# CHAPTER II

# BACKGROUND INFORMATION

## Vanadium and Pharmacokinetic

Vanadium (atomic weight 50.942) is a member of the Vb group of elements in periodic table and shares several features in common with elements in Va group (nitrogen, phosphorus, arsenic). Vanadium occurs naturally in the trivalent state as relatively insoluble salts, the average concentration in the geosphereis 110 p.p.m.. The element is found in a variety of plants and animals, many of which are ingested by human being (Schroeder, Balassa, and Tipton, 1963). It is probably an essential trace element for animals. To date, these elements have not been shown essential for man. However, it seems probable that they have an essential function in human nutrition and metabolism (Hopkins and Mohr, 1974; Nielsen and Sandstead, 1974).

Vanadium forms somewhat labile chelated, bonding to oxygen in higher valence states and to nitrogen in lower. It is a strong reducing agent. In solution vanadium forms oxyanions that resemble the more familier phosphate compounds. Vanadate exists in different forms depending on the pH. The precise structure of inorganic orthovanadate oxyanions is not known, since the compounds form large aggregates at concentrations in the millimole rate. In biological fluids oxyvanadium exists principally in two redox states. Vanadium (V) (vanadate, $HVO_4^{2-}$) is formed spontaneously in atmospheric oxygen from vanadium (IV) (vanadyl, $VO^{2+}$). Vanadate reductase in cell membrances may facilitate reduction to the vanadyl state. The strong binding of vanadyl to phosphates which appears to be protected from oxidation to vanadate is,

at least in part, the basis for intracellular vanadium accumulation and distribution. Moreover, vanadium can be held in its reduced vanadyl form in the presence of relatively strong reducing agents, e.g., sulfite, epinephrine, norepinephrine, DOPA, dopamine, ascorbic acid suggest the possibility that the metal may be a natural cofactor in a biological reaction involving the compound. There is evidence to indicate that intercellular vanadium is principally in the vanadyl form, whereas in extracellular fluids it is principally in vanadate form (Schroeder, Balassa, and Tipton, 1963; Grantham, 1980; Nechey et al., 1986).

Oxyvanadium compounds are absorbed from intestine, respiratory or percutanous and eliminated principally by the kidney. However, because most of the ingested vanadium elimination is via the feces. Most mammalian tissues contains traces of vanadium; the kidney, however, contains the highest concentration, especially in the cortex. Vanadium is also found in substantial amounts in fat, liver and bone, the quantities being related to the size of these tissue reservoirs (Bogden et al., 1982).

## Action of Vanadium Compounds on Enzymes

Vanadium is both a physiologically and pharmacologically active substance by low concentration. A functional role, however, has not been identified. It has been suggested that vanadate may serve as a regulator or modifier of many enzymes and thus play a vital role in intermediary metabolism (Nechay et al., 1986).

### 1. Inhibition of Phosphoenzyme Ion-Transport ATPases

This class of intrinsic membrane enzymes has two principal conformations called $E_1$ and $E_2$. In the $E_1$ conformation the enzyme appears to be the catalytic

portion, since it is phosphorylated by ATP at binding site of high affinity and in the $E_2$ conformation is phosphorylated by $P_i$ at binding site of low affinity. The latter reaction is called "back door" phosphorylation. The acceptor site is the beta-carboxyl group of the same aspartyl residue in both cases. All members of this class are inhibited by vanadate (Nechay et al., 1986).

