ELECTROLYTE AND MINERAL METABOLISM[1,2]

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This review is composed of two main parts, one dealing with electrolyte metabolism, the other with mineral metabolism. The section on electrolyte metabolism includes discussions on the movement of ions across cell membranes, the binding of ions by cell constituents and the relation of these events to cell metabolism in the presence and in the absence of drugs. The section on mineral metabolism deals with recent findings relating to the metabolism of the fluoride ion and of metals, particularly trace metals, and also includes discussions on chelation and calcification. Virtually all of the material which serves as a basis for this review was published in the period from 1959 to 1962.

ELECTROLYTE METABOLISM

The growing interest in and importance of the distribution of ions in tissues and cells can be indicated by the increasing frequency of publications devoted almost entirely to this subject since 1959. (1 to 5). Of special interest to pharmacologists has been the demonstration that electrolyte shifts may alter the potency of, or in turn be influenced by, low levels of a variety of drugs.

While the characteristic distribution of electrolytes in the intra and extracellular compartments is factual, the interpretation of the causes of this distribution must still be regarded as controversial. Two schools of thought have emerged which may be designated as the membrane permeability school and the sorption school. The former group places emphasis on the selectivity of the cell membrane as the factor that determines which ions are intracellular and which are extracellular [Conway (6)]. The sorption group emphasizes the fact that the binding of selected ions by cell constituents could also account for the distribution of electrolytes found intra and extracellularly [Ling (7), Dreisbach (8)].

Membrane Selective Permeability

The physical basis of membrane discrimination is thought to be a restricted passage of ions as influenced by pore size [Solomon (9)], as well as,

[1] The survey of the literature pertaining to this review was concluded March, 1962.

[2] Abbreviations used in this chapter include: ACh (acetylcholine); ATP (adenosine triphosphate); DNA (deoxyribonucleic acid); DNP (dinitrophenol); DOC (deoxycorticosterone); LSD (lysergic acid diethylamide)

by a pump mechanism [Ussing (10)] which actively drives out ions which get past the membrane barrier. The active energetic requirements for such ion pumping are formidable, the major fraction of oxygen consumption being given over to this purpose [Lassen & Thaysen (11)]. Sodium ion particularly is actively pumped out of cells, and if this ion is replaced by choline, the oxygen consumption of such systems will not rise as when it is present [Rasmussen *et al.* (12)]. Ion pair formation is thought to be a requirement of ion transport [Mullins (13)] but only one member of the required pair need be actively carried. In the renal tubules of the rat water and chloride have been shown to be passively absorbed along with the primary sodium effect [Windhager & Giebisch (14)].

In addition to pore size a variety of factors has been suggested which can contribute to permeability. These include (*a*) relative organization of acid mucopolysaccharides of the dermis [Myers, Bishop & Scheer (15)], (*b*) the presence of fixed negative charges in the lining of the pores which restrict anionic more than cationic mobility [Tasaki *et al.* (16)], (*c*) ability of the membrane to secrete lactic acid and lower the pH sufficiently to allow anions to pass as lipid soluble uncharged units, thereby avoiding the aqueous pore barrier [Clarkson, Rothstein & Cross (17)], (*d*) complex formation and the passage through a pore free section of the membrane [Charley *et al.* (18)], and (*e*) existence of specific activators, such as lysine and lactose enhancement of calcium absorption by the ileum [Lengemann & Comar (19)].

Activators may act either by complexing or by altering the membrane protein configuration. The well-known action of antidiuretic hormone on the permeability of toad bladder to water has been shown to be related to the binding of the hormone to SH containing proteins of the membrane. The attachment site is apparently on the serosal side while the site of action manifests itself on the mucosal side [Fong *et al.* (20)]. A chain reaction of SH, S-S exchanges resulting in a configurational change across the thickness of the membrane is believed to lead to the increased water permeability observed.

Ionic Exchange

In some instances membrane selectivity can be related to a linked exchange between two different cations. Reducing the concentration of one member of the pair inhibits the uptake of the other member. While this mechanism may not at first glance seem to be a membrane controlled discrimination, the specific pairing of the two exchangeable ions is most likely tied to either membrane structure or metabolism. Walker *et al.* (21) have found that in the renal tubule, low levels of Na^+ in the lumen inhibit K^+ secretion. These authors observed that the distribution of Na^+ and K^+ concentrations in the tubular lumen was such as to suggest that Na^+ reabsorption is necessary to obtain K^+ secretion.

Taking advantage of the ability of carefully isolated calf lens to restore its original electrolyte composition after storage in the cold for 24 hr, Kinoshita

et al. (22) found a one for one exchange of Na^+ for K^+ which required both oxygen and glucose. Briggs & Holland (23) found that ventricular fibrillation is associated with a replacement of K^+ by Na^+ although not at a one for one level. Findings such as these suggest either that the exchange mechanism may be a singular nonspecific one, or that direct exchange does not account for all ionic shifts observed in a tissue.

Schultz & Solomon (24) have observed an interchangeability of K^+ and H^+ in *Escherichia coli* cultures. Aging cultures lose K^+ while the medium is becoming acidified. The low pH of 4.5 inhibits K^+ uptake by the cells.

Curran & Gill (25) find that Ca^{++} interferes with Na^+ entry through the outer surface of frog skin with a resulting diminution of intracellular Na^+. The reduced intracellular Na^+ in turn retards Na^+ for K^+ exchange at the inner surface of the skin; the net effect appearing as one of Ca^{++} inhibiting K^+ uptake. The effect of Ca^{++} on K^+ influx is correspondingly smaller when the external Na^+ is reduced which implies that some minimal Na^+ uptake is independent of the Ca^{++} effect and is not linked to K^+ exchange. Exchange may therefore account for only a portion of Na^+ and K^+ shifts in a given tissue. Exchanges other than Na^+ for K^+ have been found. Rothstein (26) using yeast cultures has shown an exchange of K^+ for H^+, the medium becoming acidified as K^+ uptake increases.

Abundant examples exist in which the ionic exchange concept does not apply. Gantt & Synek (27) reported that DOC in rats lowered the renal loss of Na^+, while not altering the K^+ over a wide dosage range. Rabbit hearts which are in experimental cardiac arrest, continue to lose K^+ throughout the "dead" period and during resuscitation as well. Na^+ and Ca^{++} increase only during the period of resuscitation and do not correspond in time with K^+ loss [Gomoll & Sherrod (28)]. In nerve, a fast carrier system for Na^+ and a slower one for K^+ have been shown by Wright & Ooyama (29) to be capable of independent initiation. Humphrey (30) has determined that K^+ efflux from the isolated rabbit heart is not an exchange with K^+ in solution, since it occurs in K^+ free solutions as well. It may, however, be a Na^+ for K^+ exchange, or other heterionic exchange process.

Selective Binding of Ions by Cell Constituents

Surfaces other than cell membranes have been found to show selectivity and ability to accumulate electrolytes against a concentration gradient. Winters *et al.* (31) have used isolated mitochondria to demonstrate active sulfate accumulation. While a source of energy was necessary, oxidative phosphorylation did not have to be concomitant with transport of the anion.

Large blood vessels have been shown by Sawyer *et al.* (32) to transport ions actively. The activity is confined to the intimal and sub-intimal layers but the direction is opposite in the aorta to that of the vena cava.

