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THYMUS GLAND, VITAMIN D AND SEASONAL INFLUENCES ON ELECTROLYTE METABOLISM

City University of New York PH.D. 1981

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THYMUS GLAND, VITAMIN D AND SEASONAL INFLUENCES ON ELECTROLYTE METABOLISM

by

Danielle Desroches

A dissertation submitted to the Graduate Faculty in Biology in partial fullfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1981

Danielle Desroches

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

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ABSTRACT

Male hooded rats were thymectomized (TMX), sham-thymectomized (SHAM), parathyroidectomized-thymectomized (PX-TMX), parathyroidectomized-sham thymectomized (PX-SHAM), thyroparathyroidectomized-thymectomized (TPX-TMX) or thyroparathyroidectomized-sham thymectomized (TPX-SHAM) at 7-8 weeks and maintained in individual metabolism cages. They were given tap water and either Purina Chow (regular diet) and/or vitamin D-deficient diet for control and injection periods of 3 weeks each. Urinary electrolytes were measured weekly and the values were corrected for food intakes. Serum calcium (Ca) and inorganic phosphate (P) were measured prior to and following vitamin D treatment.

All surgical groups on the regular diet excreted more chloride (Cl) but less inorganic phosphate, sodium, potassium and calcium during April-August than during November-March TMX, PX-TMX and TPX-TMX animals excreted more chloride calcium, sodium and phosphate than SHAM, PX-SHAM and TPX-SHAM groups respectively during July-August, but thymectomy had little effect duringthe winter months. Parathyroidectomized rats (PX-SHAM, PX-TMX, TPX-SHAM, TPX-TMX) excreted more chloride, potassium and calcium than TMX and SHAM in July-August. SHAM animals had the highest serum Ca and P concentrations in both summer and winter. Parathyroidectomy and thyroparathyroidectomy reduced Ca and elevated serum P levels more markedly in the summer. TMX and PX-TMX rats had lower

serum Ca concentrations than SHAM and PX-SHAM animals respectively in both winter and summer. 400 IU vitamin D_3 (D_3) 3x weekly in April-August increased Cl excretion in SHAM only, but it decreased Ca excretion in PX-SHAM, TPX-SHAM, PX-TMX and TPX-TMX rats. This dose also elevated serum Ca levels of all groups. 4,000 IU D_3 3x weekly tremendously increased Ca, P and Cl excretion and serum Ca levels in all PX groups. During November-March, 400 IU D_3 3x weekly increased Cl and Ca excretion in SHAM, PX-SHAM and TPX-SHAM but not thymectomized rats, while 800 IU D_3 increased Cl but decreased Ca excretion in TMX and SHAM rats.

TMX and SHAM rats, when fed a D-deficient diet during April-August, excreted more P, but less Cl, K than when fed the regular diet. The serum concentrations of Ca and P were not greatly affected by the deficient diet. 800 IU D_3 3x weekly to D-deficient TMX and SHAM elevated serum Ca and Cl excretion values but lowered Ca, P, Na and K excretion.

These findings show influences of the thymus gland on electrolyte metabolism, on seasonal variations in responses to vitamin D administration and on responses to surgical manipulations of the endocrine system that affect Cl, Na, K as well as Ca and P metabolism.

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ABBREVIATIONS AND SYMBOLS

ADH	Antidiuretic Hormone
CT	Calcitonin
cAMP	Cyclic Adenosine 3'5' Monophosphate
GCC	Glucocorticoids
PTH	Parathyroid Hormone
D, D_3	Vitamin D_3, Cholecalciferol
1α-OH-D_3	1α- Hydroxyvitamin D_3
25-OH-D_3	25- Hydroxyvitamin D_3
25HCC	25- Hydroxycholecalciferol
1,25-$(OH)_2$-D_3	1α,25- Dihydroxyvitamin D_3
24,25-$(OH)_2$-D_3	24,25- Dihydroxyvitamin D_3
1,24,25-$(OH)_3$-D_3	1α,24,25- Trihydroxyvitamin D_3
25,26-$(OH)_2$-D_3	24,26- Dihydroxyvitamin D_3
25-OHase	25-Hydroxylase
24-OHase	24-Hydroxylase
1α-OHase	1α- Hydroxylase
Ca	Calcium
$[Ca^{2+}]_{in}$	Intracellular calcium concentration
P	phosphorus
Cl	Chloride
Na	Sodium
K	Potassium
ECF	Extracellular Fluid
PX	Parathyroidectomized
TPX	Thyro parathyroidectomized
TMX	Thymectomized
SHAM	Shamthymectomized

INTRODUCTION

I - Objectives

In recent year, most of the research on the thymus gland has been directed towards elucidation of the role of that organ in the development and maintenance of cellular immunity (258, 305, 307, 316). The immune response can be permanently damaged if the thymus gland is removed very early in life (eg. during the first 48 hours after birth in rats or mice). However, if the surgery is performed only days later, only minor damage is inflicted (68, 113, 206). The thymus grows rapidly long after the critical period for influences on the immune system seem to be completed (203). It exhibits exquisite sensitivity to glucocorticoids and to the gonadal hormones secreted later in life. Reproducible effects of thymectomy on a variety of physiological functions of adult animals have been described (23, 39, 58, 182, 183, 184, 189, 233, 256, 276). The findings point to some kind of thymus function which may be unrelated to its role in cellular immunity.

Several kinds of observation support the concept that the thymus gland markedly influences the metabolism of calcium, inorganic phosphate, sodium, potassium, chloride and water (188). Findings consistent with a physiological role in the regulation of calcium metabolism

include the following:

1- Very young salamander larvae exhibit symptoms of hypocalcemic tetany if they are fed thymus tissue. As they mature and develop parathyroid glands, the salamanders become resistant to the thymus effects (309).

2- Weak, fragile skeletons, reduced bone calcium content and elevated serum phosphate concentrations have been described in tadpoles, puppies, rabbits and mice subjected to thymectomy at early ages (224, 267).

3- Calcitonin (99, 170) and proteins of higher molecular weight that depress plasma calcium concentrations (95, 208) have been isolated from thymus glands. A thymus-derived protein has been reported to reverse the effects of thymectomy in young rabbits. Glucagon (95, 170), which is known to stimulate the secretion of calcitonin (297), has also been identified.

4- Thymectomy seems to impair the ability of rats to clear the plasma of excess calcium following injection of calcium gluconate (79) and to enhance the suceptibility to development of cardiovascular necrosis following induction of hyperparathyroidism (161, 162, 189, 190).

5- Patients suffering from myasthenia gravis often show inflammation of the thymus gland and abnormal production of thymopoetin. Anomalous patterns of calcium and phosphorus metabolism have been found in such patients (113).

The thymus gland also exerts profound influences on the renal excretion of sodium, potassium, chloride and water (133); and thymectomy impairs responses to salt loading. Although adrenocortical steroids rapidly promote involution of the gland, at least some thymectomy influences on electrolyte metabolism are exaggerated by adrenalectomy (39).

The thymus gland seems to synthesize and secrete sulfated mucopolysaccharides (49) and it influences plasma concentrations of heparin (81). Heparin has been shown to modify both sodium excretion (185) and bone mineralization (118). It has been reported that heparin injections affect electrolyte excretion in thymectomized rats.

Thymus gland cells are also markedly sensitive to gonadal steroids (44, 64, 278), somatotropin (64, 181, 232, 298, 309), thyroxine (64, 191) and parathyroid hormone (64, 131), all of which influence electrolyte metabolism.

The present study was designed to investigate some thymus gland influences on the metabolism of calcium and phosphate and related electrolytes, and to elucidate possible relationships of the gland with the hormones most frequently implicated in calcium and phosphate metabolism.

The hormones most directly involved in maintaining calcium homeostasis in terrestrial vertebrates are parathyroid hormone (PTH) (77, 100, 137), cholecalciferol (D_3)

and its metabolites (21, 71, 101, 160, 218, 220, 288, 295, 297) and calcitonin (CT) (10, 221, 275, 297). In addition, calcium and phosphorus metabolism are influenced by gonadal and adrenocortical steroid hormones, somatotropin, thyroxine, prolactin, and other components of the endocrine system (10, 64, 105, 172, 221, 244, 258).

II - The importance of maintaining calcium homeostasis.

Calcium (Ca^{2+}) is one of the most biologically important cations. It is a key factor in the regulation of numerous cellular events in a variety of cells.

Calcium, the intracellular "messenger" is the coupling factor for excitation-contraction and excitation-transmission (74, 172). It plays a crucial role in cardiac muscle physiology (90, 319). Acute elevation of calcium concentration enhances cardiac contractility, impairs the relaxing phase and can lead to systolic arrest. The calcium transport system of the sarcoplasmic reticulum is a major participant in the process of force development (259), and in the regulation of the contraction-relaxation cycle of skeletal muscles (78, 287). It is essential for displacing troponin from the actin filament and allowing formation of the actomyosin complex. Hypercalcemia impairs the ability of acetylcholine to promote depolarization of the skeletal muscle membrane (74). In smooth muscles (72, 280), although the role of the sarcoplasmic reticulum has not been unequi-

vocally established, there is evidence for calcium participation in the relaxation system and activation of smooth muscle contraction. Calcium is also a current-carrier in excitation secretion processes at presynaptic endings. Its presence is critical for the release of neurotransmitter substances (26, 141, 239, 303) and in the propagation of nervous impulses (35, 50). Such roles cannot be demonstrated for cells bathed in calcium-free media. A rise in intracellular $[Ca^{2+}]$ triggers transmitter release. This leads to a large influx of Ca^{2+} following depolarization. Some of that calcium is sequestered in mitochondria and other organelles. Eventually all the excess Ca^{2+} which enters must be extruded. Na^{+}-Ca^{2+} exchange appears to play an important role here (26, 259).

Calcium is involved in intercellular and intracellular communication. It regulates the permeability of cell-cell membrane channels. Such channels are "open" when cytosol $[Ca^{2+}]$ (Ca^{2+}in) is low or normal, and "closed" when the $[Ca^{2+}]$ is elevated (167). The changes affect the transfer of hydrophilic molecules from one cell interior to another.

The cytosol calcium is a modulator of enzyme activity. It affects several regulatory enzymes including adenylate cyclases (34), guanylate cyclases (54), phosphodiesterases (165), phosphorylase kinases (205). In fact, the effects of calcium ions and cyclic AMP overlap to a great extent. Several laboratories have shown the requirement for Ca^{2+}

(and for cAMP) in the stimulation and profileration of lymphocytes (116) and its critical involvement in mitogen action (248). The importance (247) of calcium is seen, after capacitation, in the acrosome reaction and following egg fertilization when a high rise in $[Ca^{2+}]$ in triggers an enzyme kinase that helps provide the energy for the synthesis of many cell constituents needed by a rapidly dividing embryo (193).

Ca^{2+} prepares cells for entry into mitosis (131, 139). It plays a role as a membrane-to-nuclear signal. It affects assembly of microtubules (279). Calcium seems to provide the dominant trigger for exocytosis of several hormones, such as vasopressin, oxytocin (303), insulin (178). It also participates in the regulation of prostaglandin biosynthesis (246), and the activity and degradation of parathyroid hormone (115, 123, 229).

The extensive evidence presented above, and others found in the literature (35, 322), for calcium's role in cellular regulation in such a variety of systems raise the question of how this cation produces its diverse effects. Recently, the discovery of calmodulin has brought some new insights to such an array of functions (146, 149, 193, 320). Calmodulin (193), a peptide molecule with a molecular weight of approximately 17,000, is believed to be an intracellular calcium receptor for the binding of calcium ions when the Ca^{2+} concentration is raised in response to a particular signal or stimulus. Following

some conformational change in the protein molecules, the Ca-peptide complex affects cell activities by binding directly to certain enzymes or by affecting calcium itself or other regulators including cAMP. So far, several enzymes, including phosphodiesterases and adenylate cyclases, have been linked to calmodulin. It is even believed that calmodulin is one of the four chains that constitute phosphorylase kinase of skeletal muscles. More and more evidence is accumulating for the involvement of calmodulin-calcium complexes in several of the systems of which calcium is known to be a regulator, including smooth muscle contraction, neurotransmitter release, mitosis, sperm activation and sperm-egg fusion (135, 193). Calmodulin has been identified in all nucleated cells and it mediates the "messenger" function of calcium.

Powerful intracellular regulatory mechanisms control the level of calcium in the cytosol. The intracellular calcium concentration of unstimulated cells is about 10^{-7} to 10^{-8}M, while that of the blood plasma is 10^{-3}M. Even in stimulated cells, the cytosol Ca^{2+} concentration rarely rises above 10^{-6} to 10^{-5}M. The precise regulation of $[Ca^{2+}]_{in}$ depends on the concerted operation of specific calcium pumping systems located in the plasma membrane and in such cellular organelles such as endoplasmic reticulum, sarcoplasmic reticulum and more especially in the mitochondria. While the plasma membrane pumps and the low permeability of

the cell membrane constitute long-term control, the mitochondrial transport system is of central importance in the short-term and overall regulation of intracellular calcium (43). The influx of Ca^{2+} into mitochondria is probably mediated by a carrier, via a "passive electrophoretic uniport." Much of the Ca^{2+} is sequestered along with phosphate in an inactive but recruitable complex conveniently represented as $Ca_3(PO_4)_2$. The mitochondria are capable of returning calcium ions to the cytosol when the need arises, such as when the cell pH is high or cytosol calcium is low. This probably is accomplished via a Na^+-dependent-Ca^{2+} releasing system.

The constancy of calcium in the extracellular fluid and blood plasma is important for the maintenance of the regulatory system of the cytosol. Such constancy is achieved by a complex set of interrelated hormonal and non-hormonal controls. The three main hormone systems involved are PTH, calcitonin and vitamin D_3. Together, they help maintain the calcium content of blood plasma within very narrow limits, at about 10 mg/100 ml, and they protect against development of hypocalcemia. Other hormones affect calcium homeostasis either directly or indirectly.

The importance of proper calcium balance can be appreciated from examination of effects of calcium depletion. Acute or severe hypocalcemia can lead to the development of

tetany and the associated convulsions, muscle spasms resulting in death (80, 243), while chronic or milder conditions lead to mental retardation in children. Hypocalcemia, which may arise from parathyroidectomy or rickets in animals, can lead to formation of some types of cataracts (8).

Well balanced diets and normocalcemia provide sufficient amounts of calcium to satisfy the mineralization of bone.

Elevation of calcium concentration above the normal range leads to calcification of soft tissues of the vascular, digestive and renal systems. Severe hypercalcemia can lead to renal failure due to deposition of calcium salts within the tubules, particularly if there is concomittant hyperphosphatemia such as in the case of vitamin D toxicity.

Hypercalcemia can also reset arterial baroreceptors, can lead to deposition of calcium within the aorta reducing its elasticity; and it impairs physiological vasodilation, adjustments of blood pressure and heart rate, and body temperature (35, 153).

III - Relationships between metabolism of calcium and metabolism of other ions.

Phosphate ion: The metabolism of calcium is intimately related to that of phosphate. Calcium forms poorly ionized complexes with inorganic phosphate; and the amount of calcium ion free to enter body cells is dependent on concentration of plasma phosphate (187).

Hyperphosphatemia retards calcium entry into cells but it facilitates deposition of calcium salts in bone and soft tissues (51).

Hypophosphatemia impairs cellular uptake, sequestration of calcium, and bone mineralization. Phosphate depletion leads to augmented intestinal calcium absorption, increased mobilization of calcium from previously formed bone, and enhanced urinary excretion of calcium. Renal handling of phosphate is associated with calcium transport in various parts of the nephron (106, 291).

The hormones known to regulate calcium metabolism also influence the metabolism of phosphate (106). They are discussed later.

Sodium ion; Sodium interacts with both calcium and phosphate in several ways. Transport of sodium ions across plasma membranes is affected by calcium. Calcium is extruded from cells by a sodium - dependent process (303a). Martin and DeLuca (192) demonstrated the requirement for sodium for the expulsion of calcium across the basal-lateral membrane of the intestinal cells. In the absence of sodium, calcium

accumulates in the intestinal villi structures engaged in Ca transport.

Renal handling of sodium, phosphate and calcium are interdependent in the proximal tubule. However, they are dissociated and regulated by different mechanisms in the more distal portions of the nephron (106, 134, 291). Because of the close association of these ions, the assessment of the influence of any factor on urinary calcium excretion must take into account any simultaneous changes in urinary sodium excretion.

Clearance studies have shown that sodium administration leads to volume expansion of extracellular fluid, renal vasodilation, and decreased sodium, calcium, phosphate reabsorption (291). This results in increased urinary excretion of all three ions.

Chloride and Potassium, other Factors: Renal handling of chloride is closely associated with the handling of sodium. It is believed that sodium is actively reabsorbed in proximal, distal, collecting ducts, and that chloride transport depends on this active sodium transport. However, recent studies with diuretics show that chloride, (not sodium), is actively transported by ascending limb of loop of Henle (40). The interrelationships between chloride excretion and the excretion of calcium and phosphate have not been extensively investigated. Only calcitonin has been reported to somehow affect chloride excretion via its effect on sodium (140, 187).

No mention of vitamin D_3 influences of chloride excretion are reported in the literature.

Potassium movements across plasma membranes are related to those of sodium. Potassium excretion depends in part upon establishment of sodium-dependent electrochemical gradients in renal tubules (221), but it can be dissociated from Na^+ transport under certain conditions (4, 24).

Water reabsorption mechanisms have recently been directly linked to active transport of chloride ions by the cells of the loop of Henle (40).

Metabolic acidosis associated with low bicarbonate content of blood, and alkalosis associated with elevated bicarbonate ions in blood, also influence renal excretion of calcium phosphate, potassium and sodium (291).

IV - Calcium regulating hormones

Parathyroid Hormone (PTH)

The term PTH designates a group of chemically and biologically related species-specific peptides secreted by the parathyroid glands. For purposes of discussion, PTH will be treated as a single entity. It plays essential roles in the regulation of the metabolism of calcium, phosphorus and other minerals. The hormone elevates the total calcium content of the blood plasma and the fraction thereof that is present in ionic form. It also lowers the plasma concentration of inorganic phosphate. The physiological effects are attributed primarily to influences exerted on mobilization of calcium from bone and soft tissues, renal mechanisms for conservation of calcium and excretion of phosphate, and (indirectly) on the intestinal absorption of calcium and phosphate. The actions of PTH on its primary target organs, namely bone, kidney make this hormone a potent hypercalcemic agent (77, 81, 100, 204, 209).

a) Action on bone: PTH is probably the major physiological regulator of bone resorption (137, 228). It directly stimulates osteoclastic activity and osteocytic osteolysis. The actions on the skeleton are mediated, at least in part, via a rise in cAMP levels in bone tissues (212, 227). In addition, when present at physiological doses, PTH permits extensive remodeling of bone while maintaining a normal calcium ion activity (209). There is evidence suggesting that low concentrations of PTH promote bone formation (225, 247, 315).

b) Action on kidney: PTH affects the kidney in several ways. It inhibits calcium reabsorption in the proximal tubule, but it promotes calcium reabsorption in the distal portions of the nephron (106). Under physiological conditions and following administration of small doses, the net effect is usually calcium conservation, since distal effects tend to predominate. However, chronically elevated PTH levels promote the development of hypercalcemia and the consequent appearance of high concentrations of calcium in the glomerular filtrate. PTH also promotes development of phosphaturia via inhibition of phosphate reabsorption in the proximal tubule. This effect of PTH involves a parallel inhibition of sodium reabsorption (106, 207). Usually, there is no net change in sodium excretion, because inhibitory effects of PTH on reabsorption in the proximal tubule are offset by more distal actions of mineralocoticoids. PTH also affects the renal excretion of hydrogen and bicarbonate ions (187, 209, 210, 231).

c) Action on intestine: PTH also indirectly elevates plasma calcium via both activation and induction of the kidney enzyme, 25-OH-D_3-1-hydroxylase (69, 94, 121). This enzyme is needed for the conversion of 25-(OH)-vitamin D_3 to 1,25-$(OH)_2$-D_3, the primary metabolite involved in intestinal calcium absorption. The mechanisms of action of PTH on the renal enzyme have not been established, but there are reasons to believe that they involve stimulation of adenylate cyclase. cAMP and dibutyryl cAMP are known to stimulate the production

of 1,25-$(OH)_2$-D_3 (155). Biosynthesis of 1,25-$(OH)_2$-D_3 occurs mainly in the proximal tubule (290). PTH has been shown to accumulate in this region (29, 87, 245).

The parathyroid glands serve as major sensors of plasma calcium concentration. Hypocalcemia leads to augmented secretion of PTH, and this is followed by increased production of 1,25-$(OH)_2$-D_3 (69). The latter supports PTH actions on bone and kidney. The restoration of blood calcium level to normal leads to suppression of PTH release and of production of 1,25-$(OH)_2$-D_3. (Figure I).

Enzymes affecting PTH activation and degradation are widely distributed (47, 268). There is evidence for a Ca-dependent intracellular pathway for the degradation of preformed PTH and also of pro-PTH and pre-pro-PTH (115). The half-life of the circulating hormone in blood plasma is about twenty minutes (199, 273).

Responses to the hormone are age-dependent, and the level of the circulating PTH has also been found to decrease with age (138, 244). Furthermore, PTH actions on kidneys surpass those exerted on bone in older individuals (137) of at least some species.

Evidence is accumulating for neuronal regulation of PTH secretion (36, 119).