The enzymatic features of $Na^+$-$K^+$ATPase may normally undergo a consecutive series of reactions in which ATP is bound to a high affinity site ($E_1$.ATP), the enzyme is phosphorylated by the nucleotide in the process of binding to three intracellular sodium ion [$E_1$.P.(3Na)], and is dephosphorylated as two extracellular potassium ion is bound [$E_2$.(2K)], thereby liberating sodium ion to the extracellular medium. Potassium ion is released to the inside as intracellular ATP is bound with relatively low affinity to the enzyme (Grantham, 1980). Vanadium potently inhibits $Na^+$-$K^+$ATPase from variety of tissues and species same as a deadend transition state analog of organic phosphate. It combines reversibly with the sodium pump possibly at binding sites of high and low affinity from the cytoplasmic side of the enzyme by it enters the reaction sequence via the back door and forms a stable inactive complex in a reversible reaction with the $E_2$ conformation of the enzyme in a stoichiometry of one vanadate bound per active center for phosphorylation, slowing the conformational change to [$E_1$.K.ATP]. Increasing extracellular potassium ion and magnesium ion concentrations and reducing extracellular sodium ion concentrations potentiate the inhibition caused by vanadate. On the other hand, cytoplasmic vanadate slows the operation of the pump by altering the affinity for extracellular potassium and by locking the dephosphoenzyme in a configuration that markedly delays rephosphorylation. Marked slowing of the catalytic rate by an elevation of the extracellular potassium concentration in the physiologic range is a cardinal feature of the vanadilized sodium pump. If the inhibition of $Na^+$-$K^+$ATPase by vanadate has a physiological role, the degree of inhibition could

be controlled by altering the amount of available vanadium, by altering the fraction of vanadium in the fully oxidized form, or by altering the local extracellular potassium concentration (Grantham and Glynn, 1979; Grantham, 1980; Werdan et al., 1982; Noel and Souto Pardon, 1989).

At the present time the simplest single criterion for membership in the family of phosphoenzyme ion-transport ATPases appears to be an aspartyl residue at the active site of phosphorylation. Vanadate not only has been inhibiting $Na^+$-$K^+$ ATPase but it also has been inhibiting other phosphoenzyme ion-transport ATPases in the following : $Ca^{2+}$ATPase of sarcoplasmic reticulum, the high affinity ATP-dependent $Ca^{2+}$ efflux in human red cell membrane (Rossi, Garrahan, and Rega, 1981), $Ca^{2+}$-$Mg^{2+}$ATPase of the ascites and human red cell plasma membranes, $Mg^{2+}$ATPase of the ascites plasma membrane, and it is potentiated by intracellular magnesium ion and potassium ion but extracellular calcium concentrations antagonizes the inhibitory effect of vanadate, the $K^+$ATPase of ***Escherichia coli*** and hog gastric mucosal cell membranes (O'Neal, Rhoads, and Racker, 1979; Wierichs, Hagenmeyer, and Bader, 1980), the $H^+$-$K^+$ATPase of gastric mucosal cell membranes (Rabon and Reuben, 1990) and rat cortical and medullary collecting tubules in distal nephron segments (Garg and Narang, 1988; Dafnis et al., 1992), $H^+$ATPase of the turtle bladder cell membrane (Arruda, Sabatini, and Westenfelder, 1981).

### 2. Inhibition of the Other Enzymes

Acid and alkaline phosphatases of the ***Escherichia coli*** which have been implicated in the control of protein phosphotyrosine content of phosphotyrosine protein phosphatase and ***Helix pomatia*** aryl sulfatase can be inhibited by vanadate (Stankiewicz and Gresser, 1988). The cytoplasmic motility contractile protein,

dynein ATPase is selectively inhibited by vanadate, and actomyosin ATPase can be inhibited by vanadate even though it does not require a convalent phosphoenzyme intermediate (Kobayashietal., 1978). The inhibition of cathepsin D, the ATP-activated lysosomal enzyme proteolysis, at acid pH by vanadate has no relationship to inhibition of proteases and/or ATP dependence of such enzymes (Pillai and Zull, 1985). Vanadate has been found to be a potent inhibitor of both the glucose-6-phosphate phosphohydrolase and the glucose-6-phosphate phosphotransferase in both microsomal preparations and in situ using permeable isolated hepatocytes (Singh, Nordlie, and Jorgenson, 1981).

