Comments on Inorganic Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455155

The New Biochemistry of Vanadium

Kenneth Kustin[a]; Ian G. Macara[b]
[a] Department of Chemistry, Brandeis University, Walthum, Massachusetts [b] Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts

To cite this Article Kustin, Kenneth and Macara, Ian G.(1982) 'The New Biochemistry of Vanadium', Comments on Inorganic Chemistry, 2: 1, 1 — 22
To link to this Article: DOI: 10.1080/02603598208078107
URL: http://dx.doi.org/10.1080/02603598208078107

The New Biochemistry of Vanadium

In the short time since 1977, when it was discovered that vanadate is a potent inhibitor of the sodium pump (Na,K-ATPase), vanadium has become a valuable and widely used probe of enzyme function and mechanism. In the V(V) oxidation state, as diamagnetic vanadate, it mimics phosphate and interacts with a large class of phosphatases and phosphotransferases. In the V(IV) oxidation state, as paramagnetic vanadyl, it behaves as a transition metal ion and can replace other such ions in metalloproteins. Because the potentials of V(V) and V(IV) lie within limits tolerated by living systems, vanadium can also participate in many biological electron transfer reactions. The wide variety of effects reported in the literature that are observed when vanadium is added exogenously to enzymes, cells and tissues can be largely explained by consideration of the principles of aqueous inorganic vanadium chemistry. The true role of endogenous vanadium in living systems remains obscure, however.

INTRODUCTION

Until very recently, vanadium was viewed by the biochemist as a rather obscure and exotic element. Although found in extraordinarily high concentrations within certain species of sea squirt (tunicate)[1] and mushrooms of the genus *Amanita*[2] and recognized as an essential trace element in other organisms,[3] vanadium had no known biological function. No vanadium-containing enzymes were known, and the effects of exogenous vanadium on metabolism were not understood. About five years ago, however, vanadate was found to act as an extremely potent inhibitor of the Na,K-ATPase,[4] which is a key enzyme, universally present in eukaryotic organisms, that maintains the membrane potential and sodium gradient across the plasma membrane. This effect on the enzyme was discovered when vanadate was identified as the inhibitory impurity

Comments Inorg. Chem.
1982, Vol. 2, Nos. 1–2, pp. 1–22
0260-3594/82/0202–0001/$06.50/0

present in a commercial preparation of ATP. Since the ATP was prepared from horse muscle, it was suggested that endogenous vanadium might function as a physiological regulator of sodium pump activity. This hypothesis provoked a considerable effort to analyze the effects of vanadium *in vivo*, and although it is now known that the element plays no role in regulation of the Na,K-ATPase,[5] the physiological responses to exogenously supplied vanadium continue to excite interest. Moreover, vanadate has proven to be a valuable probe of the catalytic mechanism of the purified Na,K-ATPase[6] and of other phosphohydrolases and phosphotransferases. It has very recently been shown to mimic insulin when added to fat cells[7] and skeletal muscle[8] *in vitro*, and can be expected to shed new light on the means by which peptide hormones trigger specific cellular responses.

Vanadium(IV) is paramagnetic and has been used successfully as an environmentally sensitive EPR probe for several metalloproteins.[9] In the form of vanadyl–nucleotide complexes, the element has even found use in molecular biology, to inhibit cellular ribonucleases that digest messenger RNA.[10] Despite these advances, however, the function of endogenous vanadium remains obscure, even in those organisms in which it occurs at much greater than trace levels.

Nonetheless, many of the physiological effects of subtoxic doses of vanadium are explicable by a consideration of the aqueous inorganic chemistry of the element. It is likely that such consideration will also provide clues to vanadium's biological role. The present article will therefore cover in some detail the following aspects of vanadium chemistry: (1) the aqueous chemistry of the V(IV) and V(V) oxidation states, and its relation to other transition metal ions, and of oxoanions, such as phosphate; (2) the observed physiological effects of vanadium; (3) the properties and location of endogenous vanadium.

1. THE SOLUTION CHEMISTRY OF VANADIUM

A. Hydrolysis of Aquo-Vanadium Species

Standard potentials at $[H^+] = 1$ M (Table I) are unambiguous about which ions are associated with the *formal* oxidation states II, III, IV, V; namely, V^{2+}, V^{3+}, VO^{2+}, VO_2^+. This connection breaks down as one moves to higher pH and considers different vanadium concentration

TABLE I
Standard reduction potentials[a]

Redox couple	E_0 (volts)
$V^{3+} + e^- = V^{2+}$	−0.255
$VO^{2+} + 2H^+ + e^- = V^{3+} + H_2O$	0.34
$VO_2^+ + 2H^+ + e^- = VO^{2+} + H_2O$	0.999

[a]All activities equal one, temperature 25°C. From G. Charlot, A. Collumeau and M. J. C. Marchon, *Selected Constants of Oxidation-Reduction Potentials of Inorganic Substances in Aqueous Solution* (Butterworths, London, 1971).

ranges. At moderate concentrations, ≳ mmol, each of the vanadium ions hydrolyzes and precipitates as the pH is raised to neutrality (Figure 1). Some of the precipitates then redissolve as anionic species at alkaline pH. *Vanadous ion*, V^{2+}, will slowly reduce water to H_2. It is of no biological significance and will not be considered further. *Vanadic ion*, V^{3+}, is appreciably hydrolyzed to VOH^+ at pH 2.2. Beyond that pH it dimerizes and precipitates. Water-soluble species exist in neutral and

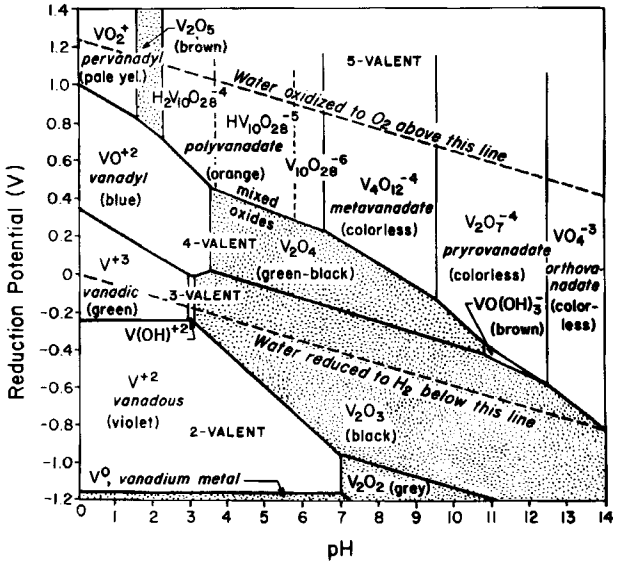


FIGURE 1 Vanadium equilibria in water. Stippled areas are solid phases. Each region is labeled with most stable species. [Adapted with permission from M. Pourbaix, *Atlas of Electrochemical Equilibria in Aqueous Solutions* (Pergamon, New York, 1966), Figure 3b, p. 92.]

basic conditions but are little understood, since they are very easily oxidized and highly unstable.[11] *A priori* one would not therefore expect V(III) species to be of any biological importance. Nonetheless V(III) has been detected at high (>mM) concentrations within the blood cells of certain species of tunicate, by EXAFS[12] and magnetic susceptibility[13] measurements on intact cells. Presumably, the oxidation state of the vanadium is stabilized by complexation, but the ligand is unknown.