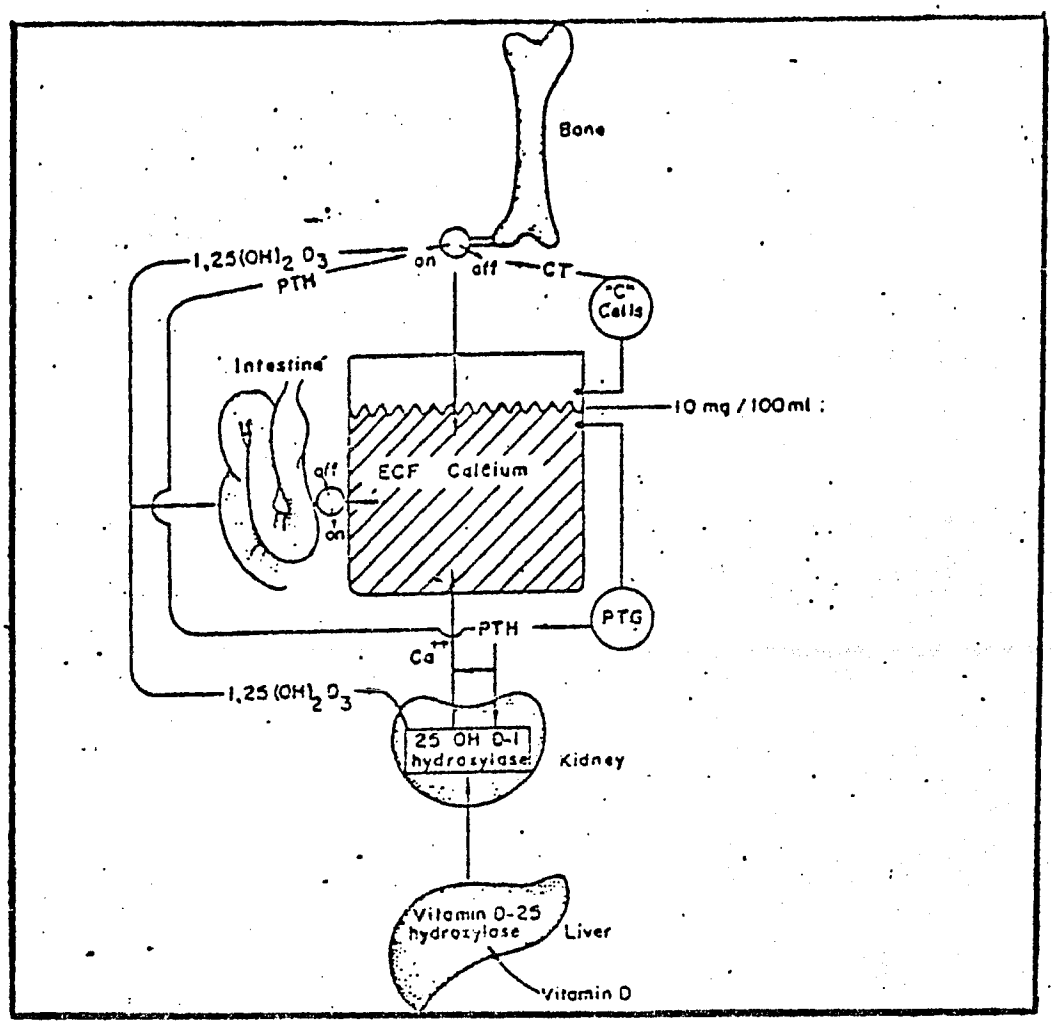

FIG.1: MECHANISMS WHICH REGULATE SERUM (ECF) CALCIUM CONCENTRATION.

Parathyroid glands monitor hypocalcemia while parafollicular cells or "C" cells of the thyroid monitor hypercalcemia. In response to hypocalcemia, PTH is secreted, which stimulates the kidney to produce 1,25-$(OH)_2$-D_3. 1,25-$(OH)_2$-D_3 initiates (turns on) intestinal calcium absorption by itself and together with PTH stimulates (turns on) the mobilization of calcium from bone. In addition, both PTH and 1,25-$(OH)_2$-D_3 improve renal reabsorption of calcium. These mechanisms therefore elevate serum calcium concentration, shutting off secretion of PTH. The parafollicular cells secrete CT in response to hypercalcemia, which then inhibits the mobilization of calcium from bone, thereby bringing about a suppression of serum calcium concentration (69).

Calcitonin (CT)

Calcitonins are species-specific peptide hormones secreted by parafollicular cells of the thyroid gland of mammals, and by ultimobranchial glands of sub-mammalian vertebrates (187). Although CT has been identified in plasma samples from all mammals studied, including man (164), and although it can exert both anti-hypercalcemic and hypophosphatemic actions, there are controversies concerning its physiological functions. There are no indications that its absence in man or other mammals is life-threatening. Endogenous CT does not completely prevent hypercalcemia nor does its absence cause exaggerated hypercalcemia (136). However, CT may confer the ability to adjust (13). Failure to see hypercalcemia when no CT is present could depend upon compensatory suppression of PTH secretion.

However, under non-physiological conditions such as following the administration of large amounts of calcium by intravenous infusion, gavage or intraperitoneal injection, endogenous calcitonin prevents or decreases hypercalcemia and shows its importance as an anti-hypercalcemic hormone.

Calcitonin secretion is stimulated by hypercalcemia, by feeding, and by certain of the gastro-intestinal hormones released during meal absorption (61, 296). Secretion tends to rise after parathyroidectomy (292), and it is inhibited by somatostatin (119).

Potent preparations of CT have been shown to exert various influences on several aspects of mineral metabolism. The biological activity of the hormone varies with both the source of the hormone and the species tested. Salmon CT is by many criteria the most potent preparation at least in part, because of its resistance to degradation, and also because it binds with especially strong affinity to receptors. It is also a most potent stimulator of cAMP (122). The primary target tissues of CT are bone, kidney and possibly the intestine. CT accomplishes its effects by facilitating the removal of Ca^{++} and $HPO_4^{=}$ from the extracellular fluid (ECF) and/or by decreasing the rate of entry of these ions into the ECF (209).

a) Action on bone: The major action of CT seems to be suppression of osteoclastic bone resorption and osteocytic osteolysis (96, 187, 275). Changes in morphology and reduced osteolytic activity can be demonstrated within minutes after administration of the hormone (187). In addition, less calcium is released, and collagen synthesizing activity is augmented. The long-ranged effects of

the hormone involve changes in bone mass related to decreased mitoses of precursor cells and reduced numbers of osteoblasts (125, 187, 200). Receptors specific for CT have been demonstrated in bone (122, 228, 277, 300, 321). Although CT stimulates skeletal adenylate cyclase, its hypocalcemic effect has been shown to be dissociable from its effects on bone cAMP metabolism (213).

b) Action on kidney: Calcitonin increases the urinary excretion of numerous solutes, including calcium, phosphate, sodium, chloride and water (4, 5, 24, 48, 76, 216, 281). This action can lead to marked reduction of circulating blood volume and to depletion of body fluids. Although the effects of CT have been attributed by some to PTH antagonism (106), there is evidence for direct action of CT on kidney, via stimulation of adenylate cyclase. Such action is observed in parathyroidectomized but not in nephrectomized animals (254). This view is further supported by the demonstration of renal receptors specific for CT, at sites different from those for PTH receptors (122, 194, 195, 196).

Calcitonin decreases plasma calcium and phosphate concentrations through its dual effects on bone and kidney (140, 294). The actions on bone usually predominate when

bone turnover is rapid. The calciuria and phosphaturia produced by CT are not essential to its anti-hypercalcemic and hypophosphatemic actions. But, when bone turnover is slow, the renal effects of CT contribute significantly to the hypophosphatemia and hypocalcemia.

c) Actions on intestine: CT has been implicated in inhibition of renal-1-hydroxylase activity (243). However, findings are conflicting. Lorenc et al (168) were unable to demonstrate an effect of thyroidectomy on vitamin D metabolism in rats made hypercalcemic by high Ca or/and low phosphate diets.

Age and sex related differences in plasma concentrations of, and responses to CT administration have been found (70, 90, 230, 271).

Vitamin D and its metabolites

Vitamin D_3 is perhaps the most important single factor regulating the use of dietary calcium and a major one in controlling the concentration of calcium in the extracellular fluids (69, 70, 121). Its metabolites play essential roles in stimulating the active transport of calcium and phosphate across the small intestine, mobilization and mineralization of bones, and the renal handling of phosphate and calcium.

Vitamin D metabolism

Vitamin D can be totally synthesized from ubiquitous endogenous precursors (acetyl CoA or cholesterol) in individuals receiving adequate exposure to sunlight that provides ultraviolet radiation of appropriate wavelengths (69, 121). Seasonal variations have been reported in serum levels of vitamin D metabolites, with lowest concentrations found in the winter months and the highest values during the summer months. In fact, vitamin D may not be required in the diet during the summer months (157, 211, 289).

Cholesterol, synthesized in the liver or elsewhere from acetyl coenzyme A, is converted by an intestinal enzyme into 7-dehydrocholesterol (186). This product is then carried by the circulation to the skin. Ultraviolet irradiation of skin is believed to induce photolytic opening of the B ring and this gives rise to pre-vitamin D_3 and other photoisomers. A final temperature-dependent reaction leads to the formation of vitamin D_3 (Figure II).

Before it interacts with its target organs, vitamin D must be metabolically activated (69, 121) (Figure III). Vitamin D_3 (from dietary or endogenous sources) accumulates first in the liver, where it is converted to 25-OH-D_3. The latter is secreted into the blood stream, in which it associates with specific transport protein (94). The high-affinity binding accounts in large part for the fact that the concentrations of 25-OH-D_3 are one hundred times greater than those of

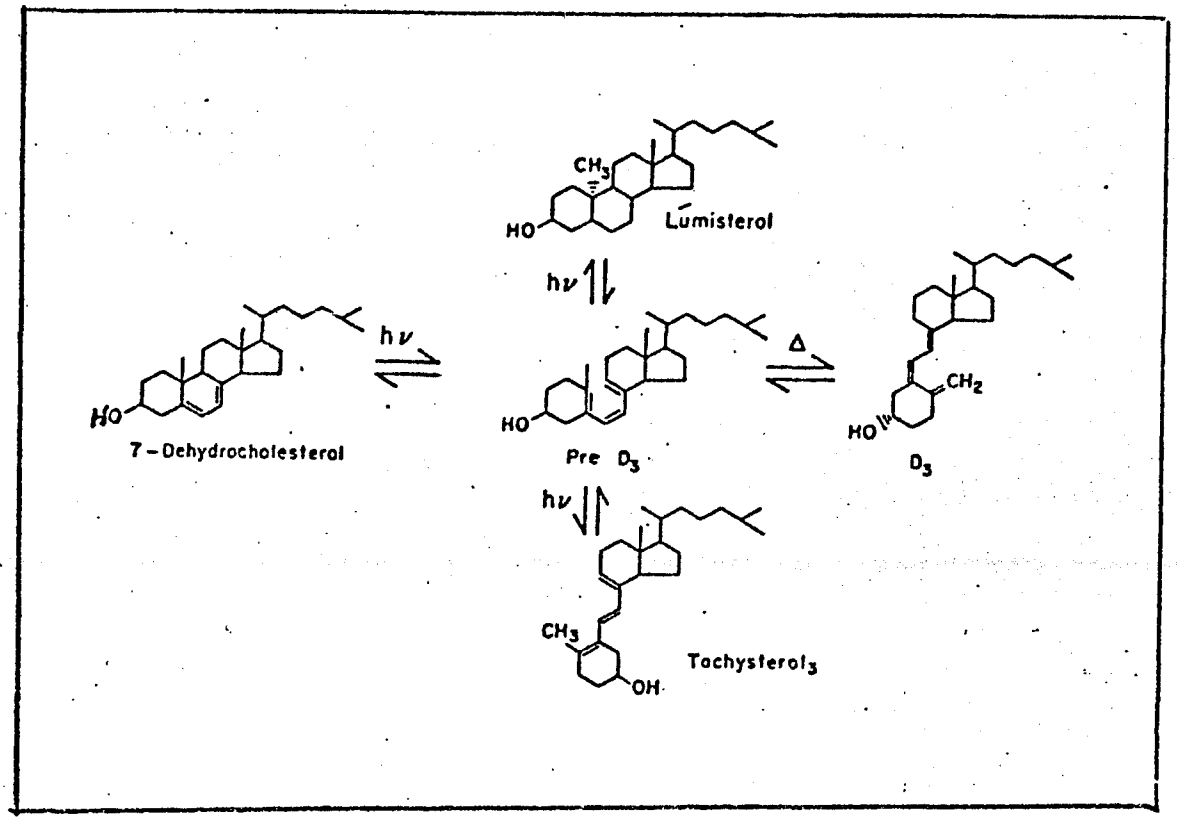

FIG. 2: Photolysis of 7-dehydrocholesterol to form previtamin D_3 and its subsequent thermal isomerization to D_3 (69).

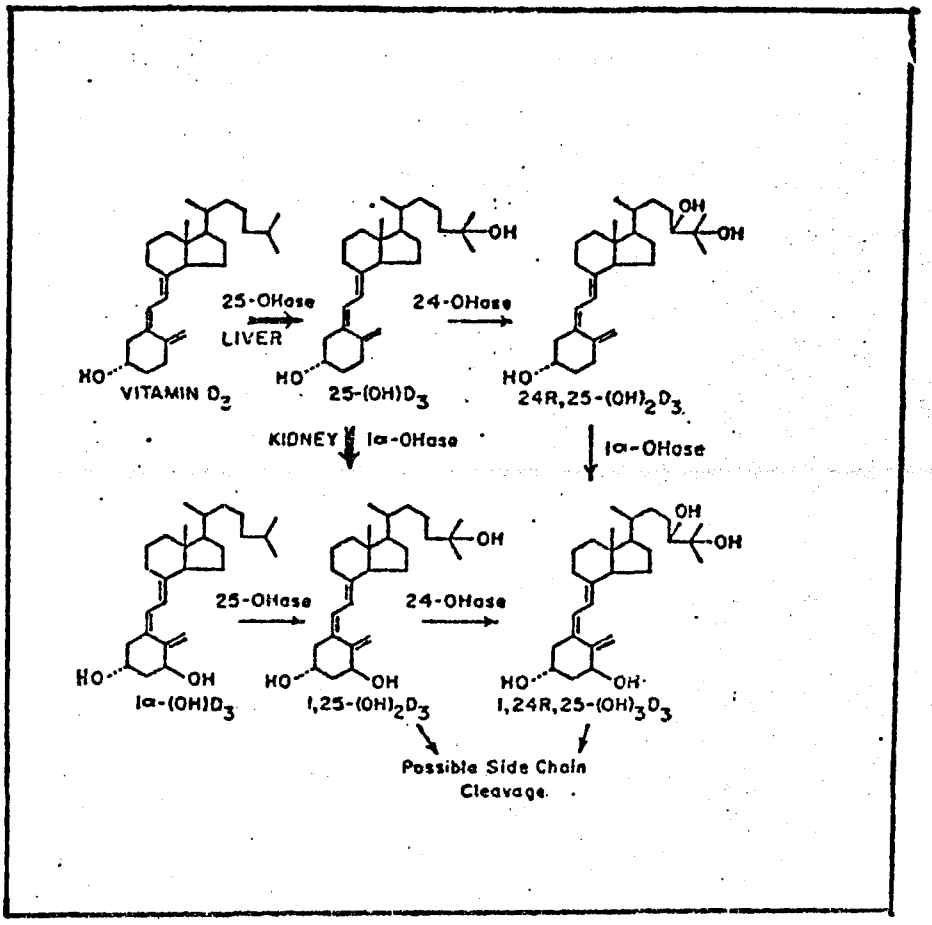

FIG.3: METABOLISM OF VITAMIN D_3 (155).

the vitamin or of any other of its metabolites. The potency of 25-OH-D_3 is 2-5 times greater than that of D_3 on mineralization of bone, elevation of serum calcium and stimulation of intestinal calcium transport (218). 25-OH-D_3 is possibly the compound which brings about the toxic effects of vitamin D_3 such as metastatic calcification (69). In addition, it may be the metabolite that facilitates the exchange of phosphate across the cell membrane in muscles, in response to an acute hypophosphatemic challenge, therefore maintaining homeostasis. Cytoplasmic binding components for 25-OH-D_3 have been found in several chick and rat tissues (313).

25-OH-D_3 is further converted in the kidney into 1,25-$(OH)_2$-D_3, the most potent metabolite known for regulation of calcium and phosphorus metabolism (102, 151, 174). It will be considered below.

In addition, 24-hydroxylation (152) can occur in kidney and other tissues, and this leads to formation of 24,25-$(OH)_2$-D_3 or 1,24,25-$(OH)_3$-D_3. 24,25-$(OH)_2$-D_3, possibly because it can be 1α-hydroxylated, is almost as active as 25-OH-D_3 and is believed by some researchers to be the predominant circulating dihydroxymetabolite under conditions of high calcium diets, normocalcemia and hypercalcemia, and when PTH secretion is depressed or absent (18, 124, 249, 251). It has little action on bone resorption and would preferentially promote bone mineralization (27, 179, 222, 226) and collagen synthesis (241). Resorbing effects on bone can only be seen at concentrations 1000x more than that of 25-OH-D_3 (226).

24-hydroxylation is also thought by many to be a mechanism for inactivation of 1,25-$(OH)_2$-D_3 (69).

1,24,25-$(OH)_3$-D_3 is about 60% as active as 1,25-$(OH)_2$-D_3 in the rat but much less so in the chick. Its exact functions remain unknown (69).

Hydroxylation can also occur at the 26 position to yield 25,26-$(OH)_2$-D_3. This metabolite, found in low concentrations in normal animals, has little effect on Ca transport and bone mobilization and mineralization (69, 251). The administration of superphysiological doses of vitamin D brings the appearance of large amounts of 25,26-$(OH)_2$-23 lactone-D_3 (266). The functions of this metabolite are still unknown.

<u>1,25-$(OH)_2$-D_3:</u> Evidence is accumulating that 1,25-$(OH)_2$-D_3 is the active and potent hormonal form of vitamin D_3 as mentioned earlier. It is 500x more effective on a molar basis than 25-OH-D_3 for calcium transport in intestine and for bone mineral mobilization (174). The responses are seen much sooner following administration of 1,25-$(OH)_2$-D_3 than of cholecalciferol or the other metabolites (70, 218). In addition, highly specific receptors for 1,25-$(OH)_2$-D_3 have been demonstrated in intestine (314), bone (180), kidney (56, 290), parathyroid glands (313). While 25-OH-D_3 and 24,25-$(OH)_2$-D_3 synthesis are not stringently regulated (except possibly for product inhibition) (69, 127), the biosynthesis of 1,25-$(OH)_2$-D_3 is strictly controlled and suffers minimal fluctuations (Figure IV). The concentrations of 1,25-$(OH)_2$-D_3 are maintained within a constant range (250) and do not show the seasonal variations reported for 25-$(OH)_2$-D_3 (211, 289). Besides being regulated

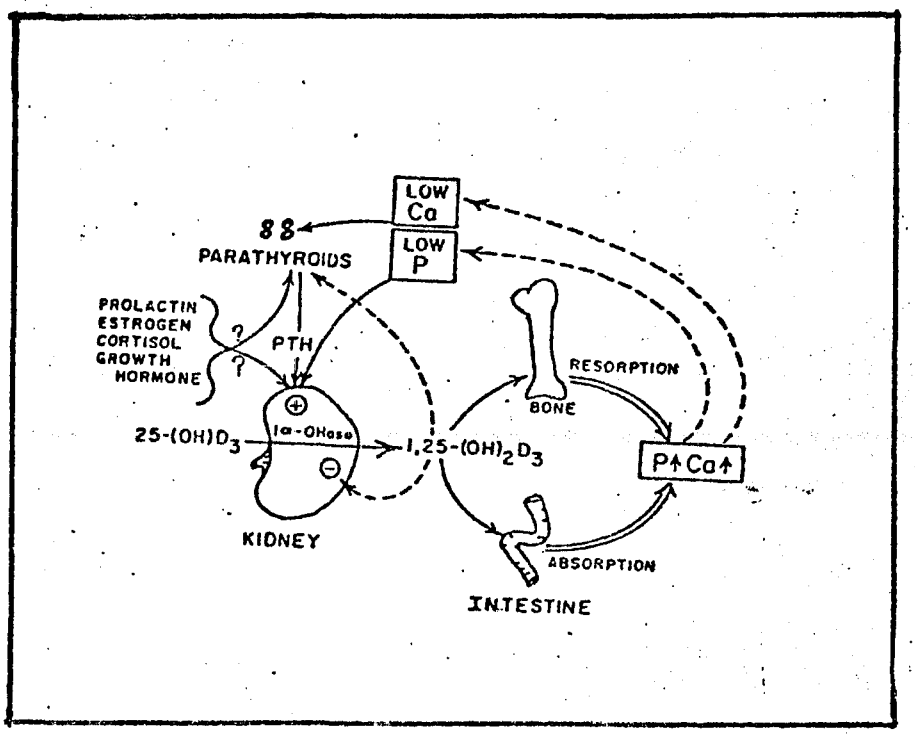

FIG.4: MODEL FOR REGULATION OF 1,25-$(OH)_2D_3$ BIOSYNTHESIS

Solid arrows indicate a positive effect; dashed arrows refer to negative feedback (155).

by PTH (171, 263), the vitamin D status of the animal (127), and dietary calcium and phosphate (250), there is evidence for feed-back regulation by 1,25-$(OH)_2$-D_3 itself (55, 56). When vitamin D_3 is administered to D_3-sufficient animals, to animals on high calcium diet, or the non-growing animals, there is no rise in 1,25-$(OH)_2$-D_3 level nor increased intestinal calcium transport, whereas the level of 25-OH-D_3 does increase. Colston and Feldman (56) demonstrated the existence of a highly specific cytoplasmic receptor for 1,25-$(OH)_2$-D_3 in kidney tubules and suggested a dual role for this receptor: on the one hand, it would be directly involved in Ca and P reabsorption; on the other, it would serve as a sensor for circulating level of 1,25-$(OH)_2$-D_3. Elevated levels of 1,25-$(OH)_2$-D_3 would inhibit the 1-hydroxylase enzyme. This induction of enzyme activity is dependent upon new protein synthesis as assessed by studies with transcriptional inhibitors. Evidence for a direct influence of 1,25-$(OH)_2$-D_3 on its own synthesis or transcriptional event is seen from the extremely rapid increase in nuclear RNA polymerases I and II activities following subcutaneous administration of the metabolite (55).