### 3. Stimulation of Enzymes Activity

Vanadate may affect other nucleotide triphosphatases and cause activation of adenylate cyclase by inhibiting a guanosine-3-thiotriphosphatase (GTPase) associated with the guenylnucleotide binding protein from isolated rat fat cells membrane (Schwabe et al., 1979), and rat adrenal membrane (Hayashi and Kimura, 1986). Vanadate has been stimulated the incorporation of [$^3$H] thymidine into acid-insoluble residues (DNA), increased the incorparation of [$^3$H] proline into collagenase-digestible protein (CDP), increased the labeling of noncollagen protein (NCP) syntheses in cultures rat calvariae (Canalis, 1985). Vanadate enhances the phosphorylation of the insulin receptor by stimulating the tyrosine kinase reaction. It has been activating rat adipocyte glycogensynthase (Tamura et al.,1984; Kadota et al., 1987). Vanadate exhibits a kinase C-independent mechanism for stimulation of the $Na^+/H^+$exchange, inhibitable by cyclosporin A (Daniel and Ives, 1987).

Vanadium compounds are versatile at forming complexes that inhibit or stimulate activity of many enzymes by specific mechanisms as inorganic phosphate.

## Cardiovascular Effects of Vanadium

Effect of vanadate on hemodynamic has been postulated to play a role in the etiology of volume-expanded, low-renin type of hypertension (López-Novoa et al., 1982; Sundet et al., 1984). It has been shown that intravenous or intracoronary-arterial infusion of vanadate reduced cardiac output, the latter resulting from both decreased heart rate and stroke volume (Inciarte et al., 1980; López-Novoa and Garrido, 1986). The decreased heart rate apparently was produced by no direct effect on the cardiac rate (chronotropic) mechanism of the heart, but some indirect mechanism, perhaps arterial baroreceptor reflex (Hom, Chelly, and Jandhyalo, 1982). The decreased stroke volume seemed unlikely entirely from a rise in afterload, in part from a fall in left ventricular preload subsequent to pulmonary vasoconstriction. A possibility was that contractility decreased, perhaps subsequent to the increased coronary resistance and decreased coronary flow and myocardial tissue $Po_2$ and pH (Inciarte et al., 1980). A striking difference in the force of cardiac contraction (inotropic) direct actions of vanadate on myocardium in isolated heart preparations strongly produced negative inotropic effects in the atria though it produced positive inotropic effects in the ventricular myocardium. On the other hand, vanadate reduced the force of contraction of stimulated left atria and spontaneously beating right atria, however, it increased the force of contraction of papillary muscles of ventricular myocardium. This disociation correlated well with changes in transmembrane potential (Borchard et al., 1979). In addition to vascular effects of vanadium increased the arterial blood pressure, the latter resulting from produced potent vasoconstriction. Total peripheral, pulmonary, coronary, and renal resistances rose and cardiac output fell by the cardiovascular actions of vanadate so blood flow to organs fell (Inciarte et al., 1980; López-Novoa and Garrido, 1986; Sánchez-Ferrer et al., 1988).

Intravenous and intrarenal arterial infusion of vanadate raised the resistance to flow and the latter was associated with reduced urine flow and glomerular filtration rate (Higashi and Bello-Reuss, 1980; Hatfield and Churchill, 1981; López-Novoa, Mayol, and Martínez-Maldonado, 1982; Benabe, Cruz-Soto, and Martínez-Maldonado, 1984).

The mechanism of the hemodynamic actions of vanadate is still not clear. It is still possible that the positive inotropic action of vanadate on ventricular myocardium caused by an increase in intracellular cyclic-AMP, however, the negative inotropic effect of vanadate in atria which occurs at concentrations seems to be due to a shortening of the potential (Borchard et al., 1979). Inhibition by vanadate of $Na^+$-$K^+$ATPase would lead to cell deplolarization in smooth muscle causing increased intracellular sodium ion and extracellular potassium ion concentration and also increased calcium influx or slowed sodium-calcium exchange causing decreased calcium ion efflux from the cell. Other differences might be related to actions of vanadate that are independent of $Na^+$-$K^+$ATPase. Vanadate also inhibited calcium pumps of sarcoplasmic reticulum, which was involved in active extrusion of intracellular calcium in red cell membrane, could also reduce calcium efflux from the cytoplasmic of muscle cell (Rossi, Garrahan, and Rega, 1981; Sánchez-Ferrer et al., 1988). The increase in cytoplasmic calcium ion from any of these mechanisms may serve as a stimulus for vasoconstriction. Vanadium was also reported to stimulate monoamine oxidase and inhibited norepinephrine uptake by autonomic nerve terminals (Schroeder, Balassa, and Tipton, 1963). On the other hand, it might released noradrenaline from adrenergic sympathetic nerve ending or increased noradrenaline concentration in the cleft between the terminal and smooth muscle cell (Inciarte et al., 1980; Sánchez-Ferrer et al., 1988). It has been suggested that vanadate can produce cardiovascular alterations by a mechanisms mediated via the autonomic sympathetic nervous system (Hom, Chelly, and Jandhyalo, 1982). These hemodynamic changes was accompanied by a decrease in

