	?
RNA polymerase	Wheat germ, bacteria, viruses	c
Reverse transcriptase	Oncogenic viruses	c
Nuclear poly(A) polymerase	Rat liver, virus	c
Terminal deoxyribonucleotidyl transferase	Calf thymus	?
Mercaptopyruvate sulfur transferase	*E. coli*	?
Class III, Hydrolases		
Leukotriene A_4 hydrolase	Human	c
Alkaline phosphatase	Mammals, bacteria	c, ca
5′-Nucleotidase	Bacteria, lymphoblast, plasma	?
Fructose-1,6-bisphosphatase	Mammals	ca
Phosphodiesterase (exonuclease)	Snake venom	c
Phospholipase C	*B. cereus*	c, ca
Cyclic nucleotide phosphodiesterase	Yeast	?
Nuclease	Microbes	?
α-Amylase	*B. subtilis*	s
α-D-Mannosidase	Mammals, plants	?
Aminopeptidase	Mammals, fungi, bacteria	c, ca
Aminotripeptidase	Rabbit intestine	c
Astacin	Crustacea	c
Meprin	Mammals	?
Enkephalinase	Mammals	?
Thermolysin	Bacteria	c
Dipeptidase	Mammals, bacteria	c
Class III, Hydrolases (*continued*)		
Angiotensin-converting enzyme	Mammals, bacteria	c
Carboxypeptidase A	Vertebrates, crustacea	c
Carboxypeptidase B	Mammals, crustacea	c
Carboxypeptidase (other)	Mammals, plants, bacteria	c
Carboxypeptidase DD	*S. albus*	c
Elastase	*P. aeruginosa*	c
Neutral protease	Vertebrates, fungi, bacteria	c
Collagenase	Mammals, bacteria	c
Protein kinase C	Mammals	s
Hemorrhagic protease	Snake venom	c
Aminoacylase	Pig kidney, microbes	?
Dihydropyrimidine aminohydrolase	Bovine liver	?
Dihydroorotase	*Clostridium oroticum*	?
β-Lactamase II	*B. cereus, P. maltophila*	c
Creatininase	*P. putida*	?
AMP deaminase	Rabbit muscle	?
Inorganic pyrophosphatase	Yeast	?
Nucleotide pyrophosphatase	Yeast	c
Adenosine deaminase	*E. coli*, mammals	?
Class IV, Lyases		
Fructose-bisphosphate aldolase	Yeast, bacteria	c
1-Rhamnulose-1-phosphate aldolase	*E. coli*	c
Carbonic anhydrase	Animals, plants	c
δ-Aminolevulinic acid dehydratase	Mammalian liver, erythrocytes	c
Glyoxalase I	Mammals, yeast	c
Class V, Isomerases		
Phosphomannose isomerase	Yeast	?
DNA topoisomerase I	*E. coli*	?
Class VI, Ligases		
tRNA synthetase	*E. coli, B. stearothermophilus*	c
Pyruvate carboxylase	Yeast, bacteria	?

c, catalytic role; s, structural; ca, coactive; ?, available information is insufficient to make an assignment.

been examined to allow for certain generalizations. Imidazole nitrogen atoms and cysteine thiol groups appear to be the predominant ligands in the catalytic and structural sites of enzymes, respectively (Tables 4 and 5).

1. Catalytic zinc atoms

One catalytic zinc atom per subunit of enzyme is the rule. Typically, it is bound to four ligands, three of which are amino acids, with His being the most frequent, followed by Glu, Asp, and Cys (Table 4, Fig. 1). Zinc is bound to three His in *Bacillus cereus* β-lactamase, *Streptomyces albus* carboxypeptidase DD, and human carbonic anhydrases I and II (121, 261, 307, 487). It is bound to two His and one Glu in carboxypeptidases A and B, thermolysin, *B. cereus* and *Bacillus thermoproteolyticus* neutral proteases, *Pseudomonas aeruginosa* elastase, and *B. cereus* phospholipase C and is bound to two His and one Asp in *Escherichia coli* alkaline phosphatase (235, 276, 334, 404, 428, 454, 496). The sole exception to the above thus far is alcohol dehydrogenase, the catalytic zinc site which uniquely contains just one His as well as two Cys (53, 244). However, in this case, too, the third zinc ligand at the active site appears to be variable and has been deduced to be Cys, Glu, or Asp in different enzymes within the alcohol dehydrogenase superfamily (254).

A water molecule is the fourth ligand at all catalytic sites. Mechanistically, the water molecule can be ionized, polarized, or displaced (523–526). Ionization or polarization provides hydroxide ions at neutral pH, while the displacement of the water leads to Lewis acid catalysis. The preferred mode and mechanism of action for each enzyme is likely determined by the properties

TABLE 4. *Zinc ligands and their spacing for the catalytic and structural zinc*

Enzyme	L_1	*X*	L_2	*Y*	L_3	*Z*	L_4	Reference No.
Class I								
Alcohol dehydrogenase	Cys	20	His	106	Cys (C)		H_2O	53
Alcohol dehydrogenase*	Cys	2	Cys	2	Cys (C)	7	Cys (C)	53
Class II								
Aspartate transcarbamylase*	Cys	4	Cys	22	Cys (C)	2	Cys (C)	234
Class III								
Carboxypeptidase A	His	2	Glu	123	His (C)		H_2O	434
Carboxypeptidase B	His	2	Glu	123	His (C)		H_2O	454
Thermolysin	His	3	His	19	Glu (C)		H_2O	334
B. cereus neutral protease	His	3	His	19	Glu (C)		H_2O	404
Carboxypeptidase DD	His	2	His	40	His (N)		H_2O	121
β-Lactamase	His	1	His	121	His (C)		H_2O	487
Phospholipase C	His	3	Glu	13	His (N)		H_2O	235
Alkaline phosphatase	Asp	3	His	80	His (C)		H_2O	276
P. aeruginosa elastase	His	3	His	19	Glu		H_2O	496
Class IV								
Carbonic anhydrase I	His	1	His	22	His (C)		H_2O	261
Carbonic anhydrase II	His	1	His	22	His (C)		H_2O	307

X, number of amino acids between ligand 1 (L_1) and L_2; *Y*, number of amino acids between L_3 and its nearest zinc ligand neighbor; *Z*, number of amino acids between L_3 and L_4. L_3 is contributed by either the amino (N) or the carboxy (C) portion of the protein.
* Structural zinc site. All others are in catalytic zinc sites.

of the three other zinc ligands and their spacing in the primary structure, as well as by the charge, hydrophobicity, hydrogen bonds, and Van der Waals forces created by the three-dimensional structure. The nature of the ligands and the degree of ionization of the bound water will significantly affect the net charge of the resulting complex, which can range from a dication to a dianion. This charge likely could play an important role in the type of reaction catalyzed and in the catalytic process itself (Table 6).

The spacing between the ligands of catalytic zinc sites is strikingly regular and has been classified as either "short" or "long" (522-526). The first amino acid ligand in the sequence, L_1, is nearly always separated from the second, L_2, by one, two, or three amino acids, the "short spacer" (Table 4). The third ligand is separated from the second by from 18 to 123 amino acids, the "long spacer" (522). The short spacer can form a bidentate zinc complex that could stabilize local and overall protein structure by imparting rigidity to the region affected, analogous to disulfide bond formation or calcium binding in some proteins. The long spacer would further stabilize the structure and help align the residues that bind substrate. Variations in the size and amino acid composition of the long spacer could contribute to generate structures that would accommodate binding of different substrates and could be involved in inducing the conformation essential to enzymatic specificity (522, 523).

These findings have proven to be of predictive value for catalytic zinc binding sites of enzymes whose structures have not as yet been determined, as is exemplified by monozinc aminopeptidases and leukotriene A_4 hy-

TABLE 5. *Zinc binding sites in proteins*

Catalytic	$(L)_3^*Zn\text{-}H_2O$	Zn_2 cluster	Zn_2Cys_6
Coactive	$(L)_3^*Zn\text{-}H_2O$ Zn-Asp-Mg	Zn_3 cluster	Zn_3Cys_9
Structural	$ZnCys_4$	Zn_4 cluster	$Zn_4\ Cys_{11}$
	$ZnCys_3His$	Zn_n finger	$(ZnCys_2His_2)_n$
		Zn_2 twist	$(ZnCys_4)_2$

$(L)_3^*$, 3 amino acid ligands of zinc.

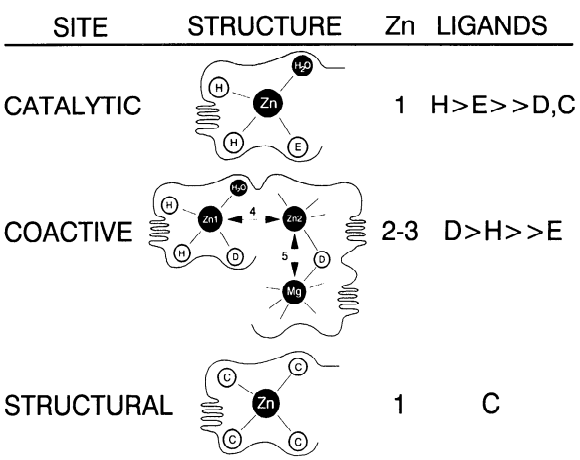

FIG. 1. Zinc binding sites in zinc enzymes are shown. Three amino acids (1 letter code) and one water molecule are the zinc ligands in the catalytic site. Enzymes with a coactive site exhibit both a catalytic zinc site, Zn1, and a separate coactive site, Zn2. In such multizinc enzymes, both sites act as a unit for catalytic activity. Zn2 sites modulate but are not essential for catalysis. These 2 Zn atoms can bind to 1 amino acid, usually aspartic acid, sharing it as a bridge. Furthermore, one of the amino acid ligands of a Zn2 site can additionally form a bridge to another, i.e., 3rd metal, in this case, a magnesium atom. In structural zinc site shown, metal is coordinated tetrahedrally to 4 Cys residues that prevent access of other ligands to the coordination sphere. In protein kinase C, Zn is coordinated to 3 Cys and 1 His residues, a thus far unique structural binding site in an enzyme. [From Vallee and Auld (526).]

TABLE 6. *Net charge of zinc binding sites in some zinc proteins*

Example	Ligands	Charge
Catalytic		
Carbonic anhydrase	$(His)_3(H_2O)$	+2
Thermolysin	$(His)_2Glu(H_2O)$	+1
Alcohol dehydrogenase	$(Cys)_2IIis(H_2O)$	0
Structural		
TFIIIA	$(Cys)_2(His)_2$	0
Protein kinase C	$(Cys)_3His$	−1
Alcohol dehydrogenase	$(Cys)_4$	−2
Glucocorticoid receptor	$(Cys)_4$	−2
Clusters		
GAL4	$(Cys)_6(Zn)_2$	−2
Metallothionein	$(Cys)_9(Zn)_3$	−3
	$(Cys)_{11}(Zn)_4$	−3

No account is taken of any partial charge from the water molecule.

drolase. A domain of ~300 amino acids in the monozinc human intestinal, rat kidney, and *E. coli* aminopeptidases contains a linear arrangement of two His and one Glu that is similar to the zinc binding site of thermolysin. The three amino acids that comprise the short spacer in thermolysin are identical to that of the aminopeptidases. The long spacer in thermolysin contains 19 amino acids, while the corresponding region in the aminopeptidases has 18 amino acids (523). This structural analysis of the zinc binding sites in those zinc enzymes predicted the presence of zinc and a hitherto unrecognized activity in yet another enzyme: leukotriene A_4 hydrolase contains an amino acid segment that is homologous to the zinc binding domain of intestinal aminopeptidase (523). On this basis, the leukotriene A_4 hydrolase was shown to contain 1 g · atom zinc/mol protein, to exhibit aminopeptidase activity, and to be inhibited by bestatin and captopril, specific peptidase inhibitors. Moreover, mutagenic replacements of leukotriene A_4 zinc ligands completely abolish its activity (211, 352, 526). This is the first proof of the identity of a hitherto unknown zinc enzyme binding site by mutagenesis.

Cobalt can replace the zinc atoms in catalytic sites. In most, but not all, enzymes, the activity is retained, although its magnitude may be either increased or decreased. The absorption, magnetic circular dichroism, and electron paramagnetic resonance spectra of catalytically active cobalt-substituted zinc metalloenzymes are unusual and do not resemble those of typical cobalt complex ions (529). They have been interpreted to indicate that the catalytic zinc sites of enzymes are asymmetric. The three-dimensional structure of the molecule, heterogeneity of the ligands, and degree of vicinal polarity of metal binding at the active sites jointly generate the atypical coordination properties that have been defined as "entatic" (534) and are believed to signify a state of tension or stress of the metal. Because denaturation abolishes both enzymatic function and these atypical spectral features, the latter are considered to be indicative of a biologically active state in which the metal is "poised for catalysis."

2. Coactive (cocatalytic) zinc atoms

A seemingly distinct category of zinc site exists in zinc enzymes that contain two or more metal atoms that function as a catalytic unit. The additional zinc (or other metal) site has been named coactive (or "cocatalytic") (525, 526). An amino acid forms a ligand bridge between two zinc or a zinc plus a different metal atom (Fig. 1). There are two such bridged metal atoms, zinc and magnesium, in alkaline phosphatase (276), two zinc atoms in phospholipase C (235), zinc and possibly magnesium in bovine lens leucine aminopeptidase (67), as well as zinc and copper in bovine erythrocyte superoxide dismutase (491).

3. Structural zinc atoms

Thus far, structural zinc atoms have been found only in three enzymes, alcohol dehydrogenase (53), aspartate transcarbamylase (234), and protein kinase C (237). In alcohol dehydrogenase and aspartate transcarbamylase, the zinc is fully coordinated tetrahedrally to four cysteines (Fig. 1). The various cysteine ligands in alcohol dehydrogenase (ADH) and aspartate transcarbamylase are separated by 2, 2, and 7 or 4, 22, and 2 amino acids, respectively (523-525).