However, such direct effects of 1,25-$(OH)_2$-D_3 upon its own synthesis are not accepted by all, and Fraser (94) proposed an alternative explanation: the influence of 1,25-$(OH)_2$-D_3 upon its synthesis is indirect and secondary to the actions on Ca and P metabolism. Factors such as Ca^{2+} concentration

and consequently PTH secretion would regulate the synthesis and the maintenance of adequate supplies of $1,25\text{-}(OH)_2\text{-}D_3$.

Defects of the regulatory processes of vitamin D metabolism have been implicated in a variety of clinical conditions (12) including postmenapausal osteoporosis (157), renal osteodystrophy (69), nephrotic syndrome (117) and osteomalacia (25).

Vitamin D metabolites act as modulators of PTH functions (219, 244), and they also directly (46) and indirectly (108, 121, 129) affect the secretion of that hormone. Influences on calcitonin secretion have also been demonstrated (92, 311). In common with PTH and calcitonin, levels of the active metabolite ($1,25\text{-}(OH)_2\text{-}D_3$) decrease with age. This may partially explain the poor ability of adult intestine to adapt to low calcium diets (7).

Much remains to be known about vitamin D metabolites. Most observers agree that $1,25\text{-}(OH)_2\text{-}D_3$ is the most active form of the vitamin. However, recent findings by Beamer et al (19) shed some doubt on the importance of 25-hydroxylation, at least in phosphate metabolism. $1\alpha\text{-}(OH)\text{-}D_3$ preparation was found to be successful in repairing hypophosphatemia in mice whereas $1\alpha,25\text{-}(OH)_2\text{-}D_3$ was not (19).

Vitamin D actions

a) Actions on intestine: Vitamin D_3 is essential for adequate absorption of dietary calcium. It improves the active transport of calcium and phosphorus in the small intestine by two independent mechanisms: Ca and P are absorbed together along the entire length of the small intestine, but in the jejunum, P transport is stimulated independently of that of calcium. The mechanisms whereby vitamin D and its metabolites stimulate intestinal Ca and P transports are matters of controversy, and several aspects of the process remain largely unknown. There is good evidence that they involve induction of synthesis of proteins for the transport of Ca, and presumably of P, across the brush border of the intestinal cells. The absorbed Ca and P accumulate in vesicles or are taken up by mitochondria (69).

Under physiological conditions, vitamin D stimulation of calcium and phosphate absorption does not lead to excessive elevation of cytosol Ca^{++} concentrations, since the minerals are rapidly transported to the mitochondrial matrix for sequestration in inactive (but readily recruitable) form. A shuttle system which seems to be sodium-dependent (69), functions to replenish cytosol Ca^{++} and to raise its concentrations under appropriate conditions. However, pharmacological doses of vitamin D can lead to the development of severe hypercalcemia.

b) Actions on bone: Vitamin D effects on bone are dose-related. Physiological amounts facilitate bone mineralization, since the influences exerted on the intestine provide the needed minerals. Additional calcium and phosphate are made available for new bone formation by actions of the vitamin on resorption of older bone that is undergoing remodeling (27, 69, 121, 179). The vitamin (at least 1,25-$(OH)_2$-D_3) may also limit the biosynthesis of collagen, and thereby favor shunting of calcium and phosphorus into the new, growing portions of the bone that are useful to the organism. It has been suggested that the growth-promoting effects are mediated indirectly via augmentation of the supply of calcium and phosphate. The ions can stimulate localized collagen synthesis (121, 240). However, there is evidence for direct effect of vitamin D on bone mineralization without changes in serum calcium and phosphorus (28, 63); and specific receptors for vitamin D metabolites have been identified (313).

Pharmacological doses of vitamin D bring about the mobilization of calcium and phosphorus from the bone fluid compartment. This leads to elevation of plasma calcium concentrations (69). This process, as well as the action on intestinal transport, is actinomycin D sensitive and may therefore depend upon an induction process. However, while a calcium-binding protein has been demonstrated in intestinal cells (69, 84, 89, 255) following D_3 administration, little is known concerning the mechanism thereby vitamin D brings

about the release of calcium from bone.

Unlike the physiological effects, the pharmacological ones are not dependent on PTH, for they are seen in parathyroidectomized and thyroparathyroidectomized animals. Large amounts of the vitamin can cause marked demineralization of bone at the same time they are causing hypercalcemia, soft tissue calcification and nephrocalcinosis (215, 311). Toxicity may develop following vitamin D administration or excessive dietary intake since this vitamin and its derivatives are stored in the body (121, 128, 311).

c) Actions on kidney: Vitamin D seems to exert direct, as well as indirect influences on the kidney. Physiological amounts promote calcium, phosphate and sodium reabsorption in intact, parathyroidectomized and thyroparathyroidectomized animals (104, 236, 286). The effects are more apparent in D-deficient animals (62). Further support for direct renal actions derives from the discovery of specific receptors for vitamin D metabolites in mouse (56), chick and rat kidneys (290, 313). Those receptors are similar to the ones found in intestine and bone (56) and are located in proximal and distal portions of the nephron.

Prolactin and Growth Hormone

Prolactin is said to modify calcium and phosphorus metabolism, particularly during lactation. Prolactin affects intestinal Ca and P absorption (175) and also renal handling of sodium and potassium (140). Administration of ovine prolactin to rats leads to augmentation of both serum calcium concentration and urinary output of calcium (173, 252).

Like prolactin, growth hormone influences the metabolism of calcium and phosphorus. It increases the rate of bone formation in young animals probably via enhancement of intestinal transport of calcium and phosphate (32, 85, 176). Chronic administration results in increased urinary excretion of calcium. In addition, growth hormone promotes phosphate and sodium retention (106, 140). Although large doses of growth hormone raise plasma concentrations of PTH, the effects on the kidney have been attributed to antagonism of PTH, influences on the renal tubules (106) and/or expansion of the extracellular fluid volume (106, 140).

Under conditions of calcium stress, such as pregnancy, lactation, and growth, it has been reported that circulating levels of 1,25-$(OH)_2$-D_3 are elevated (172). Therefore, it has been suggested that effects of growth hormone and prolactin under those conditions, are mediated by this metabolite. Evidence has been presented for prolactin and growth hormone regulation of vitamin D metabolism during pregnancy and growth. The mechanisms of action, however, have not been fully elucidated (94).

Thyroid Hormones

Thyroid hormones tend to increase the urinary excretion of calcium and phosphate. The calciuric and phosphaturic effects seem to be due chiefly to enhanced skeletal turnover. Direct renal actions on mineral ions have been found but are poorly understood (75, 106, 140).

Sex Steroids

Although estrogens seem to affect the intestinal absorption of minerals, promote bone maturation, and antagonize PTH actions on bone, the influences of the steroids on calcium and phosphorus metabolism in mammals have not been clearly defined (29, 75). The hormones evidently do not affect renal 1α-hydroxylase activity under physiological conditions;. No cyclical variations in plasma 1,25-$(OH)_2$-D_3 have been found during the course of normal menstrual cycles in women (14) and there are many indications the calciferols are not the major promotors of calcium conservation during either pregnancy or lactation in rats (147). Postmenopausal females show an increased loss of calcium into the urine (97), but estrogens effects on renal handling of calcium are variable and poorly documented (140).

There is, however, support for the concept that estrogens play special roles in the regulation of calcium metabolism in birds. During egg-laying cycles, the birds accumulate large quantities of mineral-rich medullary bone. They subsequently transfer the calcium to the liver and shell gland

and utilize it for the production of egg yolks and the formation of egg shells. Stimulatory influences of the steroids on 1-α- hydroxylase and inhibitory ones on the 24-hydroxylase have been described in studies utilizing kidney cell cultures (282). Such influences are apparently independent of PTH. However, testosterone and progesterone synergize with estrogen in the stimulation of the 1α-hydroxylase system . . . egg laying hens and maximum stimulation is achieved by a combination of moderate dosages of all three (299).

It is doubtful that such observations have relevance for mammalian physiology. Mammals do not ordinarily make the type of bone described above, nor do they have shell glands and the associated needs for massive calcium transfer. It is possible to persuade rodents to form medullary bone when they are grossly overdosed with estrogen; but the findings are purely of pharmacological interest.

Prostaglandins

Intravenous administration of prostaglandin E_2 has been reported to markedly elevate plasma calcium concentration, probably via stimulation of bone resorption (93). Prostaglandin E_2 inhibits parathyroid hormone release from parathyroid glands (103). Renal influences of prostaglandins, particularly those on sodium, made some consider those hormones as "natriuretic" (158). However, more recent evidence militates against this concept (304).

Insulin and Glucagon

Insulin deficiency is associated with disturbances in calcium metabolism (65, 264). Defective intestinal calcium transport, reduced calcium-binding-protein concentrations in the intestine, and hypocalcemia are among the abnormalities reported. Diabetic rats show low concentrations of 1,25-$(OH)_2$-D_3 which rise following insulin treatment. This observation is consistent with a stimulatory influence of insulin on vitamin D metabolism, probably exerted on the renal 1-hydroxylation step. Spencer et al recently provided in vitro evidence for the latter possibility (284).

In vitro administration of insulin supports the concept of stimulation of bone collagen synthesis, an action antagonistic to that of PTH. Administered glucagon (physiological doses) inhibits bone resorption and stimulates calcitonin secretion (42).

In pharmacological doses, glucagon substantially increases demineralization of bone and renal excretions of numerous solutes including calcium (140, 235).

Mineralocorticoids

Aldosterone and deoxycorticosterone acetate, when administered acutely, decrease sodium excretion without affecting the excretion of calcium (163, 198). However, following prolonged administration, healthy animals soon "escape" from the sodium-retaining actions and they regain the ability to excrete sodium. The expansion of extracel-

lular-fluid volume leads secondarily to increased calcium excretion (106).

Mineralocorticoids counteract the effects of PTH on sodium excretion by enhancing sodium reabsorption in the terminal parts of the nephron, and especially in the collecting tubules (106).

Glucocorticoids (GCC)

Acute administration of GCC does not appear to significantly alter calcium excretion (163). However, chronic doses lead to development of hypercalciuria (106). This effect seems to result from enhancement of bone resorption which in turn leads to an increased filtered load. It has also been suggested that the calciuria observed following high doses of GCC might be the result of inhibition of paired sodium and calcium transport in response to chronic ECF expansion, as is the case for mineralocorticoids (106).

GCC have direct effects on the parathyroid glands. GCC treatment causes increased PTH secretion and parathyroid hyperplasia in humans (98) and rats (318). In addition, a direct stimulatory effect on PTH secretion has been demonstrated in cultured parathyroid cells of rats (11).

GCC have been implicated in the regulation of vitamin D metabolism but the data are conflicting. Some laboratories report inhibitory effects of cortisone on intestinal action of $1,25\text{-}(OH)_2\text{-}D_3$ (83) while others report a stimulatory influence on 1α-hydroxylase by cortisol (283). In other experiments, no

effect of GCC on vitamin D action was observed (83, 148). However, GCC are effective in treating acute cases of hypervitaminosis D. They can decrease calcium absorption without affecting the ability of 1,25-$(OH)_2$-D_3 to induce calcium-binding protein (84, 217, 226).

V - The thymus as part of the endocrine system.

The thymus gland is comprised of lymphoid and epithelial cells, and can be rightly designated as a "lymphoepithelial" organ (67, 177, 202, 227). It develops in the embryo from branchial pouches of the pharynx, in close proximity to the precursors of parathyroid glands, thyroid gland and the cells that will later secrete calcitonin (114). In higher vertebrates, epithelial buds grow out as solid cords, pinch off and migrate to the midline of the upper thorax and become infiltrated with lymphocytes. The origin of thymus lymphocytes has long been a subject of controversy. It is now generally agreed that, in the early embryo, they come from the yolk sac and only later do they migrate from the bone marrow.

The typical thymus (202), which looks whitish-grey in some mammals, and pinkish in others, has two lobes containing very small blood vessels. Its weight varies among species, but in all, there is a division of the organ into lobules, each comprised of a cortex and a medulla. Lobular size appears to be consistent from species to species.

All known vertebrates, with the exception of hagfish, possess a thymus gland. However, hagfishes do show ability to reject homografts and may have some yet undiscovered "thymus-like" structure (2). They are also the only vertebrates that do not osmoregulate (188).

In species with long gestation periods, the thymus exerts influences on cellular immunity prior to birth.

Thereafter, it continues to grow in absolute size to the time of puberty, although the thymus weight to body weight ratio may decrease (30, 31).

The thymus undergoes two types of atrophy: one that proceeds gradually with time (age involution), and the other characterized by occasional rapid bursts that follow severe stress or GCC administration (accidental, hormonal involution) (73, 210, 260, 262, 272). In addition, there is diurnal variation in weight (262a). Such changes complicate the problems of determining the size of a normal thymus. The most accurate approximations are obtained from examination of thymuses of healthy persons who died suddenly by accident. Such studies have been reported by Boyd (30, 31) who found that the medulla, (which in most species contains concentrically arranged cells or bodies called Hassal corpuscles) begins to involute at about the age of puberty. Involution of the lymphoid cortex starts at 4 years of age. The connective tissues and fat content increase until old age.

The thymus gland is not directly affected by antigenic stimulation (202) probably because of the barrier formed by the epithelial sheath cells of the organ, and the absence of true macrophages in the cortex.

Endocrine Functions in Immunity

The phenomenom of age involution lead people to believe at one time that thymus is only important in early life. Such belief was supported by observations made following neonatal thymectomy in rodents. These are the occurence of

wasting disease, immunological deficiency, decrease in number of circulating lymphocytes, and loss of ability to reject heterologous grafts. More recently, Haltori and Brandon (120) demonstrated autoimmune-like damage in the ovaries of neonatally thymectomized rats.

But, if the effects of thymectomy are not as drastic in adult animals, they are still important. Metcalf and Miller (170) reported that thymectomized adult mice become lymphopenic and cannot cope with certain stresses to the immunological system. However, these effects develop very slowly. In addition, immune capability cannot be restored following X-irradiation of the thymus (188).

Much is known of the role of the thymus in immunity, but many controversies remain. The gland provides specific populations of lymphocytes, and the most obvious effects of thymectomy can be corrected with thymus grafts (202). The importance of humoral factors released by the gland are obvious from studies in which thymus extracts and thymus tissue placed in chambers that are impermeable to cells have been shown to restore some of the functions (206, 305). The existence of thymic hormones is widely accepted; but the multiplicity of factors identified and/or isolated is puzzling and confusing (12, 109, 169, 306, 317).

The isolated peptides show specific influences on T-cell differentiation, maturation or proliferation within and/or outside the thymus (169).

TABLE I

THYMIC FACTORS AND THYMUS DEPENDENT SERUM FACTORS

FACTOR	AUTHOR	REFERENCE
- Thymosin α 1 β 1 Fraction 5 (mixture of more than 30 peptides)	A.L. Goldstein	107
- Thymopoietin I Thymopoietin II	G. Goldstein	110
- Thymic humoral factor	N. Trainin	306
- Thymic factor	D. Amici	6
- Facteur thymique serique (FTS)	J.-F. Bach	12
- Human FTS	J. Lacovara	12
- Serum factor (SF)	A. Astaldi	111
- Prealbumin	A. White	41
- Thymic epithelium supernatants	A. Kruisbeek	150
- Thymic epithelium - produced chemotactic factor	K. Pyke	238

The Thymus in Endocrine Physiology

The observations of immunologists have been concerned primarily with the influence of thymic humoral factors on immune functions. While the recent findings suggest age related changes in T-cell functions that affect the development of autoimmune disease (12a), they provide little insight into the reasons for the rapid growth of the gland long after its major role in lymphocyte differentiation has been accomplished; nor do their observations of hormonal control of the immune system adequately explain the exquisite sensitivity of the gland to sex steroids secreted by the adult animal and to glucocorticoids (132).

Thymectomy does cause changes not related to the immune system. Thymic influences have been demonstrated on:

- Thyroid gland (57, 58, 233)
- adrenal glands (39, 58, 59, 66, 81, 184, 233, 276, 295)
- gonrads and secondary sex characteristics (144, 182, 183, 189, 190, 214, 257)
- pituitary gland (59, 60, 142, 233)
- blood glucose concentration (59)
- muscle physiology (110, 112, 143)
- calcium and phosphorus metabolism (207, 208, 234, 267)

The present study is concerned with the investigation of:

a - The role of the thymus gland in the metabolism of calcium, inorganic phosphate, and related electrolytes.

b - Vitamin D, thymus gland and seasonal interactions in the regulation of some aspects of electrolyte excretion.

c - Some interrelationships between thymus, parathyroid and thyroid glands.

MATERIALS AND METHODS

1) Experimental animals

Young adult hooded rats of a Long-Evans derived strain were bred in our laboratory. To avoid complications imposed by hormonal changes associated with ovarian cycles, only males were used. Since experimental procedures included thyroparathyroidectomy and parathyroidectomy which impair growth and are poorly tolerated by immature animals, surgery was delayed until rats attained ages of 7-8 weeks and body weights of 180-200 grams.

2) Housing

The animals were maintained in a temperature and humidity controlled room, and exposed to natural changes in environmental lighting.

3) Surgery

All surgical procedures were performed under ether anesthesia according to the methods described by Segaloff (82). One group of rats was thymectomized (TMX). Sham-thymectomy was performed on a second group (SHAM) to control for the possible effects of the deep anesthesia, cutting of the sternum, exposure of the chest cavity to atmospheric pressure and other stress. The SHAM operation was identical to the thymectomy except that the thymus gland was replaced in the thoracic cavity after the surrounding connective tissue was loosened. Additional groups of rats were thymectomized-parathyroidectomized (TMX-PX), sham-thymectomized-parathy-

roidectomized (SHAM-PX), thymectomized-thyroparathyroidectomized (TMX-TPX), sham-thymectomized-thyroparathyroidectomized (SHAM-TPX).

Following surgery, animals were housed in group cages containing wood shavings for a 2-day recovery period. They were then placed in individual metabolism cages for the duration of the study.

4) Metabolic studies

Each cage was equipped to permit monitoring of food and fluid intake, and collection of feces-free urine. The animals had continuous access to powdered food and drinking water.

5) Diet

Depending on the particular experiment, the rats were given one of the following diets, along with tap water ad libitum:

Regular diet: Purina laboratory Chow containing 1.20 gmCa, 0.86 gmP and 530 IU of vitamin D_3 per 100 grams.

Vitamin D_3-deficient diet: Purified Diet from Ralston Purina Company, containing also 1.2% Ca and 0.86% P but no vitamin D_3. The animals given this diet had been previously kept for three weeks on the Regular Diet so that the effect of vitamin D_3 deprivation could be observed.

6) Hormone administration

After a control period of three weeks during which metabolic studies were performed, selected groups of animals were injected subcutaneously three times weekly for three weeks with one of the following dosages of vitamin D_3:

"Low dose": 400 IU/injection

"Medium dose": 800 IU/injection

"High dose": 4000 IU/injection

Source of the vitamin: Vitamin D_3 Powder from Nutritional Biochemicals Corporation. 400,000 units/gram #1640.

7) Measurements

A- Body weights, and food and fluid intakes were measured on a weekly basis.

B- Measurements of urine: weekly determinations were made of urine volume, and urine content of the following electrolytes: Calcium (Ca), inorganic phosphate (Pi), chloride (Cl), sodium (Na), potassium (K)

- Calcium was measured according to the method of Kingsley and Robnett (145).
- Inorganic phosphate was measured according to the method of Fiske and Subarrow (86).
- Chloride was measured with an Orion specific ion electrode and Corning Model #7 pH meter.
- Sodium and potassium were measured by Flame Photometer (IL Model #143 equipped with #144 automatic dilutor).

C- Measurements of plasma: Samples of blood were taken from

the tail vein prior to and following D_3 treatment, permitted to clot at room temperature, and centrifuged. The serum was used for the following determinations:

- Calcium: measured colorimetrically with reagent Di (O-hydroxyphenylimine) ethane, with the aid of a commercially purchased HPE calcium kit (126).
- Inorganic phosphate: measured according to the method of Fiske and Subarrow (86), modified for the use of 50µl samples.

8) Autopsy: At the end of the experiments each animal was exsanguinated under ether anesthesia. The blood was used for determination of calcium and phosphate concentrations, sodium and potassium concentrations and hematocrit. All the animals were examined for grossly visible abnormalities including possible soft tissue calcification and completeness of thymectomy where appropriate. Endocrine glands and other pertinent organs were especially examined and their wet weights obtained with an electro-balance.

9) Data analysis: Data were pooled according to diet and vitamin D_3 treatment. Urinary electrolytes were corrected for food intakes and expressed as mg or MEQ /wk/rat/100g Food intake. In addition, since the values varied with the time of the year, the data were pooled in the following manner, based on the similarity of the results for these time periods:

a) For calcium and phosphate

"Winter" experiments: November - March

"Spring-Summer" experiments: April - August

b) Seasonal variations in chloride, sodium and potassium excretion were more marked and displayed different patterns than those for P and Ca. The data for these ions were grouped into

"Winter" - November - March

"Spring" - April - June

"Summer" - June - August

Samples for all the above time periods were collected during at least three consecutive years.

Analysis of variance and paired t tests were utilized for the statistical studies.

RESULTS

I- URINARY EXCRETIONS OF CALCIUM AND ASSOCIATED CHANGES IN SERUM CALCIUM CONCENTRATIONS.

A - Influences of surgery on seasonal variations in calcium excretion (Table 2).

Animals with intact thyroid and parathyroid glands excreted more than twice as much calcium during the "winter" than during the "summer" months. (Fig. 5, TMX and SHAM groups, summer:winter, $P<.001$).

Parathyroidectomy did not appreciably affect calcium excretion during the winter, but it did blunt the seasonal differences. Thyroparathyroidectomy also failed to appreciably affect calcium excretion in the winter. However, it totally abolished summer:winter differences in animals with thymus glands (TPX group).

Thymectomized groups (TMX, TMX-PX and TMX-TPX) all excreted more calcium than their sham operated counterparts (SHAM, PX, and TPX) during the winter. An effect of thymus deprivation on calcium excretion is apparent for the summer only when animals with intact thyroid and parathyroid glands are compared (TMX vs SHAM $P<0.05$).

B - Influences of surgery on seasonal variations in serum calcium concentrations (Table 3).