renin secretion (López-Novoa et al., 1982; Jadhav and Jandhyalo, 1983). The catecholamine and renin are hormone principally to lodge in renal vessels controlling renal hemodynamics that are indirectly actions of vanadium. A small part of the increase in total peripheral resistance could have resulted from an increase in blood viscosity subsequent to the rise in hematocrit. The latter might reflect splenic contraction and transcapillary fluid efflux.

## Renal Effects of Vanadium

Trace amounts of vanadium can be detected in most mammalian tissue; the highest concentrations are often found in the kidney, especially in the renal cortex. It is excreted in the urine and is accumulated by renal cells in the course of its elimination (Bogden et al., 1982). When infused intravenously or intrarenally into rats, it also causes a remarkable natriuresis and diuresis, though during the initial infusion there is generally evidence of cardiovascular instability (Day et al., 1980; Higashi and Bello-Reuss, 1980; Hatfield and Churchill, 1981; Westenfelder, Hamburger, and Garcia, 1981). Since profound diuresis is seen even when the GFR is unchanged, vanadate must inhibit sodium and water absorption in renal tubules rather than cause natriuresis and diuresis by processes tied only to hemodynamic alterations. In rats, vanadate inhibited primarily proximal tubules sodium, chloride, water, bicarbonate and glucose reabsorption and p-aminohippurate (PAH) secretion. Vanadate depressed free water formation and sodium reabsorption and potassium secretion along the ascending limb of Henle's loop, it would interfere with urinary concentration (Higashi and Bello-Reuss, 1980; Westenfelder, Hamburger, and Garcia, 1981; Edwards and Grantham, 1983a). Vanadate does not affect the AVP-induced increase in hydroosmotic permeability at a site distal to cyclic-AMP formation when they were perfused with hypotonic vanadate-containing solutions, but water flow was

suppressed if the tubules were preincubated with the compound in the serosal solution. Vanadate caused a rapid decrease in the lumen-negative transepithelial voltage which would be decrease free water and sodium reabsorption in the cortical collecting tubule (Edwards and Grantham, 1983b) and in toad bladder (Arruda and Westenfelder, 1983). There are, however, marked differences in the response among different species. The diuresis and natriuresis effect of vanadate appears to be easier to demonstrate in rat than other animals. The cardiovascular effects of vanadate evidently predominate in dogs and cats, to the extent that decreased GFR, oliguria and antinatriuresis are routinely seen. It has been suggested that fractional reabsorption of sodium could be decreased in proximal tubule, but the excess sodium and water reabsorption occured in distal sites of the nephron (López-Novoa, Mayol, and Martínez-Maldonado, 1982). The mechanism of the renal tubular functions of vanadate is a directly result of the actions of renal tubular cells ATPase system or indirectly interfering hormone to lodge principally in the renal tubules. It has been suggested that vanadate inhibited $Na^+$-$K^+$ATPase and $Ca^{2+}$ATPase in peritubular membrane and phosphotransferase and phosphohydrolase reaction but activated adenylated cyclase activity.

Distal renal tubular acidosis is common among Thai citizen in the Northeastern part of Thailand, representing one of the national health problems. Northeastern Thailand is the area known to have high vanadium content. Vanadium was also present in the urine of 30% of the people studied. There is a tendency to the development of potassium depletion during heat exposure in summer, inhibition of $H^+$-$K^+$ATPase in the cortical collecting tubule by vanadium is a possibility (Visith Sitprija et al., 1990; Dafnis et al., 1992). Vanadate may accentuate the effect of metabolic acidosis. Conversely, metabolic acidosis may enhance the effects of vanadate on renal acidification (Kannika Chankasem, 1991). However, inhibition of $Na^+$-$K^+$ATPase by vanadate should result in hyperkelemia, and if one of the collecting tubule $H^+$ATPase

and $Na^+$-$H^+$ exchange are also inhibited, hyperkelemia distal renal acidosis will result (Dafnis, and Sabatini, 1994).