Removal of cell membranes by storing muscle fibers in cold glycerine results in a loss of Na^+ and K^+. The ability of the muscle fiber to concentrate K^+ is lost in this preparation [Craig, Ohr & Fenn (33)]. While the glycerine

treatment may have altered cell constituents, this evidence would seem to contradict the contention that the ionic selectivity of cells is a function only of the ability of nondiffusible cytoplasmic elements to bind ions selectively. Ling (7), Lester & Hechter (34), and Troshin (35) have advanced such a concept of selective binding by internal cell constituents as the basis for electrolyte distribution. The many influences on energy yielding pathways which have been shown to alter ionic composition of cells would in this view be exerted by altering protein or other ion binding configurations to modify specific binding of K^+ or Na^+. Polyelectrolyte resins have been investigated which exhibit marked preference for K^+ over Na^+ [Woermann & Co-workers (36)] and it can be inferred that analogous selectivity may occur in cell cytoplasm. Sanui & Pace (37), however, found microsomal material to bind K^+ only slightly better than Na^+. An experimental demonstration of order of magnitude differences in K^+ and Na^+ binding is required if the sorption theory of ion selectivity is to replace the membrane permeability theory. This latter theory in itself is not at all satisfactory since the difficulty of explaining discrimination between Na^+ and K^+ by cell constituents has its counterpart in the membrane theory. Presuming that Na^+ is actively and specifically transported by carrier molecules which traverse the membrane, a satisfactory basis for the preferred carrying of Na^+ over other monovalent cations is yet to be advanced.

Energy Requirements of Transport

The requirement that the energy yielding systems of a cell must be functional [Van Bruggen & Zerahn (38)] in order to transport Na^+ has stimulated hypotheses about the linking of energy sources with the transport mechanism. High energy phosphate bonds such as arginine phosphate and ATP are clearly involved [Caldwell (39)] but the connection between these energy yielding substances and active transport is by no means clear.

Hokin & Hokin (40) have advanced a mechanism for the conversion of ATP energy into Na^+ transport. From the observation that water soluble substances in general are transported with an accompanying unique increase in P^{32} labelling of phosphatidic acid and phosphoinositide, they have implicated these phosphatides as possible carriers of Na^+. According to this view, on the inner surface of the transporting cell membrane, diglyceride kinase catalyzes the phosphorylation of diglyceride by ATP. The disodium phosphatidate which then forms is a lipid soluble complex capable of traversing the membrane to its outer surface where a phosphatase liberates Na_2HPO_4 from the diglyceride. The latter is returned to be recycled and the HPO_4 exchanges passively for Cl^-. The Na^+ is thereby actively transported and the Cl^- in contrast is transported passively with no energy expenditure. That Na^+ is the actively transported member of the pair is supported indirectly by the fact that gastric mucosa which actively transports Cl^- does not exhibit a phospholipid effect even under the influence of histamine [Eggman & Hokin (41)].

Increased incorporation of P^{32} into phosphatidic acid of brain slices occurred only when Na^+ was used in the medium and an intact membrane existed [Yoshida & Nukada (42)]. Later work by Yoshida and co-workers (43) demonstrated an increased P^{32} turnover in phosphatidic acid accompanied by a 24 per cent increase in oxygen consumption. Similar increases, however, were found with depolarizing substances such as K^+, ACh and proveratrine. The authors conclude that phosphatidic acid does not play a role as an ion carrier.

Na^+ and K^+ activation of an ATP-ase found in red blood cell membranes has been demonstrated by Post and co-workers (44). Nine almost identical characteristics of Na^+ and K^+ transport and of the enzyme have been found. It is inferred that the presence of excess Na^+ or K^+ stimulates transport processes which are linked to the energy of ATP by such an enzyme. A similar system isolated from crab nerve has been described by Skou (45).

Kanfer & Titus (46) have prepared an acetone powder preparation of a rabbit brain ATP-ase which requires simultaneous Na^+, K^+ and Mg^{++} for activation. Ouabain inhibited the enzyme by preventing the Na^+ and K^+ joint action, but had no effect on the Mg^{++} activation. In spite of this strong inhibitory effect of ouabain on the partially purified enzyme, no inhibition of ATP-ase could be demonstrated in slice preparations as measured by nucleotide PO_4 labelling.

Active sulfate transport in either direction has been claimed for renal tubules. In marine fishes, sulfate is secreted, and in mammals it is primarily reabsorbed [Berglund (47); Globus, Becker & Thompson (48)]. The interference with the transport of sulfate by thiosulfate found by Hilz, Kittler & Knappe (49) is thought by Berglund (47) to occur by way of the activation step. Active sulfate formed from ATP has been used in a model system described by Keller & Blennemann (50). Methylene blue could be carried through a nonaqueous phase by the formation of a complex which is $CHCl_3$ soluble. The complex was enzymatically formed from active sulfate and was cleaved enzymatically to release the dye. The carrier used was 5-nitro-2-hydroxyphenyl sulfate. Since thiosulfate is believed to compete for the activation step of sulfate the effect of this ion on the system would be of interest.

Sanui & Pace (37) link metabolism and transport by the exchange displacement of microsomal bound Na^+ by H^+ produced from metabolic processes.

The energy of oxidative metabolism may be indirectly involved in the formation of chelates which act as carriers. Chelation by steroid carrier molecules has been proposed by Willbrandt (51) as the mechanism of transport of Na^+ and K^+.

Rather than emphasizing ATP as the immediate source of energy and ATP-ase as the connecting link with a carrier, Conway (52) has provided evidence that poisoning oxidative phosphorylation at the molecular level with DNP actually stimulates Na^+ transport. He has advanced the concept that reduced electron carriers may also be Na^+ or K^+ carriers. The support

for the Conway *Redox Pump* hypothesis derives from skeletal muscle, while that for ATP-ase theory comes from different experimental systems, such as red blood cell ghosts and nerve fibers. This distinction may very well prove to be a critical one for it is entirely possible that a variety of carrier mechanisms exist.

An understanding of the coupling of energy requiring processes to ATP is still in the conjectural stage. ATP is able to contract aged mitochondria which are unable to either respire or maintain an ion gradient. The contraction is independent of ionic environment and has been interpreted as due to a mechano-enzyme analagous to actomyosin [Lehninger (53)]. Transport obviously is more indirectly linked to ATP than is mitochondrial contraction, and there may well be several types of such coupling for any complex of processes such as transport.

The Action of Drugs on Transport

It has been possible in the last ten years to keep cell metabolism intact while blocking active transport, a fact which reveals that it is possible to directly block the carrier mechanism. This has been done successfully with cardiac glycosides using red blood cells, brain slices, muscle and skin. Ouabain has received the most attention being effective over a dose range of $10^{-6}-10^{-4}$ *M*. In the usual experimental arrangement surviving tissues or cells are allowed to equilibrate in a saline solution during which time they lose K^+ to the medium. Raising the K^+ of the medium, or adding substrates to promote metabolism will either restore or slow down the loss of K^+. Ouabain increases the K^+ loss of such systems and its action can be reversed by the addition of K^+ [Schreiber, Oratz & Rothschild (54)]. Two rates of K^+ influx can be distinguished in mammalian heart tissue, a fast one with a half life of 9.4 min, and a slower one with a half life of 73 min. Ouabain inhibition applies only to the slower rate of K^+ influx, representing about 50 per cent of the total heart K^+.

