BACTERIOLOGICAL REVIEWS, Mar., 1966 Vol. 30, No. 1
 Printed in U.S.A.

Roles of Metallic Ions in Host-Parasite Interactions

EUGENE D. WEINBERG

Department of Bacteriology, Indiana University, Bloomington, Indiana

INTRODUCTION

For efficient metabolism and growth of plants, protists, and animals, appropriate qualitative and quantitative balances of essential metallic ions are required (26, 30, 45, 50, 89, 121). A corollary observation is that various types of abnormal or diseased metabolic states of plants and animals are associated with mineral imbalances in specific tissues or organs (58, 87, 108). Moreover, the selective biological activities of vitamins, hormones, and drugs are found in numerous instances to be either suppressed or enhanced by, and in some cases even dependent upon, the presence of specific metallic ions (22, 39, 58, 87, 96, 108). Furthermore, many examples of control by metallic ions of the synthesis of a variety of secondary metabolites and of the initiation of morphogenetic processes, especially in plants and protists, are known (134). Consequently, it logically can be predicted that metallic ion balances might be of considerable importance in the establishment of host-parasite relationships, and that adjustments between hosts and parasites could be significantly affected by distortions in the metallic ion environment. In this paper, examples of the influence of metallic ions on host-parasite interactions are described briefly; the classification to be used is based on postulated molecular sites of activity of the ions.

A successful parasite, whether virus or protist, must be able to (i) survive outside of its natural host, (ii) infect a new host, (iii) replicate within the new host, (iv) leave the host, and (v) we hope, cause little or no damage to the host. Because of the differences between viruses and protists in chemical composition, mode of replication, and metabolic capacity, the problems encountered by viruses and by parasitic protists in fulfilling the above five requirements are considerably different. This review is divided into three parts. In the first, the dependence of viruses on metallic ions for survival in vitro, for infection of new cells, and for replication is considered. The second part consists of a survey of the involvement of metallic ions in three facets of protist pathogenicity: (i) differential metallic ion growth requirements of virulent and avirulent bacterial strains, (ii) metal-dependent synthesis of bacterial and mycotic factors of virulence, and (iii) association of metallic ions with activity of bacterial and mycotic toxins. In the final part, utilization by the host of metallic ions and metal-binding substances as components of antimicrobial defense mechanisms is described.

VIRUS-HOST CELL INTERACTIONS

Stability of Free Particles

Divalent metallic ions can aid in stabilization of the tertiary structure of molecules of some proteins (41), ribonucleic acid (RNA) (41), and deoxyribonucleic acid (DNA) (35). It is possible, therefore, that viruses, at least during their extracellular existence, might make use of metallic ions to withstand such deleterious agents as heat and degradative enzymes.

The first crystalline samples of tobacco mosaic virus (TMV) contained 1.3 to 1.77% ash (111); subsequently, attempts were made to determine whether the metallic ions associated with TMV

are merely contaminants, acquired either during the sojourn in the host tissues or during purification, or are actually integral components of the complete virion (73). The evidence obtained points to the latter alternative, inasmuch as the cation composition of TMV neither bears any direct relationship to that of the host fluids nor varies in content during successive ultracentrifugal cycles of purification. The purified virus is considered to be a relatively undissociated coordination complex of RNA, protein, Ca^{++}, and Mg^{++} (73); the two metallic ions comprise 0.38% by weight of the samples. Additionally, TMV preparations have been found to be associated with Al^{+++}, Cr^{+++}, Mn^{++}, Fe^{++}, Ni^{++}, Cu^{++}, Zn^{++}, Sr^{++}, and Ba^{++} (124), and removal of Fe^{++} has been correlated with loss of infectivity (72); however, not all infective samples contain Fe^{++} (40).

Infectivity of TMV RNA is stabilized by the presence of 10^{-7} M Ni, presumably because the metallic ion interferes with the activity of an extrinsic labilizing factor (23); it is not known if such a protective mechanism is operative under natural conditions. Metallic ions apparently are chelated to the nitrogenous bases of TMV RNA and stabilize the helical structure (52). However, appropriate additional quantities of any of the ions in the group of nine cations listed above can destroy biological activity of TMV RNA at 65 C by catalyzing hydrolysis of phosphodiester bonds (52). As few as three equivalents of Fe^{+++} per mole of TNV RNA (6,400 nucleotides) can photocatalytically inactivate the nucleic acid; Ag^{+}, Hg^{++}, In^{+++}, Al^{+++}, and Cr^{+++} are inactive, and Ni^{++} and Cu^{++} have protective action (109*a*). From experiments with nucleotides and other model compounds, it appears that, under the influence of Fe^{+++} plus visible light, bases are destroyed and released from glycosidic linkage and that diester bond breakage occurs secondarily (109*a*).

In contrast to the strong affinity of TMV for alkaline earth metallic ions, the bacterial virus *Escherichia coli* T5 loses more than 90% of Ca^{++} acquired from the host cell with each successive sedimentation; after five cycles of centrifugation, there remain fewer than 10 atoms of Ca^{++} per virus particle (68). Nevertheless, the thermal stability of bacteriophages, including T5, is enhanced very markedly by 10^{-3} M Ca^{++} or Mg^{++} and is depressed by metal-binding agents (68). Presumably, the latter agents decrease the stability of the virus particles by removing metallic ions (other than Ca^{++}) from their natural sites.

Two animal viruses, mouse encephalomyelitis (97) and vaccinia (49), were reported in early studies to be associated with Fe^{++} and Cu^{++}, respectively; in neither case, however, was evidence subsequently obtained that the cation is actually a component of the virus rather than a contaminant from host cell substances. As with bacteriophages, salts of Mg^{++} enhance the stability of enteroviruses and reoviruses at a variety of temperatures. For example, 1 M $MgCl_2$ or $MgSO_4$ protects entire populations of poliovirus for 25 days at 24 C and for 1 to 2 hr at 50 C; controls show only 20% survival and complete inactivation, respectively (127). Live oral polio vaccine in tightly stoppered vials at *p*H 6.4 can be stored without loss of efficacy for as long as 1 year at 4 C, if 1 M $MgCl_2$ is included in the formulation (83). The titer of active particles in reovirus preparations is actually increased by four- to eightfold by exposure to 2 M $MgCl_2$ for 5 to 15 min at 50 to 55 C (132); however, in $MgSO_4$ solutions, the preparations are inactivated (131). Of 42 strains of enterovirus tested, all are stabilized by $MgCl_2$, whereas only five are stabilized by $MgSO_4$ (131).

Strains of such RNA viruses as Japanese B encephalitis (88), measles (98), and influenza (131) are stabilized by $MgSO_4$ and Na_2SO_4; $MgCl_2$ and NaCl are inactive. Of the four salts, NaCl is most effective in stabilizing infectious RNA obtained from encephalitis virus (88). Such DNA animal viruses as vacuolating virus (128), vaccinia virus, and herpesvirus (131) are rapidly inactivated by heat in the presence of 1 M $MgCl_2$, and use has been made of this property to free poliovirus cultures from the vacuolating virus contaminant (128). Lipid-containing animal RNA and DNA viruses are inactivated at 36 C by Fe^{+++} and Ru^{+++} chelates of substituted 1,10-phenanthrolines; nonlipid viruses are resistant to these chelates (139).

Attachment and Penetration of Particles to Host Cells

Divalent metallic ions are frequently found to be necessary for the reversible electrostatic attachment of bacterial virions to host cells (95, 101, 104, 119); usually, 10^{-3} M Mg^{++} or Ca^{++} is most effective, but often Sr^{++}, Ba^{++}, or Mn^{++} can function in such systems. There is little need for a specific cation, since any of the above ions can lessen repulsion between the virus and bacterium by neutralizing their respective negative charges (118). Such other divalent cations as Co^{++}, Ni^{++}, and Cu^{++} usually cannot be used because of their toxicity to host cells at 10^{-3} M. Monovalent ions can in some instances substitute for divalent ions, but are much less efficient; usually, concentrations in the range of 1 M are required. In such experiments, recrystallized salts must be employed, since reagent grade chlorides of Li^{+}, Na^{+}, and K^{+} are often contaminated with as much as 0.05% Mg^{++} and Ca^{++}.

The attachment of such animal viruses as poliovirus and coxsackievirus to monkey kidney cells also is enhanced by 10^{-3} M Mg^{++} or Ca^{++} (4, 77); for adsorption of fowl plague virus to chick embryo cells, optimal concentrations are 10^{-3} M for Al^{+++}, 10^{-2} M for Mg^{++} or Ca^{++}, and 10^{-1} M for Na^{+} or K^{+} (2). No examples of the need for cations in attachment of plant viruses to host cells are presently available; the lack of information in this area can be attributed to the difficulty in control of the ionic environment, inasmuch as most plant virus studies are performed with intact plants or organs rather than with cell cultures.

After attachment of the virus particle to a receptor site on the surface of the host cell, the parasite or at least its nucleic acid must be able to penetrate into the cell. Detailed studies of the mechanism of penetration of a few bacterial viruses have demonstrated that the specificity of the metallic ion requirement for penetration is much greater than for attachment. Penetration of a variety of bacterial viruses has been found often to be dependent upon an environmental supply of Ca^{++} (33, 74, 93, 125, 137), possibly for activation of the myosinlike contractile phage enzyme that aids the invasion of the nucleic acid (43). In a few cases, Mg^{++} (74) or Mn^{++} (33) can substitute for Ca^{++}; with coliphage T1, the Ca^{++}-dependent process is antagonized by Zn^{++} (1).