The structural motif in protein kinase C differs significantly from these and is the first of its kind described in an enzyme. It contains 4 g · atoms zinc/mol protein located in the noncatalytic domain of the enzyme. Each zinc atom is fully coordinated tetrahedrally to three cysteines and one histidine (237). This motif has previously been observed in DNA binding protein g32p from bacteriophage T4 (193, 194), and either X-ray or nuclear magnetic resonance (NMR) structure analysis will ultimately assess the significance of this mode of coordination.

Recognition, delineation, and generalization of the scaffolding of the catalytic and structural sites of zinc enzymes provide a novel theoretical basis for the design and synthesis of model systems. Clearly, both the catalytic and structural potential of zinc in enzymes depend, on the one hand, on the characteristics of the short and long spacers and, on the other hand, on the detail of the distances between the cysteine residues of structural zinc atoms. For catalytic zinc atoms one would expect that, minimally, models would have to mimic these features to achieve the potential of catalysis and specificity, characteristic of catalytic zinc in enzymes. Similarly, structural zinc atoms would be expected to induce and control folding of the peptide chain as well as local and overall conformation typical of a native enzyme containing a structural zinc atom. A first step in this direction has been taken with ADH, aiming at the verification of the coordination chemistry of its structural zinc atom when synthetically mimicking that of the native enzyme. The structural zinc atom of mammalian alcohol dehydrogenases is coordinated tetrahedrally by a short peptide segment containing four Cys ligands

(Cys-97, Cys-100, Cys-103, and Cys-111) and constitutes an isolated loop of the protein that contributes to subunit and substrate interactions. A 23-residue peptide has been synthesized to incorporate the loop and stoichiometrically bind zinc or cobalt in a tetrahedral coordination geometry (35). The loop domain is sufficient to bind zinc in a stable structure that mimics the metal-binding properties of the intact enzyme. The structural consequences of zinc incorporation were examined through absorption and magnetic circular dichroic spectra of the peptide. By these criteria the structure of the product is virtually identical to that of the structural site of horse and human ($\beta_1\beta_1$) liver alcohol dehydrogenases, which served as the template for the synthesis. Thus this seems to be the first instance of the design and synthesis of a structural zinc atom in an enzyme (35).

The relationship of the overall catalytic potential of zinc enzymes to the nature of the amino acid spacers and the creation of an environment for metal ligands suitable to catalysis is relevant to the design of zinc enzyme model systems in general. The design of systems based on the above considerations should lead to the synthesis of molecules with both the specificity of and capacity for catalysis as well as the requisite primary structures and conformations characteristic of zinc enzymes (526).

4. Zymogen activation (transformation of a structural to a catalytic zinc atom in matrix metalloproteases)

The collagenases and gelatinases are matrix metalloproteinases that hydrolyze the major components of the extracellular matrix. On the basis of their substrate preferences, the matrix metalloproteinases have been grouped into three classes: the interstitial collagenases, the type IV collagenases (gelatinases), and the stromelysins (562). The enzymes are synthesized as zymogens, i.e., inactive precursors. The single zinc atom in the inactive precursors of this family of enzymes is coordinated tetrahedrally to four amino acid residues as found in structural sites. The coordination sites of the metals in these proenzymes, therefore, are filled; neither water nor substrate has ready access to the metal. One of the ligands to the zinc is a highly conserved cysteine in the "activation" peptide that forms a mercaptide with the metal atom and is removed in the activation process. The cysteine appears to act like Velcro by "sticking" to the zinc atom through its SH-group and blocks it from participating in the catalytic process (524). Dissociation and/or displacement of that cysteine from the metal atom transforms the zinc from tetradentate, i.e., structural, to tridentate with respect to protein ligands, with water becoming the fourth ligand and rendering it "catalytic" (535). This ligand exchange reaction generates an enzymatically active molecule. The capability to exchange coordination sites of the metal that are not open into ones that are, so that water and substrate have ready access to the zinc atom, is another example of the adaptability and versatility of zinc chemistry to biological objectives (523, 524).

D. Zinc Proteins

In addition to the above zinc proteins, two other classes of zinc proteins, the metallothioneins and the gene regulatory proteins, have been recognized and have been studied extensively. Metallothionein has long been known to have an extraordinary metal content (328), but its function has remained elusive despite ever increasing attention (256, 257). Knowledge of the involvement of zinc in the structure and function of the gene regulatory proteins is of very recent origin (218, 357). An understanding of the relationship between zinc and the structures of these proteins is necessary to comprehend the metal's specific roles in the activities of these molecules and their overall biological importance.

1. Metallothionein

This protein was first isolated as a cadmium- and zinc-containing species from equine kidney cortex in 1957 (328). It is composed of 62 (or 61) amino acids, including 20 cysteines, and it has a molecular weight of 6,700, but cystine as well as heterocyclic and aromatic amino acids are absent. It contains 7 g · atoms of zinc and/or cadmium per mole of protein. In some instances copper, iron, or mercury has also been detected.

Metallothionein-like proteins and peptides have now been found in numerous unicellular and multicellular organisms and grouped together as a family composed of three classes (256, 257). Class I metallothioneins typically occur in mammalian organisms, and their primary structures are highly conserved. Class II metallothioneins are found in unicellular eukaryotes, such as yeast, and their primary structures bear little or no resemblance to those of the class I metallothioneins. Class III is present in plants, composed of chains varying from 2 to 11 γ-glutamylcysteinyl units collectively designated phytochelatins when glycine is the COOH-terminal residue or homophytochelatins if β-alanine is at that site (204, 206, 282, 431). Phytochelatins are believed to be synthesized from glutathione through the action of phytochelatin synthase (205).

Structural studies of native mammalian metallothionein show that the arrangement of the 7 g · atom zinc and/or cadmium/mol and the cysteine ligands of the protein is most unusual by inorganic chemical standards. The metals are present in the form of clusters (56, 186, 258, 393). Remarkably, a cluster structure has not been described hitherto for any inorganic, naturally occurring zinc and/or cadmium coordination complex and is therefore devised by and unique to biology. The zinc atoms of Zn_7-metallothionein are organized into two distinct metal clusters, Zn_4Cys_{11} (residues 33–60) and Zn_3Cys_9 (residues 5–29), with five and three cysteine residues acting as bridging ligands between two metal ions in each cluster, respectively (Fig. 2). The interatomic distance between the single thiolate-bridged zinc atoms in the Zn_2Cd_5 metallothionein crystal is 3.88 Å (237). The clusters are reminiscent of those of iron-sulfur in ferre-

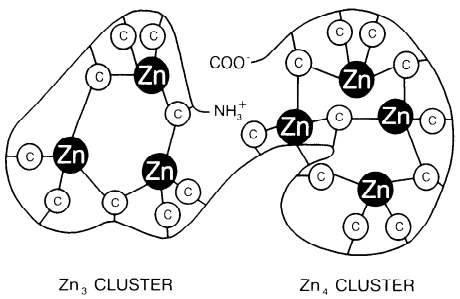

FIG. 2. Zinc thiolate clusters of metallothionein are shown. Molecule is composed of 2 clusters, the NH_2- and COOH-terminal regions containing 3 (Zn_3) and 4 (Zn_4) zinc atoms, respectively, in each cluster. Each metal is tetrahedrally coordinated to 4 thiolate bonds with some of the thiolate ligands sharing the zinc ion. [From Vallee and Auld (523).]

doxins and rubredoxins, or the molybdenum and tungsten clusters involved in oxidoreductive reactions. The specific function(s) of zinc thiolate clusters in metallothioneins, however, is not established.

A large number of physiological and pathological agents induce thionein or metallothionein synthesis in vivo. These include metal atoms (zinc, cadmium, copper, mercury, bismuth, etc.), hormones (dexamethasone, glucagon, epinephrine, norepinephrine), cytokines (interleukin-1 and -6), angiotensin II, interferon, phorbol esters, as well as physical and chemical stresses (10, 111, 256, 414, 550). Metal induction is mediated through transcriptional control of the genes coding for metallothionein with the number of their specific mRNA transcripts increasing in response to inducers, such as zinc (132). For many of these inducers, there are regulatory elements in the gene that serve to activate the transcription of the metallothionein mRNA (263, 397). The reason for the existence of so many inducers is unknown.

The function of metallothionein remains elusive, although many possibilities have been proposed. It has been thought to detoxify heavy metals, stabilize membranes, or regulate zinc and copper metabolism; it has been suggested to be a radical ion scavenger, shown to be the source of zinc for newly synthesized apoenzymes, and postulated to serve as a regulator molecule in gene expression (191, 258–260, 287, 498, 501, 511, 517, 519). Thionein, the metal-free protein, had been suggested to be the biologically active species as long ago as 1978 (517, 519, 525, 527), and experimental evidence for this suggestion has recently been reported (573, 574).

The metals in the protein are known to exchange within each cluster, suggesting metal transfer between molecules (394). Those in clusters can exchange with metals in solution or in different metallothionein clusters (373). Furthermore, metallothionein can transfer metal to glutathione (59) or thymulin (450), and the capability to take up metals from other species extends to other molecules.

Class III metallothioneins (phytochelatins) exchange their metal with apoenzymes, reconstituting them into fully active enzymes (502). The metal-free form of the protein, thionein, can remove zinc from the gene activator proteins SP1 (573) and TFIIIA (573, 574), lending support to the view that thionein (519) and metallothionein may modulate genetic processes (258, 517, 519, 527) through a redistribution of zinc among various DNA binding proteins.

2. *Gene regulatory proteins*

Many nucleoproteins directly involved with replication and transcription of DNA are now known to contain functionally important zinc atoms. The recognition that zinc is likely involved in regulation of their activity has attracted much attention and generated a large body of data that promises to contribute to an understanding of how specific genes are expressed and what the role of zinc in this process might be. Six activator proteins critical to replication and/or transcription have now been shown to contain zinc by direct analysis, and in five, the ligands to the metal have been identified (Table 7). As many as 500 (or more) are presumed to contain zinc.

TFIIIA, a transcription factor protein from *Xenopus laevis* oocytes, interacts with the 50-bp internal control region of the 5S RNA gene, thereby activating transcription by RNA polymerase III (60). It also interacts with 5S RNA itself, forming a 7S complex that is readily obtainable from the eggs of *Xenopus laevis* (410). TFIIIA has been purified to homogeneity from its 7S RNA complex, and its physical and chemical properties and functions have been characterized extensively (62, 196). It is the first transcription factor to be identified as a zinc protein. The initial publication reported the presence of 2 g · atoms zinc/mol purified protein (218, 464), whereas the 7S intact protein-RNA particles were shown to bind from 7 to 11 zinc atoms (280, 357). The difference in zinc stoichiometry was attributed to analytical and experimental parameters. However, recent data (574) suggest that the variation in metal content could also be due to physiological differences that might be brought about by the action of thionein, which can remove zinc from TFIIIA, thereby rendering the transcription factor inactive (574). Such chemical monitoring of TFIIIA action could be postulated to modulate the number of zinc

TABLE 7. *Zinc in replication and transcription regulatory proteins*

Protein	Zinc/mole	Ligands/zinc	Reference No.
TFIIIA	2	2 Cys, 2 His	218, 464
TFIIIA	7–11	2 Cys, 2 His	357
Glucocorticoid receptor	2	4 Cys	181, 233
Estrogen receptor	2	4 Cys	457
GAL4	2	4 Cys*	398, 399
g32p	1	3 Cys, 1 His	192

* In this zinc cluster, a total of 6 Cys coordinate with 2 zinc atoms such that 2 of the Cys are shared by both zinc atoms. Formally, 4 Cys are involved in the coordination of each zinc, 2 of the Cys with both metals.

atoms bound to the protein, thereby controlling the number of zinc fingers actively engaged in the transcription process at any given time, a consideration that is in need of further experimental attention.

The primary structure of TFIIIA contains highly conserved sequences comprised of 2 Cys and 2 His residues separated by variable numbers of amino acids in 9 repeat units of ~30 amino acids (62, 196). Because Cys and His residues serve as zinc ligands in a number of enzymes (see references in Refs. 523, 524), these residues in each of the conserved repeat units of TFIIIA were proposed to form tetrahedral coordination complexes with one zinc atom (357). This was suggested to generate a loop structure containing the DNA binding domain of the protein in the sequence intervening between the pairs of Cys and His residues, resulting in the "zinc finger" DNA binding motif (357). Numerous observations have confirmed the hypothesis that zinc forms a tetrahedral coordination complex with conserved cysteines and histidines of TFIIIA. Absorption spectroscopy of the cobalt complexes supports the view that the geometry of the site is tetrahedral (178). Extended X-ray absorption fine structure measurements indicate that two cysteines and two histidines are the ligands to zinc (119). Synthesis of peptide domains and NMR spectra of their zinc complexes also reveal that zinc is coordinated to cysteines and histidines and generates an intervening compact globular peptide (72, 192, 298, 374, 402). X-ray structure determination has now confirmed all of this directly (405).

The evidence is conclusive that zinc is required for DNA binding of TFIIIA. The protein will not bind to the internal control region of the 5S RNA gene if zinc is removed by chelating agents. Reconstitution with zinc, but not with cobalt, iron, copper, magnesium, or nickel, restores binding to specific DNA (218). The crystal structure of a complex containing a consensus DNA binding site and three zinc finger domains associated with 2.1–2.5 zinc (or cobalt) atoms has been determined (405). Each of the three zinc fingers is composed of an antiparallel β-sheet and an α-helix. A zinc atom is coordinated to two Cys and two His residues from the β-sheet and α-helix regions, respectively. The molecule binds in the major groove of B-DNA. The primary contacts are in a 3-bp subsite in a guanine-rich strand of the DNA. The contacts with the bases are made by conserved arginines that are located in the β-sheet region that immediately precedes the α-helix. A conserved His, the seventh residue in each of the α-helices, both coordinates the zinc atom and makes contact with the base pair.

The gene 32 protein, g32p, from bacteriophage T4 binds to and stabilizes the conformation of single-stranded DNA so that it can serve as a substrate for replication and repair enzymes (5, 193, 194). The protein also has affinity for single-stranded RNA, potentially affecting translation of its own mRNA (199). The purified protein contains 1 g·atom zinc/mol (194). The metal is tetrahedrally coordinated as indicated by visible absorption spectra of its Co(II) derivative. The ligands to the zinc atom are three cysteines and one histidine found in the region comprised of residues 77–90 (194). Removal of the zinc weakens binding to DNA considerably but does not abolish it. The zinc protein undergoes only limited proteolysis to yield a core (residues 22–253) that contains the metal and also retains DNA binding properties. In contrast, the apoprotein becomes susceptible to extensive digestion by trypsin (193). The zinc is believed to function by organizing a subdomain within residues 22–253 of g32p that is required to maintain the protein-protein interactions that are essential for cooperative binding to single-stranded DNA (193, 194).