The inhibition can be demonstrated in intact animals since K^+ is capable of reversing the action of strophanthidin. This aglycone of K-Strophanthin causes a diuresis in dogs which can be blocked by K^+ loading [Cade and coworkers (55)]. This same agent can block active Cl^- transport by gastric mucosa with no effect on Na^+ flux. In view of the nonspecificity of action it is suggested that the energy utilizing step of ion transport may be involved [Cooperstein (56)]. Gourley (57) has been able to show a similar K^+ loss with a variety of cardiac glycosides. Digoxin, ouabain and deslanoside all were able to block K^+ influx in frog leg muscle.

Since the cardiac effects of these substances is eliminated by removal of the lactone ring or saturation of the double bond, it is of considerable interest too that such changes influence the action of these agents on ion transport to a similar extent.

Insulin plus ouabain resulted in a greater loss of K^+ than ouabain alone, and it occurred without any diminution of O_2 consumption and in the pres-

ence of lactate. Insulin alone can prevent K^+ loss from a perfused rat liver according to Mortimore (58). The effect is greater in magnitude than is accountable for by glycogen formation. The action of insulin on membrane permeability apparently is such that it complements the action of ouabain but promotes permeability when used alone.

K^+ dependent activities such as the accumulation of I^{131} from NaI solutions by thyroid and I^- concentrating tissues can be inhibited by ouabain or scilliroside [Wolff & Maurey (59)]. Here too, K^+ can reverse the inhibition, as can Rb^+ and Cs^+, but Na^+ has no reversing activity. The action of the glycosides have been shown not to be on K^+ requiring enzymes such as yeast aldehyde dehydrogenase, myosin ATP-ase or pyruvate kinase. It is tempting to infer from the similarity of both the active drugs and of ions which can reverse the action that a steric relationship is involved which is an essential step in energy utilization for transport.

Another membrane phenomenon dependent on ion concentration is the absorption of sugars. Parsons & Wingate (60) indicate that one of the mechanisms of glucose transfer across the intestine is an active process requiring Na^+ or choline but prevented by Li^+ or K^+. Active galactose absorption by kidney cortex slices can be blocked by ouabain, phlorizin,[3] or replacement of Na^+ by Li^+ under aerobic conditions [Kleinzeller & Kotyk (62)]. The active transport of still another sugar, 3-methyl-glucose, across frog intestine can be blocked completely by ouabain 10^{-6} or thevetin 10^{-7} *M* [Csáky *et al.* (63)].

Brain slices will rapidly lose over 50 per cent of their K^+ when used in such an equilibration study. The loss can be partly reversed by adding substrate to the medium but complete reversal requires Ca^{++}. [Gardos (64)]. The total absence of Ca^{++} prevents any K^+ influx even in the presence of added glucose substrate. No interference with O_2 consumption or lactic acid formation was observed. Ca^{++} ions therefore promote K^+ influx, and do so by apparently acting directly on the carrier system. It would be helpful to clarify the relationship between Ca^{++} and ouabain. Clinically it is known that the two produce synergistic toxicity while *in vitro* studies suggest that they act antagonistically.

Ouabain at dose levels of 10^{-7} to 10^{-6} *M* has an inotropic effect on the embryonic chick heart but no effect on Na^+ or K^+ influx or efflux. Klein & Evans (65) therefore suggest that some other event such as Ca^{++} influx may be acted on by ouabain. Solomon (9) reports that Ca^{++} has an important role as an integral part of the cell membrane pore, and that in its absence K^+ influx does not occur. Whittembury & co-workers (66) find an antagonism between Ca^{++} and vasopressin, the former reducing equivalent pore size and the latter increasing this parameter. The suggestion that Ca^{++} may be a structural part of the membrane receives support from the work of Gilbert (67) who found that low pH removes relatively much more Mg^{++} than Ca^{++} from

[3] Phlorizin activity apparently resides in the presence of a glucose moiety in the glycoside. Galactose substitution for glucose prevents its activity [Diedrich (61)].

muscle cells. The importance of Ca^{++} to the cell was also suggested by the fact that an elevated pH increased intracellular Ca^{++} more than Mg^{++}. Shanes & Bianchi (68) found that 20 per cent of the superficial muscle fiber Ca^{++} is exchangeable only with Ca^{++} unlike the 80 per cent which undergoes heterionic exchange. Such specificity for Ca^{++} implies a chemically precise role for this action in muscle contraction. Bianchi & Shanes (69) have advanced the hypothesis that the entry of Ca^{++} into the myoplasm is the connection between surface depolarization and the contractile mechanism.

The different behavior of complexed and free Ca^{++} may explain the variations in Ca^{++} reabsorption by the renal tubules according to Walser (70, 71). Complexing anions such as $SO_4^{=}$ which increase in blood along with Ca^{++} under the action of parathyroid hormone may be responsible for some of the effects of this hormone [Bronner (72)].

The significance of K^+ in the mechanism of action of some stimulant drugs has been analyzed by Paton (73). He has advanced a theory of drug action in which response is based on the rate of drug-receptor combination rather than on the number of occupied receptor sites. The replacement of receptor K^+ by the drug ion and its subsequent replacement by intracellular K^+ which is predicted in this theory would create a situation related to the K^+ efflux studies cited above. Influx tending to restore cell potassium can, in this concept, be causally linked to the sensitivity of the reacting cell to the drug. According to Paton's view the rate of restoring cell K^+ influences the magnitude and nature of drug action since it frees more sites for subsequent combination with the drug.

The successful demonstration of a direct action of the cardiac glycosides on cation transport has naturally led to the examination of a variety of agents known to have electrolyte effects. The mercurial diuretics come immediately to mind since they appear to act by blocking tubular reabsorption of ions. White *et al.* (74) found that meralluride brought about an increased flux of Na^+ passing into the blood from the tubule cells, and concluded that the action of this agent must increase Na^+ leakage from the blood to the lumen. Jamison (75), on the other hand, found that the toad bladder which normally can pass Na^+ against a potential gradient of 20 to 40 mv cannot do so under the influence of chlormerodrin.

Angiotensin blocked the extrusion of Na^+ by kidney cortex slices and its action could be easily reversed by flushing with drug free Ringers [Leyssac *et al.* (76)]. Such a result is compatible with a superficial site of action such as blockade of the receptor site for active Na^+ transport.

The permeability of rabbit mesentery to Rb^+ is increased by 5-hydroxytryptamine. This action was blocked by salicylate but not by LSD. Lowering Ca^{++} levels of the medium increases permeability of the membrane in this instance [Berndt & Gosselin (77)].

The action of local anesthetics has been related to their ability to block ion shifts. Squid axon membranes that are excited exhibit an increased permeability to Na^+ and K^+. This increase can be blocked by both procaine

and cocaine [Shanes & co-workers (78)]. Ca^{++} has similar actions to these anesthetics only if the level of depolarization is low. Weiss, Coalson & Hurwitz (79) find that guinea pig ileum contraction is associated with an efflux of K^+. By incubating in Ca^{++} free tyrodes solution, efflux not accompanied by contraction is observed when pilocarpine is added. This again emphasizes the separation of the membrane ionic events and the contraction process. Cocaine blocks contraction and also lowers the K^+ efflux to 25 per cent of its original value. Its action would therefore seem to be on the coupling of the membrane event and the contractile mechanism, while pilocarpine appears to act on the contractile process only.