Bacteriophage T2 particles can be classified into two groups; one contains long-headed, dye-permeable, slowly sedimenting phages, and the second contains virions with the opposite features (29). Conversion from the first to the second group can occur after irreversible adsorption; however, the heads of the particles must be shortened before injection of DNA can occur. Shortening of the head protein is enhanced by components that leak from host cell cytoplasm during infection. Most effective of these is Ca^{++} (10 times as active as Mg^{++}); the percentage of short-headed forms in a medium containing 10^{-4} M Ca^{++} is 28.6, whereas in the presence of 10^{-3} M Ca^{++} the percentage is 75.0. Approximately 2 to 4% of the estimated 7×10^{-4} M Ca^{++} in host cytoplasm escapes during infection; this quantity is itself insufficient for conversion, but is probably supplemented by Mg^{++} and Na^{+} (29).

Subsequent to attachment of the tail of T2 coliphage to the host cell, tail fibers are unwound and removed, provided that Zn^{++} is present in the cell wall (66). Presumably, this metallic ion catalyzes the splitting of thiolester bonds that hold the tail fibers to each other and to the tail core. Removal of Zn^{++} from isolated walls does not interfere with phage attachment, but does preclude subsequent digestion of the wall by liberated phage enzyme; moreover, Zn^{++} can be restored to such walls, and phage enzyme digestion then occurs. The function of the restored Zn^{++} cannot be fulfilled by Mg^{++}, Ca^{++}, Mn^{++}, Fe^{++}, Co^{++}, Cd^{++}, or Hg^{++} (67).

Other than the Zn-dependent system described for coliphage T2, there is no available information concerning the extent to which metallic ions present as integral parts of bacterial walls might either aid or interfere with phage penetration. Evidence for the presence of metallic ions in walls continues to accumulate. For example, walls of corynebacterial (51) and streptococcal (105) strains contain 10.1 and 2.7% ash, respectively; walls of listerial (64) and rhizobial (53) strains contain 36 and 56%, respectively, of the Ca^{++} of the entire cells. Of the 74,000 atoms of Zn^{++} in the whole cell of *E. coli*, 4.2% are in the walls (67). Cells of *Azotobacter* that have had metallic ions removed by ion-exchange resins become muramidase sensitive; restoration of Zn^{++} but not Ca^{++} to such cells causes them to regain their resistance (100). Of the dry weight of isolated walls of *Pseudomonas aeruginosa*, 0.09% is Zn^{++}, 0.15% is Ca^{++}, and 0.20% is Mg^{++} (34). Obviously, a considerable amount of work must be done to determine to what extent bacterial viruses in general make use of Zn^{++} and Ca^{++} present in bacterial walls for penetration; it would be surprising, however, if the roles of these cations described above for T2 are unique for this strain of bacteriophage. In biological systems, Zn^{++} and Ca^{++} are usually antagonistic (*see*, e.g., 30, 50); therefore, if each cation is needed for the overall invasion process, they would most likely function either sequentially or in spatially distinct regions of the cell surface.

Replication and Release of New Particles

We must now consider a very ill-defined region with regard to mechanisms of metallic ion function at the molecular level. The majority of studies in this area have been designed and performed for applied purposes; for example, to suppress a plant viral pathogen or to accelerate the production of poliovirus in vaccine manufacture. Attempts are made to alter the metallic ion balance sufficiently so as to change the rate and extent of viral replication but to cause no grossly obvious biochemical lesions in the host cells. Operationally, the imbalance is created by (i) alteration of the quantities of metallic salts in the environment, (ii) addition of selective metal-binding agents, or (iii) addition of specific metal chelates. In most instances, the sole parameter is that of rate of appearance of new virions; it is usually assumed that a single step somewhere in the sequence of virus uncoating, enzyme induction, nucleic acid and protein synthesis, maturation, and release has

been affected. In a few cases (see below), experiments to show that the metallic ion imbalance neither inactivates extracellular virions nor interferes with attachment or penetration were performed.

As has been true in the early development of many areas of virology, the first virus to be intensively studied was TMV. The rate of virus synthesis is depressed in cells of the tobacco plant deprived of Fe^{++} (94) or Zn^{++} (46), and is accelerated in cells deprived of Mn^{++} (138). Efficient synthesis occurs even in chlorotic Mn^{++}-deficient cells; apparently, TMV formation is independent of Mn^{++}-catalyzed enzymatic processes. The minimal Zn^{++} requirement, as well as the maximal quantity tolerated, was found to be identical for normal host metabolism and for optimal virus replication, whereas low concentrations of Fe^{++} exert a greater suppression on virus production than on cell metabolism (46, 94). Interestingly, those virus particles that are produced by Fe^{++}-deficient plants contain as high a quantity of Fe^{++} as do virions synthesized by normal plants (124).

In partial disagreement with results of the study cited above (46) on Zn^{++}, there are reports that manipulation of the level of Zn^{++} can dissociate virus synthesis from host cell metabolism. For example, 10^{-3} M Cd^{++} (considered to be active by virtue of its ability to displace Zn^{++}) can suppress TMV synthesis by 70% without obvious damage to the tobacco plant (120), and 10^{-3} M Zn^{++} can stimulate production 90%, provided that bean rather than tobacco leaves are employed (142). If carnation leaves are used, 10^{-3} M Zn^{++} partially suppresses TMV synthesis (142), and complete suppression is obtained with 10^{-2} M Zn^{++} (136).

The quantity of TMV produced in cells of apical meristems of tomato roots is increased more than 100-fold by use of 6×10^{-4} M ethylenediaminetetraacetic acid (EDTA) in the nutrient solution (28). The authors suggest that EDTA, by removal of Ca^{++} and Mg^{++}, permits endogenous enzymatic degradation of microsomal granules, which in turn renders RNA from these particles more available for TMV synthesis. Inasmuch as (i) the concentration of Zn^{++} is quite critical for efficient TMV synthesis, and (ii) EDTA binds Zn^{++} 10^5 times as strongly as it combines with Ca^{++} or Mg^{++}, it is conceivable that EDTA acts simply by altering the availability of Zn^{++} to the host cells.

Attempts have been made to control the synthesis of at least two additional plant viruses, clover phyllody and a camelia pathogen, by adjustment of the metallic ion environment. In clover plants, addition of 3×10^{-4} M Zn^{++} suppresses virus synthesis while permitting normal host growth (18), and in camelia plants added Fe^{++} has a similar beneficial action (90).

A few reports are available concerning metallic ion control of various aspects of bacteriophage synthesis. A virus strain infecting *Salmonella typhi* requires specifically Ca^{++} (36) for an unknown function associated with replication; a second strain needs specifically Mg^{++} (119). In a medium containing 10^{-4} M Mg^{++}, development of *E. coli* phage T4rII in cells lysogenic for phage λ proceeds normally for 10 min but then requires a 500-fold increase in Mg^{++} for continued normal maturation; the additional quantity of cation is not needed for wild-type T4 replication (42). The Mg^{++} requirement is antagonized by K^+ and by agents that interfere with aerobic oxidation. Conceivably, these mutant phages might be causing excessive leakage either of Mg^{++} or of other substances essential for replication, and the additional quantity of the cation might be able to halt the efflux by strengthening the cell surface (42). However, general permeability changes occur to the same extent in host cells infected with either wild-type or mutant phage (9); furthermore, although there is more leakage of putrescine in cells infected by mutant phage, Mg^{++} is unable to prevent this efflux (3). The Mg^{++} requirement for rII phage replication in lysogenized cells can be replaced by polyamines (3, 9), and it is possible that, in this system, the active substances function as cofactors or stabilizers of an unknown product essential for virus synthesis (9).

Synthesis of phage by cells of a strain of *Bacillus megaterium* is suppressed by 5×10^{-6} M Cu^{++}, 5×10^{-5} M Zn^{++}, or 5×10^{-5} M Co^{++} without any obvious injury to the bacterial cells (54); in the same system, 10^{-4} M Mn^{++} is required for virus production. The latter requisite is compatible with the general need of *Bacillus* species for considerably more Mn^{++} for the synthesis of various secondary metabolites and structures than for vegetative growth (135). With a corynebacterial phage-synthesizing system, on the other hand, large quantities of Mn^{++} as well as of Zn^{++} and Cu^{++} are detrimental to virus replication (5).

The maintenance of prophage in a strain of *B. megaterium* depends on an adequate supply of Ca^{++} (25), and the induction in this species of virulent phage from prophage by reducing agents requires Cu^{++}; the latter ion is antagonized by Co^{++} (75). The inducing effect of ultraviolet light also depends on the balance of cations, and Lwoff (75) has concluded: "The absence of phage development in lysogenic bacteria is controlled by an equilibrium depending on the absolute and relative affinities of bacterial constituents for

cations, and of the absolute and relative proportion of cations in the bacteria."

As with bacteriophage synthesis, a small amount of information is available concerning the effect of alteration of metallic ion balances on animal virus replication. In monkey kidney cells, 2×10^{-4} M Al^{+++} suppresses synthesis of foamy virus, herpes simplex virus, and herpesvirus B, but does not interfere either with normal host cell metabolism or with the synthesis of poliovirus, mumps virus, influenza virus, vaccinia virus, adenovirus, or simian virus 40 (129). Liberation of polio virus from monkey kidney cells (130) and of an influenzal virus variant from chorioallantoic membrane cells (91) is facilitated by the presence of 2.5×10^{-2} M Mg^{++} and 0.9×10^{-2} M Ca^{++}.

Four groups of metal-binding compounds contain members that suppress plant or animal virus synthesis, or both, at concentrations somewhat below the cytotoxic doses. These are (i) diamidines (8) such as noformicin and amidinomycin, (ii) biguanides (109) such as the N^1-isobutyl derivative, (iii) β-thiosemicarbazones (116) such as the *N*-methylisatin derivative, and (iv) Cu^{++}-binding agents (79) such as D-penicillamine and thiourea. Information is not yet available concerning the possibilities that the compounds either may act in the form of specific metal chelates or be bound to sensitive intracellular sites by specific metal ion linkages. Transition metal chelates of substituted 1,10-phenanthrolines strongly inhibit the synthesis of new virions in allantoic membrane cells, but unfortunately are not selectively toxic; the same concentration that suppresses virus synthesis likewise inhibits host cell oxygen consumption (140).