The glucocorticoid and estrogen receptors are also zinc proteins. They are members of a multigene family that includes receptors for thyroid hormone, retinoic acid, and vitamin D_3 (142) that incorporate three polypeptide domains, each interacting with a different ligand. The first domain binds to a specific hormone, e.g., cortisol or estrogen; the second interacts with enhancerlike DNA segments; and the third binds to RNA polymerase (190). The enhancer regions are usually present in a dyad symmetry, suggesting that the interaction with the receptor involves dimerization of the protein (142). The steroid itself or the hormone binding domain is not required for DNA binding, although the hormone-receptor complex facilitates the interaction between the DNA binding domain and the enhancer region (142). The template specificity of the DNA binding domain is unaltered by the hormone binding domain. Exchange of the glucocorticoid binding domain in the glucocorticoid receptor by the corresponding estrogen binding domain from the estrogen receptor results in a hybrid molecule that retains the template specificity of the glucocorticoid receptor (142).

The DNA binding domain of the glucocorticoid receptor has been deduced by both limited proteolysis (71) and selective mutational deletion of segments of the protein (233). Deletion of a segment within the molecule containing 88 amino acids in the region of residues 410–518 results in complete loss of both DNA binding and transactivation. A fragment of 150 amino acid residues, 407–556, encompassing this DNA binding domain of the glucocorticoid receptor has been obtained in an expression vector. The soluble fraction was purified in the presence of zinc and found to contain 2 g·atoms zinc/mol. The metallo-DNA binding domain interacts specifically with the glucocorticoid response element sequence (181). Removal of zinc by chelating agents at low pH yields an apoprotein that does not bind to specific DNA fragments. Binding is fully restored by reconstitution with either zinc or cadmium, but less so with cobalt, and not with other metals (181).

The primary structure of the DNA binding domain of the glucocorticoid receptor encompasses one His and nine Cys residues (181). These cysteine-rich regions of the receptor (amino acid residues 440–525), but not the His, serve as the ligands for the 2 g·atoms zinc or cadmium/mol associated with these metalloproteins (400). Each metal is coordinated to four isolated sulfur li-

gands. There are no bridging sulfur ligands. The single Cys-500 that does not participate in metal binding can be altered by site-directed mutagenesis without affecting the receptor activity (462). The intervening DNA binding sequence is located between and anchored by the two zinc atom complexes. The DNA binding motif is helical and does not have a finger structure (221). The crystal and NMR structures of the receptor have been reported (221, 321).

While other members of the hormone receptor family of proteins exhibit sequences in the DNA binding domain that contain potential zinc-binding ligands, so far only the estrogen receptor has been shown to contain 2 g · atoms zinc/mol (443, 457).

The GAL4 protein from yeast activates the genes utilized for galactose metabolism (391). It consists of 881 amino acids, but only the NH_2-terminal 74 amino acid residues are involved in binding to the upstream activation sequence (195, 265).

Zinc is required for GAL4 function as first suggested by experiments with yeast that contained a mutant GAL4 protein in which Pro-26, found in the region intervening between Cys-14 and Cys-28, is changed to leucine. The mutant yeasts are unable to grow in medium containing galactose as the sole carbon source. This deficiency is corrected if higher than normal amounts of zinc are present in the growth medium (251). The zinc requirement is confirmed directly by studies with a fragment of the protein containing a DNA binding region, residues 1–147, obtained by the use of expression vectors. The expressed fragment binds from 1 to 1.5 g · atoms zinc/mol. Removal of the zinc by dialysis against EDTA at low pH abolishes, while reconstitution with either zinc or cadmium restores, specific DNA binding (398).

The NMR and crystal structures of GAL4 have been reported (23, 285, 329, 400). The two metal atoms of GAL4 are coordinated to a sequence that contains six cysteines (233). Cd NMR spectra reveal that the two bound metals are coordinated by the six cysteines, two of which form bridging ligands between the Cd ions (400). The resultant binuclear metal clusters are similar in principle to those observed in metallothionein (257). The structure of the DNA binding domain composed of such a cluster is clearly distinct from those of the TFIIIA and glucocorticoid receptor proteins.

The ligands that bind zinc and the resultant DNA binding motifs generated when the metal binds to the known zinc transcription/replication proteins are quite varied in the few regulatory proteins that have been examined (Tables 5 and 7, Fig. 3). The first suggestion that zinc could bind to a transcription activator in a manner that differs from that observed with TFIIIA arose from work with the transactivating *tat* protein from human immunodeficiency virus. This protein has been shown by optical absorption spectroscopy to bind 2 zinc atoms/monomer and form a metal-linked dimer, not a zinc finger structure (179, 180).

With the use of the few known examples, DNA binding proteins can be categorized into different structurally distinct groups (Fig. 3), constituted by zinc fingers (TFIIIA), "zinc twists" [glucocorticoid (GR) and estrogen receptors (ER)], and "zinc clusters" (GAL4 protein) (527). Each group likely constitutes a family of related proteins, although each will need to be examined individually. The interatomic distances between the zinc atoms in these molecules differ typically and may be, in fact, characteristic of each of the three types of groups: 27 Å for TFIIIA, 12–13 Å for GR and ER, and 3 Å for GAL4 (Fig. 3) (527). There also are important differences in the mode of binding of the zinc to these protein types. The finger types require only one zinc atom for each site, whereas the others require at least two. The DNA binding site of the glucocorticoid receptor type is located between and anchored by the two zinc atom complexes, whereas in the finger types, the DNA binding site is between two successive pairs of ligands to one zinc atom (192, 221, 400, 405). The functional significance as it pertains to, for example, DNA binding remains to be elucidated.

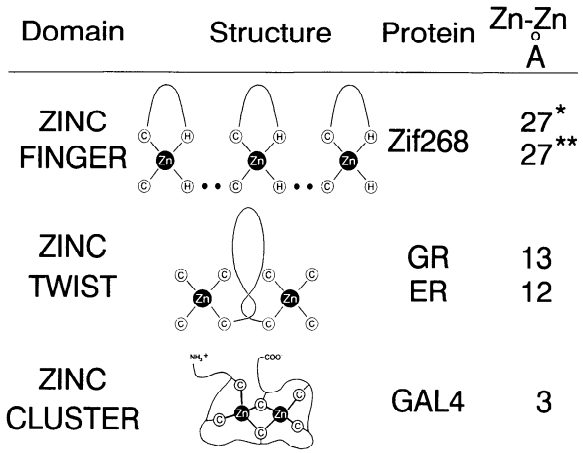

FIG. 3. Zinc binding sites in gene regulatory proteins are shown. Three distinct zinc sites are presently known. In the zinc finger type, metal is coordinated to 2 Cys and 2 His residues. Structure has been defined crystallographically using a 3-finger construct, Zif268 (405). * Zn-Zn distance from 1st zinc finger to 2nd finger; ** Zn-Zn distance from 2nd to 3rd finger. In both the zinc twist type, found in the glucocorticoid receptor (GR) and estrogen receptor (ER), and the zinc cluster type of the GAL4 protein, metal is coordinated to 4 Cys residues. Structure of resultant DNA binding domain differs for each type. [From Vallee and Auld (526).]

The major herpes simplex virus type 1 protein (HSV-1) is required for replication of viral DNA. The protein binds single-stranded DNA and protects it from nuclease action. On the basis of atomic absorption spectroscopy, it contains 1 g · atom zinc/mol that *p*-hydroxymercurimethylsulfonate can remove; however, extensive dialysis against EDTA is not successful (209). Like other single-stranded DNA binding molecules, such as g32p from bacteriophage T4 (193, 194), the apo(HSV-1) can still bind to DNA (209). The ligands to the zinc atom of the HSV-1 protein are unknown.

A summary of the ligand types to zinc proteins and enzymes and their net charges are shown in Tables 5 and 6.

Since 1985, more than 500 proteins functioning in replication and/or transcription control in viruses, bacteria, yeast, and mammals, among other organisms, have been shown to contain amino acid sequences in DNA binding domains including combinations of four or more conserved Cys and/or His residues that could serve as metal binding sites (34, 280, 485). While many of these have been categorized as zinc proteins, only those discussed above and shown in Table 7 have been confirmed to contain zinc by direct zinc analysis.

Several other regulatory proteins, SP1 (255) and ADR1 (137), among others, are believed to require zinc for function. SP1 and ADR1 are inactive when assays for either DNA binding and/or transcription activation are carried out in the absence of added metal or in the presence of chelating agents.

Zinc potentiates the interaction of the 4.5S [^{3}H]dihydrotestosterone receptor with rat prostate tumor nuclei. Cadmium, cobalt, copper, and other metals do not affect receptor binding (86). Presumably, the zinc-dependent interaction of the receptor with specific genes generates mRNA species. At least one gene product, a 23-kDa protein derived from the prostate, is known to be formed as a direct consequence of the presence of zinc (336), but the gene and its activator have not been identified and characterized.

The protein(s) that bind to the metal regulatory element(s) (MRE) of the metallothionein gene are believed to require zinc to carry out their function. An L cell nuclear factor will not bind to the MRE-d region of the mouse metallothionein-1 gene in the presence of EDTA or 1,10-phenanthroline, and zinc reverses this inhibition but cadmium does not (460). This zinc requirement pertains to at least one of the other six MREs of the metallothionein gene. The binding of a rat liver protein to the mouse MRE-a region of the metallothionein gene is also zinc dependent (459).

Transcription of histone 4 requires the interaction of H4TF-1 and H4TF-2 to an H4 promoter upstream of the transcription start site (104). Gel shift assays show that binding of both of these factors, partially purified from HeLa cell nuclear extracts, to the H4 promoter is abolished by 1,10-phenanthroline or EDTA and is restored by zinc but not by cobalt or other metals (103). These observations suggest that histone synthesis is zinc dependent, consistent with data showing that the histone content/DNA is markedly reduced in zinc-deficient *E. gracilis* (101, 340) and rat liver (74, 75).

In the past, such observations have not proved to be reliable criteria for either the presence or potential function of zinc or other metals (524, 531). Unlike for TFIIIA, g32p, GAL4, HSV-1, and GR and ER, the data demonstrating a requirement for zinc in SP1, ADR1, the testosterone receptor, and others are not matched by direct analytical information on the metal/protein stoichiometry.

IV. INTERFACE OF BIOCHEMISTRY AND PHYSIOLOGY OF ZINC

Virtually all zinc-dependent physiological observations described next preceded much of the recognition of the zinc chemistry of biological systems discussed above, and hence, there is now need for correlation and integration. As has proven to be the case for other similar metabolites, e.g., calcium and iron, seemingly disparate phenomena are prone to be recognized as due to one and the same underlying chemical event, resulting in unification of the field.

The biological essentiality of zinc implies the existence of homeostatic mechanisms that regulate its absorption, cellular uptake, distribution among intracellular compartments and macromolecules, as well as excretion. Such mechanisms are needed to maintain the broad range of biochemical functions that are dependent on zinc proteins and enzymes described above, which range from participation in intermediary metabolism (Table 3) to, e.g., membrane structure in erythrocytes and platelets (40, 83, 318) and many steps in the metabolism of the fatty acids (96), as well as the transmission of genetic information (528). The homeostatic mechanisms that regulate the amounts of zinc that are absorbed by the gastrointestinal tract, excreted from the kidneys and skin, and transported in plasma have been speculated on as indicated briefly. However, detailed knowledge of the chemical species and processes involved in each of these physiological events is surprisingly limited.

About 30–40% of the total cellular zinc is in the nucleus, ~50% is in the cytoplasm and its organelles, and the remainder is in the cell membrane and/or wall (469, 497; A. Stankiewicz, K. H. Falchuk, and B. L. Vallee, unpublished observations). Apparently, virtually all zinc in these compartments is bound to macromolecules in the form of zinc proteins/enzymes or nucleotides, RNA, and DNA; the corresponding metal-free apoproteins, apoenzymes, or nucleic acids are normally not identified in biological systems. This points to the existence of a regulatory apparatus within the cell that stores, transports between compartments, and transfers zinc from one type of macromolecule to others. Thionein and metallothionein have received increasing attention as putative components of this chain by virtue of their unique zinc chemistry, as described above. However, too little is known of such zinc regulatory systems, their molecular constituents, and their capacities to control intracellular processes through the types of molecules with which they interact.

In this regard, in microorganisms, plants, and animals, extensive pathology has been found to arise from nutritional as well as genetic disturbances in zinc metabolism. This is detailed in sections V and VI. These sections mostly cite phenomenological data, almost all of the information now available. Although we cannot indicate cause and effect relationships, whenever possible we call attention to the type of biochemistry that may be involved in the physiological events observed. Clearly, we have confidence that these physiological findings will ultimately be explicable in terms of specific zinc molecules. The resultant pathology will ultimately be understood in terms of the chemical alterations in the functions of these zinc molecules, owing to, for example, changes in their zinc content, replacement of zinc by

other metals, alterations in binding sites, failure to be synthesized, or enhanced catabolism, among other possibilities.

In the systems described, however, most of the pertinent target molecules for zinc have not been isolated as yet. Among them are proteins and enzymes in sperm, oocytes, the developing embryo, dermal appendages, the gastrointestinal tract, neural tissues, components of the ocular system, including the tapetal cells of the eyes of carnivores, and many others. Both the metal-binding ligands and the zinc stoichiometries, if any, of the large class of neuroreceptors, bioamines of the brain, and hormone binding domains of steroid and other endocrine receptors, are unknown, of course, and it is not even clear that in all instances these zinc-binding molecules are zinc proteins as defined above. Where this seems likely, there is still no information on whether the metal-protein interaction is characteristic of a metalloprotein or a metal-protein complex. Moreover, the functional results and significance of the interaction of zinc with one of these classes of proteins, in particular the hormone binding domain of the endocrine receptors, remains an open question. Within the limits placed by the above reservations, we cite the physiologically relevant data largely because there is very good reason to infer a relationship between zinc and the activity of particular, biologically important molecules. Although some of these specific molecules may turn out to be zinc metalloproteins, we consider them to be putative zinc-protein complexes until definitive data become available.