The aqueous species of the paramagnetic *vanadyl,* V(IV), oxidation state have been well-characterized over a wide range of pH, largely by ESR spectroscopy.[14] The blue vanadyl ion VO^{2+} exists in equilibrium with $VO(OH)^{+}$ below pH 2. Above this pH, a soluble ESR-silent dimer, $[VO(OH)]_2^{2+}$, begins to appear, and a precipitate of $VO(OH)_2$ forms. At pH >11 the precipitate dissolves, due to the formation of the brown, ESR-active species, $VO(OH)_3^-$. Although acidic solutions of vanadyl ion are air stable, atmospheric oxidation occurs at neutral or alkaline pH. Again, like V^{3+}, vanadyl ion is detectable in tunicate blood cells.[12]

The solution chemistry of V(V) is more complex than that of the lower oxidation states. Below pH 2, the pale yellow *pervanadyl* cation, VO_2^+, predominates (Figure 2). As the pH is raised, precipitation of V_2O_5 will occur if the concentration is greater than millimolar. Above pH 3, an orange-colored solution is formed, due to the presence of a

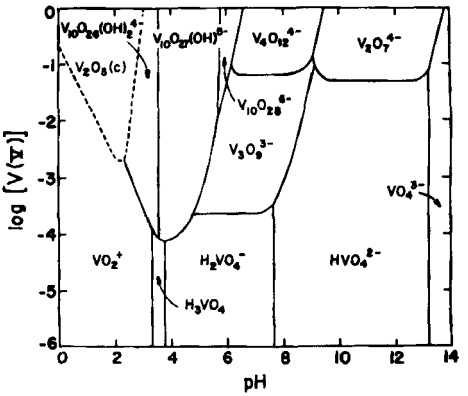


FIGURE 2 Equilibria in aqueous vanadium(V) solutions. Solid line gives equimolar amounts of neighboring species. Dashed line is aqueous solid equilibrium. Note absence of polyanions below 50 μM at all pH. (Taken with permission from Ref. 11, Figure 10.7, p. 210.)

polymeric oxoanion, *decavanadate*, $V_{10}O_{26}(OH)_2^{4-}$. Dissociation of the polymer occurs upon dilution (to <0.1 mM) or increase of the pH to neutrality, but equilibration is very slow (~2 h at 40°C) and in the preparation of monomeric "orthovanadate" it is therefore advisable either to boil the solutions or incubate them at room temperature overnight before use. Since *metavanadate*, a mixture of $V(OH)_3$, $H_2VO_4^-$, $V_3O_9^{3-}$ and $V_4O_{12}^{4-}$ which forms at pH 6–8, is colorless, the absorbance at around 310 nm can be used as a measure of the amount of contaminating decavanadate polymer remaining in solution. Over pH 9 and at concentrations much greater than millimolar, pyrovanadate $V_2O_7^{4-}$, is important but at lower concentrations the monomeric species, orthovanadate, HVO_4^{2-} and $H_2VO_4^-$, predominate (K_d for dissociation of the trimer is ~2×10^{-6} M at pH 7).[15] Under physiological conditions and at subtoxic doses only the monomeric forms of vanadate are of interest, and reports detailing the varied *in vivo* effects of metavanadate, pyrovanadate and so forth display a lack of familiarity with aqueous inorganic vanadium chemistry.

B. Vanadium Complexes

Electronic and magnetic properties of vanadium(III) complexes have been thoroughly investigated because they are easily prepared examples of a d^2 system in different, well-characterized geometries.[16] The formation, in solution, of an octahedral V(III) complex is rapid. The water exchange rate constant for $V(H_2O)_6^{3+}$ is 2.8×10^2 s^{-1} at 25°C. Due to the high formal charge of 4+, a V^{4+} ion as such does not exist in solution. However, complexes of V^{4+} have been synthesized recently, and their structures determined by x-ray crystallography.[18] The properties of these complexes are those expected for a d^1 ion in the observed octahedral field. Usually, the instability which would result from the high charge of 4+ on vanadium in aqueous solution is relieved by attachment to a π-bonded oxo ligand. The resulting VO^{2+} ion then reacts as a unit, which is analogous to the cationic species of divalent transition and alkaline earth metals forming a wide variety of stable complexes with inorganic and organic ligands. The structures of vanadyl chelates are square pyramidal, with the oxo ligand at the apex (Figure 3a),[19] or trigonal bipyramidal with the oxo ligand in the equatorial plane (Figure 3b). Although labile, vanadyl is less so than most simple divalent ions; the water exchange rate constant is only 5.0×10^2 s^{-1} at 25°C.[20]

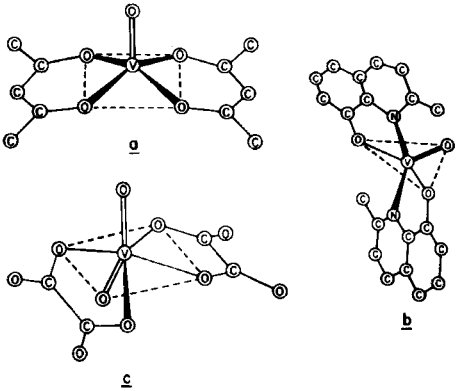


FIGURE 3 Structures of vanadium complexes. In each structure, the dashed lines connect coplanar atoms. A double bond joins vanadium and oxo ligand; hydrogens are omitted. (a) Square pyramidal bis(acetylacetone)oxovanadium(IV) complex. The vanadium lies above the square plane of oxygens of this highly symmetrical structure [R. P. Dodge, D. H. Templeton and A. Zalkin, J. Chem. Phys. **35,** 55 (1961)]. (b) Trigonal bipyramidal bis(2-methyl-8-quinolinato)oxovanadium(IV) complex. The central vanadium and the three oxygens connected by dashed lines lie in the same plane. The coordinating nitrogens form the apices of the distorted trigonal bipyramid. There are two vanadium–oxygen distances, the shorter one being to the oxo ligand [M. Shiro and Q. Fernando, J. Chem. Soc. Chem. Commun. 63 (1971)]. (c) Octahedral bis(oxalato)dioxovanadate(V) complex anion. The vanadium atom is *coplanar* with the four oxygen atoms connected by dashes. The shorter distances from vanadium to the oxo ligands displace the vanadium atom from the center of the octahedron, causing a distortion.[21a]

The weakly bound water opposite the oxo ligand exchanges much more rapidly, a specific rate of 10^8 to 10^{10} s^{-1}.