To summarize the section on replication of viruses, there are a number of observations that virus synthesis is affected by alteration of metallic ion balances in host cells. However, it is not yet possible, by changing the level of any specific metallic ion, to predictably retain normal host cell metabolism while altering a given reaction in the sequence of steps in virus replication.

BACTERIAL AND MYCOTIC PATHOGENICITY

Differential Metallic Ion Growth Requirements of Virulent and Avirulent Bacterial Strains

A few comparisons have been made of the in vitro requirements for, and tolerance to, metallic ions between virulent and avirulent bacterial strains. There has been little correlation of the observations with specific in vivo metallic ion environments; thus, it is not presently possible to predict whether the differences are causally associated with virulence and if they can be applied in the development of either preventive or therapeutic regimens. An early study was that made with *Pasteurella pestis*; nine virulent strains but not three avirulent strains were found to require 2×10^{-3} M Ca^{++}, Sr^{++}, or Zn^{++} for in vitro growth at 37 C (47). However, reduction of the concentration of Mg^{++} from 20×10^{-3} M to 2.5×10^{-3} M resulted in a decreased need for Ca^{++} by the virulent strains and an increased Ca^{++} requirement by the avirulent strains. An additional observation of the importance to *P. pestis* of the Ca^{++}-Mg^{++} balance will be described in the next section.

In the case of three pairs of avirulent and virulent group A streptococci, added Mn^{++} inhibits nucleic acid synthesis to a greater extent in avirulent than in virulent suspensions, whereas protein synthesis is largely unaffected in the former (38). With virulent suspensions, Mn^{++} exerts a general depression of respiration, nucleic acid synthesis, and protein synthesis. Each of the above effects of Mn^{++} is antagonized by an equimolar concentration of Ca^{++}.

In contrast to the inhibitory effect of Mn^{++} on virulent strains of streptococci, the same metallic ion acts synergistically with digested DNA to promote rough $\rightarrow$ smooth (R $\rightarrow$ S) population changes and to stimulate multiplication of S (virulent) cells of pneumococci (37). The metallic ion specifically depresses respiration of R cells and enhances that of S cells; it cannot be replaced by Mg^{++}, Fe^{++}, Co^{++}, Cu^{++}, or Zn^{++}, and is reversed by Ca^{++}. Growth in proteose peptone medium of mixtures of virulent and avirulent cells of various strains of *Klebsiella pneumoniae* yields predominantly avirulent cultures; however, if the broth is supplemented with 2×10^{-3} M Mg^{++}, the cultures become fully virulent (16).

When smooth populations of *Brucella suis* are grown in vitro in the presence of 6×10^{-6} M Fe^{++}, their ability to dissociate into the rough form is increased, whereas their capacity to invade tissues and to multiply rapidly in vivo is considerably reduced (133). Cells of *B. suis* grown with Fe^{++} appear to possess a factor that stimulates the inflammatory response at the site of injection; Fe^{++}-deficient cells, on the other hand, do not stimulate mobilization of host defense factors.

Metallic Ion Requirements for Synthesis of Specific Factors of Virulence

The synthesis of a wide variety of microbial secondary metabolites, i.e., substances possessing no obvious function (at least in the large quantity produced) in normal metabolism, require metallic ion environments that must be controlled more rigidly than those needed for normal vegetative growth (134, 135). Therefore, it is not surprising

that the synthesis of factors of virulence is affected markedly by slight changes in the balance of metallic ions; usually, these changes have little or no effect on vegetative-cell multiplication. As might be expected, the specific metallic ions that are most important for the synthesis of nontoxic secondary metabolites by a given microbial group are the same ions that are most critical for the synthesis of factors of virulence. The cations thus far recognized to be of importance are Fe^{++} for *Clostridium*, Mn^{++} for *Bacillus*, Ca^{++} and Mg^{++} for *Pasteurella*, and Zn^{++} for molds. Undoubtedly, further generalizations of this nature will become apparent as the syntheses of additional secondary metabolites, including factors of virulence, are examined in a greater variety of microbial groups.

The ability of subinhibitory concentrations of Fe^{++} (usually in the range of 5×10^{-6} to 1×10^{-4} M) to depress the synthesis of such factors of virulence as α-toxin lecithinase of *C. perfringens* (86, 102), neurotoxin of *Shigella shigae* (122), and diphtheria toxin of *Corynebacterium diphtheriae* (6, 24) has long been recognized. For production of tetanus toxin by *Clostridium tetani*, the optimal concentration of Fe^{++} is 10^{-4} M; suppression of toxin synthesis occurs if this quantity is tripled (70). Additionally, Co^{++}, at concentrations 10-fold higher than those of Fe^{++}, has a depressing effect on toxin synthesis (6, 24, 122), and acts synergistically with Fe^{++} in some systems (24). *Staphylococcus aureus* grows well in a semisynthetic medium containing acid-hydrolyzed casein, L-cysteine, glucose, three vitamins, and $MgSO_4$; for optimal production of α-hemolysin and enterotoxin, however, glucose must be replaced by acetate and 1.3×10^{-4} M Fe^{++} must be added (20).

The depressing effect of Fe^{++} on diphtherial toxin production may be merely a special case of the suppression by this metallic ion of microbial porphyrin accumulation; presumably, Fe^{++}-activated enzymes convert porphyrin precursors into more complex substances such as hemes (69). Replication of *C. diphtheriae* phage β has been proposed to be necessary prior to toxigenesis; however, Fe^{++} neither interferes with ultraviolet light induction of prophage β nor with the synthesis of vegetative phage, but rather appears to suppress the subsequent conversion of a toxin precursor to the biologically active material (6).

As noted in the section on replication of viruses, strains of *Bacillus* consistently have been found to require more Mn^{++} for the synthesis of many different types of secondary metabolites than for vegetative growth (135). In unpublished experiments in this laboratory, the concentration of Mn^{++} required for synthesis of crystalline toxin of *B. thuringiensis* was found to be 10^{-5} M, which is 100-fold the quantity needed for normal growth. Likewise, formation of protective antigen (factor II) by *B. anthracis* has an elevated Mn^{++} requirement (141); however, data are not yet available concerning the concentration of Mn^{++} needed for maximal synthesis of factors I and III of anthrax toxin (110). Factor I, not toxic by itself, is a powerful chelating agent (110); conceivably, it might function to sequester a portion of the very small quantity of Mn^{++} normally bound to protein in host fluids [e.g., only 5×10^{-7} M in whole blood (26)] and, in turn, make the metallic ion available to the bacterial cells for use in synthesizing factors II and III.

Virulence antigens are produced by nonmultiplying cells of *P. pestis* in an in vitro environment containing 20×10^{-3} M Mg^{++} and no Ca^{++} (13). If 2.5×10^{-3} M Ca^{++} is added, antigen production is suppressed, and the cells begin to multiply. When 2.5×10^{-3} M Ca^{++} is present without Mg^{++}, the cells swell and ultimately lyse. The quantity of Ca^{++} that represses virulence antigen formation and permits cell multiplication in vitro is similar to the concentration present in mammalian *intravascular* fluid, whereas the amount of Mg^{++} and Ca^{++} optimal for in vitro synthesis of virulence antigens is, unfortunately, identical to that reported for mammalian *intracellular* fluid.

Zinc (II) ion is the cation whose concentration is most critical for the synthesis of desired quantities of various secondary metabolites by yeasts and molds (134), and the two fungal toxins for which metallic ion requirements have been examined show no exception to this generalization. The quantity of the wilt toxin, fusaric acid (5-butyl picolinic acid), synthesized by *Fusarium vasinfectum* in the presence of 7×10^{-6} M Zn^{++} is only 10% of that produced with 4×10^{-6} M Zn^{++}, whereas the amount of vegetative growth is the same at each concentration (61). For maximal formation of aflatoxin by *Aspergillus flavus*, 6×10^{-6} M Zn^{++} is required; when the concentration is lowered to 6×10^{-7} M, vegetative growth is unaffected but toxin synthesis is reduced by 82% (81).

Association of Metallic Ions with Activity of Factors of Virulence

The majority of such biologically active molecules as drugs, hormones, vitamins, amino acids, polypeptides, and enzymes contain sites at which metal binding can occur, and factors of virulence are not exceptions to this general observation. With substances of low and medium molecular weights, metallic ions often can serve as a link between the compound and a site on an enzyme

surface; with macromolecules, the cations also can function in the stabilization of the tertiary structure so that the polypeptide or protein is maximally active and is resistant to alteration by heat or degradative enzymes.

The activity of at least three, and possibly eight, different kinds of mycotic and bacterial toxins is associated with their metal-binding capacity; additional toxins have not yet been examined for this characteristic. Of the eight toxins, the smallest in molecular weight is fusaric acid; this compound is considered to function by altering the balance of Ca^{++}, Mg^{++}, Mn^{++}, Fe^{++}, or Cu^{++} in various regions of the host plant. The most damaging consequences of such alteration are impairment of respiration and of nonosmotic water intake (44). It may be recalled that for synthesis of fusaric acid, the metallic ion whose concentration is important is Zn^{++} (61), and this cation is not involved in the function of the toxin. In general, the key metallic ion(s) whose concentration is critical in the synthesis of various types of both pathogenic and nonpathogenic secondary metabolites is distinct from the cation(s) needed for stability or for function of such compounds (134).

The second toxin, of slightly greater molecular weight, is lycomarasmin (α-OH-α-acetaminopropionylglycylasparagine); this substance, like fusaric acid, is produced by fungal pathogens. It is associated with alteration of permeability of leaf cells of such hosts as tomato plants. Although the Cu^{++} chelate is more stable that the Fe^{++} chelate, only the latter possesses biological activity (31). The actual toxic entity is believed to be the 2:1 lycomarasmin-Fe^{++} chelate.