V. PHYSIOLOGY AND CELL AND MOLECULAR BIOLOGY

A. Gastrointestinal System

1. Absorption and excretion

Daily zinc requirements vary as a function of age, growth stage, and the extent of the loss of the element through the intestines, gall bladder, pancreas, kidneys, and skin. In humans, the requirement of zinc is ~800 μg at 1 mo of age, decreasing to ~500 μg from 4 to 12 mo of age (286). Subsequently, it increases to ~3–5 and up to 10 mg in 1- to 10-yr-old children (575). Normal adults, on the average, require 10–15 mg/day and up to 20–25 mg/day during pregnancy to meet the needs of the growing fetus (146, 419).

Zinc is absorbed in the jejunum and ileum (297), and the presence of glucose in the intestinal lumen assists its uptake, but calcium, phytic acid, and other compounds retard it (297, 508). Absorption appears to take place by both passive diffusion and (unknown) carrier-mediated processes, zinc entry increasing in relation to requirements (110, 232, 481).

A role for metallothionein in zinc uptake has both been proposed and questioned (51, 89, 164, 213, 353, 437, 480). More recently, the hypothesis that metallothionein is involved in zinc absorption has been replaced by the proposal that a different intestinal protein that comigrates with metallothionein plays such a role. Cysteine-rich intestinal protein, a 77-amino acid polypeptide containing 7 Cys and 1 His (43), binds ^{65}Zn (228). Both its postulated function in zinc absorption and the relationship to metallothionein are in need of further study. Similarly, a relationship between prostaglandin E_2 and F_2 and both intestinal zinc absorption and secretion has been proposed (475, 476). Experiments confirming the suggestion have yet to be carried out.

Zinc is excreted through the gastrointestinal tract; 2–5 mg/day are found in pancreatic secretions (30, 139). Urinary losses amount to ~500–800 μg/day, mostly through secretion from the proximal tubule (2). From 500 to 600 μg/day are found in sweat (304, 515). Turnover of the epithelial layer of the intestine and desquamation of skin account for further losses. The amount required to replenish these losses and maintain the system in balance is obtained by dietary intake.

2. Plasma transport and liver uptake

The total zinc content of plasma is usually ~100 μg zinc/100 ml, varying as a function of age, sex, pregnancy, and time of day (411). In all subjects, the plasma zinc content is higher in the morning than in the afternoon. Samples collected from subjects in the morning, following an overnight fast, contain more zinc than those obtained from nonfasting individuals. The zinc content of plasma in males is significantly higher than that in females. While that of male children is relatively low, it increases through adolescence and reaches a maximum in adulthood, an age-related difference that is less pronounced in females. Pregnancy and the use of oral contraceptives decrease the plasma zinc content. In the general population, there does not appear to be a relationship between the plasma zinc, stature, and weight of children from age 3 to 11 yr. Plasma zinc represents <1% of the total body content but serves as a primary source of the element accessible to all cells. Information on the nature of this zinc pool is quite limited; most studies on the subject were carried out several decades ago and have not been extended through the use of up-to-date methods and procedures.

A spectroscopic examination of human serum fractions detected at least three zinc proteins and suggested the existence of yet others that were below the limits of accurate zinc determination at the time they were performed (231). Resolution of the zinc-containing fractions led to the identification of α_2-macroglobulin as a zinc protein (401). That fraction accounts for ~30% of the total zinc in serum; the metal is tightly bound to the protein and does not exchange with ^{65}Zn. This zinc is necessary to enable the protein to maintain the esterolytic activity of trypsin in the presence of soybean trypsin inhibitor. The remaining 70% of the serum zinc is loosely bound to a protein fraction that elutes from gel filtration columns in the same region as albumin (143, 401). The exact number of proteins associated with this zinc fraction is not known, although it has been thought that the metal is associated with albumin (540), since

infused ^{65}Zn binds to this protein (52) and its concentration correlates with that of albumin in serum (197, 453). However, because albumin is the major serum protein and it has a high affinity for metals, including both free zinc and zinc already bound to amino acids or peptides, direct proof of the role of albumin in zinc transport is still missing.

About 99% of the total body zinc, ~2–3 g in a 70-kg human (561), is intracellular. Most tissues contain between 10 and 200 μg zinc/g wet wt. However, the eye, prostate, prostatic secretions, and sperm contain much higher amounts (39, 304, 515). Apparently, there is no tissue that functions as a special storage site analogous to, for example, bone marrow for iron. While bone contains large amounts of zinc, its nature and function are not well defined. The liver, however, appears to play a special role in zinc metabolism. Once absorbed from the gastrointestinal tract into the circulation, or after infusion into the venous system, zinc is cleared within 3 h (361, 540). It is taken up by the liver and eventually appears in the pancreas, kidneys, and other tissues, suggesting that the liver may be central to zinc transfer and distribution (139).

Zinc uptake by the liver from plasma may be among the earliest steps in the body's acute response to a number of physiological stimuli. Corticosteroids or estrogens lower plasma zinc by ~30–40%, and this persists as long as the hormones continue to be administered (143, 167, 229). Infusion of adrenocorticotropic hormone (ACTH) lowers serum zinc by as much as 50% within 3–4 h, even in adrenalectomized individuals, suggesting a direct action on plasma zinc distribution (143). All of the hormone effects are believed to be associated with an increase in zinc uptake by liver, concomitant with the uptake of amino acids, iron, and other metal ions.

B. Reproductive System

The zinc requirement of cells of the reproductive system, sperm, and oocytes, as well as those of the embryo, have received particular attention due to the extensive alterations ensuing to zinc deprivation. These cell types differentiate and proliferate extensively. These processes are zinc dependent and likely play a role in the sensitivity of these cells to zinc deprivation. An understanding of the role of zinc in the biology of these cells and the effects of zinc deficiency is relevant to the functions of the metal in all cells.

1. Sperm and oocytes

The zinc content of testicular tissue varies in different animals from ~20 to 200 μg/g dry wt, values that are in the range of those for most other organs (39, 337, 512, 515). In contrast, the content of the prostate gland, the seminal fluid, and ejaculated sperm are much higher, ranging from ~800 to 3,000 μg/g dry wt (39, 327, 338, 339). Moreover, the zinc content of sperm increases after exposure to seminal fluid, suggesting that sperm accumulate the metal as they traverse from the testicles to the urethra (291, 558).

Testicular zinc is critical to spermatogenesis. Zinc deficiency induces atrophy of seminiferous tubules in the rat (168, 339) and failure of spermatogenesis, particularly the last stages when the zinc content of maturing sperm increases (356, 512). Zinc is also involved in a number of functions of importance to sperm physiology. Before fertilization, sperm zinc must be maintained to stabilize quaternary structure of chromatin and to preserve genomic integrity (47, 240, 290–293, 436). Following contact with the oocyte, zinc removal from sperm appears to be involved in penetration and fertilization. Zinc contributes to the stable attachment of sperm head to tail, and its removal induces head-tail detachment (44). Once oocyte penetration has taken place, the spermatozoan nucleus undergoes decondensation and forms the pronucleus. This decondensation process requires reduction of the chromatin zinc content, since the metal inhibits that process (240).

Information on oocyte zinc content is more limited. The sea urchin and *Xenopus laevis* oocytes are fertilized and develop in the sea and lakes or streams, respectively, and are "closed" systems that contain nearly all the nutrients necessary for the formation of the early embryo. In contrast, mammalian embryos take up nutrients from the fallopian tube fluid beginning with the earliest divisions (107). The zinc content of the oocytes of *Xenopus laevis* (70 ng/egg) or of those of sea urchin (20 pg/egg) does not change after fertilization or during development of the embryo up to the blastula stage. The zinc content of mouse eggs, on the other hand, increases following fertilization from ~1–2 pg/egg to 7 pg/embryo (K. H. Falchuk, T. Nomizu, I. Atsuya, and B. L. Vallee, unpublished observations). Hence, mammalian embryos would be particularly sensitive to a reduction of environmental zinc, although all embryos can be targets for zinc deficiency, as described below.

2. Embryonic development

Multicellular organisms develop by a complex sequence of steps from cell division, determination, commitment, differentiation, growth, and the arrangement of cells into specific organs with specialized functions. Recognition that zinc is required for some of these developmental events has emerged as a consequence of studies of the effects of its deficiency on the embryo.

The development of embryos of fish (395), birds (46, 249, 377), and mammals (14, 15, 127, 128, 210, 241, 243, 268, 359, 397, 439, 440, 449, 549), including humans (216, 461), is altered, and their survival is placed at risk when zinc intake is reduced. The effects on the rat embryo have been examined in the greatest detail, starting with the earliest events following conception to those following implantation, and analogous effects have been observed in other species.

During normal pregnancy, the rat fetus acquires 3% of the total maternal dietary zinc from the maternal plasma (332). One or two days of a low zinc diet will reduce the plasma zinc content of a pregnant dam to ~30% of the control value (129). Similarly, administration of EDTA, D-penicillamine, or triethylenetetramine can reduce the zinc available in the plasma of pregnant rats (266, 267, 269, 277, 489). Within 4 days, the decrease in plasma zinc is followed by a 50% reduction of its amount in the rat uterine fluid (188). The effects of reduced zinc in uterine fluid or plasma on preimplantation and implanted embryos have also been studied. As early as the four-cell and persisting through the blastocyst stage, the embryos of zinc-deficient mothers differ from controls in blastomere size, degenerating cytoplasm, and abnormalities of the blastocoel cavity (242). The embryonic poles of 9.5-day-old zinc-deficient embryos are small, and their development is retarded or abnormal (432). Eleven-day-old embryos are extensively deformed, and their growth is retarded. There is marked cell death, more prominent in the neural epithelium than in mesenchymal cells (222). The resorption rate of embryos that survive to be implanted is ~30%, while 90% of those that do survive become malformed and are underweight (243). The congenital malformations involve nearly all organs, including lack of formation of particular organ systems, particularly of the head region and involving bone and brain structures, and concurrent development of additional body parts. The latter are exemplified by two fully developed lower halves of the body, including spinal cord, legs, tail, etc., in zinc-deficient fetuses. The types of malformations vary depending on the time of exposure to zinc deficiency. Early deprivation, i.e., in the first days of pregnancy, usually causes more marked defects of the head region, including eyes, facial structures, and central nervous system. Later exposure, in the first to second weeks, to zinc deficiency results in a more frequent incidence of skeletal malformations (243, 268, 439, 440, 549).

3. Zinc biochemistry in developmental biology

The observation of congenital malformations in rats described above preceded by many years the delineation of the chemistry underlying transcription. Ultimately, this led to the discovery that zinc is critical to the genetic process when it was shown that it is indispensable to the action of enzymes involved in replication and transcription (528, 569). This now provides a satisfactory rationale for the above observations that at the time that they were made encountered considerable skepticism regarding their possible cause. As the following data from cell physiology, molecular biology, and biochemistry make abundantly clear, there is now a surfeit of data that offer multiple and plausible hypotheses for these observations. Surprisingly perhaps, there seems to have been little follow-up on these pace-setting studies mostly in rats with work on other species, including humans.

Most attempts to understand the biochemical basis for the role of zinc in cell division and development were directed at defining the effects of its deficiency on chromatin composition, structure, and function and on enzymes and proteins involved with replication and transcription. Much evidence now indicates that zinc is essential to the function of some, though not all, enzymes directly involved in DNA and RNA synthesis and that, moreover, it has a direct role in the regulation of the activation of many genes (528, 569). There is less information on the effects of zinc deficiency on these enzymes, the processes that they catalyze, and how this might lead to the extensive abnormalities in developmental events and their outcome.

I) CHROMATIN. Zinc deficiency alters the amount and types of the major chromatin proteins. The five classes of nuclear histones are normally present in a 1:1 ratio by weight with DNA (284). Zinc deficiency changes the content and properties of the histones in rat brain (130), rat liver (74, 75, 544), and *E. gracilis* (101, 340). In rat liver the relative amount of the histone H1 subvariant, H1*, is reduced by 50%. Moreover, other, as yet unidentified, histone 1 variants are also affected (74, 75). In *E. gracilis*, the ratio of histone to DNA falls to ~0.4, while the capacity of acid and/or salt to solubilize these proteins from chromatin is markedly reduced, suggesting tighter binding to DNA (101, 340). The total amount of basic proteins relative to DNA remains the same, however, since another protein, a 3- to 5-kDa species, comprises the remainder of the basic proteins associated with the DNA in the nucleus (101, 340, 479). This protein is synthesized only in response to zinc deprivation. It is not formed in iron-, manganese-, or magnesium-deficient cells. The DNA content of cells deprived of these other metals increases up to fivefold compared with normal ones and is associated with histones (149). The effect of zinc deprivation also extends to nonhistone proteins of both rat liver (84) and *E. gracilis* (101). Specifically, of four nonhistone proteins with amino acid compositions and electrophoretic mobilities characteristic of the high-mobility group of proteins found in zinc-sufficient cells, only one is observed in zinc-deficient *E. gracilis*.

One consequence of the shift in the types and amounts of nuclear proteins is to alter the structure and function of chromatin (145, 528). In both liver and *E. gracilis*, the chromatin becomes more compact, as evidenced by an increased resistance to both micrococcal nuclease and deoxyribonucleases I and II (73, 479). Moreover, the normal nucleosomal arrangement of the *E. gracilis* chromatin (325) is altered (479). The result of this condensation is to reduce the transcriptional capacity of the chromatin (528). The basis for the condensation is not fully understood, although it involves the 3- to 5-kDa protein described above. Other factors that potentially act to condense the chromatin of zinc-deficient *E. gracilis* may yet be found. In particular, metals whose concentrations increase during zinc deficiency may play a role in the condensation process (147, 148, 302, 542).