Effect of Ions on Biochemical Processes

A wide variety of influences of ions on biochemical processes have been studied with the view of determining the factors which control and modify such processes. Latzko (80) observed that oxidative phosphorylation is increased to the extent that the P/O ratios are raised by 160 per cent if K^+ replaces Na^+.

It was possible for Kunitz (81) to change the nature of enzymatic action of yeast pyrophosphatase to ATP-ase by substituting Zn for Mg^{++} as the activator. Similarly the copper protein ceruloplasmin exhibits oxidase activity which can be uncompetitively inhibited by Cl^- and acetate [Curzon (82)].

Differences in strength of complexing between divalent cations and deoxyribonuclease were correlated by Desreux *et al.* (83) with activation or inhibition of the enzyme. Strong complexers were inhibitory while weaker complexing cations such as Ca^{++} were activating to the DNA-ase which catalyzes the slower splitting of phosphoester bonds.

The goitrogenic action of Ca^{++} has been localized by Gandra & Coniglio (84) to the oxidative conversion of I^- to I°. Ca^{++} has also proven to be essential for steroidogenesis in the perfused adrenal with or without ACTH [Rosenfeld (85)]. Its action has been localized to the transformations prior to the formation of pregnenolone.

Everett & Holly (86) have shown an inhibition by a variety of heavy metals on leucine incorporation into protein. No interference with soluble ribonucleic acid formation was observed. The inhibitory levels of the most active substances, selenium and zinc were comparable to the levels found in liver of rats severely poisoned by these elements.

The important but frequently overlooked phenomenon of plasma protein binding of drugs is also influenced by electrolytes. Quinidine is completely bound by human serum albumin at pH 10. Lowering the pH to 5 breaks the complex completely. Cl^- is capable of preventing the complex from forming between pH 6.0–6.8 at physiological concentrations of the anion [Conn & Luchi (87)].

Fuwa & co-workers (88) have emphasized the role of metallic ions of the first transition series (Mn^{++}, Ni^{++}, Zn^{++}, Cr^{+++}) in stabilizing the tertiary

structure of proteins by aiding in cross-linking. The alkali and alkaline earth groups are unable to aid the stability of RNA as do the heavier elements.

The ability of ions to bring about the relaxation of glycerol-treated muscle fibers has implicated them as possible physiological controllers of muscle relaxation-contraction cycles. The relaxing protein factor of Marsh which occurs naturally in muscle can be inhibited by Cd^{++} and Ca^{++}.

Polyphosphates also can cause relaxation according to Bowen & Laki (89), and in this instance no requirement for ATP is necessary and Mg^{++} lessens the magnitude and rate of the effect. The longer chains of 30 units were the most effective relaxing agents. This action would appear to be related to the Ca^{++} inhibition of the protein factor and to be distinct from the Zn^{++} system mentioned below.

Undamaged granules of relaxing factor have a strong affinity for Ca^{++} and remove it from solutions containing ATP and Mg oxalate for storage as Ca^{++} oxalate. The uptake of Ca^{++} coincides in time with the increase in ATP splitting activity and implies that the relaxation is brought about by ATP-ase when the Ca^{++} ion is sufficiently reduced in amount. The ATP-ase activity can be blocked by mersalyl [Hasselbach & Makinose (90)].

Zn^{++} in the presence of ATP can cause relaxation independently of the protein factor [Edman (91)]. The amount of Zn^{++} in muscle is sufficient to account for such an activity physiologically. Complexers of Zn^{++} (and other cations as well) such as ATP and EDTA reduce the free Zn^{++} in muscle and cause the system to contract.

Zn^{++} and mersalyl can replace each other and have an additive effect on muscle relaxation. However, the Zn^{++} is pH dependent while mersalyl which is thought to involve SH groups is not [Edman (92)]. The two agents may act at the same site but in different manner.

MINERAL METABOLISM

Calcification

The organic matrix.—In vertebrates, all organic matrices of normally calcified tissues, except dental enamel, have been demonstrated to be collagen. Collagen is a material which has crystalline features, a fact which has been believed to be critical in assessing the calcifiability of organic materials (95, 100). Recent work by Schiffmann & Martin (101), however, has shown that the crystallinity of a protein matrix per se may not be an important factor in calcification, for these investigators have demonstrated that the elastin—not the collagen—in rat aorta is the essential organic material if calcification is to occur in this organ. The most significant feature of this finding is that elastin, unlike collagen, is a nonordered structure, i.e., it is not a crystalline-type structure as is collagen. An unpublished extension of this work (102) indicates the hydroxyapatite deposited in elastin can be observed by electron microscopy to be laid down on the elastin fibers and not on the

collagen. These findings correlate well with those of Lansing (103) who showed that the calcium content of elastin from human aortas progressively increases with age and reaches maximum levels in the 5th and 6th decades of life.

Rizzo *et al.* (104) have also published evidence that preferential calcification of a substance other than collagen may occur. Reconstituted collagen fibers, when enclosed in dialysis tubing and embedded in the peritoneal cavity of the rabbit, were found not to calcify unless the preparation was contaminated with bacteria. Further investigation revealed that hydroxyapatite did not form on the collagen fibers but in and around the bacteria. Zander, Hazen & Scott (105), and Gonzales & Sognnaes (106) have described the mineralization patterns of dental calculus. Apatite crystals are laid down on the surface of and within the bacteria in the dental plaque.

Collagen, however, still retains its exclusive position as the organic matrix in bone and dentin, and, thus, is of the utmost importance. The collagen molecule is composed of fibrils arranged in a helical fashion. The structure and amino acid composition of collagen fibrils and the nature of the chemical bonding which holds the fibrils together are the subjects of continued investigation. Martin, Mergenhagen & Scott (107) have concluded that in the formation of the collagen molecule the amino acid chains are bonded together along their lengths by covalent bonding, perhaps of imidazole moities along the chains. Further work in the same laboratory has yielded even more interesting results. It had been reported in 1960 by Orekhovich *et al.* (108) that there were two components formed during the denaturation of collagen α components and β components. Recently Piez *et al.* (109) have determined that the α- and β-chains are composed of subunits, α-1, α-2, β-1 and β-2. They find that the β-chains actually are composed of cross-linked α-chains. The structure of collagen proposed by these investigators is that each collagen molecule "contains three subunits of which one is α-2 and two are α-1." They have further proposed that as collagen matures, α-chains cross link to form β-chains.

The conditions under which collagen is extracted from tissues and the age and type of tissue have a bearing upon which components will predominate in the extract. Disease states also are of importance in this regard, and this new information on collagen structure may be helpful in determining tissue changes in collagen diseases. For example, Martin *et al.* (110) have found that collagen from lathyritic rats is deficient in fibrils of the β-type. This finding indicates that the cross-linking which normally occurs during collagen maturation is inhibited in lathyrism. In a recent study on the bond between collagen and bone salt, Cartier & Lanzetta (111) found greater numbers of ϵ-amino groups in young bone or demineralized bone than in densely calcified bone. During demineralization the liberation of pyrophosphate correlated with the formation of these free amino groups in a ratio which indicates that each molecule of pyrophosphate is bound to two amino groups of collagen.