Sham operated animals had the highest serum calcium

values in both the summer and winter. The values were lower for the summer (although the animals excreted less calcium). Parathyroidectomy reduced serum calcium levels somewhat during the winter. It more markedly lowered them during the summer, and it thereby exaggerated the summer:winter differences. Thyroidectomized rats (TPX) had serum calciums similar to those of parathyroidectomized ones (PX); but the summer depression of calcium concentrations was less marked than in animals with parathyroid glands. (Fig. 6)

Thymectomized animals had lower serum calcium concentrations than their sham operated counterparts during the winter months. The values for TMX and TMX-PX were also lower than those for SHAM and PX, respectively, in the summer. However, animals deprived of all three glands failed to show a reduction in serum calcium during the summer.

C - Effects of vitamin D administration on calcium excretion (Table 4).

The administration of 400 IU vitamin D 3x weekly significantly increased the calcium excretion for SHAM, TMX, PX and TPX groups during the winter. The effects were most marked for TMX animals. The increment for TPX-TMX animals is not statistically significant, while TMX-PX animals showed no obvious response to D_3. (Fig. 7)

Administration of the same dose of vitamin D during the summer reduced the calcium excretion in all surgical groups. The depression is statistically significant for all

TABLE 2

EFFECTS OF SURGERY ON SEASONAL VARIATIONS IN CALCIUM EXCRETION
Mg/Rat/Wk/100 Gm Food Intake: Mean ± S.E.

SURGICAL GROUP	# rats	SUMMER (S)	# rats	WINTER (W)	W/S	W:S (P-values)
SHAM	17	2.1 ± 1.2	15	5.6 ± 1.7	2.66	P << 0.001
TMX	18	3.3 ± 2.1	13	7.0 ± 3.1	2.12	P < 0.001
PX-SHAM (PX)	3	3.2 ± .9	6	5.8 ± 2.0	1.81	P < 0.05
PX-TMX	6	3.6 ± 1.4	4	6.8 ± 2.1	1.89	P < 0,05
TPX-SHAM (TPX)	7	5.6 ± 2.2	5	5.3 ± .6	.95	
TPX-TMX	7	5.4 ± 1.4	4	7.2 ± 1.2	1.33	

Comparison of Means (P values)

TMX : SHAM < 0.05
TPX-TMX : PX-TMX < 0.05
PX : SHAM < 0.03
TPX : SHAM << 0.001
TPX-TMX : TMX < 0.01
PX : TPX < 0.01
TPX-TMX : TPX < 0.03
(TPX + PX) -TMX : TPX + PX < 0.05

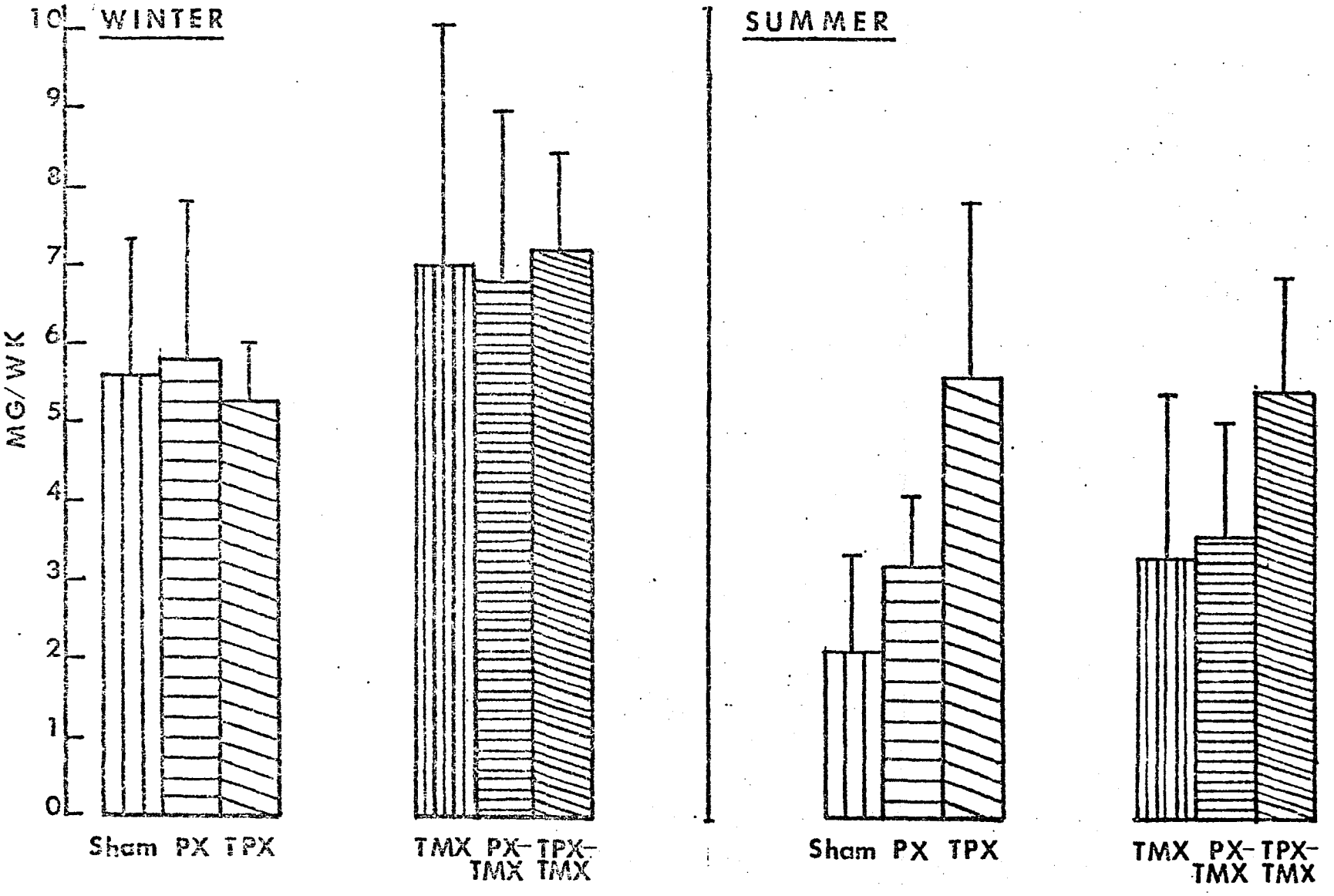

FIG.5 : RENAL EXCRETION OF CALCIUM

TABLE 3

EFFECTS OF SURGERY ON SEASONAL VARIATIONS IN SERUM CALCIUM CONCENTRATIONS

Mg % : Mean ± S.E.

SURGICAL GROUP	# rats	SUMMER (S)	# rats	WINTER (W)	W/S	W:S (P values)
SHAM	7	8.4 ± .7	13	10.1 ± .7	1.20	$P < 0.001$
TMX	7	7.1 ± .3	13	9.8 ± .7	1.38	$P < 0.001$
PX - SHAM (PX)	3	6.0 ± .6	5	9.0 ± 1.4	1.50	$P \ll 0.001$
PX - TMX	6	5.8 ± 1.5	4	8.5 ± 1.8	1.47	$P \ll 0.001$
TPX - SHAM (TPX)	6	7.9 ± 1.0	5	9.2 ± 1.8	1.16	
TPX - TMX	7	8.1 ± 1.7	4	8.2 ± 1.4	1.02	

Comparison of means (P Values)

TMX : SHAM < 0.001
PX : SHAM < 0.001
PX-TMX : TMX < 0.05
PX : TPX < 0.05
TPX-TMX : PX-TMX < 0.001

TMX+SHAM : PX+TPX 's < 0.001

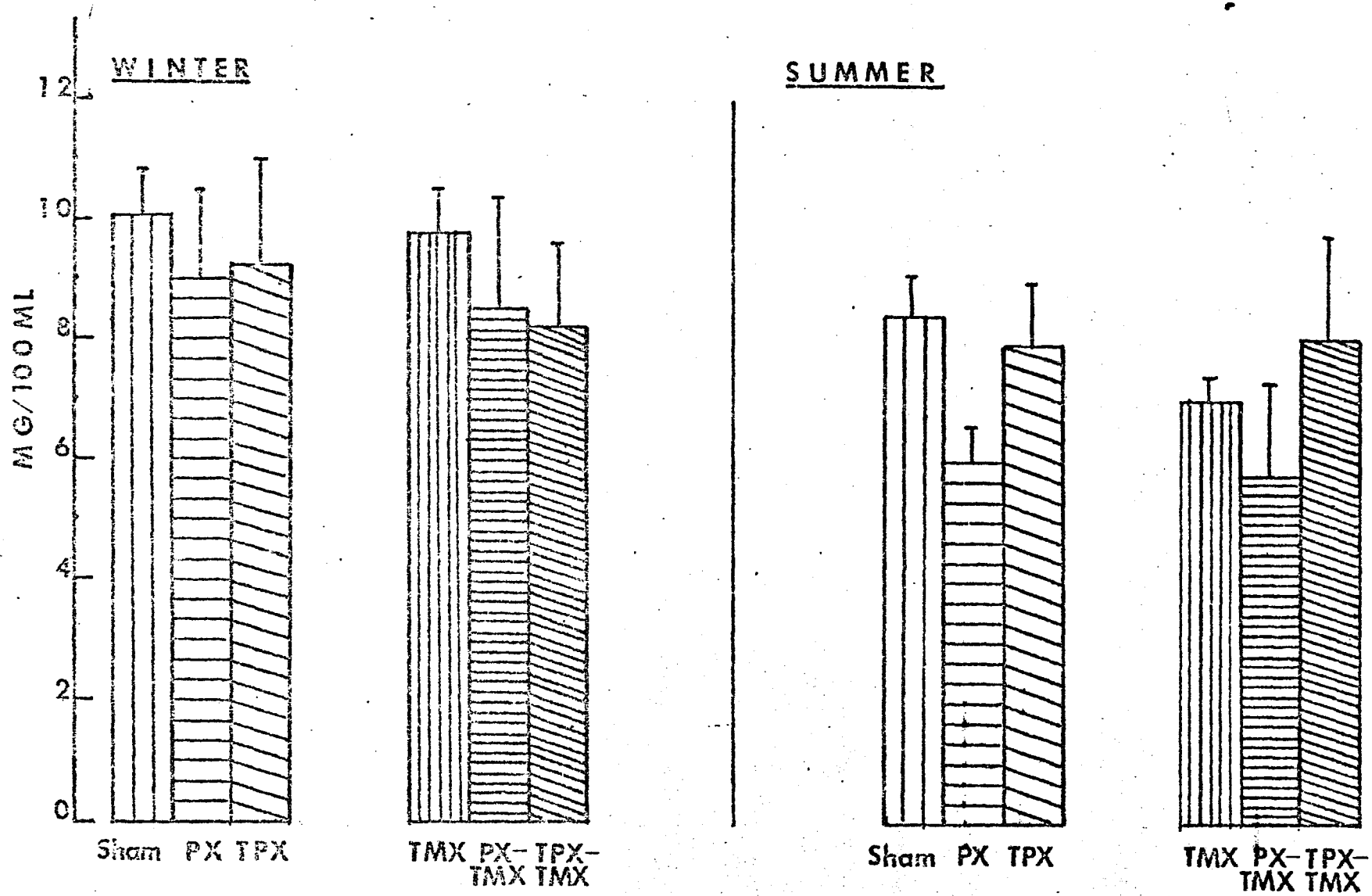

FIG.6: SERUM CALCIUM

TABLE 4

EFFECTS OF VITAMIN D_3 ADMINISTRATION ON CALCIUM EXCRETION

Mg/Rat/Wk/100 Gm Food Intake : Mean ± S.E.

	WINTER					
SURGICAL GROUP	# rats	Before D_3	After D_3 1200IU/Wk	# rats	Before D_3	After D_3 2400IU/Wk
SHAM	5	5.4 ± 1.3	8.6 ± 2.7●	8	5.3 ± 1.3	3.7 ± .5▲
TMX	5	5.7 ± 3.0	9.8 ± 5.4◆	8	7.2 ± 2.9	4.3 ± .8◆
PX-SHAM (PX)	6	5.8 ± 2.0	8.1 ± 4.4□			
PX-TMX	4	6.8 ± 2.1	6.5 ± 2.6			
TPX-SHAM (TPX)	5	5.3 ± 0.6	8.1 ± 2.2□			
TPX-TMX	4	7.2 ± 1.2	7.7 ± 1.8			

	SUMMER								
	rats	Before D_3	After D_3 1200IU/Wk	# rats	Before D_3	After D_3 2400IU/Wk	# rats	Before D_3	After D_3 12000IU/Wk
SHAM	6	2.6 ± 0.4	2.4 ± 0.9	1	3.1	2.2	3	3.8 ± 1.0	5.2 ± 0.5●
TMX	7	4.3 ± 2.4	3.1 ± 1.0	1	4.2	1.7	1	4.9	5.9 ●
PX-SHAM (PX)	1	4.3	2.1 ◇				2	2.8 ± 0.5	5.3 ± 1.5▲
PX-TMX	1	4.2	1.6 ◇				5	3.4 ± 1.5	4.9 ± 0.8▲
TPX-SHAM (TPX)	2	3.0 ± 0.7	2.3 ± 0.5◇●	1	8.6	4.3 ●	3	5.8 ± 0.8	10.6± 2.7▲
TPX-TMX	2	3.7 ± 0.5	2.4 ± 0.5◇●	2	6.1 ± 0.5	2.8 ± 0.5●	3	6.1 ± 1.4	12.3± 3.7▲

P Values (+D_3 : No D_3)

Individual surgical groups

□ P < 0.05
◆ P < 0.03
▲ P < 0.01

Combined groups

◇ PX + TPX + PX-TMX + TPX-TMX P < 0.01
● TMX + SHAM < 0.03

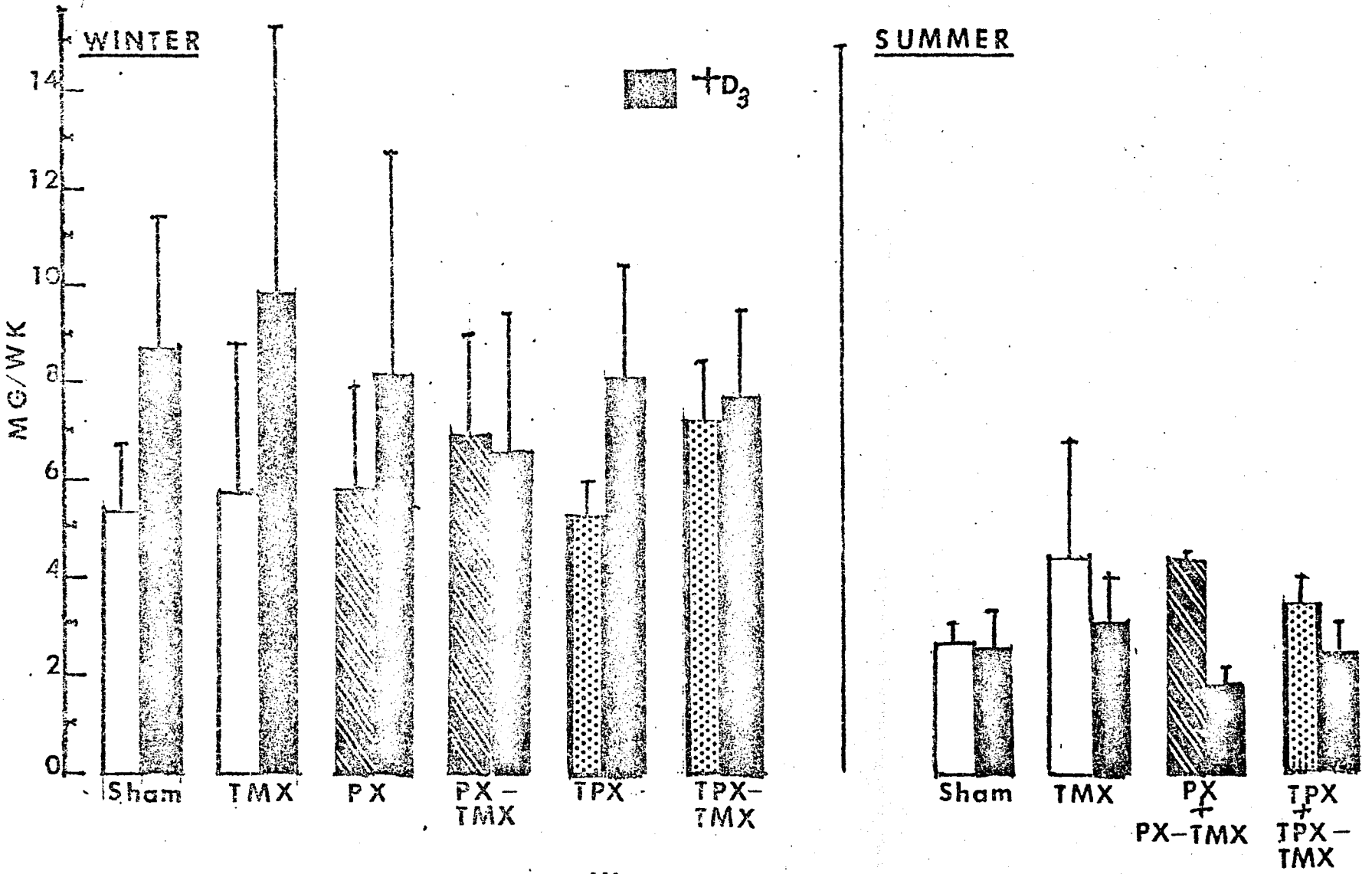

FIG.7: EFFECT OF D_3 1200 IU/WK ON CALCIUM EXCRETION

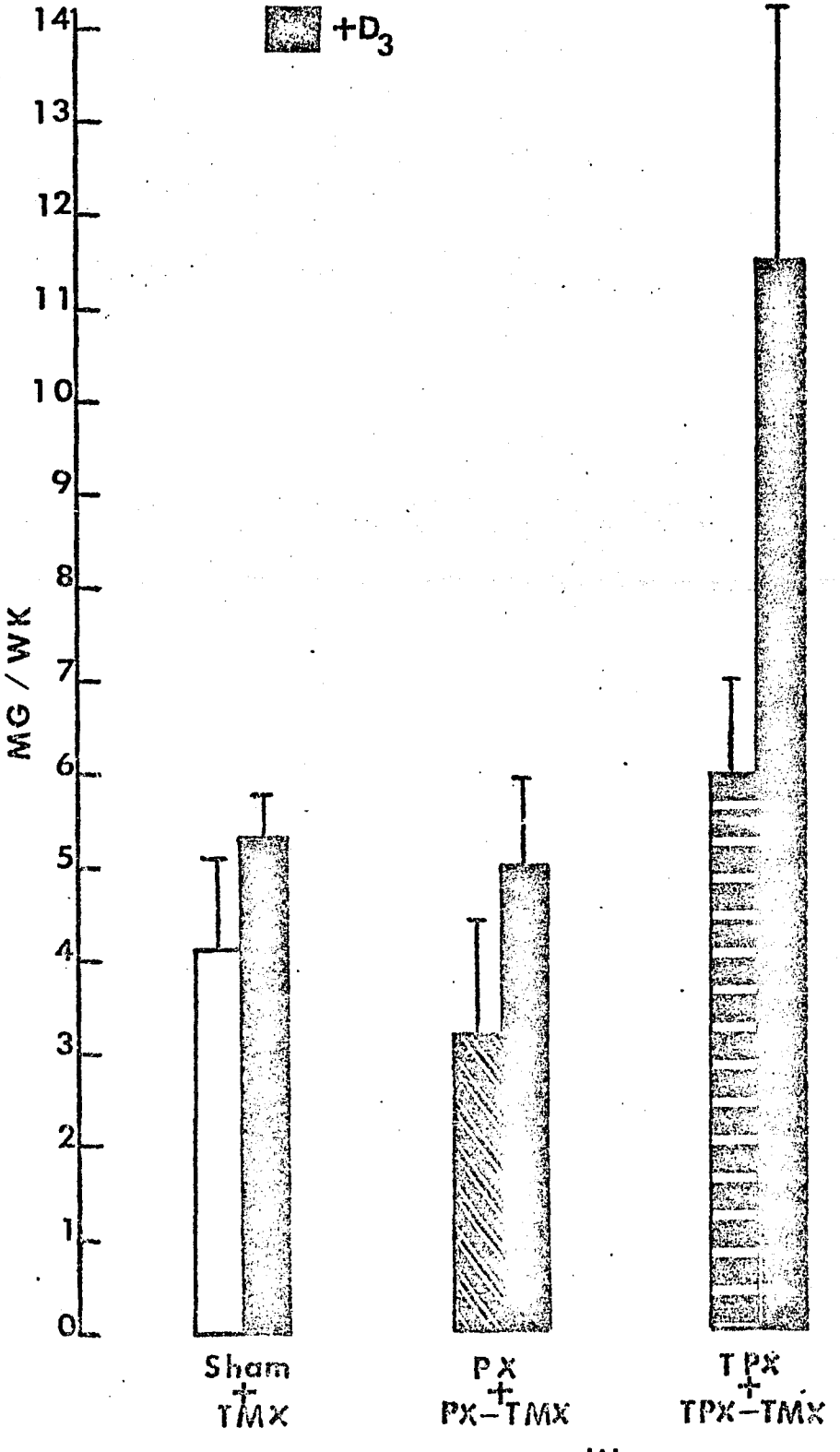

FIG.8 : EFFECT OF D_3 12,000 IU/WK ON CALCIUM EXCRETION

animals without parathyroid glands (PX, TMX-PX, TPX and TMX-TPX). (Fig. 7)

By contrast with the above, the administration of 800 IU 3x weekly lowered calcium excretion of all rats tested (SHAM and TMX) during the winter, and it also markedly lowered calcium excretion for all groups tested during the summer (SHAM, TMX, TPX, TPX-TMX).

On the other hand, when a very large dose (4000 IU 3x wkly) was given during the summer, it markedly increased calcium excretion for all surgical groups. (Fig. 8)

D - Effects of vitamin D on serum calcium concentrations (Table 5).