Complexes of the V(V) pervanadyl cation form readily under acidic conditions, and display a distorted octahedral geometry with *cis* dioxo ligands (Figure 3c). As for vanadyl complexes, the VO_2^+ entity is retained in the complex, both units being stabilized by $d\pi$–$p\pi$ bonding.[21] Unlike $V(H_2O)_6^{2+}$ and $VO(H_2O)_4^{2+}$, for which the rate of complex formation is controlled by the water exchange rate constant, V(V) complex formation occurs by a different mechanism, judging by the few examples studied.[22] However, bimolecular complex formation rate constants are high, being around 10^4 M^{-1} s^{-1}. It should be emphasized that *negatively* charged vanadate ion readily forms complexes, in particular with *cis* diols, a feature shared by other oxoanions such as molybdate and tungstate.[23] This behavior is not shared by phosphate, however, with which

vanadate bears a closer resemblance in other properties than it does with metal-containing oxoanions.

C. Comparison of Vanadates and Phosphates

Phosphate and vanadate monoanions are very similar in size and geometry. X-ray data in the solid and Raman in solution show these ions to be tetrahedral.[19] The data are clearest for VO_4^{3-} and $H_2VO_4^-$ among the vanadates.[11] At low concentrations and above pH 4, vanadate and phosphate both exist as protonated monomers. The ionization constants of these monomers are close enough to show similar distribution diagrams in the physiological pH range (Figure 4), with one important difference. Mononegative, diprotonated vanadate ($H_2VO_4^-$) is the predominant V(V) species at low concentrations under physiological conditions [ionic strength 0.17 M (NaCl), 38°C and pH 7.4]. The predominant P(V) species at the same conditions is HPO_4^{2-}. In contrast with P(V) and V(V), neither sulfate, chromate, nor molybdate are protonated at physiological pH (Table II). With similar charge, degree of protonation, size and geometry (Table II) orthovanadate is an excellent phosphate analog. Thus vanadate might be expected to compete with phosphate in many biochemical processes, and such competition has indeed been observed. Quantitative differences in behavior are attributable in part to the difference in the predominant ionic species present under physiological conditions.

Qualitative differences appear only when polyanionic species are considered. In most vanadate polyanions, for example, coordination about the vanadium is octahedral, a geometry that is never observed for polyphosphates or their esters (such as ATP). Both linear and cyclic polyphosphates exist, of unrestricted chain length, but polyvanadates are of less clearly defined structure and only those oligomers containing 2, 3, 4 and 10 units have been identified. Analysis of polymeric vanadium species is hampered by their kinetic lability. Polyphosphates are thermodynamically unstable, but *kinetically inert*. Polyvanadates, on the other hand, are thermodynamically stable and will form spontaneously in solution under appropriate conditions of pH and concentration. They occur, however, as equilibrium mixtures that decompose upon dilution because of the kinetic lability of the V–O bond.[22,24] A similar argument explains the absence of stable vanadate analogs of phosphate esters such as ATP. Vanadate forms complexes with alcohols

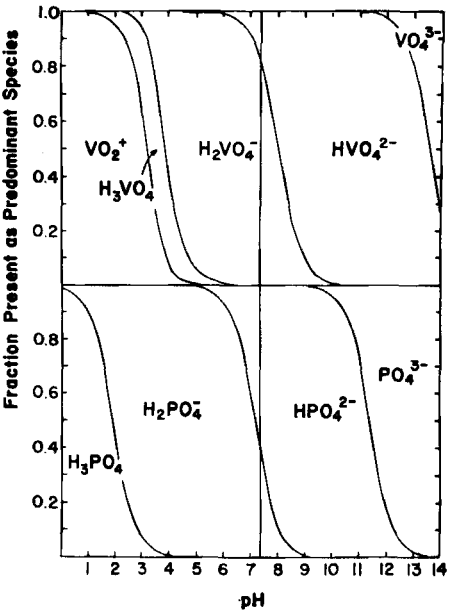


FIGURE 4 Comparison between vanadium(V) and phosphorus(V): distribution diagrams in aqueous solution. Each curve is the sum of the fractions of different species present, starting with either VO_2^+ or H_3PO_4 at the farthest left, and adding one species at a time as one proceeds to the right of the diagram. Thus, the first phosphate curve is the fraction present as H_3PO_4, the second is the sum of the fractions present as H_3PO_4 and $H_2PO_4^-$, and so on. A vertical line drawn through the diagram is cut by the curves into segments proportional to the various fractions. Each region of the diagram is labeled with the species corresponding to the fraction of phosphorus(V) or vanadium(V) given by the vertical line segment in that region. A vertical line at pH 7.38 ($[H^+] = 4.17 \times 10^{-8}$ M) has been drawn through both diagrams. The fractions present as individual species have been calculated from the data in Table II. Let total phosphorus(V) = P_i, and total vanadium(V) = V_i, then the main fractions at pH 7.38 are: $[H_2PO_4^-]/P_i = 0.396$, $[HPO_4^{2-}]/P_i = 0.604$, $[H_2VO_4^-]/V_i = 0.814$, $[HVO_4^{2-}]/V_i = 0.186$, as can also be determined nomographically from the diagram.

rather than esters, particularly with *cis* diols, and will accommodate a variety of geometries.

An explanation for these different chemistries may be found in the underlying electronic structures. For phosphorus, *d* orbitals do not need to be invoked to explain bonding and electronic spectra.[25] For vanadium, on the other hand, as for other transition metals, *d*-orbital participation is required, generating closely-spaced electronic energy levels. Hence less destabilization occurs in going from coordination number 4 → 5 → 6.

TABLE II

Comparison between vanadate, phosphate and other oxoanions

Acid ionization constants at 25°C and ionic strength 0.17 M[a] for V(V) and P(V)

$$\frac{[H^+][H_{i-1}MO_4^{-(4-i)}]}{[H_iMO_4^{-(3-i)}]} = Q_i(-\log Q_i); \qquad i = 3,2,1$$

Reaction	M = V[b]	M = P[c]
$VO_2^+ + 2H_2O \rightleftarrows H_3VO_4 + H^+$	5.01×10^{-4} (3.3)	. . .
$H_3MO_4 \rightleftarrows H^+ + H_2MO_4^-$	1.74×10^{-4} (3.76)	1.15×10^{-2} (1.94)
$H_2MO_4^- \rightleftarrows H^+ + HMO_4^{2-}$	9.53×10^{-9} (8.02)	6.34×10^{-8} (7.20)
$HMO_4^{2-} \rightleftarrows H^+ + MO_4^{3-}$	2.86×10^{-14} (13.54)	3.86×10^{-12} (11.41)

Predominant anions at pH 7.38[d], ionic strength 0.17 M, and 25°C (for P(V), V(V), Cr(VI) and S(VI)

Central atom	Ratio of mono-to-divalent oxoanion
P	$[H_2PO_4^-]/[HPO_4^{2-}] = 1/1.52$
V	$[H_2VO_4^-]/[HVO_4^{2-}] = 4.38/1$
Cr[e]	$[HCrO_4^-]/[CrO_4^{2-}] = 1/27.3$
S[b]	$[HSO_4^-]/[SO_4^{2-}] = 1/(9.89 \times 10^5)$

Central atom to oxygen bond length in tetrahedral ion[f]

Ion	Bond length (Å)
PO_4^{3-}	1.58
SO_4^{2-}	1.58
VO_4^{3-}	1.66
CrO_4^{2-}	1.65
MnO_4^-	1.63
AsO_4^{3-}	1.65
MoO_4^{2-}	1.76
WO_4^{2-}	1.76

[a]The ionic strength is that of a 0.9% solution by weight of NaCl, which approximates that of blood plasma. See *Hawk's Physiological Chemistry*, edited by B. L. Oser, 14th ed. (McGraw-Hill, New York, 1965), p. 322. A temperature of 37°C would have been more meaningful physiologically, but accurate vanadate data are not available at this temperature.