II) REPLICATION AND TRANSCRIPTION ENZYMES. The use of methods capable of measuring the incorporation of precursors into nucleic acids led to the conclusion that zinc deficiency reduces the rate of formation of DNA and RNA (128, 131, 147, 306, 423, 447, 473, 490). The basis for this effect and its importance to the understanding of the extensive manifestations of zinc deficiency is not defined, even though it is known that some, but not all, the enzymes involved in nucleic acid synthesis contain functionally essential zinc. The deoxynucleotidyl terminal transferase of thymus gland was the first of this group suggested to contain functional zinc. It is inhibited by a number of chelating agents, including 1,10-phenanthroline, EDTA, and others (79, 444). Zinc appears to act in the activation process of the enzyme (80).

The multifunctional *E. coli* DNA polymerase catalyzes polymerization as well as 3′,5′- and 5′,3′-exonuclease activity with distinct substrate binding and catalytic sites (116, 182, 283, 389). The polymerase function of the enzyme does not require zinc; it remains fully active in preparations with less than stoichiometric metal content (161, 545). The 3′,5′-exonuclease activity, however, is thought to depend on divalent cations such as zinc, magnesium, or manganese (31, 283). The crystal structure of the enzyme and its complex with both DNA substrate and nucleoside product reveal a binding site with high affinity for zinc and another for magnesium, consistent with a mechanism requiring metals to carry out its 3′,5′-exonuclease activity. However, the above conclusion remains to be verified through examination of enzyme preparations with variable zinc content.

The avian, murine, and simian oncogenic viral DNA polymerases (reverse transcriptases) contain from 1 to 2 g · atoms zinc/mol enzyme, depending on the species (20, 21). The metal is functionally essential, since incubation of all three enzymes with chelating agents of widely different structures inhibits their polymerization activities.

The single RNA polymerase in prokaryotes and the three classes typical of eukaryotes are all zinc dependent. All but one of these enzymes contain 2 g · atoms zinc/mol protein (19, 154–156, 294, 458, 546), whereas one, from wheat germ, contains 7 g · atoms zinc/mol protein (408). The metals in the RNA polymerases have been shown to be essential to catalytic activity, since many structurally different chelating agents inhibit polymerase function. The inhibition can be reversed by dilution of the chelating agent or addition of zinc to the reaction mixture. Inhibition is due to the chelating properties of the various agents used and is not an artifact of metal chelate-induced template hydrolysis (341).

The only known zinc enzymes or proteins of the nucleus that have been isolated from a zinc-deficient cell line are from *E. gracilis*. The cells of this organism contain a single RNA polymerase (150, 155), which is a zinc metalloenzyme containing 2 g · atoms of the metal per mole protein. It is a type II polymerase based on its chromatographic behavior with various ion exchange and DNA affinity columns and its inhibition profile with 1,10-phenanthroline, all of which are characteristic. However, unlike the RNA polymerase II of zinc-sufficient *E. gracilis* and that of other eukaryotic cells, it is very insensitive to the inhibitor α-amanitin (155). The enzyme is functional in that mRNA transcripts unique for zinc-deficient organisms are formed by this RNA polymerase II. In vitro translation of these mRNA transcripts results in the synthesis of proteins also unique for zinc deficiency (94). *E. gracilis* cells further contain a 26-kDa nuclear zinc endonuclease that can enhance the transcription of RNA polymerase of this organism. Remarkably, when isolated from zinc-deficient cells, this same protein contains copper and is inactive (M. Czupryn, K. H. Falchuk, A. Stankiewicz, and B. L. Vallee, unpublished observations).

III) GENE REGULATORY PROTEINS. Zinc not only participates in the action of enzymes that carry out transcription, but it is also a component of the proteins that regulate this process. The recognition of this function is based on three types of observations: *1*) zinc induces the formation of a number of proteins, *2*) zinc-deficient and -sufficient cells produce different types of transcripts, and *3*) the actions of several molecules directly involved in gene activation are zinc dependent (see sect. III*D2*).

A large number of proteins are being recognized as essential to the activation of developmental genes. The amino acid sequences of most of these have been deduced from the corresponding cDNA sequences (477). Many of them, such as the *Drosophila Kr* protein involved with the gene that determines the size of the gap in the segment pattern of the abdomen, contain putative metal binding sites of the zinc finger type. A mutation in one of the (putative) Cys ligands to the zinc in the DNA binding domain of the *Kr* protein results in an animal that lacks a number of abdominal as well as thoracic segments, a malformation believed to be the consequence of a nonfunctional activator protein (433). A mutation of the Cys ligand to the zinc could lead to either an apoprotein or a species with altered zinc binding properties. Consequently, the mutant molecule might be unable to bind to specific DNA sequences and fail to activate the developmental gene.

However, this and other proteins with putative zinc binding sites in their DNA binding domains have not been available in pure form and in sufficient amounts to determine whether they are indeed zinc proteins, whether zinc deficiency affects their function, and if it does, what their role in the phenotypic abnormalities of zinc deficiency might be. Two types of alterations in the function of nuclear proteins, mediated by the amount and types of metals they contain, have been identified. These might be relevant to the possible effects of zinc deficiency on the metabolism of transcription activators and are reviewed briefly.

The presence or absence of a metal can determine whether a nuclear protein acts as an activator or repressor and whether it is active or inactive. Such proteins include the gene regulatory protein MerR, the mercuric ion responsive protein that controls bacterial mercury-resistant genes (227, 429), and *Fur*, the protein that con-

trols bacterial iron uptake (22, 551). MerR has a dual function: it acts as a metal receptor and as a transcription regulator. The apoprotein represses the gene to which it binds, whereas the mercury-containing protein activates it. The *Fur* protein, on the other hand, appears to be inactive in the absence of metal and becomes a repressor in its presence.

The replacement of one metal by another in a nuclear protein can lead to functional alterations. To our knowledge, the only example in which another metal, Cu, replaces the zinc of a nuclear protein as a consequence of zinc deficiency is found in *E. gracilis*. Normally, a 26-kDa nuclear protein contains 1 g · atom zinc/mol and activates transcription. When isolated from zinc-deficient organisms, it contains copper and is inactive (M. Czupryn, K. H. Falchuk, A. Stankiewicz, and B. L. Vallee, unpublished observations). The significance and mechanism of this potentially general phenomenon are not known and require further investigation.

Which, if any, of the many regulatory proteins involved in development and which of the possible alterations in their function will prove to involve the biological role of zinc and its deficiency are not obvious at present. The zinc requirement for normal development and proliferation and the congenital malformations or other manifestations of zinc deficiency cannot be ascribed to specific functions of the protein products of any of the genes that are activated by the zinc proteins just described (g32p, GR or ER, or GAL4). Nor is it known what potential role the genes regulated by TFIIIA-type proteins might play; surely there are other transcription factors involved in cell division and developmental processes that must be sought out. Their identification, together with the emergence of basic information on the role of zinc in their activities, the genes they regulate, and an understanding of the effects of zinc deficiency on their function will further the elucidation of the mechanism(s) underlying this most important aspect of developmental biology.

C. Neural System

1. Neuroreceptors

The distribution of zinc in the brains of the rabbit, rat, dog, guinea pig, and other vertebrates was studied early by histochemical techniques using dithizone (165, 331), followed by silver sulfide stains (503), and later by atomic absorption spectroscopy (Ref. 91 and references therein).

In the mammalian brain, zinc is distributed unequally; the highest amounts are in the hippocampus and cerebral cortex (91). The zinc content of the rat hippocampus increases from birth to the third week of life, when adult values are reached (92, 126). Results for human brains are analogous, but the change occurs over a longer period, i.e., the first 5 yr of life (541). The highest concentrations of zinc are encountered in the giant boutons of the mossy fiber system of the hippocampus and cerebral cortex, which consists of presynaptic swellings of unmyelinated axons that make synaptic contact with pyramidal cells (45, 91, 217).

Twenty-five percent of the zinc localized in the cytoplasm of the mossy fiber boutons (226) is dialyzable and considered to be loosely bound; dialysis does not remove the remainder (219, 124). The presence of dialyzable zinc has led to the suggestion that the zinc content in the various cerebral areas may vary over time, consistent with measurements of uni- and bidirectional ^{65}Zn fluxes between blood and brain. For the first 30 min after injection of ^{65}Zn into blood, its movement is thought to be bidirectional, but later, the flux is primarily unidirectional, i.e., from blood into brain. Once zinc is in the brain, it is distributed between several rapidly exchanging compartments thought to reflect dynamic states whose nature is undefined (426).

Normal brain tissue contains a 6.8-kDa protein that exhibits a 70% sequence homology to human metallothionein II. It differs from metallothionein in that a Thr is inserted at residue 5, and six residues (Glu-Ala-Ala-Glu-Ala-Glu) are inserted at residues 55–60. The brain protein acts as a growth inhibitory factor for neuronal proliferation in vitro. The amount of this protein is decreased in brains of patients with Alzheimer's disease (510). This finding has been thought to account for the increased regenerative process that accompanies the extensive degeneration of hippocampal and cortical neural tissues in the brains of patients with Alzheimer's disease. It is unknown whether the growth inhibitory protein contains zinc and what its relationship to the function of neural metallothionein might be.

The inhibition of binding of peptides and other ligands to neuroreceptors is one of the neural functions proposed for zinc. While much of the information described below seems to support such an action, the specificity of the effects of the metal and its physiological significance or lack of it remain to be established. It should be noted that, like other metal atoms, zinc can bind nonspecifically to many amino acid residues, over and above those now known to bind zinc specifically in zinc enzymes and proteins (523).

The opioids and their receptors have been examined extensively in this regard (220, 383, 482, 483). A potential role for zinc in the function of these molecules was proposed based on the distribution of the metal in brain on the one hand, and the opioid peptides on the other. In the rat and guinea pig, the distribution of enkephalins determined by immunological staining techniques is identical to that of zinc (124, 483) and led to an examination of the effect of zinc on opiate-receptor interactions. Binding of ligands, such as [^{3}H]enkephalinamide, to opiate receptors in the hippocampus, cerebral cortex, basal ganglia, and other areas of the forebrain is inhibited completely by 10–100 μM zinc, a concentration range compatible with that normally encountered in these regions of the brain (25, 81, 482). The presence of zinc increases the dissociation constant of the opioid receptor for naloxone, i.e., it reduces receptor affinity (220, 383, 482, 483). Scatchard plots show that a zinc concen-

tration of 30 μM, close to the concentration at which ligand binding is inhibited by 50%, does not alter the number of binding sites, however. The inhibitory effect of zinc is potentiated when dialyzed tissues are utilized; it can be completely reversed by 1 mM histidine. Histidine itself neither affects the receptor affinity nor number of binding sites (220) at its physiological concentrations in brain tissues, 50–150 μM (493).

Zinc inhibits binding of ligands to each of the various classes of opioid receptors, μ, δ, ϵ, and κ, although to different extents. Thus physiological concentrations of zinc inhibit binding of a number of opioid agonists to the μ-type receptor but have little effect on the δ and classes. In all instances, however, the presence of zinc increases the receptor dissociation constants, while the maximum number of binding sites remains unchanged. However, these effects do not appear to be unique for zinc. Micromolar concentrations of other metals, including cadmium, copper, and mercury, but not cobalt, molybdenum, or nickel, also inhibit the binding of ligands, e.g., naloxone, to the opiate receptor by reducing ligand affinity (383, 494). The inhibition is believed to involve SH-groups, based on the reversal of inhibition by disulfide reducing agents, e.g., dithiothreitol, which also binds metals, of course (383, 482, 483). Furthermore, dithiothreitol, but not its oxidized form, reverses the analgesia induced by copper (II) ions, a thiol oxidant, when injected into the cerebral ventricles of mice (330).

Zinc has also been shown to affect the muscarinic acetylcholine receptors in rat brain. These receptors appear to be localized in the striatum, cerebral cortex, and hippocampus (375), areas of high zinc concentration (124). [^{3}H]quinuclidinyl benzilate, an antagonist of the receptor, has a single binding site. Binding of the antagonist is inhibited by zinc, lithium, and copper (375). The mode of inhibition may be mediated through activation of a noncompetitive endogenous inhibitor that decreases both the affinity and number of binding sites (120).

Zinc is a potent modulator of the binding of amino acids to receptors involved in neurotransmission. Zinc reversibly inhibits the association between γ-aminobutyric acid (GABA) and its receptor in the lobster muscle, depressing the GABA-evoked conductance increase. The metal binding site differs from that of picrotoxin, since the two antagonists act independently of one another (468). Similarly, the GABA-induced chloride current in frog dorsal root ganglion neurons is blocked by zinc, but also by cadmium, cobalt, copper, manganese, and nickel. As observed in the lobster muscle (4, 571), the inhibition is noncompetitive with picrotoxin. There are at least two GABA receptor subtypes composed of combinations of three different subunits, α, β, or γ. Receptors composed of α- and β-subunits are very sensitive to zinc ions, whereas those containing a γ-subunit are insensitive (125). Zinc is also thought to be essential for the synthesis of proteins required for the formation of GABA and other neurotransmitters as well as for pyridoxal 5-phosphate (134, 135). It inhibits *N*-methyl-D-aspartate, a major modulator of synaptic transmission in the central nervous system. This inhibition in hippocampal neurons is not voltage sensitive, in contrast to that by magnesium, suggesting two different sites of interaction for these cations (557). The interrelationships between these various zinc-dependent processes in the neural system remain to be established, particularly since many other transition metals can substitute for zinc in some of the actions cited.

2. Neurotransmission

Neurotransmission is another function postulated for zinc in the mossy fiber boutons. This is based on the belief that cytoplasmic zinc is complexed to biogenic amines that are involved in neurotransmitter function (248) and that zinc ions can be released from hippocampal slices following electrical stimulation (17, 236) and from nerve terminals (568). Experimentally, this is supported by the fact that removal of some of the zinc from the fibers by exposure to chelating agents abolishes transmission through the synapses (91). Conversely, iontophoretic application of the metal ion induces slow, prolonged increases in firing rates of neurons as well as bursts of high-frequency discharge (568).

Except for the metallothionein system cited with respect to Alzheimer's disease, the state of zinc chemistry in biology in general does not illuminate the mechanistic bases underlying the neurobiological phenomena that seemingly involve zinc in particular.