The injection of 400 IUD_3 3x weekly slightly elevated the serum calcium concentrations of SHAM and TMX animals in the winter, but had little influence on the calcium levels of the other surgical groups. By contrast, the same dose given in the summer substantially raised the calcium concentrations of all of the groups while it maintained the differences associated with parathyroidectomy and thyroparathyroidectomy.

The rises in serum concentrations during the summer months were associated with reduced renal calcium excretion.

Removal of the parathyroid glands impaired the ability of exogenous D to elevate the serum calcium concentrations. However, thyroparathyroidectomized animals had post-treatment values closer to those for the SHAM and TMX groups

TABLE 5

EFFECTS OF VITAMIN D_3 ON SERUM CALCIUM CONCENTRATIONS
MG % : Mean ± S.E.

		WINTER				
SURGICAL GROUP	# rats	Before D_3	After D_3 1200IU/Wk	# rats	Before D_3	After D_3 2400IU/Wk
SHAM	5	10.3 ± 0.6	10.7 ± 0.4	8	10.0 ± 0.7	10.5 ± 0.5 ●
TMX	5	9.5 ± 0.7	10.7 ± 2.1	8	9.9 ± 0.7	10.6 ± 0.3 ○
PX-SHAM (PX)	5	9.0 ± 1.4	8.4 ± 2.9			
PX-TMX	4	8.5 ± 1.8	8.5 ± 1.8			
TPX-SHAM (TPX)	5	9.2 ± 1.8	8.8 ± 1.2			
TPX-TMX	4	8.2 ± 1.4	8.8 ± 1.2			

		SUMMER							
SURGICAL GROUP	# rats	Before D_3	After D_3 1200IU/Wk	# rats	Before D_3	After D_3 2400IU/Wk	# rats	Before D_3	After D_3 12000IU/Wk
SHAM	6	8.3 ± 0.6	11.4 ± 2.4 ◆	1	9.4	10.4	3	8.3 ± 0.1	8.8 ± 0.2
TMX	7	7.1 ± 0.3	10.7 ± 0.8 ○	1	10.2	11.9	1	7.8	9.4
PX-SHAM (PX)	1	5.6	7.3				2	6.3 ± 0.6	10.0 ± 0.9
PX-TMX	1	4.8	6.1				5	5.9 ± 1.6	9.2 ± 0.3 ○
TPX-SHAM (TPX)	2	7.7 ± 1.8	10.4 ± 1.6	1	8.7	13.3	3	7.8 ± 0.9	11.9 ± 1.7
TPX-TMX	2	7.0 ± 2.8	8.4 ± 1.0	2	9.6 ± 0.1	11.5 ± 1.6 ■	2	8.0 ± 0.1	10.0 ± 0 ●

P VALUES (+D_3 : NO D_3)

Individual surgical groups

◆ $P < 0.02$
● $P < 0.01$
○ $P < 0.001$

Combined surgical groups

■ TMX + SHAM $P < 0.05$

treated during the summer.

The administration of 800 IU 3x weekly elevated the serum calcium concentrations of SHAM and TMX animals treated during the winter and summer. This rise was associated with a reduction in excretion.

The administration of 12,000 IU/wk of vitamin D_3 in the summer increased serum calcium levels of all rats. As in the case of urine excretion of Ca following that dose, the rise in serum Ca was most significant in animals deprived of parathyroids and/or thyroid glands. The findings are consistent with pharmacological influences of the vitamin D which should be (and are) more apparent in animals deprived of the main regulatory organs of calcium homeostasis.

E - Effects of vitamin-D (dietary) deprivation - Effects of 2400 IU/wk D_3 in D-deficient animals. (Table 6)

TMX and SHAM animals fed a D_3-deficient diet in the summer had Ca excretion values similar to those obtained when the same animals were maintained on a normal diet. The Deficient diet brought the serum concentrations of TMX animals in range of those of SHAM.

The administration of 800 IU 3x weekly of vitamin D_3 to D_3-deficient TMX and SHAM reduced the excretion of calcium to values below those that can be detected by Colorimetic method. (value $<$ 0.5mg %) (Fig. 9). There was a concomittant rise in serum calcium concentrations to values higher than those observed in similar rats on a normal diet

TABLE 6

EFFECTS OF DIETARY D_3 AND 2,400 IU/WK D_3 ADMINISTRATION ON CALCIUM EXCRETION AND SERUM CALCIUM CONCENTRATION (SUMMER)

A- CALCIUM EXCRETION (Mg/Rat/Wk/ 100 Gm Food Intake : Mean ± S.E.)

SURGICAL GROUP	# RATS	CONTROL DIET (CD)	D_3- DEFICIENT DIET (DF)	D_3-DEFICIENT DIET + 2400 IU/WK ($+D_3$)	P VALUES
SHAM	8	1.3 ± 1.0	2.2 ± 1.8	< 0.5	DF:$+D_3$ < 0.001
TMX	9	2.2 ± 1.4	2.4 ± 1.4	< 0.5	DF:$+D_3$ < 0.001

B- SERUM CALCIUM CONCENTRATION (Mg % : Mean ± S.E.)

SURGICAL GROUP	# RATS	CONTROL DIET (CD)	D_3-DEFICIENT DIET (DF)	D_3-DEFICIENT DIET + 2400 IU/WK ($+D_3$)	P VALUES
SHAM	8	8.4 ± 0.7	8.2 ± 0.6	13.4 ± 5.0	DF:$+D_3$ < 0.05
TMX	9	7.1 ± 0.3	8.2 ± 0.3	13.9 ± 4.0	CD:DF < 0.001 DF:$+D_3$ < 0.01

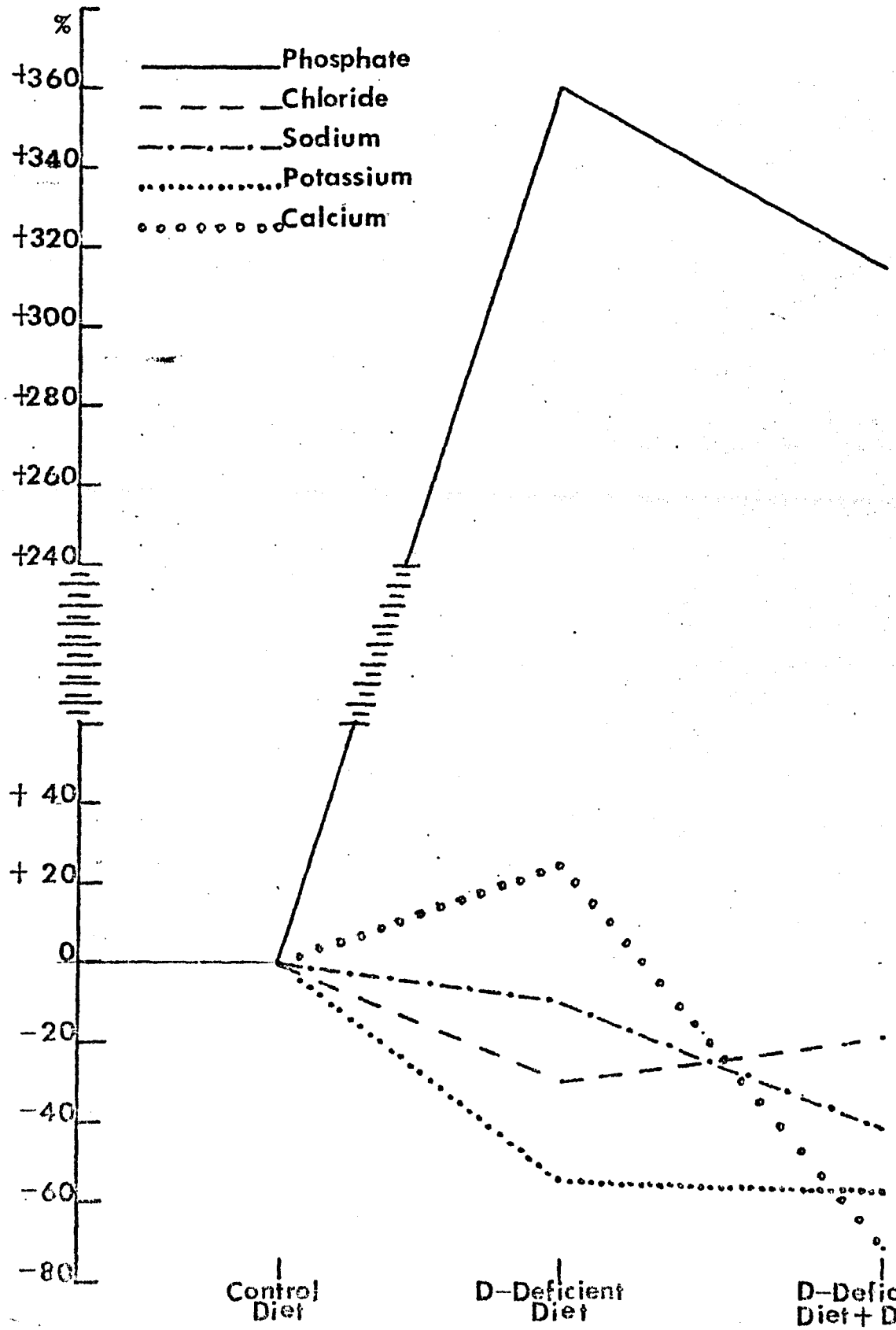

FIG.9 : Effect of D-Deficient Diet and administration of 2400 IU/WK D_3 ($+D_3$) on renal electrolyte excretion in SHAM+TMX rats

(? X, 63% higher in SHAM).

I: TION OF PHOSPHATE AND ASSOCIATED CHANGES
SPHATE CONCENTRATIONS.

A: surgery on seasonal variations in phosphate
ble 7).

ntact thyroid and parathyroid glands excreted
a: sh phosphate during the "winter" than during
t: s. (Fig. 10)

tomy decreased phosphate excretion. The
e: ignificant in the winter.($P<0.01$). But,
t: differences were still observed in PX rats.
roidectomized animals had phosphate excre-
ficantly lower than those of intact animals
ever, this was not observed in the summer.
nimals tend to show higher values than
summer differences were blunted, but not
removal.
not significantly affect phosphate
VMX animals tended to have higher ex-
SHAM animals.

surgery on seasonal variations in serum
ntrations (Table 8).
winter:summer differences were found

TABLE 7

EFFECTS OF SURGERY ON SEASONAL VARIATIONS IN PHOSPHATE EXCRETION
Mg/rat/Wk/ 100 Gm Food Intake: Mean ± S.E.

SURGICAL GROUP	# rats	WINTER (W)	# rats	SUMMER (S)	W/S	W:S (P values)
SHAM	5	81.1 ± 12.3	17	44.6 ± 16.8	1.82	$P < 0.01$
TMX	5	90.8 ± 13.9	18	47.2 ± 20.3	1.92	$P < 0.01$
PX - SHAM (PX)	6	58.9 ± 25.0	3	35.0 ± 14.9	1.68	$P < 0.05$
PX - TMX	4	58.7 ± 20.0	6	39.8 ± 18.8	1.47	$P < 0.05$
TPX - SHAM (TPX)	5	58.8 ± 20.3	7	52.8 ± 22.1	1.11	
TPX - TMX	4	66.9 ± 24.0	7	47.0 ± 10.7	1.42	

Comparison of means (P Values)

TMX+SHAM : TPX+TPX-TMX < 0.01
TMX+SHAM : PX +PX-TMX < 0.01
WINTER rats : SUMMER rats < 0.001

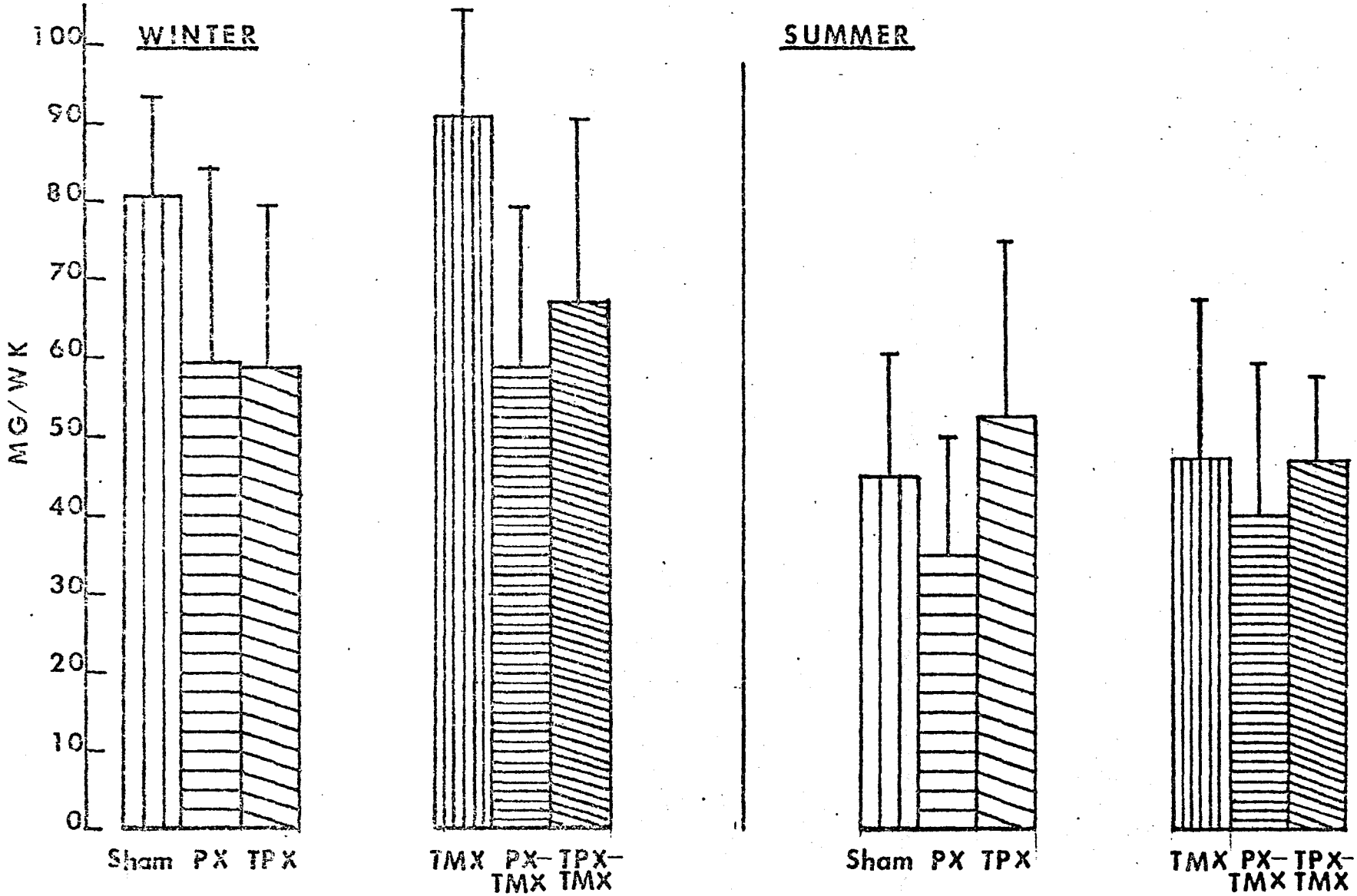

FIG.10 : RENAL EXCRETION OF PHOSPHATE

TABLE 8

EFFECTS OF SURGERY ON SEASONAL VARIATIONS IN SERUM PHOSPHATE CONCENTRATIONS

Mg % : Mean ± S.E.

SURGICAL GROUP	# rats	WINTER	# rats	SUMMER
SHAM	13	8.4 ± 0.8	7	8.2 ± 1.2
TMX	13	7.9 ± 1.3	7	8.0 ± 0.6
PX-SHAM (PX)	5	9.8 ± 1.3	3	8.4 ± 2.2
PX-TMX	4	9.6 ± 0.8	6	9.7 ± 1.7
TPX-SHAM (TPX)	5	9.3 ± 1.6	6	9.1 ± 1.2
TPX-TMX	4	9.5 ± 0.6	7	8.4 ± 1.3

Comparison of mean (P Values)	PX : SHAM < 0.05 PX-TMX : TMX < 0.05 TPX-TMX : TMX < 0.01 TPX : SHAM < 0.05 TMX+SHAM : PX's < 0.01 TMX+SHAM : TPX's < 0.01	PX-TMX : TMX < 0.05

for any of the surgical groups. (Fig. 11)

Parathyroidectomy elevated serum phosphate during the winter in animals with and without thymus gland. It also increased summer values, most significantly for the animals without thymus gland (TMX : PX-TMX $P<0.05$).

Thyroparathyroidectomized animals had serum phosphate concentrations comparable to those of just parathyroidectomized ones during the winter, summer months.

The phosphate concentrations for just thymectomized animals tended to be lower than those of SHAM animals. However, PX-TMX rats had values significantly higher than TMX or PX-SHAM, SHAM during the summer. ($P<0.05$)

C - Effects of vitamin D administration on phosphate excretion (Table 9).

The administration of 400 IU D_3 3x weekly for three weeks, during the winter, increased the phosphate excretion of PX rats, but had little influence on TMX, SHAM and TPX groups. (Fig. 12)

No animals were given 800 IU D_3 during a comparable "winter" period (November-January). However, SHAM and TMX rats received such a dose (3x weekly) in late winter (February-March). Although no similar seasonal variations were observed for the other ions, the March animals (16 rats) had much lower P excretion values than the other "winter" rats prior to vitamin D treatment. (Table 9, Special Group) Following D_3 administration the P excretion values were increased

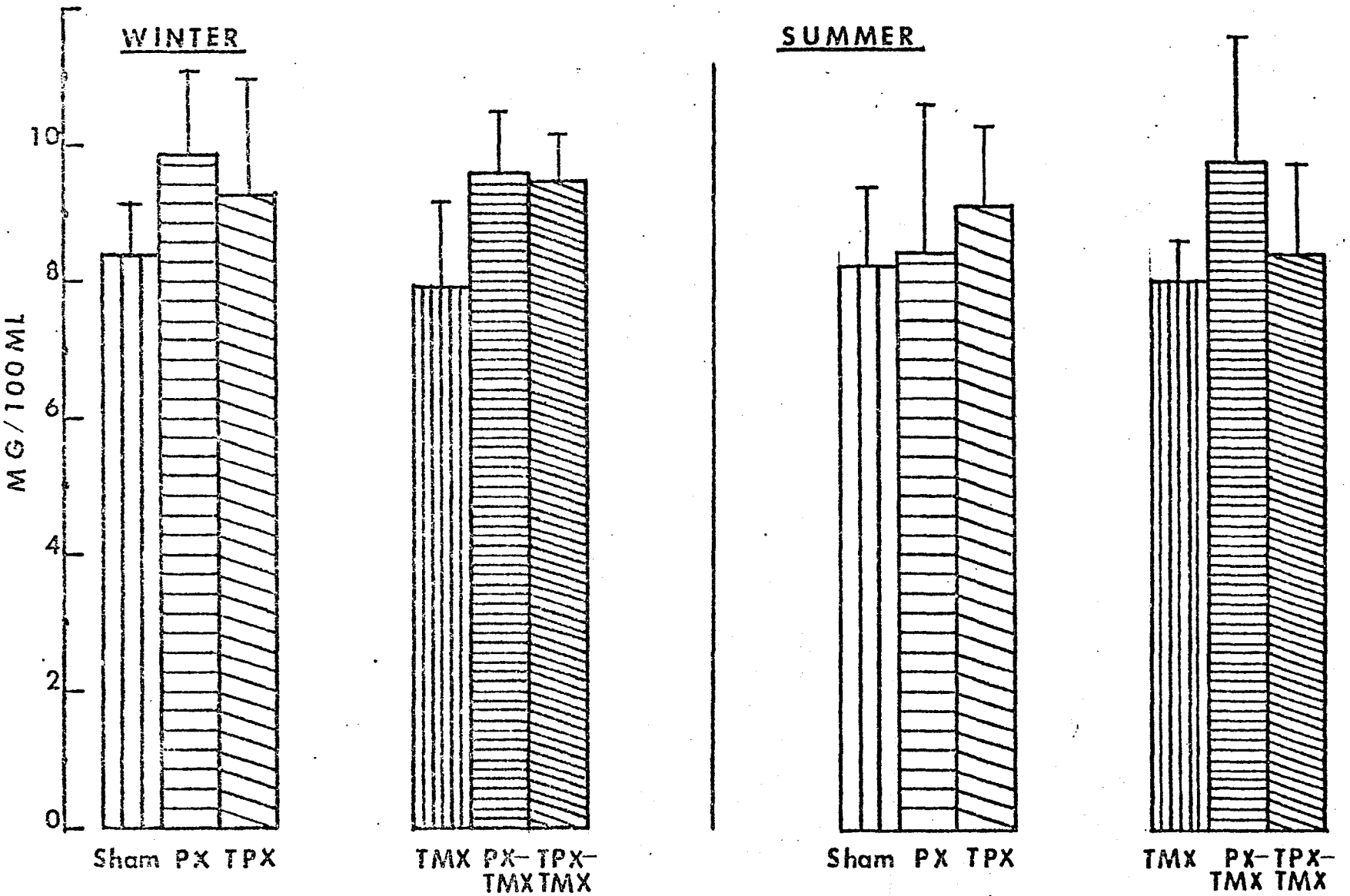

FIG.11 : SERUM PHOSPHATE

especially in SHAM rats. (Fig. 13)

During the summer, the administration of 400 IUD_3 3x weekly for three weeks increased phosphate excretion in rats with thyroid glands. It was most effective in just thymectomized rats. However, as in the winter, it tended to decrease phosphate excretion in TPX animals and to raise it in the two PX rats. (Fig. 12)

Administration of twice the dose (800 IU D_3) of the vitamin to TPX, TMX and SHAM animals resulted in a decrease in phosphate excretion during the summer.