[b]Ref. 11.

[c]The phosphoric acid ionization constants were computed as follows, zero ionic strength ionization constants at 25°C [J. R. Van Wazer, *Phosphorus and Its Compounds* (Interscience, New York, 1958), pp. 480–482], were adjusted to 0.17 M ionic strength using an empirical formula determined at 38°C [J. S. Elliot, R. F. Sharp and L. Lewis, J. Phys. Chem. **62,** 686 (1958)].

[d]Average value of venous and arterial blood; B. L. Oser, Footnote a.

[e]Ref. 11, p. 215.

[f]Bond lengths may vary according to environment. Values for P–O, S–O, V–O and Cr–O bond lengths are from A. L. Dingley, K. Kustin, I. G. Macara and G. L. McLeod, Biochim. Biophys. Acta **649,** 493 (1981). The Mn–O bond length is from G. J. Palenik, Inorg. Chem. **6,** 503 (1967). The remaining bond lengths are from R. L. Van Etten, P. P. Waymack, D. M. Rehkop, J. Am. Chem. Soc. **96,** 6782 (1974).

(See, for example, the bonding diagram developed for CrO_4^{2-} and MnO_4^-.[26]) When vanadate binds at the phosphate site of phosphohydrolase enzymes, therefore, it is able to adopt a geometry closely resembling the trigonal bipyramidal transition state that occurs during the hydrolysis of phosphate esters.[15] Since the transition states are very tightly bound by enzymes, relative to the binding affinities for substrates or products, vanadate can act as an excellent inhibitor of phosphatase enzymes.

In addition to permitting the formation of complexes, the electronic structure of vanadium also allows the existence of several stable oxidation states, interconversion between which can occur rapidly via a series of one-electron steps.[27] The potentials are such that redox reactions between V(III), V(IV) and V(V) can occur *in vivo*, and there is indeed considerable evidence for the intracellular reduction of vanadate to V(IV) in several types of cells. Intriguingly, reduction converts the vanadium from a phosphate analog to an analog of transition metals with partially occupied *d* orbitals, and it is possible that this unique behavior is related to the element's biological role. Phosphorus, on the other hand, participates in no biological redox reactions. The P(IV) state is too unstable to exist in aqueous solution and conversion to P(III) is extremely slow since it involves a two-electron reduction and considerable molecular rearrangement.

2. BIOCHEMICAL AND PHYSIOLOGICAL EFFECTS OF VANADIUM

Three types of behavior can be predicted from a consideration of the aqueous chemistry of vanadium. First, as vanadate, the element will compete with phosphate at the active sites of phosphate-transport proteins, phosphohydrolases and phosphotransferases. Second, as vanadyl, it will compete with other transition metal ions for binding sites on metalloproteins and for small ligands such as ATP, and, third, it will participate in redox reactions within the cell, particularly with relatively small molecules that can reduce vanadate nonenzymatically, such as glutathione (GSH).

The physiological effects of exogenously added vanadium must therefore be expected to be complex, and different tissues will respond in different ways because of differences in transport properties, redox

potential and enzyme activities. Clearly, *in vitro* studies using purified enzymes cannot be extrapolated directly to the situation *in vivo*. Nonetheless, analysis of the effects of vanadium *in vitro* are proving invaluable in the elucidation of a number of enzyme mechanisms, and these will be described before consideration of more physiological studies.

A. Inhibition of Phosphohydrolases and Transferases

The first evidence that vanadium can act as a potent inhibitor of enzyme-catalyzed phosphate ester displacement reactions was provided by Lienhard and co-workers, using ribonuclease (RNase).[15] Lienhard had suggested that uridine complexes of V(IV) and V(V) might resemble the trigonal bipyramidal structure of the transition state for RNase-catalyzed hydrolysis of uridine 2′,3′-phosphates. In a careful and detailed study, both free V(IV) and the uridine-V(IV) and V(V) complexes—but not free V(V)—were shown to be potent competitive inhibitors of RNase ($K_i \sim 10^{-5}$ M; see Table III). It was not possible to determine, however, whether inhibition was due to adoption of a transition state structure or simply to coordination of the V(IV) by imidazole groups at the active site. Both Zn(II) and Cu(II) inhibit RNase by the second mechanism.[28]

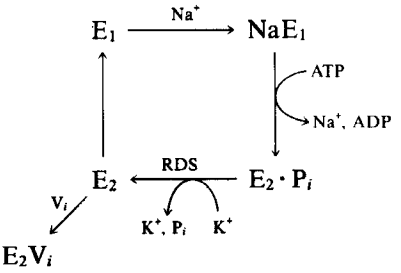
The transition state analog hypothesis has also been invoked to account for the potent inhibition of acid[29] and alkaline phosphatases[30] by vanadium (Table III). Again, however, Zn(II) will inhibit phosphatase activity, presumably through coordination with active site imidazoles, so that the mechanism of the effect of V(IV) and V(V) remains ambiguous. Moreover, it is not known what V(IV) species were involved in inhibition, since under the conditions reported, VO^{2+} would be extensively hydrolyzed. Clearly, x-ray crystallographic or EXAFS data will be required to resolve the issue.