Hydroxyapatite.—Many "foreign" metal ions are taken up by bone

hydroxyapatite, and the degree of discrimination of apatite crystals against these "foreign" ions depends in large measure to the degree of perfection of the hydroxyapatite crystal structure. Likins *et al.* (112, 113) have shown, for example, that the discrimination of apatite against strontium depends upon the degree of crystallinity (crystal perfection) of the apatite, and that large, slowly grown apatite crystals are more perfectly formed than rapidly grown crystals. This principle of the degree of crystallinity also applies to the lowered chemical reactivity of apatite caused by fluoride (see section on fluoride) and to the increased susceptibility of dental enamel to carious attack when the enamel contains high amounts of carbonate [Sobel (114), Trautz (115), Brudevold (116)].

Naturally occurring inducers and inhibitors of calcification.—A further finding of importance in the study by Schiffmann & Martin already described (101) is that rat aorta incubated in aged serum mineralized quicker than when incubated in fresh serum. This suggests the presence in rat serum of labile inhibitors of calcification such as those described previously by Fleisch & Neuman (117). These latter investigators report that certain polyphosphates can inhibit calcification and that naturally occurring inhibitors of calcification are inactivated by alkaline phosphatase. The authors now believe that the phosphatase preparation used was contaminated with pyrophosphatase which was actually responsible for the effect observed. Goldhaber (118) has detected the presence of an osteogenic inducer in bone implants in mice. This substance has not been identified, but its structure is small enough to allow it to pass through a millipore filter. The possibility of cellular activity was ruled out by immunizing the host animals to donor cells prior to implanting the millipore filter chamber containing bone.

Additional information on calcification is contained in several recent reviews (93 to 95) and books (96–100).

Fluoride

Because of its widespread use to decrease the incidence of dental caries the fluoride ion is the object of continued investigation and the subject of regular literature reviews. Five informative reviews on fluoride which appeared during 1961 and 1962 are those of Cox (119), Armstrong (120), Hodge (121), Chen, Terepka & Hodge (122), and Taylor *et al.* (123). A book on fluorine and dental health was published in 1959 (124).

Placental transfer of fluoride.—Only within the past few years have studies relating to the placental transfer of fluoride yielded conclusive results. Studies on humans by Gedalia & co-workers have shown that the urinary fluoride levels of the mother drop progressively during the course of pregnancy (125), and that the fluoride content of fetal bone simultaneously increases (126). In this same series of investigations the blood level of fluoride was found to be less in pregnant women than in nonpregnant women and less in maternal blood and cord blood than in the placenta (127). During pregnancy the amount of fluoride in the mother's urine dropped from 0.53

ppm in the fourth month to 0.29 ppm in the ninth month. The level of fluoride in the femurs of the offspring increased from 0.25 mg/100 gm in the third month of fetal life to 13.0 mg/100 gm in the ninth month of fetal life. Shortly before delivery placental tissues were found to contain 15 ppm of fluoride; the blood of the mothers contained 0.09 ppm of fluoride; and the blood of nonpregnant women living in the same area contained 0.18 ppm fluoride. In conclusion, there can be little doubt that fluoride is transmitted to the fetus from the mother's circulation despite the presence of a placental barrier.

It has not been determined whether dental benefits accrue from fluoride which is provided to the fetus. One can speculate, however, that there would be little or no benefit gained *in utero* which could not be obtained from the postnatal consumption of fluoride since only a portion of the deciduous and none of the permanent teeth are calcified at birth. This viewpoint is supported by the results of a recent study in rats which showed that no protection against dental caries is provided to the offspring by the administration of fluoride to the pregnant mothers (128).

Reaction between fluoride and hydroxyapatite.—The reaction between fluoride and hydroxyapatite remains the subject of intensive investigation. Zipkin & Posner (129) have collected x-ray diffraction data from samples of bone which contained varying amounts of fluoride and have concluded that fluoride improves the crystallinity of bone apatite by increasing probably both crystal size and crystal perfection. These more perfect, larger, and therefore more closely arranged crystals are likely to be less chemically reactive than the smaller, less perfect and more widely spaced bone apatite crystals not exposed to fluoride.

The nature of the ionic exchanges between fluoride and hydroxyapatite which produce these improved structures is becoming clearer. Both the carbonate and citrate content of apatite have been found to vary inversely with the fluoride content (129 to 131) and the fluorapatite formed when low concentrations of fluoride and hydroxyapatite are reacted is due, at least in part, to a substitution of fluoride for hydroxyl ions in the apatite structure.

Malaowalla & Myers (132) have shown that the most important variables in the reaction between fluoride and hydroxyapatite are the concentration of fluoride and the pH at which the reaction takes place. In general, fluorapatite is more likely to form in the presence of low concentrations of fluoride. Calcium fluoride is more likely to form as the fluoride concentration in the solution increases. The pH of the solutions in both cases increases as the reaction proceeds, mainly because of the liberation of hydroxyl ions in the case of fluorapatite formation and from the liberation of phosphate ions in the case of calcium fluoride formation. At high concentrations of fluoride (300 to 3000 ppm) a low pH favors the formation of calcium fluoride. This is so because at a low pH, such as 5, more calcium ions are released from hydroxyapatite; and it is more likely that the solubility product of calcium fluoride will be exceeded. These facts have practical importance in the use of

fluoride salts applied topically to reduce the susceptibility of teeth to decay; and may partly explain why single applications of 8 per cent aqueous solutions of stannous fluoride, which has a pH of about 2.6, seem to provide more protection than repeated applications of 2 per cent aqueous solutions of sodium fluoride which has a pH approaching neutrality.

Cadmium

In an assessment of the significance of cadmium in biological tissues, Schroeder & Balassa (133) concluded that cadmium is important only as a poison. It is ingested regularly by man and animals as a contaminant of foods. Because it concentrates in the kidney, tissue levels in that organ are higher than in other organs.

Sea foods and grains contain higher amounts of cadmium than other types of foods, and the kidneys of individuals whose diet includes large amounts of these foods contain cadmium in amounts greater than average. The kidneys of the average resident in the United States contain about 10 mg of cadmium. This amount is not known to be detrimental to health.

Zinc and cadmium are antagonistic. Kagi & Vallee (134, 135) have demonstrated the existence of a cadmium-containing protein (metallothionein) in the renal cortex of the horse. Metallothionein complexes zinc as well as cadmium, but cadmium is bound much more tightly than zinc. Cotzias, Borg & Selleck (136), in studying the zinc pathway in the rabbit, found cadmium to be the only metal capable of causing a delay in zinc clearance. Competition between cadmium and zinc has also been shown for binding sites on human and bovine albumins [Perkins (137)], and in the testes and dorsolateral prostate of the rat. Cadmium accumulates in the latter organs and produces sterility; this effect is antagonized by zinc [Gunn, Gould & Anderson (138, 139)].

Chromium

Schwarz & Mertz (140) have reviewed their interesting experiments in which they have demonstrated the biological importance of chromium. In the rat, trivalent chromium (glucose tolerance factor) is essential for normal glucose utilization.