In all groups treated with 4000 IU D_3 3x weekly during the summer, phosphate excretion increased tremendously: PX rats showed 108% increased, TMX and SHAM 78%, while TPX animals had 29% increase following the vitamin administration. (Fig. 14)

D - <u>Effects of vitamin D administration on serum phosphate concentrations (Table 10).</u>

Administration of 400 IUD_3 had little influence on serum phosphate concentrations during the winter. However, during the summer, the vitamin treatment increased serum phosphate values of SHAM rats, while it decreased those of TPX rats. D_3 raised the serum concentration of the only PX-SHAM treated with this dosage during the summer. However, the animal had a very low value prior to the treatment. By contrast, the one PX-TMX observed at the same time had a higher serum P concentration which was lowered by the vita-

TABLE 9

EFFECTS OF VITAMIN D_3 ADMINISTRATION ON PHOSPHATE EXCRETION
Mg/Rat/Wk/100 Gm Food Intake: Mean ± S.E.

	WINTER			SPECIAL GROUP (FEBRUARY-MARCH)		
SURGICAL GROUP	# rats	Before D_3	After D_3 1200IU/Wk	# rats	Before D_3	After D_3 2400IU/Wk
SHAM	5	81.1 ± 12.3	92.6 ± 14.5	8	36.2 ± 11.6	47.7 ± 6.3♦
TMX	5	90.8 ± 13.9	86.5 ± 16.4	8	39.5 ± 15.7	47.7 ± 11.6◊
PX-SHAM (PX)	6	58.9 ± 25.0	70.2 ± 30.4●			
PX-TMX	4	58.7 ± 20.0	69.9 ± 20.6○			
TPX-SHAM (TPX)	5	58.8 ± 20.3	53.0 ± 13.3			
TPX-TMX	4	66.9 ± 24.0	57.8 ± 23.5			

	SUMMER								
SURGICAL GROUP	# rats	Before D_3	After D_3 1200IU/Wk	# rats	Before D_3	After D_3 2400IU/Wk	# rats	Before D_3	After D_3 12000IU/Wk
SHAM	6	55.3 ± 12.4	58.6 ± 8.9○	1	40.2	35.8	3	50.8 ± 6.9	80.4 ± 10.4○
TMX	7	57.8 ± 11.3	66.9 ± 8.0●	1	49.7	21.6	1	40.9	101.9
PX-SHAM (PX)	1	46.1	48.8				2	29.4 ± 16.0	77.0 ± 6.9▽
PX-TMX	1	19.2	20.0				5	44.0 ± 17.7	85.3 ± 14.9▽
TPX-SHAM (TPX)	2	50.1 ± 0.1	36.9 ± 20.0■	1	57.7	46.7	3	46.9 ± 1.0	53.1 ± 11.0▲
TPX-TMX	2	45.2 ± 25.6	27.4 ± 21.5■	2	48.9 ± 4.7	43.4 ± 1.2◊	3	46.9 ± 25.2	67.7 ± 22.7▲

P VALUES ($+D_3$: No D_3)

Individual surgical groups
- ■ P < 0.1
- ● P < 0.05
- ♦ P < 0.01

Combined surgical groups
- ◊ TMX + SHAM < 0.05
- ○ TMX + SHAM < 0.01
- ▽ TMX + SHAM < 0.001
- ▲ TPX's + PX's < 0.001

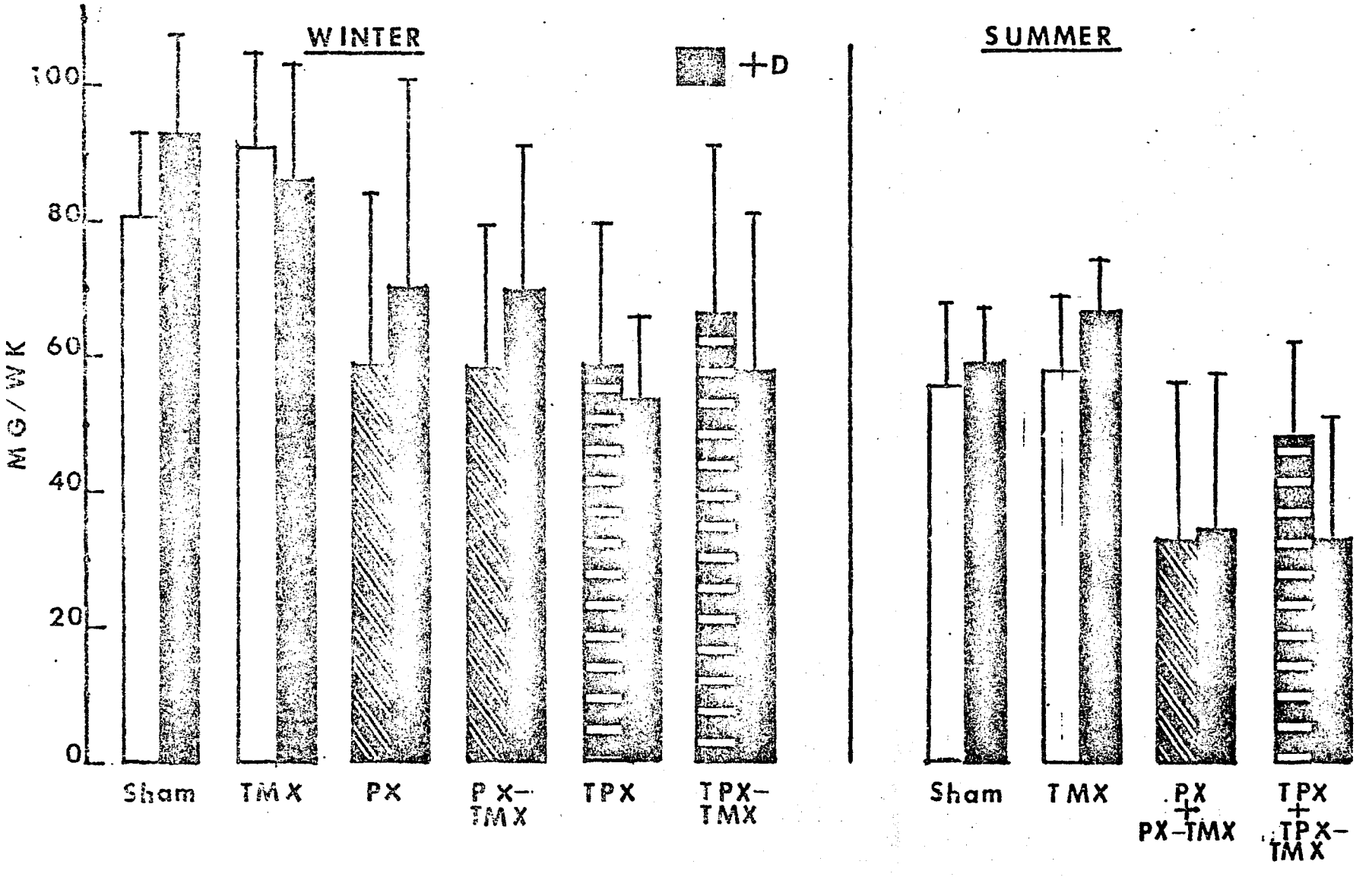

FIG.12: EFFECT OF D_3 1200 IU/WK ON PHOSPHATE EXCRETION

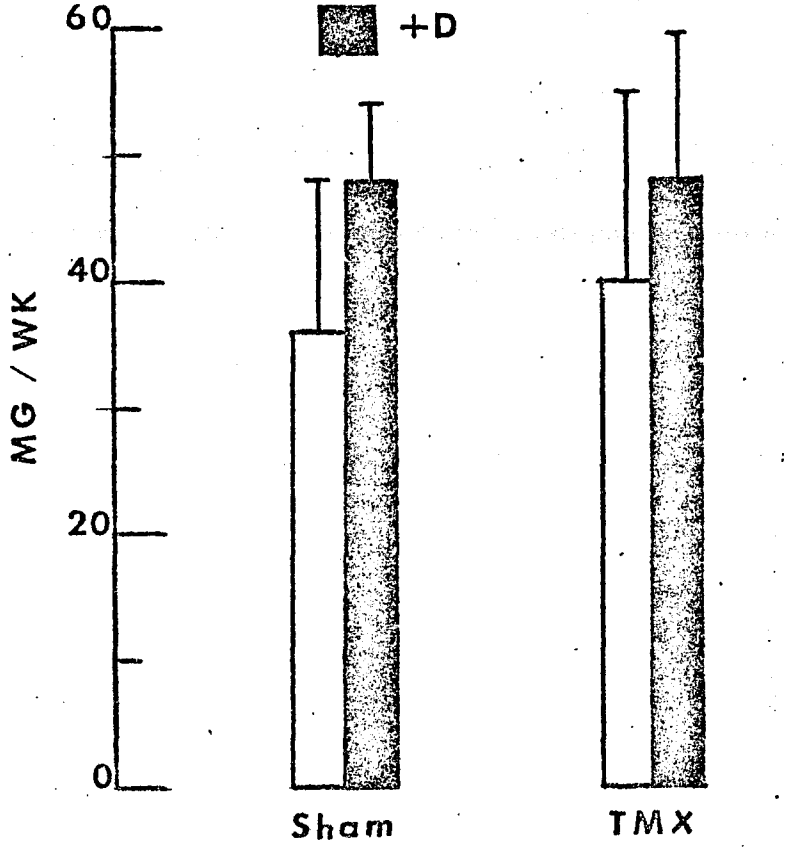

FIG. 13: EFFECT OF D_3 2400 IU/WK ON PHOSPHATE EXCRETION

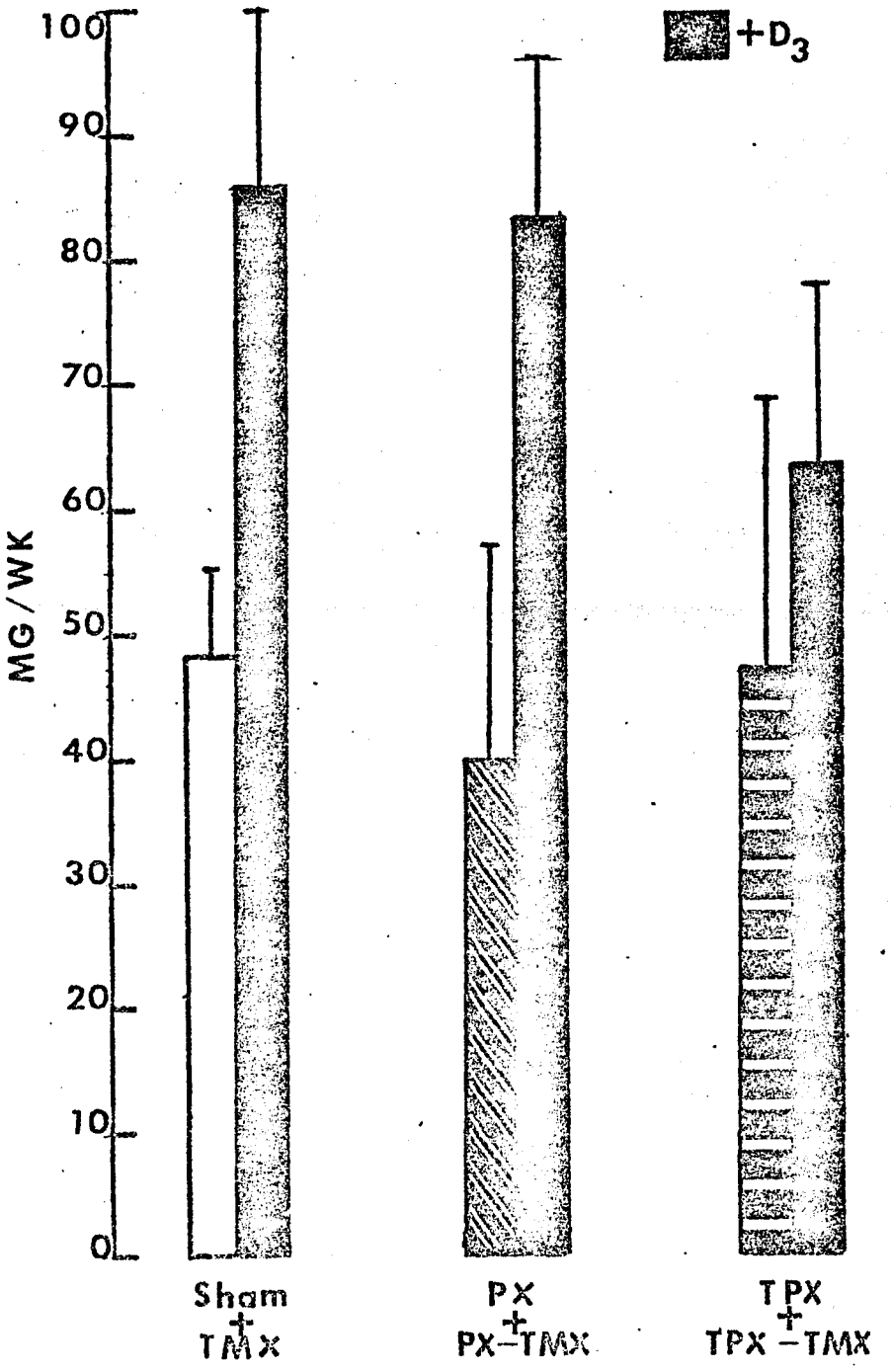

FIG. 14 : EFFECT OF D_3 12000 IU/WK ON PHOSPHATE EXCRETION

TABLE 10

EFFECTS OF VITAMIN D_3 ON SERUM PHOSPHATE CONCENTRATIONS

Mg %: Mean ± S.E.

	WINTER					
SURGICAL GROUP	# rats	Before D_3	After D_3 1200IU/Wk	# rats	Before D_3	After D_3 2400IU/Wk
SHAM	5	8.1 ± 0.7	7.8 ± 0.7	8	8.7 ± 0.8	8.7 ± 1.0
TMX	5	7.2 ± 1.7	8.0 ± 0.8	8	8.4 ± 0.9	8.7 ± 0.9
PX-SHAM (PX)	5	9.8 ± 1.3	9.4 ± 0.6			
PX-TMX	4	9.6 ± 0.8	10.2± 1.0			
TPX-SHAM (TPX)	5	9.3 ± 1.6	9.0 ± 0.8			
TPX-TMX	4	9.5 ± 0.6	10.2± 2.4			

	SUMMER								
SURGICAL GROUP	# rats	Before D_3	After D_3 1200IU/Wk	# rats	Before D_3	After D_3 2400IU/Wk	# rats	Before D_3	After D_3 12000IU/Wk
SHAM	6	8.0 ± 1.1	8.9 ± 0.7●	1	9.7	8.9	3	8.5 ± 0	7.6 ± 1.8 ♦
TMX	7	8.0 ± 0.6	8.0 ± 0.8	1	9.2	9.8	1	9.0	6.4
PX-SHAM (PX)	1	6.2	9.2				2	9.5 ± 1.6	8.1 ± 1.1
PX-TMX	1	12.2	11.2				5	9.2 ± 1.4	9.1 ± 0.7 □
TPX-SHAM (TPX)	2	8.4 ± 1.5	6.6 ± 0.6	1	8.7	7.9	3	7.6 ± 1.2	7.1 ± 0.6
TPX-TMX	2	8.9 ± 1.8	7.4 ± 0.9 ○	2	9.2 ± 0.1	9.4 ± 0.4	2	9.3 ± 1.3	8.2 ± 0.1

P VALUES (+D_3 : No D_3)

Individual group

● : < 0.05

Combined surgical groups

♦ TMX + SHAM < 0.05

○ TMX + SHAM < 0.03

□ PX's + TPX's < 0.05

min treatment.

800 IUD_3 administered 3x weekly had little effect on serum phosphate during both winter and summer months.

4000 IUD_3 (3x weekly) administration decreased serum phosphate concentration in all summer rats.

E - Effects of dietary D_3 deprivation on phosphate excretion and on serum phosphate concentrations - Effects of D_3 administration (Table 11).

Only animals with intact thyroid and parathyroid glands were fed a vitamin D_3-deficient diet, during the summer. In both TMX and SHAM rats, the phosphate excretion values were more than four times higher than the ones of animals fed a normal diet. The serum phosphate concentrations showed a slight decrease compared to those of animals on a normal diet.

The administration of 800 IUD_3 3x weekly to summer TMX and SHAM rats fed a D_3-deficient diet was followed by a decrease in phosphate excretion rates compared to the rates prior to vitamin D treatment. The vitamin treatment was more effective in TMX rats. (Fig. 9)

The injected animals had also lower serum phosphate levels.

TABLE II

EFFECTS OF DIETARY D_3 AND 2400 IU/WK D_3 ADMINISTRATION ON PHOSPHATE EXCRETION AND SERUM PHOSPHATE CONCENTRATION (SUMMER)

A- PHOSPHATE EXCRETION (Mg/Rat/Wk/100 Gm Food intake: Mean ± S.E.)

SURGICAL GROUP	# RATS	CONTROL DIET (CD)	D_3-DEFICIENT DIET (DF)	D_3-DEFICIENT DIET + 2400 IU/WK (+D_3)	P VALUES
SHAM	8	34.8 ± 8.2	156.0 ± 9.1	145.6 ± 15.5	ND:DF $\ll$ 0.001 DF:+D_3 < 0.05
TMX	9	32.6 ± 7.2	154.0 ± 14.7	133.6 ± 20.6	ND:DF $\ll$ 0.001 DF:+D_3 < 0.01

B- SERUM PHOSPHATE CONCENTRATION (Mg %: Mean ± S.E.)

SURGICAL GROUP	# RATS	CONTROL DIET (CD)	D_3-DEFICIENT DIET (DF)	D_3-DEFICIENT DIET + 2400 IU/WK (+D_3)	P VALUES
SHAM	8	8.2 ± 1.2	7.8 ± 0.1	6.8 ± 0.5	DF:+D_3 $\ll$ 0.01
TMX	9	8.0 ± 0.6	7.8 ± 0.4	6.9 ± 0.4	DF:+D_3 $\ll$ 0.01

III - URINARY EXCRETION OF SODIUM

A - Seasonal variations (Table 12, Fig. 15).

Rats with intact thyroid and parathyroid glands had low sodium excretions in the spring. There was little difference between winter and summer values. This pattern for sodium excretion is the inverse of the one observed for chloride excretion (highest values in spring).

PX animals were not studied in the spring. However, PX animals tended to have higher sodium excretion values in the summer than in the winter. Three TPX animals were observed in the spring and their sodium excretion values were higher than those obtained for TPX rats at other times of the year.

B - Effect of surgery (Table 12, Fig. 15).

Removal of the parathyroid glands decreased sodium excretion during the winter and summer months. The reduction was most significant in TMX animals. (Winter $P < 0.03$, summer $P < 0.05$)

Thyroparathyroidectomized animals had excretion values similar to those of animals with parathyroid and thyroid glands (TMX + SHAM). However, TPX animals had higher sodium excretion rates than PX animals, during the winter and summer. Removal of thyroid was most effective in the winter months. (TPX : PX $P < 0.01$)

Thymectomy slightly increased sodium excretion during the winter and spring in animals with thyroid and parathyroid

TABLE 12

EFFECTS OF SURGERY ON SEASONAL VARIATIONS IN SODIUM EXCRETION
Meq/Rat/ Wk/ 100 Gm Food Intake: Mean ± S.E.

SURGICAL GROUP	# RATS	WINTER(W)	# RATS	SPRING(SP)	#RATS	SUMMER(S)	P VALUES ☆
SHAM ☆	15	10.4 ± 2.3	7	9.0 ± 0.7	10	10.0 ± 1.6	W:SP < 0.001
TMX ☆	13	12.0 ± 2.1	11	9.5 ± 1.2	7	11.4 ± 1.4	SP:S < 0.01
PX-SHAM (PX)	6	8.6 ± 0.9			3	9.8 ± 0.2	
PX-TMX	4	8.8 ± 2.0			6	9.2 ± 2.0	
TPX-SHAM (TPX)	4	11.0 ± 2.8	1	13.7	6	10.8 ± 2.7	
TPX-TMX	5	11.3 ± 2.1	2	12.2 ± 0.3	5	10.3 ± 1.5	

Comparison of Mean (P Values)

PX's : SHAM+TMX < 0.01
PX-TMX. : TMX < 0.05
PX's : PTX's < 0.01

TMX : SHAM <0.05
PX-TMX : TMX <0.05

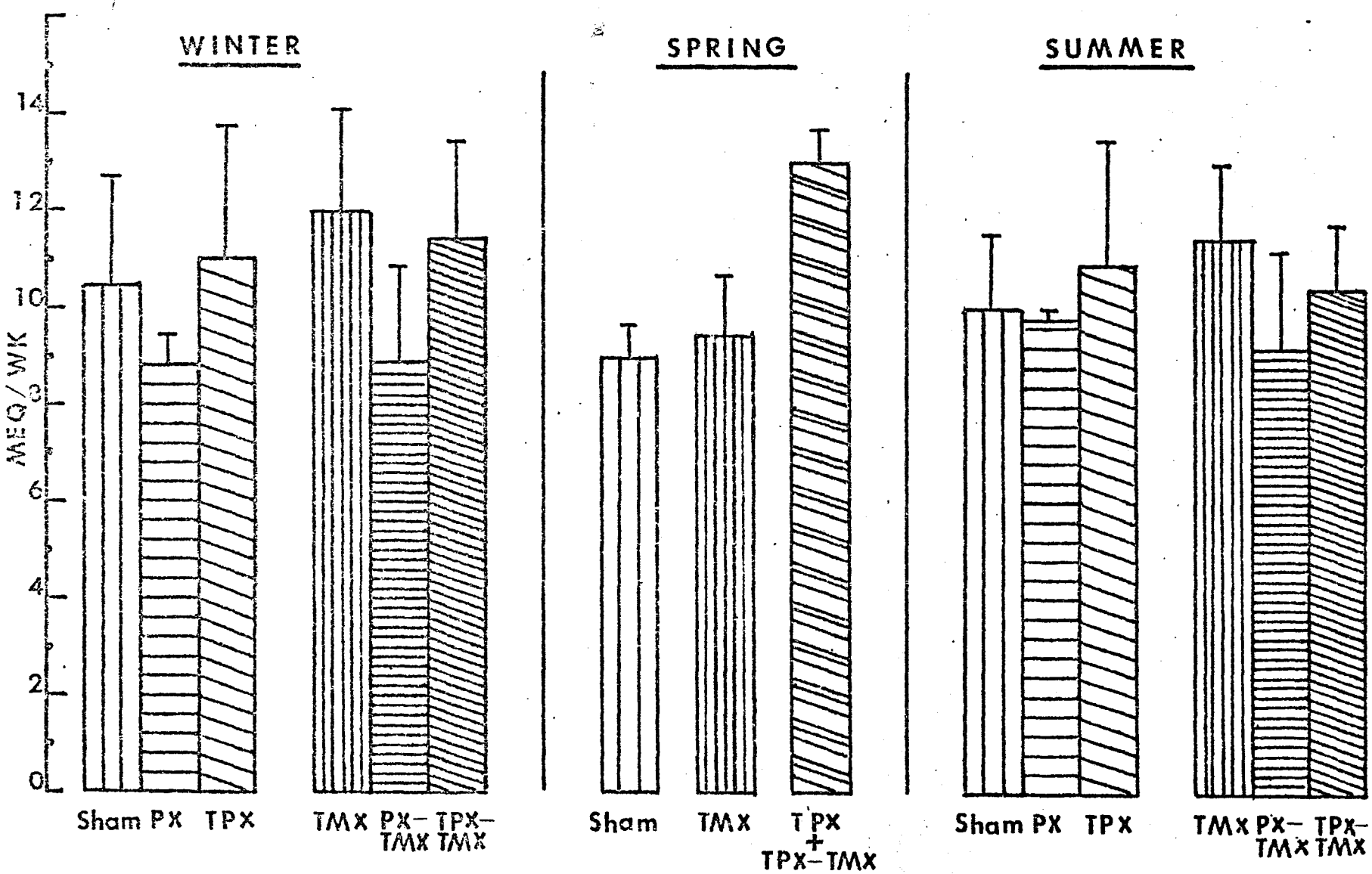

FIG. 15: RENAL EXCRETION OF SODIUM

glands. It significantly elevated sodium excretion during the summer in similar animals. ($P<0.05$) However, this effect of thymus removal was not apparent in PX nor TPX animals.