D. Endocrine System

A number of syndromes in humans and higher vertebrates give clear evidence that zinc deficiency results in endocrinological changes, particularly in growing males. In addition, the recurrent references to the effects of zinc on fertility and fecundity (13, 241) are consonant with the evidence that zinc is critical to the function of hormone receptors.

1. Growth hormone

Growth hormone and zinc in the secretory granules of the pituitary gland are present in a 1:1 ratio (98, 303, 500), although the role of zinc in these granules is unknown. However, zinc binds to growth hormone and induces its dimerization (98), and thereafter, the hormone no longer interacts with the receptor (99). Moreover, the dimer is more resistant to denaturation, suggesting that it is more stable in the granules and/or plasma (98). Growth hormone also binds to the prolactin receptor, and the presence of zinc enhances binding 8,000-fold (97).

2. Steroid binding domain of hormone receptors

The steroid and DNA binding domains of the hormone receptors have been resolved by limited trypsin

digestion. The role of zinc in the DNA binding domain was discussed in section III*D2*. Zinc and other metals also interact with sites of the hormone binding domains. The estradiol and progesterone receptors from calf uterus bind to iminodiacetate-Sepharose chelate columns containing nickel, zinc, and copper, in order of decreasing affinities (245–247, 351). The receptors will not bind to iron or cadmium chelate columns. Similarly, the 3,5,3′-triiodo-L-thyronine (T_3) and the androgen receptors bind zinc (295, 308, 317, 563).

In at least two cases, the binding of zinc to these receptors induces a change in their physicochemical properties. Thus zinc causes aggregation of purified T_3 receptors in a concentration-dependent manner with a half-maximal concentration of 200 μM (308). It also appears to bring about similar aggregation of the androgen receptors as the sedimentation coefficient of rat Dunning prostate tumor cytosol receptors is increased by a zinc concentration of 130 nM (563).

In some instances, however, occupancy of the metal binding site by zinc does not affect the interaction of the hormone with its receptor. The estrogen receptor has a single class of high-affinity steroid binding sites. The dissociation constant value is unchanged when the receptor is immobilized on Zn(II)-iminodiacetate-Sepharose, suggesting that the association of zinc with the pertinent metal binding site in the protein does not influence the steroid binding domain of the receptor (246). Correspondingly, the zinc-induced aggregation of h-TRb1, the human placental *c-erba* protein, does not affect its ability to bind T_3 (308). There are reports, however, that describe zinc inhibition of hormone binding to cellular extracts containing the androgen (295), thyroid (486), and estradiol (351) receptors or to the purified receptors themselves (317). The basis for the different results when studies use purified receptors for T_3 compared with nuclear extracts has been discussed (308). The conclusions may not pertain when pure receptors are used, and divergent results are obtained. It is apparent that further data will be needed to reconcile the contradictory findings.

E. Ocular System

1. Retina

The zinc content of the eyes and some of its components in fish and herbivorous and carnivorous vertebrates has been tabulated (515) and reviewed recently (136, 262). The amount of zinc in some of the tissues of the eye is very high, in some animals more than twice that found in most other body tissues, 570 μg/mg compared with 10–200 μg/mg (215). In fish, the iris and choroid tissues have the highest content, while in vertebrates, particularly carnivores, the choroid tissues and the tapetum lucidum cellulosum are particularly rich in this metal. This is not the case for the tapetum lucidum fibrosum, the equivalent tissue in the eyes of herbivorous animals.

Some of the ocular zinc content reflects a number of known zinc enzymes, retinol dehydrogenase, the lysosomal enzyme α-mannosidase of the retinal pigment epithelium, the ciliary body carbonic anhydrase, the corneal collagenases, and the lens leucine aminopeptidase (Table 3). Inhibition by chelating agents of the corneal zinc collagenases has proven effective in the treatment of corneal ulcerations (36). In macular degeneration, the leading cause of loss of vision in the aged, the zinc-rich retinal pigment epithelium is abnormal. Zinc treatment of patients with this disease has been claimed to decrease accumulation of degenerated visual pigment and to improve visual acuity (374a). In human eyes, an age-dependent reduction in the specific activity of α-mannosidase, a zinc metalloenzyme, has been proposed to play a role in the pathogenesis of this disease, and zinc has been found to increase its activity in supernatants of ocular tissues (570). The suggestion that zinc might be of therapeutic value in the prevention and treatment of macular degeneration requires further confirmation and is under study.

Ever since Bliss (48) first reported the effect of alcohol dehydrogenase of horse liver on the interconversion of vitamin A_1 alcohol and aldehyde, there has been a lively discussion regarding the presence or absence and role of this or a similar related enzyme in the visual process. The description of a syndrome of night blindness resistant to vitamin A, associated with reduced plasma zinc content and accompanying the manifestations of postalcoholic cirrhosis has accentuated the debate (364, 403, 532, 533). Because zinc is needed to synthesize retinol binding protein in the liver, it was proposed that decreased mobilization of vitamin A might be the basis for night blindness in patients with cirrhosis (470, 471). On the other hand, retinal dehydrogenase activity is reduced in zinc deficiency, and that has been correlated with the associated night blindness (238, 365, 366, 532, 533). Replenishment with zinc has been thought to reverse the night blindness in the absence of any change in vitamin A plasma levels. However, this reversal is accompanied by a return of the decreased retinal dehydrogenase activity to normal (238, 365, 366).

2. Tapetum lucidum

Zinc comprises 10–20% of the weight of the tapetum lucidum cellulosum (554). Atomic emission spectrometric and histochemical analyses of the eye tissues of both the ferret and the cat localize the high concentration of zinc in the tapetum lucidum cellulosum mainly to the rod membranes of the tapetal cells. No other metals appear to be present in measurable quantities (484, 505). In addition, the tapetal cell readily takes up zinc. After an injection of ^{65}Zn, the tapetal cells of ferrets contain the highest amount of the label, when compared with all other tissues (504).

Zinc is believed to play a role in the maintenance of the tapetal membrane structures and is postulated to increase the effectiveness of twilight vision, although

this is entirely conjectural. In Siamese cats, the zinc content of the rod membranes is decreased (555), as it is in common cats following dietary depletion of taurine (484). In both cases, the regular arrangement of the tapetal rods and their membranes is markedly disrupted. Infusion of dithizone promptly stains the eyeground pink, suggesting the formation of a zinc dithizonate complex, leading to edema, hemorrhage, and finally retinal detachment (409).

Histochemical and cytochemical studies using a silver sulfide method suggest that zinc in the paraplasmic tapetal rods exists as a mercaptide bound to cysteine in a 1:1 ratio (281, 554). It is not clear, however, whether the cysteine is present as a free amino acid or as a constituent of a peptide. In the cat, but not the dog, cysteine may be part of a protein of ~6.4 kDa whose amino acid composition differs from that of metallothionein (93), although the purity of the protein was not verified and the zinc stoichiometry was not reported. It would seem surprising to encounter free cysteine in the eye, since SH-groups are very susceptible to oxidation, a chemical property of questionable physiological value to vision. Both the nature of this remarkable complex and its presumable function remain to be determined.

F. Immune System

The thymus glands of pigs and rats atrophy in response to experimental zinc deficiency. In Friesian cattle, this phenomenon is associated with an inherited disorder of zinc metabolism (63, 64, 299, 427, 465). In 6-wk-old mice fed a zinc-deficient diet for 4 wk, the thymus gland atrophies by >50% and then consists of a few thymocytes within a mass of connective tissue and epithelial cells (174). It is incapable, moreover, of responding normally to various standard antigens or defending against specific types of infections. The response of mice to dinitrofluorobenzene (176) and of guinea pigs to tuberculin antigen (349) is reduced markedly. In the rabbit, both epithelial and stromal herpes simplex keratitis are more severe when zinc is deficient (157). Host resistance to parasitic infestation is markedly impaired. A dose of *Trypanosoma cruzi*, which would normally be sublethal, causes the death of 80% of zinc-deficient mice who also exhibit a 50-fold higher parasitemia than pair-fed controls (173). In such zinc-deficient rats, the expulsion of *Trichinella spiralis* (159) and *Strongyloides ratti* is impaired (158).

Methods to identify different T lymphocyte populations with specific functional properties (helper, memory, suppressor, and effector cells), surface markers (presence or absence of surface immunoglobulins in B and T cells, respectively, and T cell receptors to sheep red blood cells), blastogenic response to mitogens, lymphokine production, etc., have allowed a correlation between the effects of zinc and its deficiency on the cellular and humoral immune responses and changes in the cell types that are affected. The major impairment in zinc-deficient organisms appears to arise from effects on T lymphocytes whose numbers are reduced, and on macrophages, whose function is altered. The function of the remaining lymphocytes appears to be normal (87).

The capacity of zinc-deficient mice to produce anti-sheep red cell plasmacytes is greatly diminished, but this is reversed on administration of normal thymocytes to the zinc-deficient animals before immunization (174). Their cytotoxic T killer cell activity toward allogenic tumors and natural killer cell function are decreased (160, 177, 184).

Collectively, these findings suggest that zinc deficiency affects T but not B cell function. This has been confirmed by direct analysis of lymphocytes in zinc-deprived mice, revealing a reduction in the numbers of T cells and T cell subsets, as well as reduced proliferation and response to T cell-dependent antigens [dinitrophenol (DNP)-human albumin] but not to a T cell-independent antigen, DNP-Ficoll (367). In vitro cultures of T cells in zinc-deficient media do not proliferate normally, in contrast with B cells (166). Zinc deficiency also affects the immune memory response. Deficient mice produce 43% immunoglobulin G anti-sheep red blood cell plaque-forming cells/spleen. Primed splenocytes transferred from zinc-deficient to -sufficient, but irradiated, mice do not respond normally to standard challenges. The memory response can be restored, albeit only partially by reversing the zinc-deficiency state (115). The offspring of Swiss Webster mice made "moderately" zinc deficient while pregnant exhibit depressed immunity for 6 mo after birth; their second and third filial generations remain partially immunocompromised despite a normal dietary intake of zinc (29).

The effects of zinc deficiency on macrophage function have been examined in mice infected with *T. cruzi*. The percentage of mouse peritoneal macrophages associated with the parasite, the number of *T. cruzi* in each macrophage, as well as the ability to kill the trypanosome are reduced. All of the latter are reversed by pretreatment with zinc but not with copper, manganese, or nickel (564).

Corresponding studies of immune function in zinc-deficient humans are limited to individual case reports, although the conclusions drawn appear to be similar. Abnormal immunity in humans is observed in cases of acrodermatitis enteropathica (see sect. VI*B*), characterized inter alia by an aplastic thymus where neither lymph nodes nor spleen exhibit germinal centers or immature and mature plasma cells in paracortical areas (105). Functionally, the total number of T cells is depressed, a delayed hypersensitivity type of response to dinitrochlorobenzene is absent, and blast transformation in response to T cell mitogen stimulation is depressed (7, 78, 388, 406). The effects on the T cell subsets also vary. Helper T cells (CD4+ cells) and the activity of natural killer cells are reduced. In contrast, circulating T suppressor cells (CD8+) and monocyte cytotoxicity are increased (8).

The basis underlying the diverse and extensive effects of zinc on immunity is much more limited than that for the above systems. Thymic atrophy and the at-

tendant loss in immunity in the first 28 days of feeding mice a zinc-deficient diet does not appear to be due to the decreased food intake accompanying the deficiency. However, after 32 days of such a diet, this becomes an important contributory factor and must be considered in the interpretation of the results of such studies (320). The data available on the T cell subsets indicate that the pathology relates to a decrease in the number of cells; those lymphocytes that survive in zinc-deficient animals function normally. The decrease in T cells has been shown to be due, in large part, to the increase in adrenal steroids that accompanies the stress of zinc deficiency. Adrenalectomy protects mice from the thymic atrophy accompanying zinc deprivation and suggests that it is secondary to adrenal hormone stimulation. However, it does not completely normalize the ability to mount an antibody-mediated response, indicating that these are alternate effects that account for the decrease of lymphocytes (114). The macrophage data point to an actual functional alteration induced directly by zinc deficiency (175). The alterations are postulated to be the result of processes of the oxygen burst and H_2O_2 production needed to kill parasites, which was thought to be zinc dependent (87).

Both clonal proliferation and differentiation are sensitive to zinc deficiency, as described for embryonic cells. It is likely, then, that the understanding of the biochemical basis underlying the problems with both processes in embryonic cells rendered zinc deficient may also pertain to immunocytes.

VI. PATHOLOGY

A. Adverse Effects of Excess Zinc

The chemical basis of metal toxicity is poorly defined and, hence, leaves it a largely phenomenological subject. Quite the same, it is tempting to relate toxicological manifestations to the physical chemical properties of metal, but unfortunately the opportunities for meaningful correlations are very limited. "Toxicity" is a biological not a chemical concept of course. It is used loosely biologically and is often not understood well chemically. Given the state of the art, it would seem imperative both to identify and evade erroneous premises and their experimental pursuit. It would also be appropriate to point out that the biological effects of metals are commonly presented as a continuum, extending from physiology to pharmacology to toxicology and pathology. The chemistry thought to underlie each of these way stations is generally assumed to follow a similar progression. However, as point of fact this is not the case. While the chemical identities and structures of many physiological, functionally distinct zinc proteins are now known, the consequences of toxicological metal-protein interactions are not. Hence, the resultant pathology cannot be explained in terms of the chemical realities.

None of the many physiologically important zinc proteins is implicated in toxicological manifestations. Furthermore, storage disorders comparable to those associated with, for example, copper in Wilson's disease or iron in hemochromatosis are unknown in the case of zinc. In fact, the homeostatic mechanisms regulating zinc absorption and retention operate with such efficiency that zinc overload is extremely unlikely (12, 37). The induction of the low-molecular-weight peptide thionein by means of transition and other metals could readily result in the scavenging of up to 7 mol zinc/mol thionein. Hence, thionein at least becomes a critical candidate for the regulation of zinc metabolism. Clearly, a metal that is known to be essential to the inheritance of the genetic endowment and the induction of development, growth, and differentiation could not easily be intended to be deleterious to the perpetuation and evolution of the species. Instead, one would expect zinc to be regulated carefully to ensure the preservation and continuity of life. Such long-term effects of zinc on evolution would be quite distinct from those on short-term effects such as metabolic pathways of proteins, carbohydrates, fats, and nucleic acids and their metabolism. In fact, zinc is the only pre-, post-, and transitional element that has proven to be essentially nontoxic. It is neither carcinogenic, mutagenic, nor teratogenic (301), as has been established through long periods of observation (12, 37).