The six other toxins thus far associated (actually or theoretically) with metallic ions are bacterial products active against mammalian cells. The *C. perfringens* α-toxin, a lecithinase, requires either Zn^{++} or Co^{++} for both in vitro and in vivo activity; Mn^{++} is approximately 4% as active as Zn^{++} (85). Again, it may be noted that the crucial cation in the synthesis of the toxin (Fe^{++}) is not involved in the activity of the metabolite. Indeed, Fe^{++}, as well as Cu^{++}, depresses the activity of the toxin, presumably by displacement of the essential cations from the molecule (57). The sphingomyelinase and phospholipase factors in the toxin of *S. aureus* are each activated by Co^{++} or Mg^{++}, and are suppressed by Ca^{++} (31*a*).

The third of the bacterial toxins is factor I of *B. anthracis*. This powerful chelating substance has an ash content of 10 to 30%, depending on the quantity of metallic ions to which it is exposed during purification (110). The specific cation with which it is associated in the natural disease process is not yet known; nevertheless, it is quite unlikely that such a powerful metal-binding substance could function in mammalian tissues as an unchelated species or that the metal binding ability is irrelevant to its biological function.

The fourth and fifth bacterial toxins associated with metallic ions are the protein exotoxin produced by *C. diphtheriae* and the lipopolysaccharide endotoxin of *E. coli*. The action of each of these is decreased markedly by Fe^{++} and, as with *C. perfringens* toxin, the postulated mechanism involves competition by this cation with as yet unknown metallic ions for sites on the toxin molecules (57).

Of the many responses of host animals to *E. coli* endotoxin, a consistent and striking effect is hypoferremia. For example, after a single intraperitoneal injection of 1 mg/kg of endotoxin, serum Fe^{++} is reduced in 24 hr to 40% of its normal value in mice and to 19% in guinea pigs (21). A single intraperitoneal injection of 100 μg in rats causes a significant decrease in Fe^{++}-binding capacity of serum in 12 hr (63), a reduction in serum Fe^{++} to 24% of the normal quantity, and a marked elevation of the metallic ion in the liver, bone marrow, and erythrocytes (62). With endotoxin from *E. coli*, a linear relationship between reduction in serum Fe^{++} level and logarithm of the intraperitoneal dose per mouse is obtained between 0.001 and 10 μg; with *Brucella abortus* endotoxin, the corresponding values are between 1.0 and 100 μg (4*a*). In addition to being dose-dependent, the response is specific and reproducible, and, accordingly, has been applied to the bioassay of entotoxin (4*a*). The importance of Fe^{++} and Fe^{++}-binding capacity in antimicrobial defense mechanisms of the vascular and reticuloendothelial systems of mammals will be discussed in the next section.

The sixth bacterial toxin is the murine toxin of *P. pestis*. The uptake of Ca^{++} and inorganic phosphate by 6 mg of rat heart mitochondrial protein is inhibited 48% by 2 mg of toxin (60); in the presence of 10^{-4} M EDTA, toxin inhibition of uptake is only 17.5%. Murine toxin induces, and EDTA prevents, mitochondrial swelling; apparent suppression of uptake of ions by the toxin may actually be a loss of accumulated ions by distorted mitochondria. Information is not yet available concerning possible activation of murine toxin by a specific metallic ion or competition for this ion by EDTA.

HOST DEFENSE MECHANISMS

Infectious Diseases of Animals

Iron (II) is the metallic ion that presently appears to be most critical in determining whether

an infectious agent is to be permitted to multiply in mammalian host tissues. For example, the Fe^{++}-binding β-1 globulin component (transferrin; siderophilin) of human serum is, under normal conditions, approximately one-third saturated with Fe^{++}. The unsaturated remainder is free to combine with additional environmental Fe^{++}, and can bind as much as 3.6×10^{-5} M. Such yeasts as *Candida* (17) and such bacteria as *Staphylococcus*, *Salmonella*, and *Pasteurella* (55) can grow in the presence of serum or plasma only if sufficient Fe^{++} (usually 10×10^{-5} M) is provided to give an excess of the cation over the unbound Fe^{++}-binding capacity of transferrin. Of other divalent metallic ions tested (Mg^{++}, Ca^{++}, Mn^{++}, Co^{++}, Ni^{++}, Cu^{++}, Zn^{++}), none can substitute for Fe^{++} (55). The Fe^{++}-binding capacity of cord blood and of serum of the newborn during the first 2 weeks of life is almost completely saturated with Fe^{++}, and possibly the low resistance of the newborn to candidiasis is associated with this circumstance (17).

The yeast-inhibitory proteinlike factor in mouse ascites fluid is neutralized by addition of Fe^{++} (112), and intraperitoneal infections of *Klebsiella* can be produced in mice if Fe^{++} is injected with the bacterial cells (80). Virulence of *Klebsiella* for guinea pigs (21) and of *Listeria* for mice (113) is enhanced 1,000-fold if the injection contains 2×10^{-3} M Fe^{++}.

Nonpigmented mutants of *Pasteurella* are virulent for mice by the intraperitoneal route only if injected simultaneously with 4×10^{-4} M Fe^{++} (14). The possibilities that the cation affects (i) either mobilization or ingestive capacity of phagocytes, (ii) antibody formation, or (iii) toxicity of the bacteria have been eliminated; probably, the mutant strains are less able to obtain sufficient Fe^{++} from host fluids for metabolism and growth than are the more virulent wild strains (14). Similarly, strains of *Pasteurella* that cannot produce pesticin I, coagulase, and fibrinolytic factor are enhanced in virulence for mice if injected with 7×10^{-4} M Fe^{++}, provided that either intraperitoneal or subcutaneous routes of inoculation are employed (12). If the bacteria are injected intravenously, the cation is not required for virulence. Pesticin I presumably has iron-binding sites (e.g., its bacteriocinic action is suppressed by Fe^{+++}), and has been suggested as a possible candidate for the postulated substance that can transfer host Fe^{++} to cells of virulent strains (12).

Patients with hypogammaglobulinemia have high levels of total and unsaturated transferrin in the absence of Fe^{++} deficiency; conceivably, the elevated transferrin values represent a compensatory defense mechanism (79). Transferrin reduces the rate of synthesis of picornavirus and adenovirus in mammalian cell cultures from 0.2 to 2.0% of that of control cultures (79), but it is not known whether the protein has this activity in the natural mammalian hosts. Although the antimycotic and antibacterial action of transferrin is reversed only by Fe^{++}, the antiviral activity is not affected by this cation; rather, Cu^{++} and, to a lesser extent, Mn^{++} suppress the latter action of the protein (79).

In a variety of infectious and toxemic states, serum Fe^{++} is observed to decrease, whereas uptake of the cation into reticuloendothelial cells is greatly increased (57); in fact, the host-protective capacity of endotoxin has been attributed to its ability to induce a hypoferremia (21). A dual role of Fe^{++} in macrophages has been proposed: first, activation of lysosomal enzymes during phagocytosis of particulates, and second, interaction with and neutralization of toxins released from these particulates during their subsequent breakdown (56).

In acute infectious diseases, a moderate decrease in serum Zn^{++} has been observed (123), whereas in the course of most infections and autoimmune diseases the serum levels of Cu^{++} (19, 99) and of Mn^{++} (99) increase. The hypercupremia is so characteristic that it could be used as an indication of the presence of such diseases. A postulated mechanism of action of antipyretic salicylates is that these drugs transport the excess serum Cu^{++} back to cellular sites from which the cation originally had been released (107). This theory implies that the high value of serum Cu^{++} is disadvantageous to host defense, and it may be recalled that Cu^{++} does suppress the antiviral action of transferrin. Copper (II) ion, rather than Fe^{++}, is believed to be the cation transported by salicylates, inasmuch as each of the pharmacologically active members of the series can, at physiological *p*H values, combine with Cu^{++}; in contrast, not all of the active compounds can bind Fe^{++} (107).

As we have seen, by depriving potential pathogens of sufficient available Fe^{++} for growth, serum can be bacteriostatic. Additionally, it is advantageous to the host for serum to be bactericidal. Studies with *E. coli* and other enteric bacteria indicate that the "cidal" action of normal human serum requires two steps: (i) a sensitization stage that occurs within a few minutes and needs a factor in undiluted serum, and (ii) a second stage for which more time is needed as well as a factor that is present in highly diluted serum (84). The factor in undiluted serum apparently is complement, and it is known that, for the hemolytic (and bactericidal) action of guinea pig complement with immune serum, 1.5×10^{-4} M Ca^{++} is

needed for the reaction with C'1 and 5×10^{-4} M Mg^{++} for the reaction with C'2; the two cations are antagonistic, and the optimal proportion may vary with complement of different animal species (59). The first stage of the "cidal" action of normal human serum is suppressed by 4×10^{-2} M EDTA which, in turn, is neutralized by 5×10^{-2} M Ca^{++} or Mg^{++} (84).

The possession of sufficient serum Ca^{++} and Mg^{++} for the activation of complement is not necessarily an unmixed blessing for the host. A third useful antimicrobial attribute of serum, in addition to bactericidal and bacteriostatic actions, would be that of inactivation of such microbial metabolites as endotoxin. Endotoxin-detoxifying component (EDC), a humoral factor distinct from complement, properdin, and antibody, is suppressed by 10^{-2} M Ca^{++} or Mg^{++} as well as by Ba^{++} and Mn^{++}; Fe^{++}, Co^{++}, and Cu^{++} are inert (103). The normal serum level of Ca^{++} is 0.25×10^{-2} M, whereas the intracellular content is significantly less; EDC, therefore, may function more efficiently in intracellular than in intravascular environments.

A recently discovered host factor, pacifarin, is a nutritional entity capable of enhancing the ability of mice to survive infection with *Salmonella typhimurium* (106). The compound, active at 0.8 μg per g of dietary intake, is neither a vitamin nor an antibiotic, and is active only when the inoculum consists of a mixture of virulent and avirulent bacteria. With a uniformly virulent inoculum, no mice survive; with a uniformly avirulent inoculum, all mice survive. If the compound is added to the semisynthetic diet, animals inoculated with a mixed suspension survive, whereas in the absence of pacifarin the mice succumb to the disease.