## Toxicology of Vanadium

Vanadium is widely distributed in the environment and occurs in various concentrations in soil, water, air, plants, and animal tissues. Natural sources of airborne vanadium are belived be continental dust and marine aerosols. Vanadium and its compounds possess many valuable chemical and physical properties that have resulted in increased production and use in recent years and thus interesting in the toxicology of vanadium. Toxic effects in humans and animals under natural conditions occur infrequently. In human, vanadium toxicity is almost always associated with industrial processes, whereas in animals the only reported effects of a "natural" vanadium toxicity from the use of contaminated phosphate in diets for chicks (Nechay et al., 1986).

The major effects of vanadium in humans after industrial exposure are primarily irritations to the mucous membranes of the eyes, nose, throat, and respiration tract. Bronchitis and bronchospasm are characteristic symptoms, and pneumonia occasionally develop. Likewise, vanadium has been shown to produce gastrointestinal distress by cramping and diarrhea, fatigue, cardiac palpitation, and kidney damage as well as other physiological effects such as disturbances of the central nervous system, cardiovascular changes, and metabolic alterations (Zenz, Bartlett, and Thiede, 1962).

In general, the toxicity of vanadium is high when given by injection, low by the oral route, and intermediate by the respiratory tract. Toxicity also varies considerably with the nature of the compound, but it increases as valency increases, the pentavalent vanadium being the most toxic (Nechay et al., 1986).

## Role of the Renal Nerve and Hormone on Renal Functions

### 1. Innervation of the Renal Vessels

The renal nerves travel along the renal blood vessels as they enter the kidney. They contain many sympathetic efferent fibers and a few afferent fibers of unknown function. There also appears to be a cholinergic innervation via the vagus nerve, but its function is uncertain. The transmitter crosses the cleft and at those synaptic juntions where acetylcholine is an excitatory mediator, it acts on receptors on the membrane of the postsynaptic cell to increase the permeability of the membrane to sodium ion. In sympathetic ganglia, small amounts of acetylcholine stimulate postganglionic neurons and large amounts block transmission of impulses from pre- to postganglionic neurons. The sympathetic preganglionic innervation comes primarily from the lower thoracic and upper lumbar segments of the spinal cord, and the cell bodies of the postganglionic neurons are in the sympathetic ganglion chain, in the superior mesenteric ganglion, and along the renal artery. The sympathetic fibers are distributed primarily to the afferent and efferent arterioles. However, noradrenergic nerve fibers also end in close proximity to the renal tubular cells and the juxtaglomerular cells (Muller and Barajas, 1972). Adrenergic receptors is initially classified as alpha and beta receptors according to hemodynamic actions of different catecholamine. Alpha receptors have been subdivided into alpha-1 and alpha-2 on the basis of organ selectivity of alpha-agonist drug, whereas beta receptors have been subdivided into beta-1 and beta-2 on the basis of organ selectivity of beta-agonist drug. The alpha-1 receptors produce their effects by increasing intracellular calcium ion via activation of phospholipase C, whereas alpha-2 receptors produce their effects by inhibiting adenylate cyclase and thus decreasing intracellular cyclic AMP. The effect of both types of beta receptors stimulation are brought about by activation of adenylate cyclase via $G_s$, with a conseuent increase in

intracellular cyclic AMP. In noradrenergic neurons, there are, alpha-2 receptors are generally presynaptic sites that, when activated, decrease norepinephrine release, whereas the beta-2 receptors are generally presynaptic sites that facilitate norepinephrine release. The two subtypes of alpha and beta receptors occur in the postsynaptic structures (Starke, 1977).