The mechanism of action of all of the vanadium sensitive phosphohydrolases described so far involves an enzyme-phosphate intermediate in the catalytic cycle, a correlation that is maintained by vanadium-sensitive ATPases. Inhibition by vanadate was first described for the Na,K-ATPase.[4] As mentioned earlier, Na,K-ATPase is a plasma membrane protein that couples the net displacement of charge from one side of the membrane to the other [electrogenic transport of, in this case, Na^+ (efflux) and K^+ (influx)] to the hydrolysis of ATP (see simplified diagram below). The enzyme can exist in two states: E_1 and E_2. State E_1 binds Na^+, ATP and Mg^{2+}. The Na^+ ion promotes hydrolysis of

TABLE III
Inhibition of enzymes by vanadium

Enzyme	Probable form of E · V complex	$K_{1/2}$ for inhibition (μM)	Reference
Phosphohydrolases			
Alkaline phosphatase	E-His · V(V)	2	30
	E-His · V(IV)	0.4	
Acid phosphatase	E-His · V(V)	0.2	29
Ribonuclease	$E\begin{matrix}\diagup His_{12}\\ \diagdown Lys_{44}\end{matrix}$ V(V) · U	10	15
	$E\begin{matrix}\diagup His_{12}\\ \diagdown His_{119}\end{matrix}$ V(IV)	60	
Staphylococcal nuclease	E · dT · V(IV) · Ca	0.08	72
Na^+,K^+-ATPase	K · E_2-Asp · V(V) · Mg	0.004	31
Ca^{2+}-ATPase (plasma membrane)	(K) · E_2-Asp · V(V) · Mg	1.5	33, 34, 35
Ca^{2+}-ATPase (sarcoplasmic reticulum)	(K) · E_2-Asp · V(V) · Mg	5	33, 58
H^+-ATPase (fungal)	(K) · E_2-Asp · V(V) · Mg	0.5	36, 37
H^+,K^+-ATPase (gastric mucosa, colon epithelium)	K · E_2-Asp · V(V) · Mg	0.04	33, 70
H^+-ATPase (*Mycobacterium phlei*	?	5	71
Myosin ATPase	E_2 · ADP · V(V) · Mg	~0	39, 42
Dynein 1 ATPase	?	0.5	40, 41
G protein GTPase (regulatory subunit of adenylate kinase)	E_2 · GDP · V(V) · Mg?	$>10^3$?	43
Phosphotransferases			
Adenylate kinase	E · V_4O_9 · Mg?	<1	44
Phosphofructokinase	FbP · E · V(V) · Mg?	3	45
Phosphoglycerate mutase (2,3-bisphosphoglycerate-dependent)	BPG · E · V(V) · Mg?	5	46

the ATP and formation of an E_2–P_i (P_i = phosphate) intermediate. The intermediate then binds K^+, which promotes the breakdown and release of the phosphate (and Mg^{2+}) in the rate determining step (RDS) and reversion to E_1:



Vanadate binds to the phosphorylation site on Na,K-ATPase. Binding is inhibited by Na^+ or ATP, and facilitated by Mg^{2+} and K^+.[31] Once bound, the vanadate (V_i) traps the enzyme in the E_2 state. The off rate for vanadate from the enzyme is very slow, especially at low temperatures (0.045 h^{-1} at 4°C), and this fact has been exploited in a number of elegant studies of the catalytic mechanism.[6,32]

The Ca^{2+}-ATPases from sarcoplasmic reticulum[33] and the plasma membrane,[34,35] the K^+,H^+-ATPase from gastric mucosa,[33] the fungal H^+-ATPase isolated from *Saccharomyces cerevisiae*[36] and *Neurospora crassa*[37] are all rather similar and are closely related to Na,K-ATPase, both in structure and their mechanisms of ion transport. It is likely that they have a common evolutionary origin. All produce an E–P_i intermediate in the catalytic cycle, and all are inhibited by vanadate (Table III). In contrast, the bacterial and mitochondrial[33] H^+-translocating ATPases, for which no E–P_i intermediate is detectable, are insensitive to vanadate.

Inhibition by vanadate is very specific. Neither chromate, tungstate nor molybdate, nor a variety of polyphosphates, had any effect upon the activity of the *N. crassa* H^+-ATPase.[37] Nor does V(IV) appear to be a good inhibitor, at least of Na,K-ATPase.[38] The Mg^{2+} ion is universally required to facilitate inhibition, and it is likely that vanadate and adenine nucleotide binding are mutually exclusive.

The "translocator" ATPases—dynein ATPase (a part of the motor that drives eukaryotic flagellae) and myosin ATPase (from muscle)—present a slightly different picture which points to an interesting variation

upon the mechanism outlined above. The effect of vanadate on myosin ATPase was overlooked by many investigators because of the slow rate of onset of inhibition ($k_{app} \sim 0.3\ M^{-1}\ s^{-1}$ at 25°C), which has been interpreted as a conversion to an inactive E · ADP · V_i complex.[39] The dissociation rate for this complex is almost infinitely slow. Both dissociation and association of vanadate with dynein ATPase, on the other hand, are rapid[40] but as with myosin ATPase, the inactive species appears to be a ternary enzyme–nucleotide–vanadate complex. Both ATPases therefore appear to function by a similar mechanism which differs from that of the ion-transport ATPases in that the rate-determining step is the release of ADP and P_i from an E–P_i–ADP intermediate rather than the release of P_i from an E–P_i intermediate:

$$\begin{array}{l} \qquad\qquad\qquad\qquad\quad (E_2\text{–}V_i\text{–ADP}) \\ \qquad\qquad\qquad\qquad\quad \text{or} \qquad\quad \text{RDS} \\ \text{translocator: } E_1 + ATP \rightleftharpoons E_2\text{–}P_i\text{–ADP} \longrightarrow E_1 + ADP + P_i, \\ \text{transporter: } E_1 + ATP \rightharpoonup ADP + E_2P_i \overset{\text{RDS}}{\underset{\text{or}}{\rightleftharpoons}} E_1 + P_i. \\ \qquad\qquad\qquad\qquad\quad (E_2V_i) \end{array}$$

Na^+ accelerates the release of vanadate from Na,K-ATPase about 50-fold, by forcing the enzyme back into the E_1 state.[6] One might therefore predict that addition of actin to myosin-V_i-ADP will in a similar manner force the myosin into the ATP binding state and reverse the vanadate inactivation. Preliminary evidence for this effect has very recently been reported by Magid and Goodno.[42]

One surprising effect of vanadate, that can however be explained by a mechanism similar to that for inhibition of myosin ATPase, is the activation of adenylate cyclase.[43] This membrane-bound enzyme synthesizes cyclic AMP, which acts as a "second message" for several hormones. Cyclic AMP is removed by a phosphodiesterase, but the observed activation is not the result of inhibition of the esterase. Rather, vanadate appears to act through the so-called G protein, which is a GTP phosphohydrolase. G protein acts as the link between hormone receptors and adenylate cyclase. When GTP is bound, it activates adenylate cyclase; when GDP is bound, it does not. The G protein slowly hydrolyzes GTP to GDP + P_i. However, if vanadate is bound to E · GDP to form a stable, ternary E · GDP · V_i complex, the G protein would presumably behave as though GTP were bound, and activate adenylate cyclase.

A number of the physiological effects of vanadate are likely to be the result of this activation.

Of the enormous variety of phosphotransferases used by living organisms only three have so far been reported to be inhibited by vanadate: adenylate kinase,[44] phosphofructokinase,[45] and phosphoglycerate mutase[46] (Table III). It is probable that many others are susceptible. Adenylate kinase is of interest since it is more sensitive to decavanadate than to the monomeric species. The enzyme catalyzes the reaction:

$$\text{MgATP} + \text{AMP} \rightleftharpoons \text{MgADP} + \text{ADP}.$$

Phosphate exchange is direct, and the x-ray structure[47] shows the substrate phosphates to be situated between the adenine sites in a pocket lined with positively-charged residues. The oligomeric vanadate (probably $V_4O_{12}^-$ rather than $V_{10}O_{28}^{6-}$) binds in this pocket, with an affinity more than 500 times that of orthovanadate, and blocks the binding of both substrates.