The active pacifarin compound is a strong Fe^{++}-binding substance (106); a preliminary hypothesis of its mechanism of action is that it adjusts the available concentration of a specific metallic ion so that multiplication of avirulent cells is favored over that of virulent organisms. Growth of the former in some way then leads to suppression or extinction of the latter. The critical cation may not necessarily be Fe^{++}; it will be recalled that for differential growth of virulent and avirulent cells of *Pasteurella*, the concentrations of ions of group II of the periodic table (Mg^{++}, Ca^{++}, Sr^{++}, Zn^{++}) as well as of Fe^{++} are important. For the pyogenic cocci, the level of Mn^{++} appears to be crucial. Nevertheless, in the contest between the establishment of a bacterial or mycotic disease and the successful suppression of the disease by animal hosts, Fe^{++} is the cation whose concentration in host fluids at present appears to be most important.

Infectious and Ectoparasitic Diseases of Plants

Two metallic ions, Ca^{++} and Cu^{++}, are considered to have key roles in antimicrobial defense mechanisms of plants. The former ion stabilizes pectic substances of such hosts as tomato and potato plants (27), bean tissue (7), and apple fruit (126) so that polygalacturonases of fungal wilts and bacterial blights are unable to hydrolyze host polymers. Not only is integrity of the pectic substances preserved, but also growth of the pathogens is slowed because of lack of accumulation of such useful sources of carbon as galacturonic acid. Tomato plants contain as little as 25% of the normal quantity of Ca^{++} in their stems when grown in Ca^{++}-deficient environments, and their pectic material is readily hydrolyzed by enzymes of *Fusarium oxysporum* (27). When bean hypocotyls are infected with *Rhizoctonia solani*, Ca^{++} accumulates at the site of infection; pectins in the affected zone are demethylated and then form insoluble salts with the metallic ion (7). The salts are resistant to hydrolysis by fungal enzymes and play a major role in confining the pathogen to lesions of limited size. During the first 2 weeks of outgrowth, hypocotyls are susceptible to fungal infection. Conversion of pectin to calcium pectate occurs normally during the next 2 weeks, and is accompanied by the development of natural resistance to *R. solani* (7).

Immature apple fruit contains a fungal-resistant pectin-protein-metallic ion complex. As the fruit matures, the complex is altered; there is a reduction in Ca^{++} content of 71% and a corresponding increase in susceptibility to digestive enzymes of fungal pathogens (126). At the same time, there is a decrease in Fe^{++} and Cu^{++} content of 90 and 73%, respectively; the extent to which these two metallic ions contribute to the ability of the polymer to withstand enzymatic degradation is not clear.

The second metallic ion important in plant antimicrobial defense is Cu^{++}. Inactivation of such pathogens as *Erwinia amylovora* (15) and cucumber mosaic virus (92) is believed to be accomplished by quinones produced by Cu^{++}-activated enzymatic oxidation of polyphenols. These quinones may also inhibit fungal polygalacturonase activity (7). Additionally, Cu^{++} suppresses peroxidase activity of host tissues, thus permitting peroxide to accumulate at sites of infection to concentrations that are germicidal (15). The incidence of fire blight in apple trees can be markedly reduced by trunk injection of Cu^{++} (15).

There are, of course, many formulations containing Cu^{++}, Zn^{++}, Ni^{++}, or Mn^{++} that can be used on plants to reduce the incidence of fungal

and arthropod pests. Since these preparations generally act directly against the parasites and have no effect on host tissue, they will not be included in this discussion. However, a possible exception might be cited. EDTA chelates of Fe^{++} and Mg^{++}, and to a lesser extent of Mn^{++} and Zn^{++}, reduce by as much as 90% the fecundity of spider mites on leaves of bean, sweet potato, hop, and strawberry (114). Inasmuch as longer intervals between addition of the chelates and exposure of the female mites to the leaves produce greater reductions in fecundity, an alteration in host tissue by the chelates is postulated. Presumably, the subsequent adverse effect of feeding on altered tissue would occur via the intestinal flora of the mites; of relevance are the observations that (i) tetracycline chelates of Ca^{++}, Mg^{++}, and Mn^{++} interfere with intestinal digestion in mosquitoes (115), (ii) silkworm larval intestinal contents are germ-free, possibly because of the high Ca^{++} content of mulberry leaves (11), and (iii) dietary Mn^{++} is required for the maintenance and ovarian transfer of symbiotic bacteroids in the cockroach (10).

DISCUSSION

That closely related microbial strains often differ considerably in the ease with which they can invade and multiply in a given host strain is a familiar yet nonetheless surprising phenomenon of host-parasite interactions. Equally common and unexpected is the observation that similar kinds of hosts often differ considerably in natural resistance to infection and to pathogenesis by viral or protist parasites. Numerous factors of invasiveness, pathogenicity, and host resistance have been described. However, "to determine precisely the part that a particular cell component plays in the pathogenicity of a bacterial species may be very difficult" (78); also, mechanisms of resistance "are multiple, are in many instances still understood poorly or not at all, and probably differ from microbe to microbe, from person to person, and from time to time" (48).

Some of the reasons for these differences may be associated with observations cited in this review. For example, a critical distinction between infective and noninfective microbial strains may be possession or production by the infective strain of a metal-binding agent (ligand) that successfully competes with host ligands for a specific metallic ion. This ion would be required either for replication of the parasite or for the synthesis or activation of factors involved in subsequent invasiveness or pathogenicity.

A critical distinction between susceptible and resistant hosts might be possession or production by the resistant host of sufficient quantities of ligands that can successfully withhold essential metallic ions from potential parasites in various tissues, fluids, or cells. The most obvious example of a ligand of this nature is transferrin, but other serum proteins (e.g., ceruloplasmin, transmanganin) as well as other defense factors (e.g., pacifarin, interferon) might also have such a function.

A considerable amount of additional information is needed concerning levels of metallic ions and of ligands in cells, tissues, and fluids of hosts with enhanced susceptibility to infection (e.g., newborn infants and diabetic patients) as well as of hosts during varying stages of specific infectious diseases; the data on metallic ions could then be compared with the values for "standard man" that are being accumulated in the excellent studies of Tipton and Cook (117). Specimens from resistant and susceptible host species should also be compared for differences in metallic ions and in ligands. An example of a possibly unique ligand may be contained in the observation that serum from guinea pigs (but not from mice), when injected intraperitoneally into mice with *Pasteurella* mutants plus Fe^{++}, renders the cations unavailable to the bacteria (14).

Studies on interactions between animal parasites and their hosts with regard to the roles of metallic ions and ligands are likewise indicated. Preliminary reports include the following observations. Preacetabular glands of *Schistosoma mansoni* cercariae contain Ca^{++} for activation of invasive enzymes; invasiveness, but not other cercarial activities, is depressed by 10^{-3} M EDTA (71). *Clonorchis sinensis* accumulates 2×10^{-3} M Cu^{++} for activation of polyphenol oxidase which catalyzes formation of quinones; the latter substances are then linked to protein to yield a quinone-tanned scleroprotein for use in eggshells of the fluke (76).

Would it be practical to interfere with viral or protist parasitism by altering the available levels of specific metallic ions in host tissues, fluids, or cells by administration of ligands? At least two obstacles (we hope, temporary) must be dealt with in such attempts. The first is that there is insufficient knowledge concerning not only the tolerance of the host to fluctuations of ions and ligands but also to the complete spectrum of events that might ensue upon deliberate interference with the homeostatic mechanisms for the various cations.

The second obstacle is that synthetic metal-binding agents do not usually have the necessary specificity for a particular metallic ion; the order of strength of binding is routinely: $Cu^{++} > Ni^{++} > Co^{++} > Zn^{++} > Cd^{++} > Fe^{++} > Mn^{++} > Mg^{++}$ (82). It is not yet possible to purposefully design a ligand that would have the

appropriate stability constant for a specific cation, have lower constants for other cations, diffuse to the desired anatomical site, and function effectively at the *p*H reaction of the desired site. Nevertheless, synthetic compounds are in use that do function, at least in part, by virtue of their metal-binding (and possibly metal-transporting) ability. Good examples of these are the salicylates that possess antimicrobial, analgesic, antipyretic, antirheumatic, and hypoglycemic activities (39).

Many naturally occurring products of plants, microorganisms, and animals sequester or transport a specific metallic ion with great efficiency; unfortunately, the majority of these compounds have not yet been extracted or characterized. Some of these substances should have considerable pharmacological usefulness in metabolic as well as in infectious diseases. An example of one such product is desferri-ferrioxamine B which is the first satisfactory therapeutic agent capable of removing iron deposits from tissues in hitherto lethal diseases such as hemochromatosis and siderosis (65).

Dubos (32) suggested that the time has arrived to consider "whether useful practices of disease control can be derived from the fact that peaceful coexistence with pathogens often occurs in nature. This approach will require that the determinants of infection be separated conceptually from the determinants of disease; its objective will be to understand and control the processes which are responsible for converting infection into overt disease." Knowledge of metallic ion requirements for various facets of host-parasite interactions should undoubtedly be of considerable aid to such an approach.

SUMMARY

Numerous observations have been made concerning the importance of the metallic ion environment in host-parasite interactions. In host-virus associations, the ions of groups IIA and IIB of the periodic table are generally involved in stability of extracellular plant virions, adsorption of bacterial and animal viruses to host cells, and liberation of animal virus particles from host cells. Penetration of bacterial virus nucleic acid involves Ca^{++} and Zn^{++}. Many metallic ions affect virus replication, but it is not yet possible to predict for a given system the type of alteration of the metallic ion environment that would lead to an interruption of virus synthesis without interfering with host cell metabolism.