Small amounts of trivalent chromium enhance markedly the uptake of glucose by epididymal fat *in vitro* and, when fed to rats deficient in chromium, increase the rate of removal of glucose from the blood. The relation of chromium to diabetes has not yet been determined and only a few facts are available which are of help in assessing the degree of importance of chromium in nutrition and metabolism. In 1949, Stickland (141) reported that chromium activates phosphoglucomutase, and recently Langenbeck, Augustin & Schafer (142) found that trypsin is activated by chromium. Other recent studies on chromium include its toxic effects on the chick (143), and its excretion in the dog (144).

Cobalt

The erythropoietic effect of cobalt seems to be the main reason for its pharmacological importance. Eriksen, Eriksen & Haavaldsen (145) recently have provided data which suggest a mechanism for the polycythemic effect of cobalt. This metal causes an inhibition in the formation of heme because of its inhibiting effect on the formation of tetrapyrollic intermediates. The authors suggest that the polycythemic effect of cobalt may be secondary to its heme-inhibiting effect.

The hyperglycemic effect of cobalt may be related to an action on the adrenal cortex. Following its administration, cobalt is rapidly taken up by the adrenals and pancreas (146).

Cobalt also has a goitrogenic effect. This action of cobalt is reversible but, nonetheless, mitigates against its use to stimulate erythropoiesis (147).

In some plants, both legumes and non-legumes, cobalt is necessary for the fixation of nitrogen (148).

Copper

Adelstein & Vallee (149) recently have published a comprehensive review of copper metabolism.

Copper is bound by proteins and has been identified as a component of cerebrocuprein I in the brain, erythrocuprein in red blood cells, hepatocuprein in the liver and milk cuproprotein. It is a component of a number of enzymes, including cytochrome oxidase, phenol oxidase and β-mercaptopyruvate trans-sulfurase. The cuproprotein, ceruloplasmin, appears to be the chief transporting substance of copper in the blood.

Altered copper metabolism is characteristic of certain diseases of humans and animals. While there is no clear-cut documentation of copper deficiency in humans, the daily copper requirement for the human adult is thought to be about 2 mg. Copper is absorbed from the upper part of the gut and is transported rapidly to various body organs where it is stored and taken up by cuproproteins. It is excreted chiefly by the bile.

Adelstein & Vallee (149) have classified the disorders in which copper metabolism is altered as those which are inheritable and are characterized by a failure to synthesize a cuproenzyme, those which are characterized by hypocupremia and those which are characterized by hypercupremia. Albinism and hepatolenticular degeneration are inheritable disorders which are characterized by a failure of synthesis of a cuproenzyme. The enzyme is phenol oxidase (tyrosinase) in the case of albinism, and probably ceruloplasmin (82) in the case of hepatolenticular degeneration. Disorders characterized by hypocupremia include the "dysproteinemias" of infancy, kwashiorkor, sprue, celiac disease, the nephrotic syndrome and multiple sclerosis. Hypercupremia occurs in a variety of disorders. These include biliary cirrhosis, infectious diseases, collagen diseases, myocardial infarction, lymphomas, leukemia, neoplastic diseases, anemias and schizophrenia. Adelstein &

Vallee state that "with the possible exception of biliary cirrhosis, in which a defect in the biliary excretion of copper has been postulated, the cause of the elevations is unknown."

Shields *et al.* (150) report that the concentration of erythrocuprein in erythrocytes of patients with various diseases remains fairly constant. Thus, the elevations of copper in the blood of these persons probably is due to an increase in ceruloplasmin concentration.

In copper deficiency disease in chickens, the most striking symptom is massive hemorrhage, both subcutaneous and internal. O'Dell *et al.* (151) believe that this hemorrhage is probably due to a defect in the elastic tissue of blood vessels and conclude that copper is an important nutrient for the proper metabolism of connective tissues.

Iron

The absorption, transport, and tissue uptake of iron are still the basis of many studies. A number of these studies support the hypothesis that the movement of iron in the body is governed by the number and the character of iron binding sites, and that the demand for iron, therefore, determines its metabolic fate. Saltman & Charley (152), for example, have demonstrated that the uptake of serum iron by rabbit liver cells is greatly enhanced if the liver cells are depleted of iron. In the same experiment these workers showed that the iron uptake by cells can be enhanced also by adding iron to serum which is low in iron. Saltman & Charley conclude, therefore, that the movement of iron from serum into the tissue is compatible with the hypothesis that equilibrium binding is operating. That absorption of iron from the gut may be similarly controlled is indicated by the study of Stewart & Gambino (153) in which the rate of elimination of intravenously administered Fe^{55} was measured simultaneously with the absorption of Fe^{59} from the gut.

Transport of iron in the blood also appears to be enhanced in situations of increased demand, for Morgan (154) has shown that plasma transferrin levels increase in the rat and rabbit following hemolytic or hemorrhagic anemia.

Iron absorption is increased during acclimitization to high altitudes and during hypoxia (155). However, the accelerated erythropoiesis which takes place under these circumstances may not necessarily be the sole factor responsible for the augmentation of iron absorption, because Mendel (156) has presented evidence that hypoxia and accelerated erythropoiesis may enhance iron absorption independently.

While iron metabolism is closely related to iron demand, factors other than those related to demand operate. Iron absorption, for example, is facilitated by ascorbic acid (157) and by bile (158). Dawson *et al.* (159) have reported that pyridoxine was beneficial in alleviating iron-refractory hypochromic anemia and high serum iron levels in two patients.

Evidence relating to the alleged interaction between iron and copper has been reviewed by Matrone (160).

Magnesium

Magnesium is an essential trace element, but the precise reasons for its essential nature are unclear. The symptoms of magnesium deficiency vary from species to species, but the most common symptoms are metastatic calcification and hyperirritability of the neuromuscular and central nervous systems (161). Both the depressant effect of magnesium and its presence in relatively large amounts in bones and teeth are well known. These two facts and the metabolic interactions observed between magnesium, calcium, and phosphorus would appear to provide some basis for studies designed to determine the metabolic reactions for which magnesium is essential.

Insofar as magnesium metabolism is concerned, bone is a critical tissue. Because bone contains relatively large amounts of magnesium, this tissue serves as a magnesium reservoir when the diet is deficient in magnesium (162, 163). Bone, also, appears to depend upon magnesium for its normal development, for bone abnormalities occur in some species deprived of adequate amounts of magnesium during growth (161).

Investigation of magnesium metabolism is made difficult by the metabolic interactions between magnesium, calcium, and phosphorus. Calcium and magnesium are antagonists. The demonstration of this antagonism on the neuromuscular system is well known, and there is ample evidence that this antagonism also exists on a nutritional basis (161). Calcium accentuates the symptoms of magnesium deficiency and, when dietary phosphorus is low, magnesium causes loss of calcium from the body. Phosphorus also antagonizes magnesium, both by accentuating magnesium deficiency and by counteracting the effects of excess magnesium. The antagonism of magnesium by phosphorus appears to be related primarily to decreased magnesium absorption. The enhancing effect of vitamin D on metal absorption is not specific for calcium and applies as well to magnesium and other elements (see section on vitamin D). This fact, of course, has an obvious bearing upon these antagonisms.

The mechanism of action of novobiocin appears to be related to its ability to complex magnesium. Brock has reported that all cellular processes affected by novobiocin are magnesium-dependent (164).

Various diuretics cause an increase in the urinary excretion of magnesium (165). This drug-induced depletion of body stores of magnesium has been reported to produce the symptoms of magnesium deficiency in humans.