### 2. The Actions on Renal Functions

Stimulation of the renal nerves caused renal vasoconstriction in both cortex and medulla and a marked decrease in renal blood flow. This effect is mediated by alpha-1 adrenoceptors which respond preferentially to catecholamine released from sympathetic nerve endings and to a lesser extent by postsynaptic alpha-2 adrenoceptors which respond primarity to circulating and actions of catecholamine (Kopp, Bradley, and Hjemdahl, 1983). In addition to producing renal vasoconstriction, stimulation of the renal nerves and latter associate to rise in catecholamine levels increases renin secretion from the juxtaglomerular cells, primarily via a beta-1 adrenergic receptors that is probably located on these membranes of cell (Osborn, Gerald, and Thames, 1981). In addition, stimulation of the renal nerves increases the reabsorption of sodium and water in the tubules. Since these actions are independent of the renin-angiotensin system, postaglandins, a consequent reduction in hydroosmotic pressure in the capillaries which may also play a role in glomerulotubular balance and can, at least in part, they are probably due to a direct action of catecholamines on the renal tubules. It is still unsettled whether the effects on sodium and water reabsorption are mediated via alpha- or beta- adrenoceptors, and they may be mediated by both (Young et al., 1984).

## Role of the Sodium Pump and Calcium Ion Channels on Renal Functions

### 1. The Sodium Pump

There is abundant evidence to show that the sodium pump, $Na^+$-$K^+$ ATPase, is a unique enzyme that spans principally in the basolatral cell membranes in different tissues, at least in part, in renal vasculature and all renal tubular segments which are found in highest concentrations in the medullary ascending limbs of Henle's loop. Each sodium pump unit is activated to transport maximally when intracellular sodium ion, magnesium ion, and ATP and extracellular potassium ion are present in appropriate concentrations. In a single pump cycle the enzyme appears to pass through a consecutive series of phosphoryl transfers and conformational changes tied to the exchange of three intracellular sodium ions for two extracellular potassium ions as one molecule of ATP is hydrolyzed. Active cation transport is dependent on the availability of these substrated to the pump, although intracellular sodium ion is the principal one that varies in the physiologic range to a sufficient extent to alter the pump rate. The transport rate may also be modulated by increasing the density of pumps in the membranes, a relatively slow process dependent on the synthesis of additional catalytic units, or more rapidly by substances that change the affinities of the enzyme for sodium ion or potassium ion. The pump generated a gradient for sodium between cytoplasm and extracellular fluid that favors diffusion of sodium ion into the cells from both the luminal and basolateral surfaces. There may be some sodium absorbed passively in conjunction with transtubule gradients of other molecule into cells. Thus the $Na^+$-$K^+$ATPase provides the energy for seconary active transport processes utilizing the potential energy stored in the electrochemical gradients, such as the $Na^+$-$H^+$-exchange, thereby playing a major role in transmembraneous and transepithelial transport processes of many inorganic and organic constituents (Grantham, 1980).

The $Na^+$-$H^+$ antiport is an ubiuitous ion-transport system which serves a wide range of important functions, including regulation of intracellular pH and volume, and the reabsorption of sodium and bicarbonate by the proximal tubule. The stiochiometry of the antiporter is 1:1 and it is therefore electrically neutral. The antiporter can mediate exchange in both directions, dependent upon the direction of the electrochemical gradients. The antiport mediates exchange of an external sodium ion for an internal hydrogen ion, due to the steep inward electrochemical gradient for sodium ion. Besides mediating $Na^+$-$H^+$exchange, the antiporter is able to mediate $Na^+$-$Na^+$, $Na^+$-$NH_4^+$ exchange. Amiloride in micromolar concentrations has been found in most studies to inhibit $Na^+$-$H^+$ exchange (Grinstein and Rothstein, 1986).

In general two classes of sodium ion channels have been identified. Voltage-gated sodium ion channels are found in the cell membranes from excitable tissue like muscle and nerve, whereas the typical amiloride-sensitive sodium ion channels are mostly found in the apical membrane of the pars recta of the proximal tubule and the collecting duct. Both aldosterone and vasopressin have been found to increase the activity of the channels. Part of these effects may be mediated through changes in intracellular calcium ion, cAMP, and pH, all of which have been shown to influence channel activity (Garty and Benos, 1988).