Only a few observations have been made concerning differences in tolerance to and requirements for specific metallic ions between virulent and avirulent protists. In contrast, there are many observations that the synthesis of specific factors of virulence depends on the level of a specific metallic ion(s) whose nature varies with the microbial genus. The key ions are identical to those needed for the synthesis of a variety of avirulent secondary metabolites by cells of the particular genus. Some factors of virulence need a specific metallic ion for activity; several are known to be strong metal-binding substances; and a few have been observed to cause marked shifts in the normal distribution of metallic ions in host tissues and fluids.

The antimicrobial power of mammalian fluids is depressed by Fe^{++} and enhanced by an increase in Fe^{++}-binding capacity; the dietary defense factor, pacifarin, is a strong Fe^{++}-binding substance. The bactericidal power of serum as well as the endotoxin-detoxifying component are affected by levels of Ca^{++} and Mg^{++}. The antimicrobial defense mechanisms of plants are dependent on Ca^{++} and Cu^{++}; the former stabilizes pectic substances against destruction by microbial polygalacturonases, and the latter activates polyphenol oxidases which catalyze the formation of antimicrobial quinones.

Thus, not surprisingly, the varied roles of metallic ions in host-parasite interactions are quite similar to the structural and catalytic roles of such ions in free-living macro- and microorganisms. Yet, there is sufficient uniqueness of roles of key metallic ions in many specific host-parasite systems so that the balance could be tipped in favor of either host or parasite by subtle alteration of the metallic ion environment. Practical applications of these phenomena have been made in a few situations; as selective metal-binding agents increasingly become available, such applications should proliferate.

ACKNOWLEDGMENT

This work was supported by Public Health Service research grant AI-06268-01 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. ADAMS, M. H. 1959. Bacteriophages. Interscience Publishers, Inc., New York.
2. ALLISON, A. C., AND R. C. VALENTINE. 1960. Virus particle adsorption. III. Adsorption of viruses by cell monolayers and effects of some variables on adsorption. Biochim. Biophys. Acta **40**:400–410.
3. AMES, G. F., AND B. N. AMES. 1965. The multiplication of T4rII phage in *E. coli* K12 (λ) in the presence of polyamines. Biochem. Biophys. Res. Commun. **18**:639–647.
4. BACHTOLD, J. G., H. C. BUBEL, AND L. P. GEBHARDT. 1957. The primary interaction of poliomyelitis virus with host cells of tissue culture origin. Virology **4**:582–589.

4a. BAKER, P. J., AND J. B. WILSON. 1965. Hypoferremia in mice and its application to the bioassay of endotoxin. J. Bacteriol. **90**:903–910.
5. BARKSDALE, L., L. GARMISE, AND K. HORIBATA. 1960. Virulence, toxinogeny, and lysogeny in *Corynebacterium diphtheriae*. Ann. N.Y. Acad. Sci. **88**:1093–1108.
6. BARKSDALE, L., L. GARMISE, AND R. RIVERA. 1961. Toxinogeny in *Corynebacterium diphtheriae*. J. Bacteriol. **81**:527–540.
7. BATEMAN, D. F., AND R. D. LUMSDEN. 1965. Relation of calcium content and nature of the pectic substances in bean hypocotyls of different ages to susceptibility to an isolate of *Rhizoctonia solani*. Phytopathology **55**:734–738.
8. BENNETT, L. L., JR. 1965. Phthalanilides and some related dibasic and polybasic compounds: a review of biological activities and modes of action, p. 23–89. *In* F. Homburger [ed.], Progress in experimental tumor research, vol. 7. T. Karger, Basel.
9. BROCK, M. L. 1965. The effects of polyamines on the replication of T4rII mutants in *Escherichia coli* K12 (λ). Virology **26**:221–227.
10. BROOKS, M. A. 1960. Some dietary factors that affect ovarial transmission of symbiotes. Proc. Helminthol. Soc. Wash. D.C. **27**:212–220.
11. BROOKS, M. A. 1963. Microorganisms of healthy insects, p. 215–250. *In* E. A. Steinhaus [ed.], Insect pathology, vol. 1. Academic Press, Inc., New York.
12. BRUBAKER, R. R., E. D. BEESLEY, AND M. J. SURGALLA. 1965. *Pasteurella pestis*: role of pesticin I and iron in experimental plague. Science **149**:422–424.
13. BRUBAKER, R. R., AND M. J. SURGALLA. 1964. The effect of Ca^{++} and Mg^{++} on lysis, growth, and production of virulence antigens by *Pasteurella pestis*. J. Infect. Diseases **114**:13–25.
14. BURROWS, T. W. 1963. Virulence of *Pasteurella pestis* and immunity to plague. Ergeb. Mikrobiol. Immunitaetsforsch. Exptl. Ther. **37**:59–113.
15. BUSHONG, J. W., D. POWELL, AND P. D. SHAW. 1964. Influence of copper gradients upon various apple leaf and twig constituents as related to fire blight incidence. Phytopathology **54**:713–717.
16. CARDINALE, M., C. V. HUBBARD, AND G. M. FUKUI. 1965. Effect of growth temperature and media on virulence of *Klebsiella pneumoniae*. Bacteriol. Proc., p. 67.
17. CAROLINE, L., C. L. TASCHDJIAN, P. J. KOZINN, AND A. L. SCHADE. 1964. Reversal of serum fungistasis by addition of iron. J. Invest. Dermatol. **42**:415–419.
18. CARR, A. J. H., AND J. L. STODDART. 1963. The ameliorating effect of zinc on symptoms of phyllody virus (strawberry green petal) in white clover. Ann. Appl. Biol. **51**:259–268.
19. CARTWRIGHT, G. E. 1950. Copper metabolism in human subjects, p. 274–314. *In* W. D. McElroy and H. B. Glass [ed.], Copper metabolism. The Johns Hopkins Press, Baltimore.
20. CASMAN, E. P. 1958. Serologic studies of staphylococcal enterotoxin. U.S. Public Health Rept. U.S. **73**:599–609.
21. CHANDLEE, G. C., AND G. M. FUKUI. 1965. The role of iron in endotoxin-induced non-specific protection. Bacteriol. Proc., p. 45.
22. CHENOWETH, M. B. 1956. Chelation as a mechanism of pharmacological action. Pharmacol. Rev. **8**:57–87.
23. CHEO, P. C., B. S. FRIESEN, AND R. L. SINSHEIMER. 1959. Biophysical studies of infectious ribonucleic acid from tobacco mosaic virus. Proc. Natl. Acad. Sci. U.S. **45**:305–313.
24. CLARKE, G. D. 1958. The effect of cobaltous ions on the formation of toxin and coproporphyrin by a strain of *Corynebacterium diphtherieae*. J. Gen. Microbiol. **18**:708–719.
25. CLARKE, N. A. 1952. Studies on the host-virus relationship in a lysogenic strain of Bacillus megaterium. II. The growth of Bacillus megaterium in synthetic medium. J. Bacteriol. **63**:187–191.
26. COMAR, C. L., AND F. BRONNER [ed.]. 1964. Mineral metabolism, an advanced treatise. Academic Press, Inc., New York.
27. CORDEN, M. E. 1965. Influence of calcium nutrition on fusarium wilt of tomato and polygalacturonase activity. Phytopathology **55**: 222–224.
28. CROWLEY, N. C., AND J. B. HANSON. 1960. The infection of apical meristems of tomato roots with tobacco mosaic virus after treatment with ethylenediamine tetraacetic acid. Virology **12**: 603–606.
29. CUMMINGS, D. J., AND L. M. KOZLOFF. 1962. Various properties of the head protein of T2 bacteriophage. J. Mol. Biol. **5**:50–62.
30. DAVIS, G. K., R. T. WASSERMAN, R. M. FORBES, B. L. O'DELL, G. C. COTZIAS, G. MATRONE, R. F. MILLER, AND R. W. ENGEL. 1960. Symposium on interaction of mineral elements in nutrition and metabolism. Federation Proc. **19**:635–677.
31. DIMOND, A. E. 1955. Pathogenesis in the wilt diseases. Ann. Rev. Plant. Physiol. **6**:329–347.
31a. DOERY, H. M., B. J. MAGNUSSON, J. GULASEKHARAM, AND J. E. PEARSON. 1965. The properties of phospholipase enzymes in staphylococcal toxins. J. Gen. Microbiol. **40**: 283–296.
32. DUBOS, R. 1965. The evolution of microbial diseases, p. 20–36. *In* R. J. Dubos and J. G. Hirsch [ed.], Bacterial and mycotic infections of man. J. B. Lippincott, Philadelphia.
33. DUKES, P. P., AND I. M. KOZLOFF. 1959. Phosphatases in bacteriophages T2, T4, and T5. J. Biol. Chem. **234**:534–538.
34. EAGON, R. G., G. P. SIMMONS, AND K. J. CARSON. 1965. Evidence for the presence of ash and divalent metals in the cell wall of *Pseudomonas aeruginosa*. Can. J. Microbiol. **11**:1041–1042.