Magnesium in the serum of humans with muscular dystrophy appears to be bound to macromolecular components of the blood (166). The ratio of free to bound calcium in these patients is within normal limits. This finding is interesting in view of the antagonism of magnesium and calcium on the neuromuscular system.

Manganese

Four review articles by Cotzias and co-workers (167–170) provide some basis for a logical interpretation of much of the data gathered from experi-

ments on the metabolism of manganese. No longer is the interest of investigators solely in the toxic effects of manganese, for most of the studies on this metal in recent years have been concerned with its importance as an essential trace element. In fact, Cotzias (168) regards manganese as being more than a mere trace element and "indeed a precious metal as far as the economy of the body is concerned."

The symptoms of manganese deficiency have been presented in the reviews cited and will not be dealt with here. These manifestations include impaired growth, bone deformation and dysfunctions of the reproductive and central nervous systems.

Although manganese absorption can be altered by a number of nutritional factors, e.g., excessive calcium intake, once manganese is in the body proper it appears to follow a highly specific metabolic pathway. No metals other than manganese have been found which compete for the manganese pathway. Bertinchamps & Cotzias (171) have found a protein in human plasma which binds trivalent manganese and serves as a means of manganese transport in the blood. This protein is a β_1 globulin which Bertinchamps & Cotzias have named "transmanganin." Borg & Cotzias (172) have evidence which indicates that one or more of the porphyrins found in animal and human tissues contain manganese instead of iron.

The information being gathered on the metabolism of manganese includes facts other than those related strictly to its metabolic pathway. Hughes & Cotzias (173), for example, have reported data which indicate that the growth-maintaining action of manganese is mediated through the pituitary-adrenal axis. Hegde, Griffith & Butt (174) have made the interesting finding that serum manganese rises significantly following myocardial infarction. This discovery has practical usefulness in diagnosing myocardial infarction and in distinguishing myocardial from pulmonary infarction. Massive pulmonary infarction differs from myocardial infarction in that the former results in a rise of aluminum as well as manganese in the serum.

Molybdenum

Molybdenum is of interest because of its poisoning effect on livestock and, more recently, because of its possible emergence as an essential trace element [Miller & Engel (175)]. The biochemical defects occurring in molybdenum poisoning are, for the most part, unknown, but impaired copper metabolism is involved. Even less is known about molybdenum's role as an essential nutrient, although there is some evidence which indicates that it may be essential for optimum growth of certain animals. The only indication that molybdenum may have importance in human nutrition is found in the data of Ludwig, Malthus & Healy (176), and of Losee, *et al.* (177). These investigators have provided evidence that molybdenum, perhaps in association with other elements, may make the teeth of animals and humans more resistant to caries.

SELENIUM

Selenium is an essential trace element for many species of animals, but selenium deficiency has not been demonstrated in man. The active form of this element in animal tissues and in nutrients is a selenium-containing organic substance which has been named "Factor 3." The exact chemical nature of Factor 3 and its precise role in intermediary metabolism are unknown. Biological studies on selenium are made difficult because of the complex chemical problems associated with its analysis and because of its presence in naturally occurring materials in minute quantities (178).

Factor 3 deficiency occurs in at least 10 species of animals (179–182). The symptoms are numerous and vary from species to species, but the symptom which occurs most commonly is muscular dystrophy. Liver necrosis is also common in animals suffering from Factor 3 deficiency and its prevention in the rat has served as a convenient means of bioässay of Factor 3 [Schwarz (179)]. Lagace (183) has pointed out that active selenium compounds are not only of prophylactic value in treating Factor 3 deficiency, but also are of therapeutic value in treating the deficiency once it is established.

The symptoms of Factor 3 deficiency in animals resemble those of vitamin E deficiency, and metabolic interactions occur among Factor 3, vitamin E, and the sulfur amino acids (179, 184). Sulfur amino acids and vitamin E both lower the requirement for Factor 3. Schwarz & Foltz (185) have related this effect of sulfur amino acids to their sparing effect on the vitamin E requirement. While vitamin E deficiency and Factor 3 deficiency resemble each other they are distinct entities. There are, for example, several vitamin E deficiencies which are not affected by Factor 3, including muscular dystrophy in the rabbit, encephalomalacia in the chick, and resorption sterility in the rat (179).

In an effort to establish the chemical identity of Factor 3 Schwarz (179) has bio-assayed semi-purified preparations of tissue Factor 3 and has compared these results with those obtained in bio-assays of synthetic substitutes of Factor 3. These experiments indicate that the daily requirement of Factor 3 for the rat is that amount which contains about 0.1 μg of selenium. The ED_{50} for the rat expressed in terms of μg of selenium per hundred grams of diet is 0.7 μg per cent. While many organic and inorganic selenium compounds can prevent Factor 3 deficiency, none have been found which are as active as Factor 3 preparations from animal tissue. The most potent synthetic substitute tested by Schwarz and his co-workers is γ,γ'-di-selenodivaleric acid. The ED_{50} of this compound in the diet of the rat is 1.4 μg per cent, i.e., it is half as potent as Factor 3. Initial clinical trial of γ,γ'-di-selenodivaleric acid in children with a form of kwashiorkor showed promising results. However, Factor 3 deficiency in man has not yet been demonstrated unequivocally.

Vitamin E and at least some sulfur amino acids and selenium compounds exhibit antioxidant activity. The tissues of Factor 3-deficient animals fed

vitamin E, cystine or selenium dioxide exhibit less auto-oxidation than animals whose diets are not supplemented [Bieri (186); Zalkin *et al.* (187)]. Bieri *et al.* (188) recently have suggested that the substitution of selenium for sulfur in amino acids may yield a new protein with remarkable anti-oxidant properties.

Zinc

Because of economic factors the dietary requirement of zinc has been studied in poultry more than in other species. The level of zinc required for the normal development of bones and feathers in turkeys is 70 ppm. For chickens, the dietary zinc requirement is 35 ppm (189). Presumably, the difference in zinc requirements between turkeys and chickens is related to the fact that turkeys grow more rapidly than chickens. The source of dietary protein is an important factor in the development of zinc deficiency in animals. Zinc deficiency can be produced easily if raw soybeans are used as a protein source, (190 to 192), and this effect cannot be antagonized by zinc if the amount of soybeans in the diet is too great. In the turkey, for example. zinc added to the diet relieved the deficiency when the soybean content of the diet was 20 per cent but not when soybeans constituted 35 per cent of the diet [Linerode, Waibel & Pomeroy (191)]. Zinc deficiency in hens results in poor hatchability of eggs and abnormal development, particularly abnormal skeletal development of the offspring [Kienholz *et al.* (192); Blamberg *et al.* (193)].

In the rat, zinc is concentrated in the dorsolateral prostate more than in any other tissue. Since their initial discovery of this fact, Gunn, Gould & Anderson have demonstrated that the capacity of the rat dorsolateral prostate to concentrate zinc can be used as a laboratory tool to study various aspects of reproduction. For example, this phenomenon can be used as a specific bio-assay technique for interstitial cell-stimulating hormone (194, 195), and can be used to demonstrate seasonal variations in the function of reproductive and related endocrine systems (196, 197). The ability of the rat dorsolateral prostate to concentrate zinc is 60 to 85 per cent higher at certain times of the year than at other times of the year (198). This seasonal variation in zinc concentrating ability correlates with changes in the responses of rats to various hormones and is believed to be a manifestation of an archaic reproductive cycle. These findings are significant because they "emphasize the importance of hidden seasonal rhythms in endocrine experimentation, even in the laboratory rat housed under constant environmental conditions" (196).