It appears therefore that the parathyroid and thyroid glands are involved in mechanisms affecting sodium excretion, and that the thymus gland in some way protects against excessive changes in the intact animals.

C - Effect of vitamin D on sodium excretion (Table 13).

The administration of 1200 IU D_3 weekly for three weeks increased sodium excretion in TMX, SHAM, PX during the winter and summer months. TPX animals had a slight reduction in excretion rates in the winter, whereas the rates were raised in the summer after the vitamin treatment. SHAM rats were the most affected by the vitamin during both winter and summer. (12% increase in winter, 28% increase in summer) (Fig. 16)

The administration of 2400 IU D_3 weekly during the winter to TMX and SHAM rats slightly but significantly increased sodium excretion. ($P<0.05$) Similar doses given in the spring reduced the excretion rates not only of TMX, SHAM but also of TPX animals. (Fig. 17)

The injection of 12,000 IU D_3 weekly during the summer, increased sodium excretion especially in PX ($P<0.001$) and TPX animals ($P<0.01$) (Fig. 18)

The findings suggest an inhibition of sodium reasorp-

TABLE 13

EFFECTS OF VITAMIN D_3 ADMINISTRATION ON SODIUM EXCRETION

Meq/Rat/Wk/ 100 Gm Food Intake: Mean S.E.

	WINTER						SPRING		
SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk
SHAM	5	11.9 ± 3.7	13.3 ± 3.4 ♦	8	10.1 ± 0.9	10.6 ± 1.2 ●	1	11.7	9.0
TMX	5	13.1 ± 3.1	13.7 ± 1.9 ♦	8	10.8 ± 1.0	11.1 ± 0.5 ●	1	9.2	9.0 ♦
PX-SHAM (PX)	6	8.6 ± 0.9	9.2 ± 1.1						
PX-TMX	4	8.8 ± 2.0	10.5 ± 0.3						
TPX-SHAM (TPX)	5	11.0 ± 2.8	9.8 ± 2.3				1	13.7	11.8
TPX-TMX	4	12.0 ± 2.2	11.4 ± 2.9				2	12.2 ±.3	10.9 ± 0.4

	SUMMER					
SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 12000IU/Wk
SHAM	6	8.7 ± 1.4	11.1 ± 1.3 ▽	3	11.2 ± 0.9	11.0 ± 1.1
TMX	7	9.8 ± 1.4	10.4 ± 1.3 ♦	1	11.7	12.6
PX-SHAM (PX)	1	9.8	13.5 ■	2	9.8 ± 0.3	11.6 ± 0.5 ○
PX-TMX	1	6.2	11.1 ■	5	9.8 ± 1.6	11.3 ± 0.3 ○
TPX-SHAM (TPX)	2	9.2 ± 0.0	10.0 ± 1.4 ♦	3	10.1 ± 1.0	11.0 ± 4.0 ▽
TPX-TMX	2	8.8 ± 0.4	10.4 ± 0.8 ♦	3	11.3 ± 1.0	14.0 ± 1.6 ▽

P VALUES (÷D_3 :No D_3)

Individual Groups

▽ $p < 0.01$

○ $p < 0.001$

Combined surgical groups

● TMX + SHAM < 0.05

♦ TMX + SHAM < 0.02

■ PX's + TPX's < 0.05

♦♦ TPX's + TMX + SHAM = 0.05

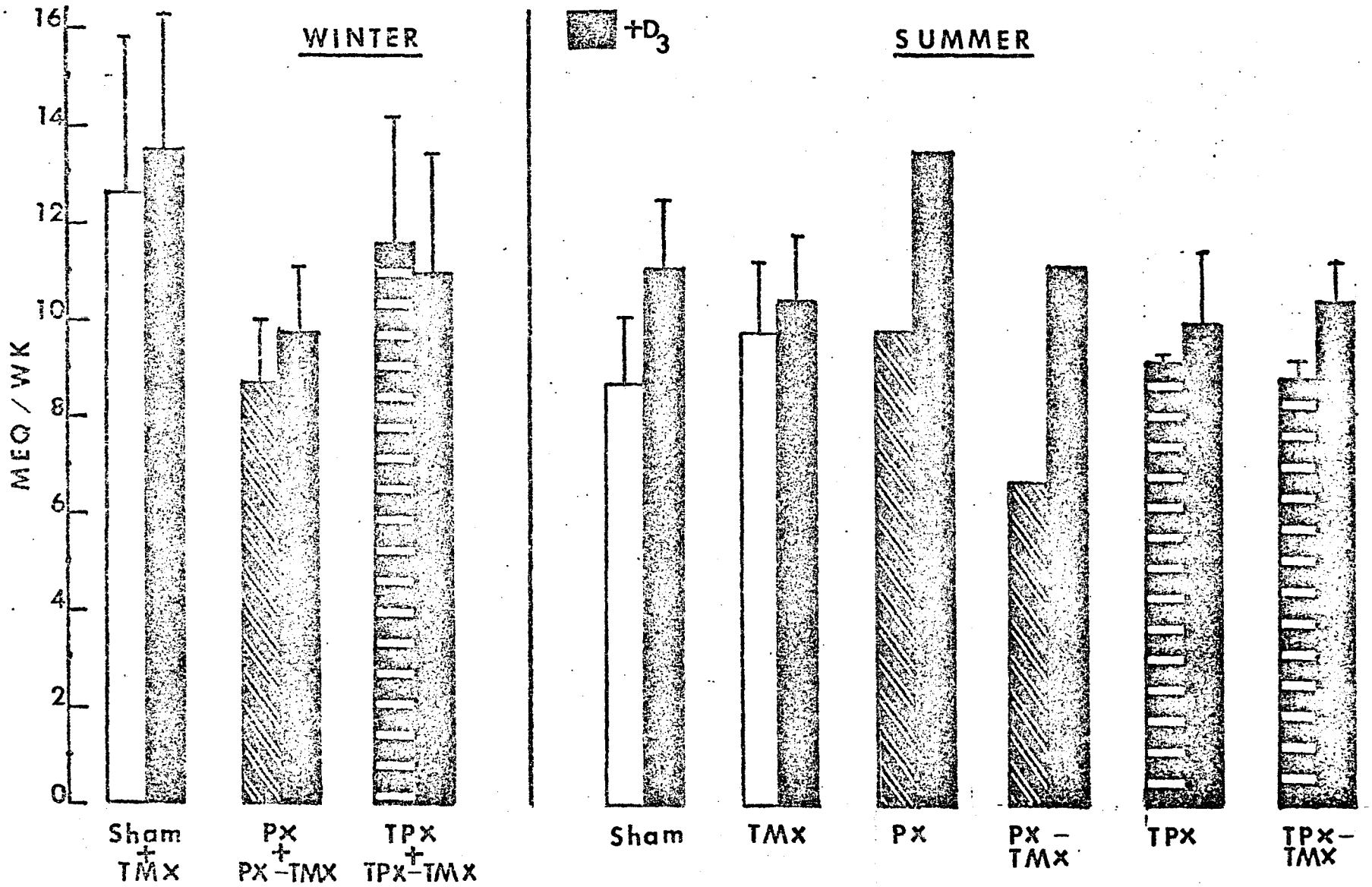

FIG. 16 : EFFECT OF D_3 1200 IU/WK ON SODIUM EXCRETION

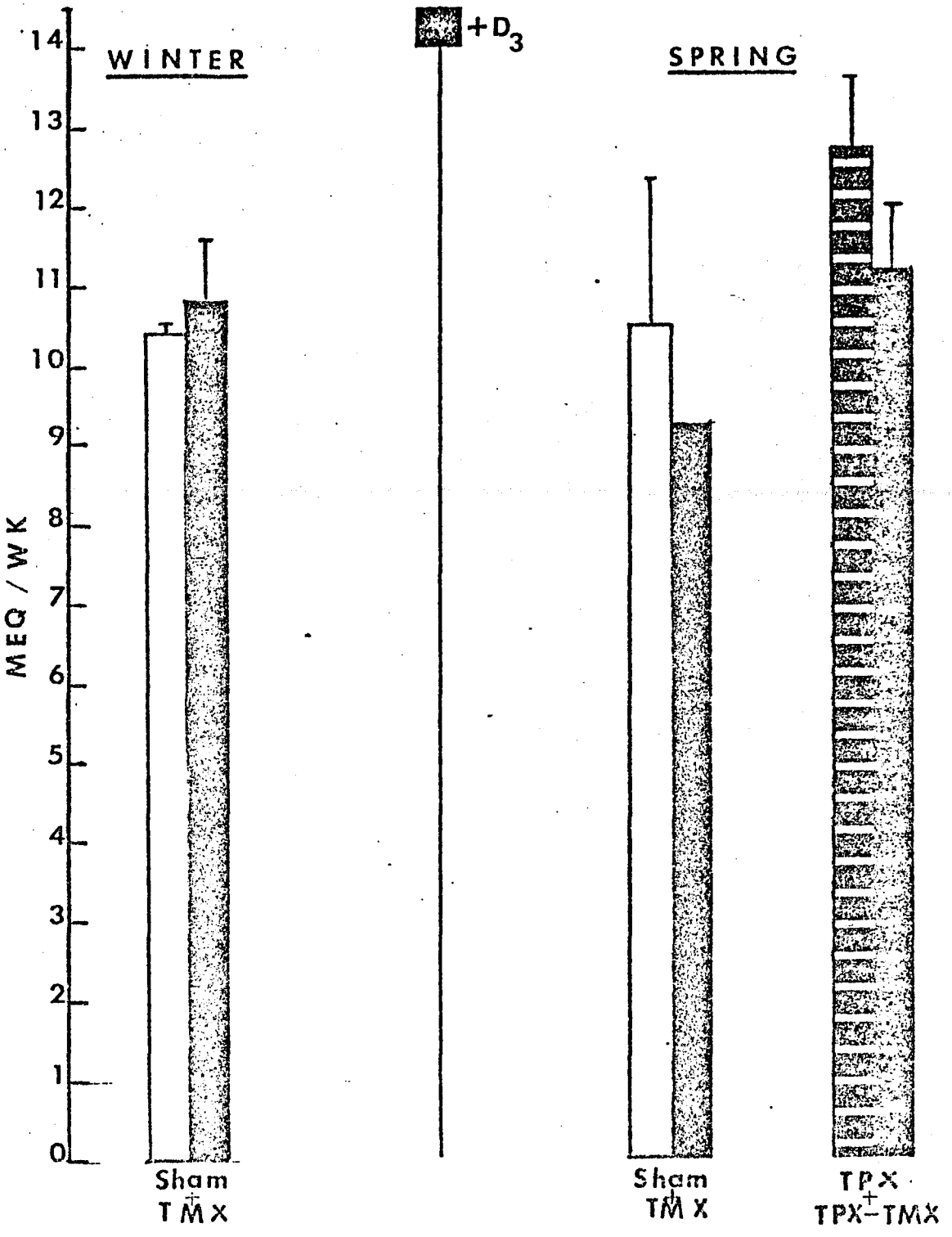

FIG.17: EFFECTS OF D_3 2400 IU/WK ON SODIUM EXCRETION

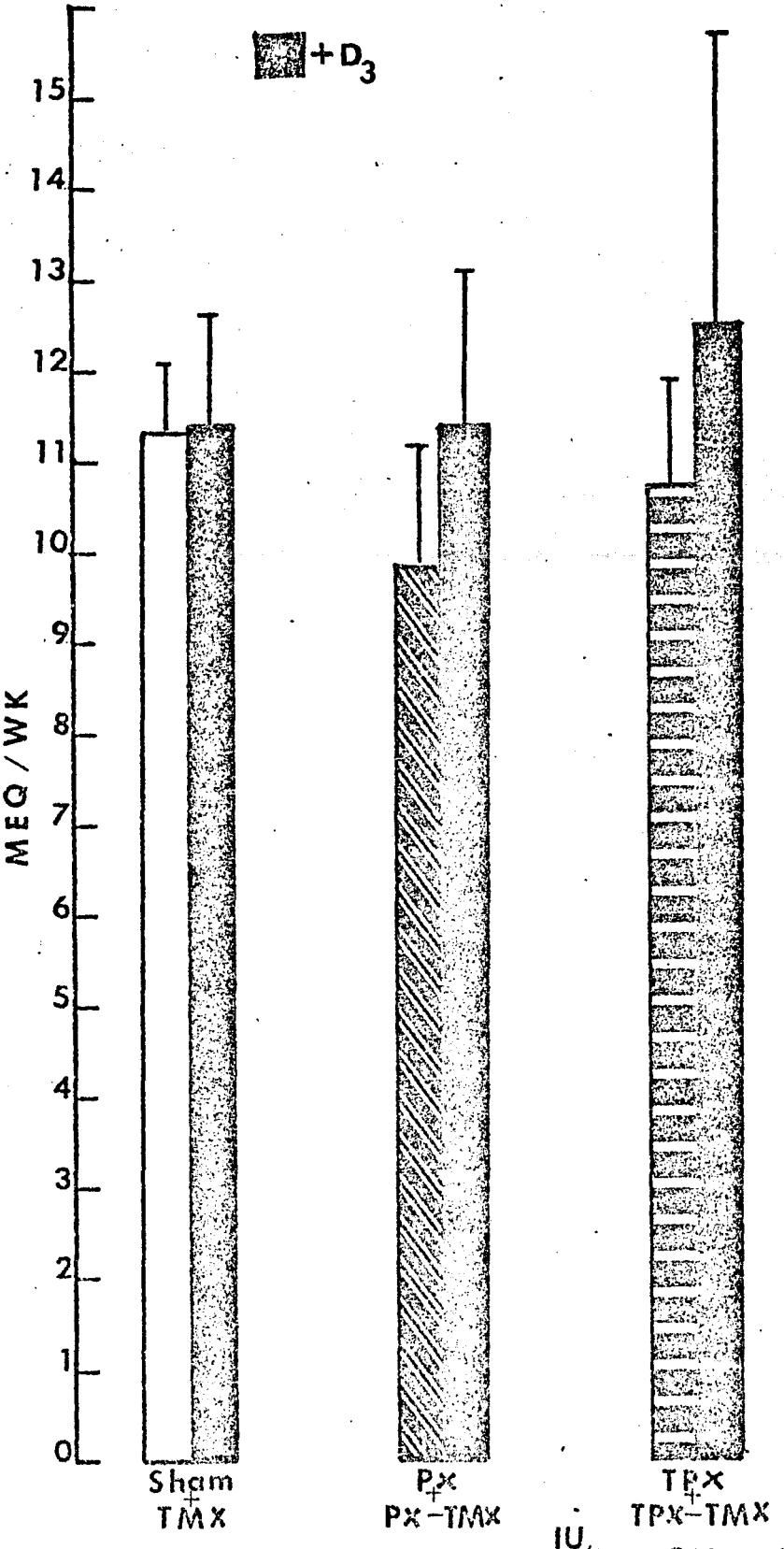

FIG.18 : EFFECT OF D_3 12,000 IU/WK ON SODIUM EXCRETION

tion by exogenous vitamin D in winter and summer rats. Somehow, this effect is interfered with in TPX animals in the winter and in all animals in the spring.

D - Effect of dietary D_3 deprivation - Effect of 2400 IU/wk D_3 in D-deficient animals (Table 14).

TMX and SHAM rats showed no change in their excretion of sodium when switched from a normal diet to a D_3-deficient diet for three weeks during the spring; however similar animals had reduced excretion rates when fed a D_3-deficient diet during the summer. (Fig. 9)

The administration of 2400 IU D_3 weekly to D_3-deficient animals (TMX + SHAM) decreased the excretion of sodium during both spring and summer months.

These findings suggest a complex role for exogenous vitamin D on sodium excretion. Such influence seems to depend on the vitamin D status of the animals prior to the exogenous administration.

TABLE 14

EFFECTS OF DIETARY D_3 AND 2400 IU/WK D_3 ADMINISTRATION ON SODIUM EXCRETION
Meq/Rat/ Wk/ 100 Gm Food Intake: Mean ± S.E.

SPRING

SURGICAL GROUP	# RATS	CONTROL DIET (CD)	D_3-DEFICIENT DIET (DF)	D_3-DEFICIENT DIET + 2400IU/Wk D_3 (+D_3)	P VALUES
SHAM	2	8.8 ± 0.5	9.8 ± 0.3	8.5 ± 1.1	TMX + SHAM (DF): +D_3 < 0.05
TMX	3	9.4 ± 1.6	9.2 ± 1.4	6.9 ± 0.6	

SUMMER

SURGICAL GROUP	# RATS	CONTROL DIET (CD)	D_3-DEFICIENT DIET (DF)	D_3-DEFICIENT DIET + 2400IU/Wk D_3 (+D_3)	P VALUES
SHAM	6	10.6 ± 1.2	9.5 ± 1.5	6.5 ± 1.5	CD:DF < 0.05 DF:+D_3 < 0.01
TMX	6	11.2 ± 1.1	9.2 ± 0.8	8.0 ± 0.7	CD:DF < 0.01 DF:+D_3 < 0.02

IV - URINARY EXCRETION OF POTASSIUM

A - Seasonal variations (Table 15, Fig. 19).

Animals with and without thyroid and parathyroid glands showed little variations in potassium excretion during winter as compared with summer months. However, TMX and SHAM rats studied during the spring months had excretion values lower than those obtained during both winter and summer. A similar pattern was obtained for sodium excretion.

B - Effect of surgery (Table 15, Fig. 19).

Removal of the parathyroid glands did not appreciably influence potassium excretion during both winter and summer, although PX-TMX animals tended to have lower excretion values than TMX groups.

Comparisons of thyroparathyroidectomized animals with the parathyroidectomized ones reveal higher values for animals deprived of thyroid glands (and parathyroids) more especially during the winter. ($P < 0.01$)

Thymectomized animals with intact thyroid and parathyroid glands (TMX) excreted more potassium than SHAM during the summer months. ($P < 0.03$) A similar tendency was seen also in winter. However, no significant effect of thymectomy on parathyroidectomized or thyroparathyroidectomized animals is observed.

The findings do not point to important roles for thymus, parathyroid and thyroid glands in the regulation of potassium excretion. However, they do suggest some fine

TABLE 15

EFFECTS OF SURGERY ON SEASONAL VARIATIONS IN POTASSIUM EXCRETION

Meq/Rat/Wk/ 100 Gm Food Intake: Mean ± S.E.