35. EICHHORN, G. L. 1962. Metal ions as stabilizers or destabilizers of the deoxyribonucleic acid structure. Nature **194**:474–475.
36. FILDES, P., D. KAY, AND W. K. JOKLIK. 1953. Divalent metals in phage production, p. 194–210. *In* P. Fildes and W. E. van Heyningen [ed.], The nature of virus multiplication. Cambridge University Press, London.
37. FIRSHEIN, W. 1962. Effect of manganese and enzymatic deoxyribonucleic acid digests on population changes and respiration of pneumococci. J. Bacteriol. **84**:478–484.
38. FIRSHEIN, W., AND E. M. ZIMMERMAN. 1964. Differential toxicity of manganese for virulent and avirulent group A streptococci. J. Gen. Microbiol. **36**:249–255.
39. FOYE, W. O. 1961. Role of metal-binding in the biological activities of drugs. J. Pharm. Sci. **50**:93–108.
40. FRAENKEL-CONRAT, H. 1959. Infectivity of tobacco mosaic virus, p. 429–457. *In* M. Burnet and W. Stanley [ed.], The viruses, vol. 1. Academic Press, Inc., New York.
41. FUWA, K., W. E. C. WACKER, R. DRUYAN, A. F. BARTHOLOMAY, AND B. L. VALLEE. 1960. Nucleic acids and metals. II. Transition metals as determinants of the conformation of ribonucleic acids. Proc. Natl. Acad. Sci. U.S. **46**:1298–1307.
42. GAREN, A. 1961. Physiological effects of rII mutations in bacteriophage T4. Virology **14**: 151–163.
43. GAREN, A., AND L. M. KOZLOFF. 1959. The initiation of bacteriophage infection, p. 203–236. *In* M. Burnet and W. Stanley [ed.], The viruses, vol. 2. Academic Press, Inc., New York.
44. GAÜMANN, E. 1958. The mechanics of fusaric acid injury. Phytopathology **48**:670–686.
45. GILBERT, F. A. 1957. Mineral nutrition and the balance of life. Univ. Oklahoma Press, Norman.
46. HELMS, K., AND G. S. POUND. 1955. Zinc nutrition of *Nicotiana tabacum* L, in relation to multiplication of tobacco mosaic virus. Virology **1**:408–423.
47. HIGUCHI, K., L. L. KUPFERBERG, AND J. L. SMITH. 1959. Studies on the nutrition and physiology of *Pasteurella pestis*. III. Effects of calcium ions on the growth of virulent and avirulent strains of *Pasteurella pestis*. J. Bacteriol. **77**:317–321.
48. HIRSCH, J. G. 1965. Host resistance to infectious disease, p. 170–180. *In* R. J. Dubos and J. G. Hirsch [ed.], Bacterial and mycotic infections of man. J. B. Lippincott, Philadelphia.
49. HOAGLAND, C. L., S. M. WARD, J. E. SMADEL, AND T. M. RIVERS. 1941. Constituents of elementary bodies of vaccinia. IV. Demonstration of copper in the purified virus. J. Exptl. Med. **74**:69–80.
50. HOEKSTRA, W. G. 1964. Recent observations on mineral relationships. Federation Proc. **23**: 1068–1076.
51. HOLDSWORTH, E. S. 1952. The nature of the cell wall of *Corynebacterium diphtheriae*. Isolation of an oligosaccharide. Biochim. Biophys. Acta **9**:19–28.
52. HUFF, J. W., K. S. SASTRY, M. P. GORDON, AND W. E. C. WACKER. 1964. The action of metal ions on tobacco mosaic virus ribonucleic acid. Biochemistry **3**:501–506.
53. HUMPHREY, B., AND J. M. VINCENT. 1962. Calcium in cell walls of *Rhizobium trifolii*. J. Gen. Microbiol. **29**:557–561.
54. HUYBERS, K. 1953. Cationic reversion of induced phage development in *Bacillus megatherium*. Ann. Inst. Pasteur **84**:242–253.
55. JACKSON, S., AND B. C. MORRIS. 1961. Enhancement of growth of *Pasteurella pestis* and other bacteria in serum by addition of iron. Brit. J. Exptl. Pathol. **42**:363–368.
56. JANOFF, A. 1964. The role of iron in macrophages. J. Theoret. Biol. **7**:168–170.
57. JANOFF, A., AND B. W. ZWEIFACH. 1960. Inactivation of bacterial exotoxins and endotoxins by iron. J. Exptl. Med. **112**:23–24.
58. JOHNSON, L. A., AND M. J. SEVEN [ed.]. 1961. Biological aspects of metal-binding. Federation Proc. vol. 20, suppl. 10.
59. KABAT, E. A., AND M. M. MAYER. 1961. Experimental immunochemistry, 2nd ed. Charles C Thomas, Publisher, Springfield, Ill.
60. KADIS, S., A. TRENCHARD, AND S. J. AJL. 1965. Effect of plague murine toxin on the uptake of calcium and inorganic phosphate ions by heart mitochondria. Arch. Biochem. Biophys. **109**:272–278.
61. KALYANASUNDARAN, P., AND L. SARASWATHIDEVI. 1955. Zinc in the metabolism of *Fusarium vasinfectum*. Nature **175**:945.
62. KAMPSCHMIDT, R. F., AND M. I. ARREDONDO. 1963. Some aspects of endotoxin upon plasma turnover in the rat. Proc. Soc. Exptl. Biol. Med. **113**:142–145.
63. KAMPSCHMIDT, R. F., AND H. F. UPCHURCH. 1964. Effect of endotoxin upon total iron-binding capacity of the serum. Proc. Soc. Exptl. Biol. Med. **116**:420–422.
64. KEERER, R. F., AND M. L. GRAY. 1960. Antigenic and related biochemical properties of *Listeria monocytogenes*. J. Bacteriol. **80**:683–692.
65. KELLER-SCHIERLEIN, W. 1963. Iron-containing antibiotics and growth factors. J. Gen. Microbiol. **33**:ii–iii.
66. KOZLOFF, L. M. 1958. Some reactions during bacteriophage invasion, p. 185–199. *In* E. Broda and W. Frisch-Niggimeyer [ed.], Biochemistry of viruses. Pergamon Press, New York.
67. KOZLOFF, L. M., AND M. LUTE. 1957. Viral invasion. II. The role of zinc in bacteriophage invasion. J. Biol. Chem. **228**:529–536.
68. LARK, K. G., AND M. H. ADAMS. 1953. The stability of phages as a function of the ionic environment. Cold Spring Harbor Symp. Quant. Biol. **18**:171–183.

69. LASCELLES, J. 1961. Synthesis of tetrapyrroles by microorganisms. Physiol. Rev. **41**:417–441.
70. LATHAM, W. C., D. F. BENT, AND L. LEVINE. 1962. Tetanus toxin production in the absence of protein. Appl. Microbiol. **10**:146–152.
71. LEWERT, R. M., AND D. R. HOPKINS. 1964. Histochemical demonstration of calcium in preacetabular glands of cercariae and the role of calcium ions in invasiveness. J. Parasit. **50**(suppl.):30.
72. LORING, H. S., Y. FUJIMOTO, AND L. F. ENG. 1959. Ultracentrifugal fractionation and iron distribution in infectious nucleates from tobacco virus. Proc. Natl. Acad. Sci. U.S. **45**:287–293.
73. LORING, H. S., Y. FUJIMOTO, AND A. T. TU. 1962. Tobacco mosaic virus—a calcium magnesium coordination complex. Virology **16**: 30–40.
74. LURIA, S. E., AND D. L. STEINER. 1954. The role of calcium in the penetration of bacteriophage T5 into its host. J. Bacteriol. **67**:635–639.
75. LWOFF, A. 1952. The nature of phage reproduction, p. 149–174. *In* P. Fildes and W. E. van Heyningen [ed.], The nature of virus multiplication. Cambridge Univ. Press, London.
76. MA, L. 1963. Trace elements and polyphenol oxidase in *Clonorchis sinensis*. J. Parasitol. **49**:197–203.
77. MCLAREN, L. C., J. J. HOLLAND, AND J. T. SYVERTON. 1960. The mammalian cell-virus relationship. V. Susceptibility and resistance of cells in vitro to infection by Coxsackie A9 virus. J. Exptl. Med. **112**:581–594.
78. MACLEOD, C. M., AND A. W. BERNHEIMER. 1965. Pathogenic properties of bacteria, p. 146–169. *In* R. J. Dubos and J. G. Hirsch [ed.], Bacterial and mycotic infections of man. J. B. Lippincott, Philadelphia.
79. MARTIN, C. M., AND J. H. JANDL. 1960. Inhibition of virus multiplication by transferrin, p. 335. *In* M. J. Seven and L. A. Johnson [ed.], Metal binding in medicine. J. B. Lippincott, Philadelphia.
80. MARTIN, C. M., J. H. JANDL, AND M. FINLAND. 1963. Enhancement of acute bacterial infections in rats and mice by iron and their inhibition by human transferrin. J. Infect. Diseases **112**:158–163.
81. MATELES, R. I., AND J. C. ADYE. 1965. Production of aflatoxin in submerged culture. Appl. Microbiol. **13**:208–211.
82. MELLOR, D. P., AND L. MALEY. 1949. Stability constants of internal complexes. Nature **159**:370.
83. MELNICK, J. L., AND C. WALLIS. 1963. Effect of pH on thermal stabilization of oral poliovirus vaccine by magnesium chloride. Proc. Soc. Exptl. Biol. Med. **112**:894–897.
84. MICHAEL, J. G., AND W. BRAUN. 1964. Analysis of sequential stages in serum bactericidal reactions. J. Bacteriol. **87**:1067–1072.
85. MOSKOWITZ, M. 1960. Protection against *Clostridium perfringens* toxin by metal-binding compounds, p. 205–208. *In* M. J. Seven and L. A. Johnson [ed.], Metal-binding in medicine. J. B. Lippincott Co., Philadelphia.
86. MURATA, R., T. YAMADA, AND S. KAMEYAMA. 1956. Production of alpha toxin of *Clostridium perfringens*. I. Preparation of the reproducible peptone medium for the production of the toxin of high potency. Japan. J. Med. Sci. Biol. **9**:81–91.
87. MYERS, H. M., AND L. C. HENDERSHOT. 1963. Electrolyte and mineral metabolism. Ann. Rev. Pharmacol. **3**:307–334.
88. NAKAMURA, M., AND Y. UENO. 1964. Infectious ribonucleic acid (RNA) of Japanese B encephalitis (JBE) virus; high yields of RNA and its stabilization. Proc. Soc. Exptl. Biol. Med. **117**:700–704.
89. NASON, A. 1958. The function of metals in enzyme systems. Soil Sci. **85**:63–77.
90. NORTH, C. P., A. WALLACE, G. F. RYAN, AND R. T. MUELLER. 1959. Amelioration of virus symptoms in camellia with iron. Virology **8**: 131–134.
91. PADGETT, B. L., AND D. L. WALKER. 1964. Enzymatic variants of influenza virus. III. Function of neuraminidase in the viral growth cycle. J. Bacteriol. **87**:363–369.
92. PIERPONT, W. S., AND B. D. HARRISON. 1963. Copper-dependent and iron-dependent inactivations of cucumber mosaic virus by polyphenols. J. Gen. Microbiol. **32**:429–440.
93. POTTER, N. N., AND F. E. NELSON. 1953. Role of calcium and related ions in proliferation of lactic streptococcus bacteriophage. J. Bacteriol. **66**:508–516.
94. POUND, G. S., AND G. W. WELKIE. 1958. Iron nutrition of *Nicotiana tabacum* L in relation to multiplication of tobacco mosaic virus. Virology **5**:371–381.
95. PUCK, T. T., A. GAREN, AND J. CLINE. 1951. The mechanism of virus attachment to host cells. I. The role of ions in the primary reaction. J. Exptl. Med. **93**:65–88.
96. PULLMAN, T. N., A. R. LAVENDER, AND M. FORLAND. 1963. Synthetic chelating agents in clinical medicine. Ann. Rev. Med. **14**:175–194.
97. RACKER, E., AND I. KRIMSKY. 1947. Relation of iron salts to inhibition of glycolysis by Theiler FA virus of mouse encephalomyelitis. J. Exptl. Med. **85**:715–727.
98. RAPP, F., J. S. BUTEL, AND C. WALLIS. 1965. Protection of measles virus by sulfate ions against thermal inactivation. J. Bacteriol. **90**:132–135.
99. RASKOVALOV, M. G. 1963. Dynamics of trace elements in the blood (Cu and Mn) in some infectious diseases. Soviet Med. **26**:127–132.
100. REPASKE, R. 1960. Unpublished data.
101. RIFKIND, D., AND M. J. PICKETT. 1954. Bacteriophage studies on the hemorrhagic septicemia pasteurellae. J. Bacteriol. **67**:243–246.
102. ROGERS, H. J., AND B. C. J. G. KNIGHT. 1946. The recognition of material present in horse muscle affecting the formation of *a*-toxin by a