There are two types of phosphoglycerate mutase, one of which requires 2,3-diphosphoglycerate (2,3-DPG) as cofactor, and only this type is vanadate sensitive.[46] The enzymes catalyze the isomerization of 3-PG and 2-PG: the DPG-dependent type by intermolecular transfer of phosphate between DPG and the substrates, and the other by an intramolecular transfer. An E–P_i intermediate can be detected in the DPG-dependent enzyme. Vanadate presumably mimics the transition state, forming an E · DPG · V_i ternary complex. Although an E–P_i intermediate may also be produced by the DPG-independent enzyme, the transfer occurs in a single step, without the substrate leaving the active site. There is thus no opportunity for vanadate to bind during the catalytic cycle.

B. Vanadyl–Protein Complexes

Several VO^{2+}–protein complexes have been prepared *in vitro* and studied by EPR spectroscopy using insulin,[48] carboxypeptidase,[49] bovine serum albumin,[9] transferrin[50] and apoferritin (N. D. Chasteen, personal communication). Because the topic is covered in depth by Chasteen,[9] who pioneered the technique, only a brief outline will be provided here. Vanadyl ion is a useful probe of metal-binding sites because of all the paramagnetic metal ion complexes, only those of VO^{2+} consistently

give sharp EPR spectra both in the frozen state and in solution. Moreover, since EPR parameters are known for a wide variety of VO^{2+} complexes, assignment of unknown ligands can be made with some certainty. In all of the proteins studied so far, the ligands that chelate the VO^{2+} are imidazole and carboxylic groups. The metal ions that are replaced include Zn^{2+}, Cu^{2+} and Fe^{3+}. Enzymatic activity is retained when VO^{2+} replaces the active site Zn^{2+} ion of carboxypeptidase A.[49]

C. Physiological Effects of Vanadium

The rich solution chemistry of vanadium suggests that its physiological effects are likely to be complex. It is therefore hardly surprising that the element is highly toxic.[3] Not all of the effects of exogenously added vanadium are yet understood, partly because the state of the element *in vivo* is not always known. After inoculation into whole animals, vanadium might be expected to remain as orthovanadate in plasma and other body fluids, but (after several days) it apparently occurs as a vanadyl–transferrin complex.[51] It is not known whether this complex can donate the vanadium to cells through the transferrin receptor. It is more likely that vanadium enters cells through phosphate or other anion transport systems as vanadate. Erythrocytes take up vanadate via the Band 3 protein,[52] the normal function of which is to catalyze Cl^-/HCO_3^- exchange. In tunicate blood cells an anion transport system exists that is specific for phosphate and vanadate.[53]

Within erythrocytes[5] and fat cells[54] and probably in all other cells also, the vanadate is slowly but quantitatively reduced to V(IV) by glutathione (GSH). The vanadyl ion is detectable by EPR spectroscopy. In erythrocytes it binds to hemoglobin.[38] In adipocytes it appears to be present largely as a complex with glutathione.[54] The physiological effects of intracellular vanadyl ion are unknown. However, reduction is slow, and many of the effects of added vanadium can be explained as interactions of intracellular vanadate with phosphohydrolases and transferases. Vanadate blocks chromosome movement in lysed mitotic cells, probably by inhibiting a dyneinlike ATPase.[55] Vanadate also acts as a powerful diuretic and natriuretic when injected into rats,[56] probably because it inhibits the Na,K-ATPase in the proximal tubule of the kidney. The effect diminishes with time, however, presumably as a result of reduction to V(IV).

Effects on other tissues appear to be caused by inhibition of Ca-

ATPase. In a number of cardiac muscle preparations (isolated ventricular and atrial muscles of rat, dog, cat and rabbit, and in cultured rat cardiac muscle cells) vanadate induces an increase in contractile force (positive inotropic effect); in cat and guinea pig left atrial muscle it produces a *negative* inotropic effect.[57,58] Ouabain, which specifically inhibits Na,K-ATPase, will also produce a positive inotropic effect in many cardiac preparations, but the effect of vanadate does not correlate with the inhibition of this enzyme. Indeed an *increase* in K^+ uptake *via* Na,K-ATPase has been observed.[59] Nor can it be fully explained by activation of adenylate cyclase. Although activation occurs on addition of vanadate, the consequent increase in cyclic AMP is insufficient to account for the magnitude of the positive inotropic effect.[60] Rather, we suggest that the effect is a result of inhibition of the Ca-ATPases of the sarcoplasmic reticulum and sarcolemma. Such inhibition should increase the cytosolic concentration of Ca^{2+}.

Calcium is the primary signal that triggers muscle contraction. High cytosolic Ca^{2+} will also open Ca^{2+}-sensitive K^+ channels present in the plasma membrane, increasing K^+ permeability and therefore hyperpolarizing the plasma membrane. Hyperpolarization will close Ca channels, and activate Ca efflux from the cells via the electrogenic Na/Ca antiport system. These effects will combine to decrease cytosolic Ca, thus acting as a negative feedback mechanism. The antiport system will increase cytosolic Na as Ca is removed, which will in turn activate the Na,K-ATPase. The scheme is presented in diagrammatic form in Figure 5. In support of the hypothesis, it should be noted that vanadate has already been shown to inhibit Ca-ATPase in intact squid axons[61] and to stimulate an increase in cytosolic Ca levels in skeletal muscle.[8] Such an increase has been proposed in explanation of the inhibition by vanadate of renin secretion from rat kidney slices.[62] It might also explain the remarkable observation that vanadate mimics the effects of insulin in muscle and fat cells, as measured by the increases in the rate of uptake of glucose analogs and of glucose oxidation, and by the inhibition of lipolysis.[7,8,54,63] It has been suggested that the actual mimetic species is vanadyl ion rather than vanadate.[7] The data, however, are by no means conclusive. Vanadyl sulfate was added to adipocytes buffered at pH 7.4 and equilibrated with air, under which conditions the vanadium would be immediately hydrolyzed and oxidized.[7] Moreover, no lag time was observed in the activation of glucose transport by added vanadate,[54] although reduction to V(IV) is a slow process ($t_{\frac{1}{2}} \sim 90$ min in red cells).[5]

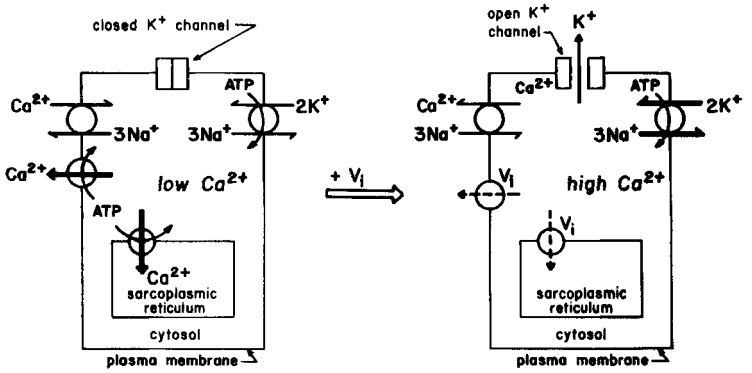


FIGURE 5 Effects of vanadate on ion transport processes in cardiac muscle cells—hypothetical mechanism for increased contractile force (positive inotropic effect) induced by vanadate. Thick arrows show active pumping; half-arrows show exchange; dashed arrows show inhibition of active pumping.