The NaCl/KCl (Na/K/2Cl) cotransport is present in the cell of medullary and cortical thick ascending limbs of Henle's loop. It has been shown to be of major importance in cell volume regulation in several cell systems, and is responsible for the major portion of NaCl reabsorption in the thick ascending limb (TAL) of Henle's loop. The Na/K/2Cl cotransporter is inhibited by loop diuretics like furosemide and bumetanide. The activity of the Na/K/2Cl cotransporter in the medullary TAL is regulated by vasopressin. The increased reabsorption of NaCl seen after vasopressin is

probably indirectly mediated through a cAMP dependent increase in the basolateral chloride ion conductance. Other hormones like glucagon, calcitonin, and parathyroid hormone, which also increase intracellular cAMP likewise lead to an increase in the transport rate (Greger, 1985).

The sodium-glucose and sodium-amino acid cotransport systems are present in the apical membrane of the renal proximal tubule. They mediate cellular uptake of their respective organic compounds, energized by the inward sodium gradient (Kinne et al., 1975).

2. Calcium Ion Channels

Electrophysiological studies have shown unequivocally that the plasma membrane of cells that respond to stimuli with changes in membrane potential channels that selectively allow the entry of calcium ions down its electrochemical potential gradient (Reuter, 1983). These channels have two main functions: to allow the participation of calcium ions currents in the rising phase of the action potential; and to allow the inflow of extracellular calcium ions that leads to the rise in cytoplasmic ions concentration and the consequent cellular response to the stimulus. Calcium ion channels are controlled by voltage-deppendent gating, that is, their opening or closing kinetics are a consequence of changes in membrane potential. The channels show both time- and voltage-dependent inactivation. Usually they open at more positive membrane potentials than the sodium pump which participate in the rising phase of the action potential. Although calcium ion channels are primarily regulated by the membrane potential, their properties are modulated by neurotransmitters, hormone, and drugs. In some cases, modulation seems to imply phosphorylation of the channels or of membrane proteins closely associated with it. Calcium ion channel are detected in



sacroplasmic reticulum and plasma membrane of smooth muscle and all renal tubular segments ; the highest activity are found in distal convoluted tubule (Doucet and Katz, 1982).

### 3. The Actions on Renal Functions

The sodium pump has been playing a major role in transmembraneous and transepithelial transport processes which serves a wide range of important functions, mediately transcellular of inorganic and organic compounds by the inward sodium electrochemical gradient. Inhibiting of sodium pump would lead to cell depolarization in excitable tissue like smooth muscle and nerve. The flow of calcium ions into cytosol that follows the stimulus is a net down an electrochemical membrane potential gradient. The probability that calcium ion channel will open increase steeply with depolarization. Inhibiting of sodium pump would lead to cell depolarization in smooth muscle by increasing calcium influx. Inhibition of calcium ion channel by vanadate could also reduce calcium efflux from the cytoplasm of muscle cell. The increase in cytoplasmic calcium may serve as a stimulus for vasoconstriction. In addition, inhibition of sodium pump and the calcium ion channel in tubular segments leads to decreasing sodium absorption and the latter resulting to alter motive force in the transtubular movement of many inorganic and organic solutes (Grantham, 1980).

## Machanism and Regulation of Renal Tubular Acidification

### 1. Bicarbonate and Reclamation and Regeneration

Levine (1990) reviewed that bicarbonate reabsorption is only one of the phenomena responsible for maintaining normal acid-base balance. When the plasma bicarbonate concentration $[HCO_3^-]_s$ is reduced below the normal range, about 25 mEq/L, the kidney reclaims all filtereded $HCO_3^-$. As the $[HCO_3^-]_s$ increases toward normal, complete $HCO_3^-$ reclamation continues until a critical $HCO_3^-$ concentration is reached. This called the bicarbonate threshold. At this $[HCO_3^-]_s$ , about 28 mEq/L, an apparent maximal reabsorptive rate ($T_{max}$), has been achieved. When the $[HCO_3^-]_s$ increases above the apparent $T_{max}$, the resultant increase in the filtereded $HCO_3^-$ load entirely excreated into the urine.