Ingestion or administration of even very large amounts of zinc compounds does not have any adverse long-term consequences (37), and any negative effects attributable to its excess are unrelated to its biological role or function.

Zinc fume fever, the one toxic manifestation of this element known, originates from an abnormal pathway of its absorption into the body. Symptoms and signs are encountered exclusively on inhalation, as contrasted with ingestion, of zinc. Smelting of zinc ores takes place at relatively low temperatures owing to the high volatility of zinc, and fumes are readily generated. Inhalation of zinc oxide fumes and vapors results in metal fume fever, also known by synonyms such as "ague" or "brass chills"; it typically presents itself with influenza-like symptoms that are accompanied by normal serum zinc concentrations. The disease is never fatal and is completely reversible (28, 270, 344). Once the fumes have been removed, such symptoms usually vanish within 48 h. This occupational disease has been eradicated almost completely by suitable ventilation of the workplace and subsequent reduction of zinc fumes in the environment. Metal fume fever has been observed also with other metals, e.g., cadmium and magnesium oxides.

There have also been very sporadic case reports of instances in which the ingestion of zinc in acid fruit juices, stored in galvanized containers, or the infusion of dialysis fluids exposed to galvanized pipes has led to acute temporary gastrointestinal and other nonspecific discomfort (49, 61, 70, 95, 189, 198, 208, 362, 451) including reversible neurological signs and symptoms (370). Zinc toxicity as defined by Bertrand (38) owing to excessive ingestion of zinc salts has not been reported.

The occurrence of copper deficiency, accompanied by anemia, in some cases of prolonged supplemental zinc therapy (172, 417, 421) has been referred to erroneously as zinc toxicity (172). It is, in fact, an example of a "conditioned deficiency" (141). This term describes the induction of a deficiency of one nutrient (in this case copper) by the ingestion of another that acts as the "conditioning factor" (in this case zinc). Thus zinc here is the conditioning factor, which renders copper deficient, but zinc itself is not toxic in the conventional sense. Discontinuation of zinc administration restores normal copper metabolism. In a different example pertinent to the metabolism of zinc, calcium can be a conditioning factor for zinc, inducing its deficiency either in plants or swine. Thus, in these instances, calcium induces zinc deficiency (508). The mechanisms in these and many other similar cases of conditioned deficiencies are not known.

B. Zinc Deficiency

Zinc deficiency is the most significant pathological state involving abnormalities in the metal's metabolism. This can be due to inadequate dietary intake, increased requirements or excretion, conditioned deficiency (141), or genetic causes (Table 8). Spontaneous deficiency has been observed in a number of species and has been induced in a variety of others (168, 170, 171, 296, 319, 378, 422, 425, 506, 508, 515) as mentioned above. We here summarize some of its features in humans.

Zinc deficiency in humans was first described in populations of limited size subsisting on a diet consisting of unleavened bread and beans, virtually devoid of any animal protein or variety but rich in phytic acid. The afflicted individuals practiced geophagia and showed some, but not all, of the characteristic manifestations of zinc deficiency (422). All individuals affected were males who exhibited lethargy, rough skin, absent pubic and axillary hair, hypogonadism, undescended testes, dwarfism, anemia, and hepatosplenomegaly. Administration of iron supplements cured the anemia, whereas the other findings were reversed only by zinc supplementation, which constituted the principal criterion to identify zinc deficiency as the cause of the syndrome.

TABLE 8. *Human zinc deficiency*

Inadequate intake or absorption with normal requirements
Low dietary zinc content
Presence of agents in diet that bind zinc and prevent absorption (phytates)
Excessive metals (Ca, Cu) in diet competing for absorption pathways
Postsurgical removal of small bowel
Malabsorption syndromes
Total parenteral nutrition without zinc supplementation
Standard intake with increased requirements or excretion
Chelation therapy (penicillamine)
?Cirrhosis
?Burns
Genetically induced
Acrodermatitis enteropathica

The growth retardation associated with zinc deficiency is similar to that observed in hypopituitarism (446). However, administration of growth hormone does not reverse zinc deficiency and its effects (323, 424).

In subsequent studies, patients who had undergone surgical removal of their small intestines and were given intravenous hyperalimentation therapy for several months but without zinc supplementation developed severe dermatological lesions including alopecia and acrodermatitis (16, 264). Zinc administration reversed all the lesions within 2–4 days.

Similarly, chronic diarrheal diseases associated with malabsorption (207, 348), including regional enteritis (343), coeliac sprue (472), cystic fibrosis (24), and disaccharide malabsorption (140), have been found to be associated with some of the signs and symptoms of zinc deficiency.

The use of the chelating agent penicillamine in the treatment of Wilson's disease has also been reported to cause parakeratotic lesions akin to those seen in swine and typical of a conditioned zinc deficiency; these, too, were reversed by zinc supplementation (279).

Acrodermatitis enteropathica is a genetic zinc deficiency syndrome in humans transmitted as an autosomal recessive trait. The disease was initially reported in children with dermatitis and malabsorption (54), and its manifestations were described subsequently in greater detail (105). The syndrome includes total alopecia and symmetrical, erythematous, and vesicopustular dermatitis localized to areas around the mouth, eyes, nostrils, and on the extremities. Diarrhea, growth retardation, and emotional disturbances are also characteristic. There may be ophthalmological disorders, such as blepharitis, conjunctivitis, photophobia, and/or corneal opacities. If untreated, the disease is fatal.

The disorder is associated with decreased absorption of ingested zinc. Normal adult individuals absorb 60–70% of a standard dose of ^{65}Zn taken orally and eliminate ~0.7%. Children suffering from this disease take up only ~3% of the amount administered, far lower than adults with the same illness who absorb 15–40% of the dose (313). Jejunal mucosal biopsies from patients with acrodermatitis enteropathica exhibit the same decreased uptake of ^{65}Zn when compared with biopsies from normal individuals (18). The signs and symptoms often appear in infants with this trait when weaned from human to cow's milk, suggesting that in addition to an intrinsic intestinal defect, other variables might contribute to decreased zinc absorption. This had been attributed to the presence of a zinc binding ligand in human milk purported to facilitate intestinal absorption (315). However, subsequent studies have claimed that the poor absorption of zinc from cow's milk may actually be due to the high phosphate content associated with bovine casein, precipitating zinc and reducing its bioavailabiliy (372).

Excepting dermatitis, all other symptoms of acrodermatitis enteropathica vary with age. The symptoms due to the gastrointestinal and central nervous systems are more marked and frequent in infancy; the decreased growth, alopecia, and frequent infections are often observed in preadolescent children; while in adolescents spontaneous remissions have been noted (55).

The diagnosis is usually made based on the clinical picture. Serum, urine, and hair zinc content may be reduced by ~50% (536), but this is not specific, since serum zinc may or may not be reduced and can also be lowered in many acute illnesses (143). Variant types of the disease characterized by a normal serum zinc content have been reported (288, 324). Decreases in ^{65}Zn absorption and of intestinal alkaline phosphatase, abnormalities in the ultrastructure of small bowel biopsies and reversal of all clinical findings on zinc administration have all been seen (50, 323, 536). Almost antithetically, before this disorder was known to be related to zinc, it was treated with Diodoquin, a halogenated derivative of the chelating agent 8-hydroxyquinoline (122). Once zinc deficiency was recognized to be part of the etiology of the disorder, oral zinc supplementation was instituted (26).

"Genetic zinc deficiency" also occurs in cattle and mice. In Adema disease of cattle, the deficiency causes increased abortions (350, 553), and in mice, lethal milk mutation leads to early death (412). The surviving offspring of cattle exhibit a high frequency of congenital malformations of nearly all organs similar to those in rats on zinc-deficient diets (241). The mouse pups are normal at birth, but nursing on the milk of mice homozygous for the mutation causes zinc deficiency that is manifest in dermatological abnormalities, including hyperkeratosis, parakeratosis, and pustular and/or crusting red scaly plaques. Infections with bacteria or fungi are common. Transport of zinc from maternal blood to milk seems to be defective (412).

An analogous syndrome in humans has been reported. The zinc content of breast milk of two mothers whose infants showed "acrodermatitis" and decreased serum zinc content was very low despite normal maternal values in plasma and dietary zinc supplementation. The low breast milk value was again proposed to result from a defect in transport of zinc from blood to milk (576).

A number of other syndromes and physiological alterations have been ascribed to zinc deficiency. Some of the children of alcoholic mothers exhibit congenital malformations similar to those described for animals subsequent to experimentally induced zinc deficiency. The malformed organs in this "fetal alcohol" syndrome include the heart, kidneys, genitalia, and facial structures (112, 252).

A decreased dietary zinc intake has been proposed to account for the aging process, including senile dementia (68, 69). It was postulated that aging represents the "accumulation of genetic errors in somatic cells" resulting from an increased rate of DNA injury not repaired owing to loss of efficiency of zinc-dependent DNA repair systems. The loss of intracellular zinc was thought to underlie the entire process. This hypothesis, when first proposed by Burnett (68), attracted a great deal of attention and generated much discussion but virtually no scientific data. Thus there is no experimental evidence to confirm or deny that either aging or the fetal alcohol syndrome are the consequence of zinc deficiency.

C. Zinc Deficiency and Organ Pathology

The available information regarding the effects of zinc deficiency on the physiology and biochemistry of a number of target organ systems is very varied. In general, cells from intestinal, dermatological, and gonadal tissues that normally undergo rapid turnover and frequent proliferation appear to be most sensitive to zinc deprivation. Therefore, pathological consequences of zinc deficiency predominate in these tissues. Those relating to the immune system and to embryological development have already been addressed; others are presented below.

1. Dermatology

Fine et al. (162) have reviewed the role of zinc in the integument. The skin undergoes continuous renewal through a combination of cellular proliferation and differentiation. The process is initiated by the proliferation of the basal cells that migrate upward through the epidermis, where they differentiate from cells that synthesize a number of structural proteins into others that are quiescent and act as a barrier between the environment and the organism (162). In zinc-deficient rats, diminished subcutaneous tissue, nonscarring alopecia, and denuded skin have been noticed (168). In swine, there are hyperkeratotic plaques and increased skin fragility, i.e., parakeratosis (508). The parakeratosis of lambs and calves is particularly prominent in the acral areas, accompanied by brittle wool, hoof distortion due to altered keratin hardening, and perioral and periorbital alopecia (358).

In humans, the dermatological findings comprise elbow, knee, perianal, intergluteal, and acral erosions, which are frequently symmetrical. Vesicles and/or pustules, and later, hyperkeratotic plaques develop in the same areas. While alopecia is commonly significant, scarring is not. Remaining hair is sparse, brittle, and dull, and nails may become atrophic. Histologically, there is psoriaform dermatitis with epidermal hyperplasia, parakeratosis, scattered dyskeratotic cells, tortuous papillary dermal capillaries, and sparse superficial lymphohistocytic perivascular infiltrate (3, 162). The skin is edematous and exhibits focal necrosis in the dermis (105).

2. Gastroenterology

The epithelium of the intestinal mucosa also undergoes continuous renewal and is in constant need of zinc.

Like the dermal epithelium, it is sensitive to decreases in plasma zinc. Most biopsies from patients with zinc deficiency do not reveal pathological abnormalities of the intestines, but in some with acrodermatitis enteropathica, the gastrointestinal tract develops lesions that exhibit flattening of intestinal villi and mucosal ulcerations. There is lymphocytic infiltration of the lamina propia (105). Electron microscopy of the duodenum reveals ovoid or rhomboid lysosomal-like inclusion bodies within the Paneth cells that contain electron-dense myelinlike material embedded within a filamentous or crystalline matrix (314) that disappears on treatment with zinc (50). Similar findings have been described in biopsies from patients with zinc deficiency secondary to hyperalimentation in the absence of supplemental zinc (488).

In cirrhosis, plasma zinc is chronically decreased associated with reductions in the content of the metal in other tissues (532, 533). The amount of zinc bound to α_2-macroglobulin is increased, while that associated with albumin is decreased (453). Despite the reduced plasma zinc, the urinary zinc excretion is markedly increased. The manifestations were thought to reflect a conditioned zinc deficiency (141).

3. Neurology

Zinc deficiency affects both the central and peripheral nervous systems. The central nervous system ailments are manifest with behavioral disorders pertaining to integration of learning, memory, and emotional stability and implicating abnormal functions of the zinc-rich hippocampal structures (463). Pregnant zinc-deficient animals have difficult deliveries and behave abnormally, e.g., ignoring and not caring for the neonates (13). Their offspring do not learn as well as the controls (445). Halas (212) has summarized the range of behavioral abnormalities relating to zinc deficiency in humans and other mammals.

Peripheral nervous system dysfunctions accompanying zinc deficiency have been reported for several species. In the guinea pig, altered posture and locomotor function as well as hypersensitivity and hyperalgesia have been observed. These are attributed to peripheral neuropathies, since the conduction velocity of sciatic nerve preparations of such animals is reduced (379, 381).

4. Endocrinology

Zinc deficiency leads to profound physiological alterations in gonadal function. Hypogonadism was observed in the earliest studies of zinc deficiency in the rat (168) and later in humans (422). Atrophy of the seminiferous epithelium, decreased spermatogenesis, and reduced output of testosterone by the Leydig cells are characteristic of the testes in zinc-deficient animals (355, 356, 390). Testicular atrophy is likely due to a primary effect on Leydig cells, not to a failure of hypothalamic-pituitary axis function. In zinc-deficient rats, levels of both luteinizing hormone and follicle-stimulating hormone are normal, and the response to gonadotropin-releasing hormone is either normal or increased (300, 342, 442).

Zinc also plays a critical role in the reproductive physiology of the female, albeit not as prominent as in the male. As in the male, secondary sexual characteristics fail to develop (441). However, delayed parturition is the most striking effect on female reproduction (13), probably the consequence of delayed luteolysis and decreased formation of the gap junctions in the uterus required for its regulated contractions (133, 322). The condition is neither associated with a decrease in the amount of estrogen in the pregnant animal nor in the total number of receptors (65, 66).