Zinc is found in bones and teeth. Its concentration in the femur of the rat made zinc-deficient by feeding soybean protein can be enhanced by feeding lactose or salts of ethylenediamine tetracetic acid [Forbes (190)]. Zinc is taken up by newly forming bone in the monkey but not by pre-existing calcified bone [Haumont & Vincent (199)]. This finding is surprising in view of the uptake of zinc by the enamel of erupted teeth (116), and is not in

agreement with the study of Rubini *et al.* (206) on zinc metabolism in mice. rats and dogs.

Zinc is present in a bound form in many soft tissues and fluids, and it may be important as a constituent of certain enzymes. Valentine, Tanaka & Fredricks (200) have demonstrated that 10^{-4} *M* zinc but not magnesium will restore the lost activity of the alkaline phosphatase of human leukocytes. Vallee, Rupley & Coombs (201) have found the reversible binding of zinc by carboxypeptidase to provide a useful model for studying metalloenzymes and metal-enzyme complexes in which the metal is loosely bound.

Histamine forms a stable complex with heparin and zinc, but a relatively unstable complex with heparin alone [Kerp & Steinhaeuser (202)]. Thus, the presence of zinc in the granules of mast cells may have significance in stabilizing heparin-histamine complexes. In man, plasma zinc quickly becomes non-dialyzable after injection, indicating that it becomes protein bound (203). In human semen zinc is present in the bound form and migrates with the β-2 globulin fraction (204). Zinc enhances the antibiotic activity of dimethylchlortetracycline *in vitro*, but the activity of this antibiotic is inhibited by copper, nickel, ferrous iron and magnesium (205).

Zinc is present in skeletal muscle in appreciable amounts and may play a role in muscle contraction. On the basis of evidence presented by Edman (91, 92), it has been postulated that the chelation of zinc by a constituent of the muscle fiber is related to the contraction of the muscle, and that muscle relaxation occurs when zinc is released by the chelator. For further discussion see the section called Effects of Ions on Biochemical Processes.

Cadmium competes with zinc for binding sites. This is discussed in the section on cadmium.

The Effect of Vitamin D on the Absorption of Cations

That vitamin D enhances the intestinal absorption of Ca^{++} is well known. Recently Dowdle, Schachter & Schenker (207), and Harrison & Harrison (208) have been able to demonstrate such an effect on everted intestinal loops. Vitamin D supplemented animals had two-fold better absorption than depleted animals. The segment adjacent to the pylorus was the most active portion of the intestine. A temperature effect from 10° C to 25° C was observed but the general improvement in diffusion did not apply to sodium. Engstrom & DeLuca (209) have found that vitamin D acts to cause a release of Ca^{++} by kidney mitochondria. The effect was absent when Sr^{++} was used and could be demonstrated by both *in vivo* and *in vitro* vitamin D. Inactive steroids such as ergosterol, cholesterol and 7-dehydrocholesterol had no effect. These data suggest a generalized action of vitamin D on Ca^{++} transport.

Some recent studies indicate that this effect of vitamin D is not specific for calcium. Worker & Migicovsky (210, 211) have found that vitamin D stimulates the uptake by the bones of the chick of all of the metals in Group IIa of the periodic table---beryllium, magnesium, calcium, strontium and

barium—and two of the three metals in Group IIb—zinc and cadmium, but not mercury. This effect has been interpreted as being due to an increased absorption of these metals from the gut caused by vitamin D. Other investigators have reported similar findings, particularly for magnesium. Hanna (212), and George *et al.* (213) found that vitamin D stimulates magnesium absorption from the gut, and that this effect is accompanied by an increased urinary excretion and decreased fecal excretion of magnesium. The effect of vitamin D feeding on the level of magnesium in the plasma or serum is inconsistent, probably because of the nearly simultaneous occurrence of enhanced gastrointestinal absorption and enhanced urinary excretion of magnesium (213).

Chelation

In the past few years, interest has risen in the phenomenon of chelation in biological systems, and this has been the subject of three recent symposia (214, 215, 216). Some of the most important developments in this field relate to trace metals as natural components of biological substances, particularly enzymes and other proteins. It is likely that biochemical information such as this will form the basis upon which pharmacologists and clinicians will be able to modify biological chelate systems with predictable effects on the whole animal. Until now such predictable effects are largely confined to creating nonspecific metal deficiencies, to ridding the body of poisonous metals, including radioactive metals, and to producing interesting but curious remissions of porphyrias, scleroderma and certain cardiovascular diseases.

The actions of some drugs have been related to their metal binding ability. Kohn (217) has reported that tetracycline, and naturally occurring macromolecules, may be linked together by divalent cations. This finding suggests a mechanism by which tetracyclines inhibit the growth of microorganisms. The oft-reported deposition of tetracyclines in bones and teeth undoubtedly is due to the firm binding of cations by tetracyclines. Foye & Turcotte (218) have correlated the varying degrees of antibacterial, analgesic, and fungicidal effects of a series of salicylic acid derivatives with the differences in affinities of these derivatives for ferric and aluminum ions. Whitehouse (219) has offered evidence which suggests that the ability to react with metal ions in a lipid phase may be the major factor which confers anti-inflammatory properties on a compound.

Biochemical studies on the binding of metals by enzymes and other proteins continue to provide fundamental and interesting data. Vallee and his collaborators (134, 135, 220, 221) have contributed to the increasing amount of evidence that the role of metals in at least some enzymes is twofold: the involvement of the metal at the active catalytic site of the enzyme (see reference 81), and the influence of the metal in maintaining the structural configuration of the enzyme. The evidence for the validity of this hypothesis includes the simultaneous loss of structural integrity of an enzyme, and the irreversible binding of its metal component by another moiety. When the

metal is only temporarily inactivated, however, structural integrity of the enzyme is maintained and enzyme activity may be restored. Enzyme specificity, moreover, may be associated more with the protein portion of the enzyme than with the metal. It is not surprising, therefore, that the association of the same metal with a number of different enzymes results in entirely different catalytic activities, or that sometimes metal substitutes can be made in an enzyme without loss of enzyme activity.

Wacker & Vallee (222) have provided evidence that metals may play a role in maintaining the configuration of RNA. Thus, metals may have importance in the transmission of genetic information. The RNA's of different tissues were found to contain significant amounts of chromium, nickel, manganese, and cadmium. It was hypothesized that metals may link purine or pyrimidine bases through covalent bonds in the RNA structure. Holley & Lazar (223) have reported, however, that about 90 per cent of the metal ions could be removed from RNA preparations without loss of RNA activity. This is a severe test of the original hypothesis and could be interpreted as meaning that the observations of Wacker & Vallee may have been complicated by trace metal contaminants. Nonetheless, the hypothesis merits further testing. Recently Eichhorn (224) has reported that some metals act as stabilizers of RNA while other metals act as destabilizers of DNA (see also reference 88). This effect of metals on the stability of the DNA molecule was assessed by measuring the effect of 10^{-4} M concentrations of the metals on the melting curves of DNA.

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