The kidney regenerates $HCO_3^-$ by excreting acid in the form of ammonium or titratable acid. Briefly, virtually all ammonium appearing in the urine synthesized primarilly by proximal tubule cell. These cells metabolize glutamine to α-ketoglutarate generates two new $HCO_3^-$ molecules that cross the proximal basolateral membrane. Proximal tubular cell ammonium is secreted into proximal tubular fluid by an active transport process, and luminal ammonium is actively reabsorbed by the thick ascending limb in the loop of Henle. After being concentrated in the medullary interstitium, ammonia diffuses into the collecting duct lumen. With secreted protons, this ammonia reconstitutes the ammonium that appears in the final urine. Additional $H^+$ secretion in the distal nephron further reduces filtrate pH and titrates filtereded buffers from their alkaline to acid form. This generates titratable acid.

## 2. Acidification in Renal Tubular Segments

Each segment of renal tubule contains unique acid and base secretory systems that have varying response to physiologic and pathophysiologic stimuli. Levine and Jacobson (1986) and Kurtzman (1990) viewed that the proximal tubule, renal $HCO_3^-$ reclamation is principally mediated by $H^+$ secretion. Within cell $H^+$ is generated by splitting $H_2O$ into $H^+$ and $OH^-$. The $H^+$ is secreted while the $OH^-$ combines with $CO_2$ to form $HCO_3^-$. The reaction is accelerated by carbonic anhydrase in the cytoplasm. The reaction that is primarily responsible for $H^+$ secretion in the proximal tubules occurs via apical electroneutral $Na^+$-$H^+$ antiporters. This is an example of secondary active transport; extrusion of $Na^+$ from the cells into the lateral intercellular spaces by basolateral electrogenic $Na^+$-$K^+$ ATPase lowers intracellular $Na^+$, and this causes $Na^+$ to enter the cell from the tubular lumen, with coupled extrusion of $H^+$. The small remaining fraction is secreted by an apical electrogenic ATPase-driven proton pump. In the proximal (but not in the distal) tubule, most of the secreted $H^+$ reacts with $HCO_3^-$ to form $H_2CO_3$, there is carbonic anhydrase in the brush border of the cells; this facilitates the formation of $CO_2$ and $H_2O$ in the tubular fluid. The $HCO_3^-$ reclamation occurs via basolateral 1 $Na^+$-$3HCO_3^-$ symporter. The negative intracellular electric potential is the principle driving force for this electrically conductive transporter. The largest fraction of the filtereded bicarbonate load as mush as 90% is reabsorbed in the proximal convoluted more than proximal straight tubule. The smaller amounts of filtereded bicarbonate that escape proximal reabsorption are retrived by the more distal nephron. Active $H^+$ secretion in the thick ascending limb mediated by apical $Na^+$-$H^+$ antiporters and basolateral $1Na^+$-$3HCO_3^-$ symporter similar to those present in the proximal tubule (Levine, 1990). As for distal tubule and cortical collecting tubule, bicarbonate reabsorption, it is believed that the type-A intercalated cell via basolateral

$Cl^-$-$HCO_3^-$ antiporter, possessing an apical proton ATPase primarily via $H^+$ATPase and a small componant via $H^+$-$K^+$ATPase , is the major determinant of bicarbonate retrieval. The bicarbonate reabsorptive rate is thought to be modulated by the sodium current in the principal cell that primarily reabsorb $Na^+$ and secrete $K^+$, this generates a lumen-negative electrical voltage, which alters the rate of proton secretion. Sodium entry is modulated by the permease effect of aldosterone. There is also evidence for bicarbonate secretion that occurs via type-B intercalated cells with an apical $Cl^-$-$HCO_3^-$ antiporter. The energy for this antiporter is derived from a basolateral proton ATPase. This cell may play an important role in the prevention and correction of metabolic alkalosis. The outer medullary collecting tubule, which has no sodium current, secretes protons electrogenically, causing a lumen-positive transepithelial potential difference in association with bicarbonate retrieval. The inner medullary collecting duct cells can promote apical sodium-independent aldosterone-sensitive $H^+$ secretion and basolateral $Cl^-$-$HCO_3^-$ antiporter, and can show carbonic anhydrase activity.