strain of *Clostridium welchii*. Biochem. J. **40**:400–406.

103. ROSEN, F. S., R. C. SKARNES, M. LANDY, AND M. J. SHEAR. 1958. Inactivation of endotoxin by a humoral component. III. Role of divalent cation and a dialyzable component. J. Exptl. Med. **108**:701–711.
104. ROUNTREE, P. M. 1955. The role of divalent cations in the multiplication of staphylococcal bacteriophages. J. Gen. Microbiol. **12**:275–287.
105. SALTON, M. R. 1964. The bacterial cell wall. Elsevier Publishing Co., New York.
106. SCHNEIDER, H. A. 1963. A fourth strategy in the war on infectious diseases. Proc. Am. Phil. Soc. **107**:443–445.
107. SCHUBERT, J. 1960. The relationship of metal-binding to salicylate action, p. 325–328. *In* M. J. Seven and L. A. Johnson [ed.], Metal-binding in medicine. J. B. Lippincott, Philadelphia.
108. SEVEN, M. J., AND L. A. JOHNSON [ed.]. 1960. Metal-binding in medicine. J. B. Lippincott, Philadelphia.
109. SHIMIZU, Y. 1964. Effect of N^1-isobutylbiguanide hydrochloride on Sendai virus multiplication in tissue culture. J. Antibiot. (Tokyo) Ser A **17**:216–217.

109*a*. SINGER, B., AND H. FRAENKEL-CONRAT. 1965. Effects of light in the presence of iron salts on ribonucleic acid and model compounds. Biochemistry **4**:226–233.

110. STANLEY, J. L., AND H. SMITH. 1961. Purification of factor I and recognition of a third factor of the anthrax toxin. J. Gen. Microbiol. **26**:49–66.
111. STANLEY, W. M. 1936. Chemical studies of the virus of tobacco mosaic. VI. The isolation from diseased turkish tobacco plants of a crystalline protein possessing the properties of tobacco mosaic virus. Phytopathol. **26**:305–320.
112. SUMMERS, D. F., AND H. F. HASENCLEVER. 1964. In vitro inhibition of yeast growth by mouse ascites fluid and serum. J. Bacteriol. **87**:1–7.
113. SWORD, C. P. 1965. Influence of iron on experimental infection of mice with *Listeria monocytogenes*. Bacteriol. Proc., p. 67–68.
114. TERRIERE, L. C., AND N. RAJADHYAKSHA. 1964. Reduced fecundity of the two-spotted spider mite on metal-chelate treated leaves. J. Econ. Entomol. **57**:95–99.
115. TERZIAN, L. A., AND N. STAHLER. 1964. The effects of certain cations and antibiotics on blood digestion in two species of mosquitoes. J. Insect. Physiol. **10**:211–224.
116. THOMPSON, R. L., J. DAVIS, P. B. RUSSELL, AND G. H. HITCHINGS. 1953. Effect of aliphatic oxime and isatin thiosemicarbazones on vaccinia virus infection in the mouse and rabbit. Proc. Soc. Exptl. Biol. Med. **84**:496–499.
117. TIPTON, I. H., AND M. J. COOK. 1963. Trace elements in human tissue. II. Adult subjects from the United States. Health Phys. **9**:103–145.
118. TOLMACH, L. J. 1957. Attachment and penetration by viruses. Advan. Virus Res. **4**:63–110.
119. TUCKER, R. G. 1961. The role of magnesium ions in the growth of *Salmonella* phage anti-R. J. Gen. Microbiol. **26**:313–323.
120. ULRYCHOVA-ZELINKOVA, M. 1959. Cadmium ions as inhibitors of tobacco mosaic virus. Biol. Plant. Acad. Sci. Bohemoslov. **1**:135–141.
121. UNDERWOOD, E. J. 1962. Trace elements in human and animal nutrition, 2nd ed. Academic Press, Inc., New York.
122. VAN HEYNINGEN, W. E. 1955. The neurotoxin of *Shigella shigae*. 5. The effect of iron and related materials on the growth, respiration and toxin production of *Shigella shigae* and related organisms. Brit. J. Exptl. Pathol. **36**: 373–380.
123. VIKBLADH, I. 1951. Studies on zinc in blood. II. Scand. J. Clin. Lab. Invest. **3** (suppl. 2):1–74.
124. WACKER, W. E. C., M. P. GORDON, AND J. W. HUFF. 1963. Metal content of tobacco mosaic virus and tobacco mosaic virus RNA. Biochemistry **2**:716–719.
125. WAHL, R. 1946. Influence de la composition du milieu sur la bacteriophage. Ann. Inst. Pasteur **72**:73–80.
126. WALLACE, J., J. KUC, AND H. N. DRAUDT. 1962. Biochemical changes in the water-soluble material of maturing apple fruit and their possible relationship to disease resistance. Phytopathology **52**:1023–1027.
127. WALLIS, C., AND J. L. MELNICK. 1961. Stabilization of poliovirus by cations. Texas Rept. Biol. Med. **19**:683–700.
128. WALLIS, C., AND J. L. MELNICK. 1961. Cationic inactivation of vacuolating virus (SV_{40}) in poliovirus suspensions. Texas Rept. Biol. Med. **19**:701–705.
129. WALLIS, C., AND J. L. MELNICK. 1962. Supression of adventitious agents in monkey kidney cultures. Texas Rept. Biol. Med. **20**:465–475.
130. WALLIS, C., AND J. L. MELNICK. 1962. Magnesium chloride enhancement of cell susceptibility to poliovirus. Virology **16**:122–132.
131. WALLIS, C., J. L. MELNICK, AND F. RAPP. 1965. Different effects of $MgCl_2$ and $MgSO_4$ on the thermostability of viruses. Virology **26**:694–699.
132. WALLIS, C., K. O. SMITH, AND J. L. MELNICK. 1964. Reovirus activation by heating and inactivation by cooling in $MgCl_2$ solutions. Virology **22**:608–619.
133. WARING, W. S., S. S. ELBERG, P. SCHNEIDER, AND W. GREEN. 1953. The role of iron in the biology of Brucella suis. I. Growth and virulence. J. Bacteriol. **66**:82–91.
134. WEINBERG, E. D. 1962. Trace metal control of specific biosynthetic processes. Perspectives Biol. Med. **5**:432–445.

135. WEINBERG, E. D. 1964. Manganese requirement for sporulation and other secondary biosynthetic processes of *Bacillus*. Appl. Microbiol. **12**:436–441.
136. WEINTRAUB, M., J. D. GILPATRICK, AND R. S. WILLISON. 1952. The effect of certain water soluble compounds on virus infection. Phytopathology **42**:417–419.
137. WELKER, N. E., AND L. L. CAMPBELL. 1964. Induction and properties of a temperate bacteriophage from *Bacillus stearothermophilus*. J. Bacteriol. **89**:175–184.
138. WELKIE, G. W., AND G. S. POUND. 1958. Manganese nutrition of *Nicotiana tabacum* L in relation to multiplication of tobacco mosaic virus. Virology **5**:92–109.
139. WHITE, D. O., AND A. W. HARRIS. 1963. Actions of metal chelates of substituted 1,10-phenanthrolines on viruses and cells. I. Inactivation of viruses. Australian. J. Exptl. Biol. Med. Sci. **41**:517–526.
140. WHITE, D. O., A. W. HARRIS, AND A. SHULMAN. 1963. Actions of metal chelates of substituted 1,10-phenanthrolines on viruses and cells. II. Inhibition of viral multiplication. Australian J. Exptl. Biol. Med. Sci. **41**:527–538.
141. WRIGHT, G. G., M. A. HEDBERG, AND J. B. SLEIN. 1954. Studies on immunity in anthrax. III. Elaboration of a protective antigen in a chemically defined, non-protein medium. J. Immunol. **72**:263–269.
142. YARWOOD, C. E. 1954. Zinc increases suceptibility of bean leaves to tobacco mosaic virus. Phytopathology **44**:230–233.