It would appear more likely that the active species is vanadate, and that its mode of action is to couple insulin receptors by oxidation of sulfhydryl residues to form a disulfide bridge. Other oxidizing agents, such as H_2O_2, also mimic insulin. Moreover, the insulin-occupied receptor is known to be disulfide cross-linked, and that dimerization of receptors is necessary for activity. Alternatively, as mentioned above, the effect might be the result of inhibition of Ca-ATPase by vanadate.

D. Endogenous Vanadium

Only 2 nM dietary vanadium is required for normal growth in rats, and 5 nM provides optimal growth of human fibroblasts in culture. Normal tissue concentrations are about 0.1–1 μM.[3,64] It is highly likely that most of this vanadium is present as V(IV) and is complexed to small molecules such as ATP or GSH, and to metalloproteins.[5,51,54] Endogenous vanadium cannot therefore function as a regulator of Na,K-ATPase activity, nor of the activity of other phosphohydrolases. Nor is it likely to function simply as a transition metal cofactor in the form of VO^{2+}. Vanadyl ion can replace, and could therefore presumably be replaced by, other transition metal ions of similar size and charge. One is left with the possibility that the redox behavior of the element, and particularly its conversion from a cation to an anion upon oxidation at phys-

iological pH, is somehow crucial to its biological role. Resolution of the question will depend upon more sophisticated investigation than has been applied so far.

Even in the sea squirts, which accumulate vanadium to extraordinary concentrations in specialized blood cells called vanadocytes, the biological function of the element remains mysterious. We know that the cells accumulate vanadate via a specific transport system in the plasma membrane,[53] that the vanadate is reduced to V(IV) [in some species to V(III)], and that it is stored at concentrations of up to 1 M within special vacuoles together with similar concentrations of a reducing agent, tunichrome.[65] The vanadium is perhaps stabilized by chelation to small molecules since the vacuoles are not particularly acidic, contrary to earlier reports. No metalloprotein is involved, however, and the so-called "hemovanadin" described in early studies is artifactual.

The vanadocytes do not function in the transport of oxygen,[67] nor are they involved in the manufacture of acid,[66] as once suggested. The early proposal that the tunicate's high acidity or high vanadium content, or both provide a chemical defense against predators has recently been carefully investigated.[68,69] Even though the vanadocyte blood cells are not acidic, cytolysis would cause the local sea water to increase in its concentration of acid and vanadate, both of which elicit antifeedant behavior.[68] Thus, if the vanadium and tunichrome (which hydrolyzes rapidly to produce acid solutions[65]) are relatively highly concentrated in a given species, they could play a role in chemical defense. The problem with assigning chemical defense to the function of vanadium is that many species with low, but still significant (i.e., 1–10 μM in the *whole* animal, much higher in the vanadocytes) concentrations of vanadium do not show chemical defenses against predators.[69] A more generalized function should exist to account for the occurrence of vanadocytes in so many tunicate species.

The most likely function is in the manufacture of the tunic material that forms an extracellular sheath around the organism. Exocytosis of the vacuole contents is probably involved, followed by a redox reaction with the tunic components, perhaps to generate cross-links. There is no definitive evidence for this hypothesis as yet. We can hope, however, that before many more reviews on vanadium biochemistry are written, the biological function of the element in at least one type of organism will finally be understood.

This article was written with the support (I.G.M.) of a grant from the Charles A. King Trust (Medical Foundation, Inc.), and with partial support (K.K.) from research grants PCM 7824782 from NSF, and GM 08893 from NIH.

KENNETH KUSTIN
Department of Chemistry,
Brandeis University,
Waltham, Massachusetts 02254

IAN G. MACARA
Department of Biochemistry and Molecular Biology,
Harvard University,
16 Divinity Avenue, Cambridge, Massachusetts 02138

References

1. I. G. Macara, K. Kustin and G. C. McLeod, Comp. Biochem. Physiol. **63 B,** 457 (1979).
2. H.-U. Meisch, W. Reinle and J. A. Schmitt, Z. Naturforschg. **33c,** 1 (1978).
3. P. M. Harrison and R. J. Hoare, in *Metals in Biochemistry* (Chapman and Hall, London 1980), pp. 1–10. If ingested at higher than trace levels or if inhaled, the element can be toxic. See, for example, T. G. Faulkner-Hudson, *Vanadium Toxcology and Biological Significance* (Elsevier, Amsterdam, 1964).
4. L. C. Cantley, L. Josephson, R. Warner, M. Yanagisawa, C. Lechene and G. Guidotti, J. Biol. Chem. **252,** 7421 (1977).
5. I. G. Macara, K. Kustin and L. C. Cantley, Biochim. Biophys. Acta **629,** 95 (1980).
6. R. Smith, K. Zinn and L. C. Cantley, J. Biol. Chem. **255,** 9852 (1980).
7. Y. Shechter and S. J. D. Karlish, Nature **284,** 556 (1980).
8. T. Clausen, T. L. Anderson, M. Stürip-Johansen and O. Petkova, Biochim. Biophys. Acta **646,** 261 (1981).
9. N. D. Chasteen, Biol. Mag. Res. **3,** 53 (1981).
10. S. L. Berger and C. S. Birkermeier, Biochemistry **18,** 5143 (1979).
11. C. F. Baes, Jr. and R. E. Mesmer, *The Hydrolysis of Cations* (Wiley, New York, 1976).
12. T. D. Tullius, W. O. Gillum, R. M. K. Carlson and K. O. Hodgson, J. Am. Chem. Soc. **102,** 5670 (1980).
13. E. Boeri and A. Ehrenberg, Arch. Biochem. Biophys. **50,** 404 (1954).
14. J. Francavilla and N. D. Chasteen, Inorg. Chem. **14,** 2860 (1975).
15. R. N. Lindquist, J. L. Lynn and G. E. Lienhard, J. Am. Chem. Soc. **95,** 8762 (1973).
16. C. J. Ballhausen, *Introduction to Ligand Field Theory* (McGraw-Hill, New York, 1962), pp. 230–233.
17. A. M. Chmelnick and D. Fiat, J. Magn. Res. **8,** 325 (1972).
18. S. Cooper, Y. B. Koh and K. N. Raymond, J. Am. Chem. Soc., in press.