Recognition of a role of zinc in the function of hormone receptors (181, 457) now provides a specific biochemical basis for the extensive effects of zinc and its deficiency on reproduction. To function, estrogen as well as other hormones secreted by both male and female gonads and adrenal glands bind to their specific intracellular multidomain receptors. Once the hormone associates with its receptor molecule, the complex is transported from the cytoplasm into the nucleus, where it binds both to a specific DNA segment and RNA polymerase, a zinc metalloenzyme. Zinc deficiency could prevent binding of the hormone receptor complex to DNA. This would likely preclude activation of genes regulated by these receptors and thereby account for some of the endocrinological abnormalities associated with zinc deficiency. It could also affect the activity of RNA polymerase directly. Together, these could also explain the insensitivity to the action of the normal amount of estrogens in zinc-deficient animals (133).

5. Hematology

Plasma is the most accessible body fluid and the one analyzed most frequently for its zinc content. Major reductions in plasma zinc content occur during the acute phase of many disease processes (143), including myocardial infarction, hepatic or renal failure, neoplasia, etc. (108, 143, 214, 215, 515, 540). This decrease of serum zinc has been proposed to be due, in part, to effects of both ACTH and cortisol, hormones that are secreted during the acute phase of many diseases; increases in these hormones are believed to induce zinc uptake by the liver.

Yet other variables decrease zinc in plasma. Administration of endotoxin or infection by organisms such as *E. coli* increases the zinc in hepatocytes and brings about a concomitant fall in plasma zinc to 50% of the control within 12 h. The amount of zinc in the plasma partially returns to ~85% of normal within 48 h (32). This effect, very puzzling when first observed, has been found related to and mediated by interleukin-1 and is independent of glucocorticoids (32, 33, 90, 123, 200, 278, 416). Interleukin-6 also reduces the plasma zinc content.

The hepatic uptake of zinc caused by these two cytokines is associated with the formation of metallothionein in the liver (90, 456).

Plasma zinc is reduced in patients affected with chronic diseases, e.g., sickle cell anemia (see Ref. 139 for reviews). It is not clear whether the reductions in zinc are due to its uptake by the liver owing either to effects of acute-phase hormones, cytokines, increased excretion in the urine, or all of these, as is observed in a chronic illness such as cirrhosis or in acute disorders such as trauma and burns (100, 109, 309, 515); nor is it certain that the decrease occurs in all patients affected by each of these disorders. In some sickle cell patients, for example, the plasma zinc content remains normal (1).

The changes in serum zinc content observed in acute diseases seem to stem from only one of the two zinc-containing protein fractions. The zinc bound to α_2-macroglobulin remains constant, while that found in the fraction identified with albumin decreases (143). One possibility is that this loosely bound zinc serves to supply the metabolic needs of all tissues (143, 144) and that this is controlled by uptake into the liver.

In most cases of zinc deficiency the zinc content of plasma is reduced. This finding has not been sufficient to establish the diagnosis in all cases, however. There is usually a decrease in most cases of zinc deficiency, but in a number of instances it remains normal (288, 324). Of itself, the finding of a decreased plasma zinc content is not characteristic of zinc deficiency per se, since reductions can be observed in a variety of acute illnesses and in response to increases in stress hormones but without any of the known symptoms and signs of zinc deficiency (143).

Platelets contain zinc (275, 326) that appears to be involved in hemostasis. The effects on platelet function are dose dependent. Addition of physiological concentrations of zinc, 0.1–0.3 mM, to washed platelets induces aggregation. This reaction is dependent on calcium, requires the presence of fibrinogen, and is blocked by antifibrinogen antibodies, suggesting that the metal interacts with the platelet fibrinogen receptor. The effect is not dependent on extracellular ADP and does not result in thromboxane synthesis or serotonin release (230). At lower concentrations, aggregation as well as platelet activation factor and the thrombin-mediated release of serotonin are inhibited (83, 239, 376). When platelets are obtained from zinc-deficient rats, they are functionally impaired, likely due to a variety of factors, including a decrease in their response to the aggregating agent prostaglandin endoperoxidase (201). The overall consequence is a prolonged bleeding time in zinc-deficient rats (138, 201). The defect is entirely reversible when the deficient animals are given zinc, suggesting that the problem is intrinsic to the platelet itself and not to a plasma factor (138).

A function of erythrocyte zinc is to participate in the activities of carbonic anhydrase and Zn_2Cu_2 superoxide dismutase, apparently its most significant contribution (37, 360, 419, 559, 572). Of the total red cell zinc, >90% is associated with these two enzymes. The remainder is bound to other enzymes and proteins (384). Another function appears to be a role in the maintenance of the membrane state. In zinc-deficient animals, the zinc content of red cell membranes is decreased while the osmotic fragility of these cells is increased (41, 250, 380). The basis for the fragility is not known and is not reversed by the addition of zinc to the assay even though it is repaired after zinc repletion of the animal. The zinc content of red blood cells does not reflect alterations in total body zinc content (419).

Leukocytes contain ~25 times as much zinc as the equivalent number of red blood cells (515, 530). A decreased leukocyte zinc content together with a reduction in the activity of leukocyte alkaline phosphatase has been proposed as diagnostic criteria of zinc deficiency (419). The 5′-nucleotidase activity of lymphocytes is decreased in zinc deficiency (413, 420). The validity of these indexes has been and remains dubious (360).

Sensitive immunological methods capable of detecting a number of zinc-containing proteins in plasma have been described. Thus metallothionein is present both in serum and in red blood cells (57, 203, 363). In the latter, it is believed to relate to the period when the red blood cell was nucleated. Thymulin, a peptide derived from the thymus gland, another zinc protein in serum, can be detected in a similar manner (106, 420). The concentrations of both metallothionein and thymulin have been suggested to reflect the body's zinc status, although the validity of this proposal has not been confirmed experimentally.

D. Rheumatoid Arthritis

In this disease the destruction of cartilage is accompanied by degradation of its component collagen, and this has been attributed to matrix metalloproteases, enzymes that are present in both normal nonarthritic as well as rheumatoid cartilage (58, 369, 448). Conjectures that this is a causal relationship are based on a number of findings. "Proteinase" activity toward collagen, proteoglycans, and glycoproteins is found in ~10% of samples of synovial fluids (224, 225) and in the extracellular fluids of explants of synovium taken from patients with rheumatoid arthritis (223). Injection of leukocyte granules that contain many and varied proteases into rabbit joints induces arthritis (552). Immunohistochemical analysis reveals the presence of "proteases" in the cartilage surface undergoing destructive changes (565, 567) or of matrix metalloproteinase-3 (stromelysin) in synovial cells (452). Stromelysin has been localized to the lining of rheumatoid but not to that of normal synovia (387). Synovial cells obtained from patients with rheumatoid arthritis express mRNAs for interstitial collagenase and stromelysin (163).

Matrix metalloproteinases are thought to be zinc metalloenzymes (42, 253, 347, 524, 525, 535, 560), and on this basis zinc has been implicated in the pathology of this disease. The measurement of proteinase activity of tissues, however, does not suffice to confirm a role of

this class of enzymes in the pathogenesis of arthritis or of any other disease (see sect. VI*E*). Clearly, it is necessary to characterize the enzymes both by correlated activity and zinc measurements as well as by inhibition of the former with chelating agents suitable for metalloenzymes, e.g., 1,10-phenanthroline, 8-hydroxyquinoline-5-sulfonic acid, and dipicolinic acid, among others (529). Experimental data addressing these issues with enzymes from normal and rheumatoid arthritis synovial tissues and fluids are sparse (369). Very few matrix metalloproteases of human synovium have been purified and characterized (385, 387, 566); hence, their properties, particularly in terms of the metal, remain to be studied fully. The fact that matrix metalloproteinases are produced largely in the form of inactive precursors (77, 537, 538, 556) and that in synovial fluids the precursors are usually present complexed with tissue inhibitors of metalloproteinases (76) further compounds these problems. To become enzymatically active these proteases must undergo both activation and dissociation from inhibitory molecules (76). Although the activation of these zymogens has been addressed (524, 535), the process and mechanism by which it proceeds are not known. It certainly has not been examined for the synovial enzymes of rheumatoid arthritis, which is of particular interest here. Although a metalloendopeptidase and its inactive zymogen have been purified from rheumatoid synovium, and its activation by trypsin, plasma kallikrein, plasmin, and thermolysin has been observed (385, 386), the fate of these zymogens in diseased joints is unknown. One might further expect that zinc deprivation of experimental animals might also reflect in the activity of these enzymes in affected joint tissues. In the absence of such data it is left to inference and implication whether the proteolytic activity demonstrated in the synovial fluids of rheumatoid joints is the result of matrix metalloproteinase activities. Analogous considerations pertain to neoplasia, where the matrix metalloproteinases have been assigned similar roles.

E. Neoplasia: Invasiveness and Metastasis

Zinc has also been ascribed roles in the metabolism and interaction of malignant cells (455, 515). The zinc content of leukemic leukocytes is reduced (521), and zinc deficiency inhibits proliferation of transplanted tumors in host animals (118, 407). On the other hand, zinc deficiency enhances the carcinogenic effects of nitrosomethylbenzylamine (169). The decreased zinc content of some malignant cells and/or the response of neoplastic tumors to zinc deficiency has suggested a potential for therapeutic opportunities (152, 516). However, there is no known role of zinc in oncogenesis. The detection of zinc in the reverse transcriptases of animal leukemias (20, 21) first suggested that yet other zinc enzymes might be important to the development and/or maintenance of neoplasia.

Recent interest in zinc in regard to metastases has been generated by reasoning similar to that which led to speculative hypotheses regarding its role in the family of matrix metalloproteinases and their bearing on rheumatoid arthritis (see sect. VI*D*) (202, 311, 369). As in that condition, the relationship of these proteinases to neoplasia is conjectural much as we have weighted the merits of its inclusion in this zinc review on the same basis as that of rheumatoid arthritis.

Both normal and nonmetastatic and metastatic malignant cells secrete matrix metalloproteinases as biologically silent precursors into the surrounding environment where they are activated. The amount of enzyme secreted by malignant cells has been reported to exceed that of normal ones (311, 369). The collagenolytic and gelatinolytic activities attributed to active metalloproteinases are abolished or markedly reduced by EDTA (577), although the full range of chelating agents does not seem to have been examined. The amount of tissue inhibitors of metalloproteinases expressed by metastatic tumor cells is much less than that of nonmetastatic tumors (415). On this basis, the "aggressiveness" of tumors, both in vitro and in vivo, has been correlated with the synthesis of metalloproteinases, suggesting that the greater the collagenolytic activity, the more aggressive the cancer tissue (310, 312, 333, 345, 371, 392, 495, 509).

Oncogene amplification and stimulation by cytokines, thought critical to metastatic behavior, have been implicated in the production and regulation of metalloproteinases in malignant cells (305, 346, 467, 492). Nontumorigenic NIH 3T3 cells, transfected by the *ras* oncogene, express high levels of metalloproteinase mRNA together with production of gelatinase activity, and these are more chemoinvasive than the control (507). Similarly, when the Ha-*ras* oncogene is present in squamous cell carcinomas, or when these are exposed to interleukin-1, they express activated stromelysin and are more invasive in vitro (183, 333). In contrast, interleukin-6 does not affect metalloproteinase gene expression but is a potent inducer of the tissue inhibitors of these enzymes (316).

While the metalloproteinases are now thought to play a significant role in the metastatic capability of malignant cells (273, 354, 415, 435, 547), their inhibitors are believed to serve to control tumor metastases (6, 9, 113, 274, 371, 499, 548). Rat embryo cell line (4R) transfected by c-Ha-*ras*-1 secretes metalloproteinases that degrade types I and IV collagens and gelatin and are highly metastatic. A recombinant human tissue inhibitor of metalloproteinases (rTIMP) completely inhibits both the proteolytic activity and the degradation of collagen and reduces the ability of the 4R cells to colonize the lungs of nude mice by 83% (9). Addition of TIMP-2 to human fibrosarcoma HT-1080 or to the c-Ha-*ras*-1 transfected 4R embryo cells prevents invasion of the tumor of a reconstituted basement membrane (6) or smooth muscle matrices (113), respectively.

Agents of chemotherapeutic value are also able to inhibit the metalloproteinases and affect tumor invasion. The metastatic capacity of rat 13762NF mammary adenocarcinoma cells has been correlated with its pro-

duction of type IV collagenase. Treatment of these cells with all *trans*-retinoic acid, at concentrations known to induce antitumor activity, inhibits the degradation of extracellular matrix and type IV collagen by >50% (371).

As the above discussion of the matrix metalloproteinases/rheumatoid arthritis relationships indicates, a number of experimental approaches that would solidify those conjectures remain to be performed. The considerations regarding this group of enzymes relating to metastatic aggressiveness of cancer are similar to the reasoning correlating them to rheumatoid arthritis and deserve scrutiny.

VII. CONCLUSION

Since first reviewed three and four decades ago (515, 521), the state of zinc physiology has undergone dramatic changes that have fundamentally altered the understanding of the biological role of this element. The present review, while emphasizing and summarizing the nature of the new insight, also indicates the areas in which substantial progress may soon be expected and what its nature may be.

Zinc is the only of the pre-, post-, and transition elements recognized to be nontoxic while being indispensable to all forms of life and critical to transmission of the genetic message, development, growth, and differentiation. Zinc is essential to long-term preservation and perpetuation of the species through its fundamental role in the synthesis, transcription, and translation of the genetic message. On a short-term basis, it is prerequisite to the anabolism and catabolism of all essential foodstuffs and their intermediates by participating as a functionally obligatory component in the action of relevant metabolic enzymes. Similarly, it functions in hormone action by regulating the structures of their receptors. All these physiological roles have been clarified through the recognition and definition of biological zinc chemistry and its relationship to observed phenomena. As is the case for metallothionein, in some instances, the structure of biological zinc molecules is completely unique to natural products in biology and has no precedent thus far in inorganic chemistry. This review reveals that the emerging chemistry accounts for many past observations and anticipates those yet to be made.

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