19. F. A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry* (Wiley, New York, 1980) 4th ed.
20. K. Wütrich and R. E. Connick, Inorg. Chem. **6,** 583 (1967).
21. (a) W. R. Scheidt, C. Tsai and J. L. Hoard, J. Am. Chem. Soc. **93,** 3867 (1971); (b) W. R. Scheidt, D. M. Collins and J. L. Hoard, *ibid.* 3873; (c) W. R. Scheidt, R. Countryman and J. L. Hoard, *ibid.* 3878.
22. K. Kustin and D. L. Toppen, J. Am. Chem. Soc. **95,** 3564 (1973).
23. (a) K. Gilbert and K. Kustin, J. Am. Chem. Soc. **98,** 5502 (1976); (b) S. Funahashi, Y. Kato, M. Nakayama and M. Tanaka, Inorg. Chem. **20,** 1752 (1981).
24. M. P. Whittaker, J. Asay and E. M. Eyring, J. Phys. Chem. **70,** 1005 (1966).
25. K. H. Johnson, Ann. Rev. Phys. Chem. **26,** 39 (1975).
26. C. J. Ballhausen and A. D. Liehr, J. Mol. Spectr. **2,** 342 (1958).
27. N. Sutin, Ann. Rev. Nucl. Sci. **12,** 285 (1962).
28. C. A. Ross, A. P. Mathias and B. R. Robin, Biochem. J. **85,** 145 (1962).
29. R. L. Van Etten, P. P. Waymack and D. M. Rehkop, J. Am Chem. Soc. **96,** 6782 (1974).
30. V. Lopez, T. Stevens and R. N. Lindquist, Arch. Biochem. Biophys. **175,** 31 (1976).
31. L. C. Cantley, L. G. Cantley and L. Josephson, J. Biol. Chem. **253,** 7361 (1978).
32. C. T. Carilli, R. A. Farley, D. Perlman and L. Cantley, J. Biol. Chem. **257,** 5601 (1982).
33. S. C. O'Neal, R. B. Rhonds and E. Racker, Biochem. Biophys. Res. Commun. **89,** 845 (1975).
34. G. H. Bond and P. M. Hudgins, Biochim. Biophys. Acta **600,** 781 (1980).
35. H. Barrabin, P. J. Garrahan and A. F. Rega, Biochim. Biophys. Acta **600,** 796 (1980).
36. J.-P. Dufour, M. Boutry and A. Goffean, J. Biol. Chem. **255,** 5735 (1980).
37. B. J. Bowman and C. W. Slayman, J. Biol. Chem. **254,** 2928 (1979).
38. L. C. Cantley and P. Aisen, J. Biol. Chem. **254,** 1781 (1979).
39. C. C. Goodno, Proc. Natl. Acad. Sci. USA **76,** 2620 (1979).
40. I. R. Gibbons, M. P. Cosson, J. A. Evans, B. H. Gibbons, B. Huck, K. H. Martinson, W. S. Sale and W.-J. Y. Tang, Proc. Natl. Acad. Sci. USA **75,** 2220 (1978).
41. O. Makato, J. Cell Biol. **85,** 712 (1980).
42. A. Magid and C. C. Goodno, Biophys. J. **37,** 107a (1982).
43. U. Schwabe, C. Puchstein, H. Hanneman and E. Söchtig, Nature **227,** 143 (1979).
44. E. G. DeMaster and R. A. Mitchell, Biochemistry **12,** 3616 (1973).
45. G. L. Choate and T. E. Mansow, Fed. Proc. **37,** 1433 (1978).
46. J. Carreras and R. Bartrons, Biochem. Biophys. Res. Commun. **96,** 1267 (1980).
47. E. F. Pai, O. W. Sachsenheimer, R. H. Schirner and G. E. Schulz, J. Mol. Biol. **114,** 37 (1977).
48. N. D. Chasteen, R. J. DeKoch, B. L. Rogers and M. W. Hanna, J. Am. Chem. Soc. **95,** 1301 (1973).
49. R. J. DeKoch, D. J. West, J. C. Cannon and N. D. Chasteen, Biochemistry **13,** 4347 (1974).
50. (a) J. D. Casey and N. D. Chasteen, J. Inorg. Biochem. **13,** 111 (1980); (b) *ibid.*, 127.
51. E. Sabbioni and E. Marafonte, Bioinorg. Chem. **9,** 389 (1978).
52. L. C. Cantley, M. Resh and G. Guidotti, Nature **272,** 552 (1978).
53. A. L. Dingley, K. Kustin, I. G. Macara and G. C. McLeod, Biochim. Biophys. Acta **649,** 493 (1981).
54. H. Degani, M. Gochin, S. J. D. Karlish and Y. Shechter, Biochemistry **20,** 5795 (1981).
55. W. Z. Carde and S. M. Wolniak, Cell. Biol. **79,** 573 (1978).

56. W. E. Balfour, J. J. Grantham and I. M. Glynn, Nature **275,** 768 (1978).
57. U. Borchard, A. A. L. Fox, K. Greeff and P. Schlieper, Nature **279,** 339 (1979).
58. A. Schwartz, R. J. Adams, I. Gripp, G. Gripp, M. J. Holroyde, R. W. Millard, R. J. Solaro and E. T. Wallick, Basic Res. Cardiol. **75,** 444 (1980).
59. K. Werdan, G. Bauriedel, M. Bozsik, W. Krawietz and E. Erdman, Biochim. Biophys. Acta **597,** 364 (1980).
60. W. Schmitz, I. Hockbarth, H. Scholz, and E. Wetzel, Basic Res. Cardiol. **75,** 438 (1980).
61. R. DiPolo, H. R. Rojas and L. Beaugé, Nature **281,** 228 (1979).
62. P. C. Churchill and M. C. Churchill, J. Pharmacol. Exp. Ther. **213,** 144 (1980).
63. G. R. Dubyak and A. Kleinzeller, J. Biol. Chem. **255,** 5306 (1980).
64. I. G. Macara, Trends Biochem. Sci. **5,** 92 (1980).
65. I. G. Macara, K. Kustin, G. C. McLeod, Biochem. J. **181,** 457 (1979).
66. A. L. Dingley, K. Kustin, I. G. Macara, G. C. McLeod and M. R. Roberts, Biochim. Biophys. Acta, in press.
67. I. G. Macara, K. Kustin and G. C. McLeod, Comp. Biochem. Physiol. **62A,** 821 (1979).
68. D. Stoecker, Ecology **61,** 1327 (1980).
69. D. Stoecker, Mar. Ecol. Prog. Ser. **3,** 257 (1980).
70. M. C. Gustin and D. B. P. Goodman, J. Biol. Chem. **256,** 10651 (1981).
71. F. Yoshimura and A. F. Brodie, J. Biol. Chem. **256,** 12239 (1981).
72. P. W. Tucker, E. E. Hazen and F. A. Cotton, Mol. Cell Biochem. **23,** 67 (1979).