SURGICAL GROUP	# RATS	WINTER(W)	# RATS	SPRING(SP)	# RATS	SUMMER(S)	P VALUES☆
SHAM☆	15	1.7 ± 0.3	7	1.5 ± 0.2	10	1.6 ± 0.2	W:SP < 0.02
TMX☆	13	1.9 ± 0.2	11	1.6 ± 0.2	7	1.9 ± 0.1	SP:S < 0.05
PX-SHAM (PX)	6	1.7 ± 0.2			3	1.7 ± 0.0	
PX-TMX	4	1.6 ± 0.4			6	1.7 ± 0.3	
TPX-SHAM (TPX)	4	1.9 ± 0.2	1	2.4	6	2.0 ± 0.5	
TPX-TMX	5	2.0 ± 0.1	2	2.1 ± 0.0	5	1.8 ± 0.3	

Comparison of Mean (P Values)

PX : TPX < 0.01
PX-TMX : TPX-TMX < 0.05
PX's : TPX's < 0.01

TMX : SHAM < 0.03

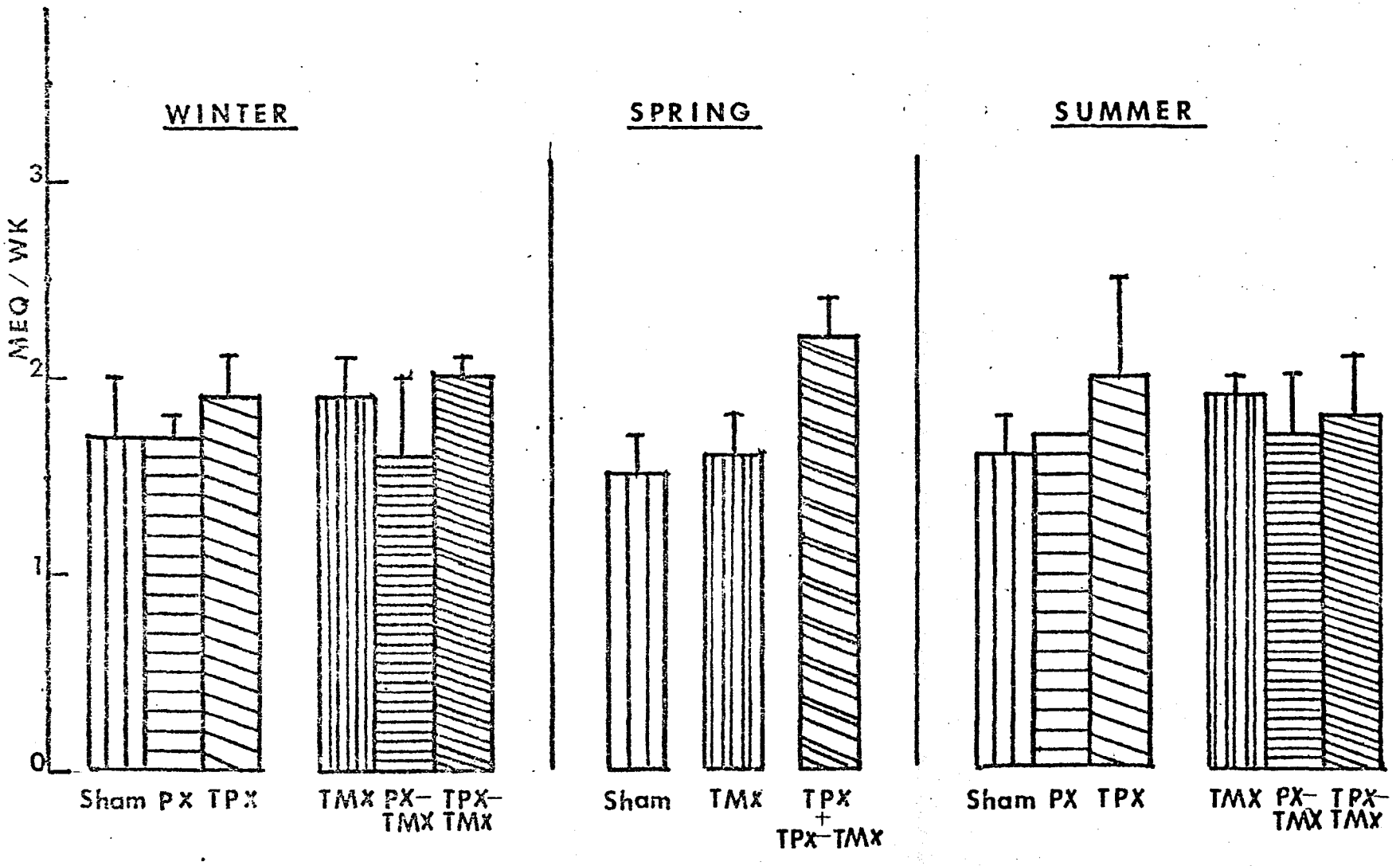

FIG. 19: RENAL EXCRETION OF POTASSIUM

control mechanisms.

C - Effects of vitamin D administration on potassium excretion (Table 16).

The administration of 400 IU D_3 3x weekly during the winter slightly increased the excretion of potassium of rats with intact thyroid and parathyroid glands. (TMX + SHAM $P<0.05$) The vitamin slightly increased the values in PX-TMX animals, but decreased those of TPX animals. Similar doses administered during the summer raised the excretion rates of all groups except of TMX rats. The increase was most significant in SHAM rats with intact thyroid and parathyroid glands ($P<0.01$) as in the case of sodium excretion. (Fig. 20)

The administration of twice the above dosage (2400 IU/wk) during the winter increased the excretion values for TMX and SHAM animals studied at that time. During the spring, the same dosage slightly decreased the values for TMX, SHAM and TPX animals. (Fig. 21)

The injection of 12,000 IU D_3/weekly during the summer increased the excretion rates of PX animals. ($P<0.02$) but had little effect on TMX, SHAM and TPX animals. (Fig. 22)

D-- Effect of dietary D_3 deprivation - Effects of 2400 IU D_3/week in D-deficient rats (Table 17).

TMX and SHAM rats fed a D-deficient diet for three weeks during the spring and summer had K excretion values

TABLE 16

EFFECTS OF VITAMIN D_3 ADMINISTRATION ON POTASSIUM EXCRETION
Meq/Rat/Wk/ 100 Gm Food Intake: Mean ± S.E.

	WINTER						SPRING		
SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 2400 IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk
SHAM	5	1.9 ± .4	2.0 ± .3 ●	8	1.7 ± .2	1.8 ± .2 ●	1	1.8	1.7
TMX	5	2.0 ± .3	2.1 ± .1 ●	8	1.8 ± .1	1.9 ± .1 ●	1	2.2	1.6 ◆
PX-SHAM (PX)	6	1.7 ± .1	1.7 ± .2						
PX-TMX	4	1.6 ± .4	1.9 ± .0						
TPX-SHAM (TPX)	5	1.9 ± .2	1.7 ± .3				1	2.4	2.4
TPX-TMX	4	2.1 ± .1	2.0 ± .5				2	2.1 ±.0	1.6 ± .1

	SUMMER					
SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 1200OIU/Wk
SHAM	6	1.5 ± .2	1.8 ± .1 ▽	3	2.0 ± .1	2.0 ± .2
TMX	7	1.7 ± .2	1.7 ± .2	1	2.0	2.2
PX-SHAM (PX)	1	1.6	2.1	2	1.7 ± .1	2.1 ± .0 ●
PX-TMX	1	1.1	1.9 ■	5	1.8 ± .2	2.2 ± .0 ⊙
TPX-SHAM (TPX)	2	1.6 ± .0	1.7 ± .2	3	2.1 ± .1	2.1 ± .0
TPX-TMX	2	1.5 ± .0	1.7 ± .1	3	2.0 ± .2	2.1 ± .4

P VALUES	Individual Groups	Combined surgical groups
	⊙ < 0.02	● TMX+SHAM < 0.05
	▽ < 0.01	■ PX's+TPX's < 0.05
		◆ TMX+SHAM+TPX's < 0.05

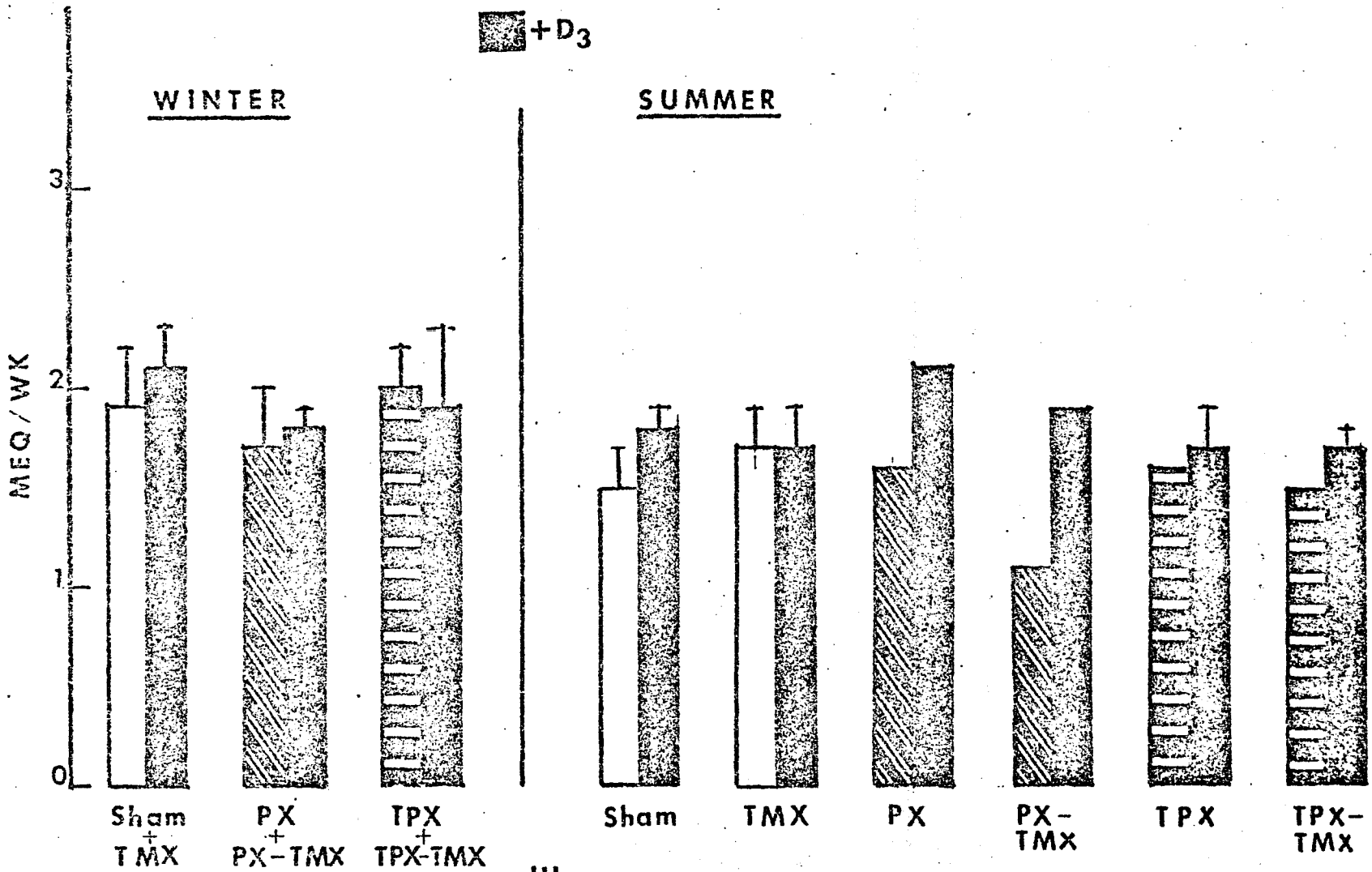

FIG. 20 : EFFECT OF D_3 1200 IU/WK ON POTASSIUM EXCRETION

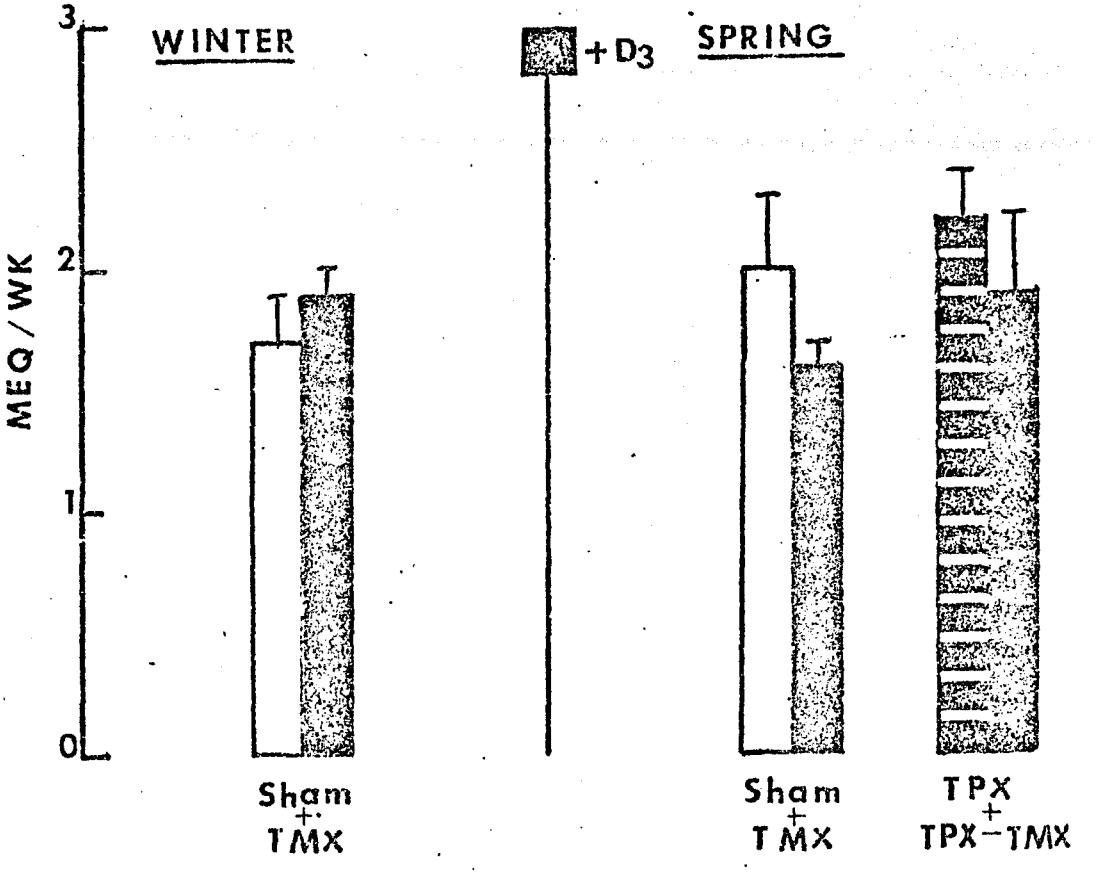

FIG. 21 : EFFECTS OF 2400 IU/WK D_3 ON POTASSIUM EXCRETION

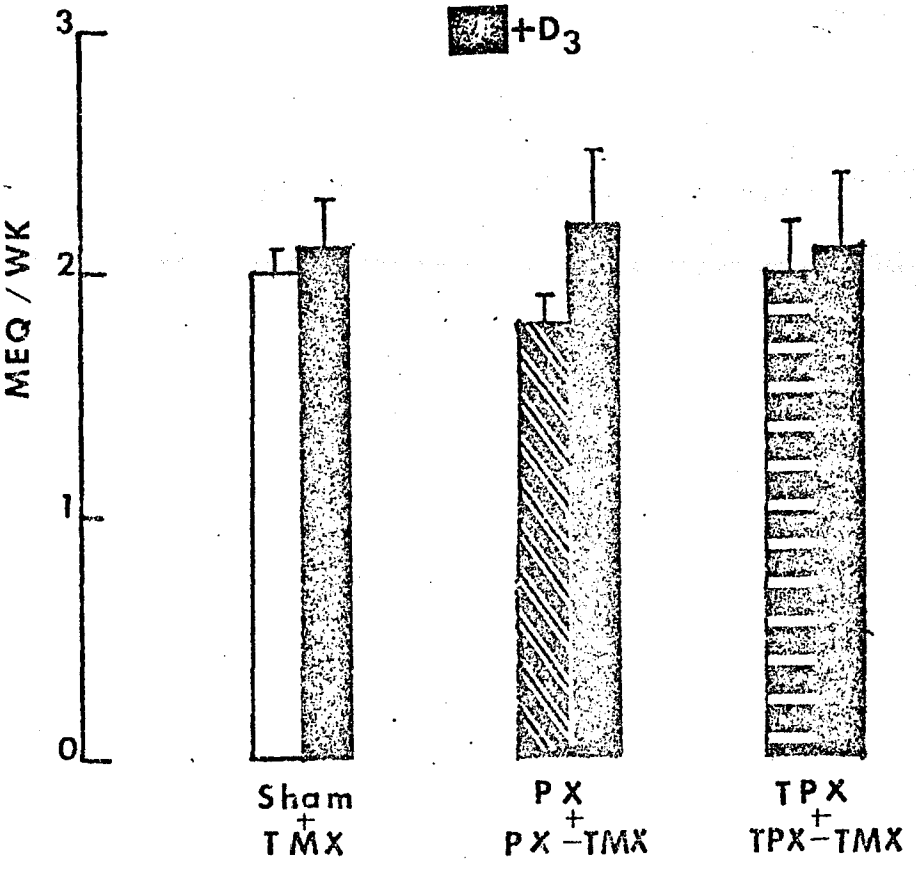

FIG. 22 : EFFECT OF D_3 12,000 IU/WK ON POTASSIUM EXCRETION

TABLE 17

EFFECTS OF DIETARY D_3 AND 2400 IU/WK D_3 ADMINISTRATION ON POTASSIUM EXCRETION
Meq/Rat/Wk/ 100 Gm Food Intake: Mean ± S.E.

SPRING

SURGICAL GROUP	# RATS	CONTROL DIET (CD)	D_3-DEFICIENT DIET (DF)	D_3- DEFICIENT DIET $+^3$2400IU/Wk ($+D_3$)	P VALUES
SHAM	2	1.6 ± 0.1	0.9 ± 0.0	0.7 ± 0.0	TMX+SHAM
					CD:DF <<< 0.001
TMX	3	1.6 ± 0.2	0.8 ± 0.1	0.6 ± 0.1	DF:+D_3 < 0.01

SUMMER

SURGICAL GROUP	# RATS	CONTROL DIET (CD)	D_3-DEFICIENT DIET (DF)	D_3- DEFICIENT DIET $+^3$2400IU/Wk ($+D_3$)	P VALUES
SHAM	6	1.7 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	CD:DF <<< 0.001
TMX	6	1.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.1	CD:DF <<< 0.001

lower than the ones observed where the same animals were fed a normal diet. In fact, the values fell by 50% in spring and 56% in summer when dietary D_3 was not provided. (No effect was seen in Na^+ excretion in spring). (Fig. 9)

The administration of 800 IU D_3 3x weekly to TMX and SHAM animals fed a D-deficient diet decreased potassium excretion during the spring months.

V - SERUM CONCENTRATIONS OF SODIUM AND POTASSIUM (At time of autopsy).

In Tables 18 and 19, serum Na^+ and K^+ concentrations at the end of each experimental period are represented. No apparent differences seem to exist among the various groups, and unfortunately, the conditions of the present study did not allow measurement of serum Na^+ and K^+ concentrations prior to D_3 treatment. However, while most values for serum Na^+ are close to the reported ones in the literature for healthy rats (135 Meq/L), the values obtained for K^+ are considerably above the normal range (4-5 Meq/L) expected for K^+. It is probable that at least the K^+ levels of the animals under investigation were affected by the vitamin D injections. D-deficient animals treated with vitamin D have slightly lower Na^+ concentrations than the non-deficient animals.

TABLE 18

SERUM SODIUM CONCENTRATIONS AT THE TIME OF AUTOPSY

Meq/L/rat : Mean ± S.E.

TIME PERIOD	EXPERIMENTAL CONDITIONS	SURGICAL GROUP (# rats) SHAM	TMX	PX	PX-TMX	TPX	TPX-TMX
A- Winter	- After 3 wks with 1200IU/Wk D_3	(4) 133.0±6.8	(4) 129.9±10.1	(5) 138.8±11.5	(4) 140.4±2.9	(5) 135.5±17.3	(4) 144.6±5.9
	- After 3 wks with 2400IU/wk D_3	(4) 129.5±6.1	(4) 128.8±8.8				
	- After 3 wks <u>without</u> 2400IU/wk D_3 ⊙	(4) 130.4±7.2	(4) 131.9±6.4				
B- Spring	- D-Deficient rats after 3 wks with D_3 2400IU/Wk	(2) 122.5±18.4	(3) 109.6±16.9				
C- Summer	- After 3 wks with 1200IU/Wk D_3	(6) 126.8±12.9	(7) 114.2±19.1	(2) 125.2±12.4	(2) 137.6±6.6	(1) 134.5	(1) 146.0
	- After 3 wks with 12,000IU/Wk D_3	(3) 134.8±3.3	(1) 137.3	(2) 134.5±0.5	(4) 136.0±6.0	(3) 98.8± 21.9	(2) 140.0±6.0
	- D-Deficient rats after 3 wks with D_3 2400IU/wk	(6) 109.2±14.0	(6) 108.3±9.6				

⊙Rats had been injected with D_3 (2400IU/wk) for 3 weeks prior to these "3 wks <u>without</u> D_3 "

TABLE 19

SERUM POTASSIUM CONCENTRATIONS AT THE TIME OF AUTOPSY

Meq/L/rat : Mean ± S.E.

TIME PERIOD	EXPERIMENTAL CONDITIONS	SURGICAL GROUP (# rats) SHAM	TMX	PX	PX-TMX	TPX	TPX-TMX
A- Winter	- After 3 wks with 1200IU/wk D_3	(4) 7.3 ± 4.3	(4) 7.6 ± 2.4	(5) 6.4 ± 1.2	(4) 6.2 ± 0.6	(5) 8.8 ± 5.5	(4) 9.0 ± 6.5
	- After 3 wks with 2400IU/wk D_3	(4) 6.6 ± 3.5	(4) 6.6 ± 5.9				
	- After 3 wks <u>without</u> 2400IU/wk D_3 ⊙	(4) 7.6 ± 0.7	(4) 7.2 ± 0.6				
B- Spring	- D-Deficient rats after 3 wks with D_3 2400IU/Wk	(2) 7.4 ± 1.6	(3) 10.3 ± 1.8				
C- Summer	- After 3 wks with 1200IU/Wk D_3	(6) 7.8 ± 2.1	(7) 9.4 ± 3.4	(2) 6.8 ± 1.3	(2) 5.8 ± 0.6	(1) 5.3	(1) 6.4
	- After 3 wks with 12,000IU/Wk D_3	(3) 6.2 ± 1.2	(1) 5.6	(2) 5.4 ± 0.5	(4) 5.9 ± 0.9	(3) 9.1 ± 1.1	(2) 5.6 ± 1.3
	- D-Deficient rats after 3 wks with D_3 2400IU/wk	(6) 6.3 ± 0.9	(6) 6.5 ± 0.6				

⊙ Rats had been injected with D_3 (2400IU/wk) for 3 weeks prior to these "3 wks <u>without</u> D_3 "

VI - URINARY EXCRETION OF CHLORIDE

A - Influences of surgery on seasonal variations in chloride excretion.

Rats with or without intact thyroid and parathyroid glands had substantially higher excretion rates in the "summer" than during the winter months. In addition, urinary chloride values were significantly highest in the spring. (Table 20, Fig. 23)

Parathyroidectomy increased chloride excretion during the winter and summer months. It enhanced the winter: summer differences significantly: rats with parathyroid glands intact showed an 18% increased excretion in the summer, whereas a 47.5% increase was observed for PX animals.

Removal of the thyroid gland did not apparently affect the chloride excretion during the winter months and summer, nor did it appreciably influence the effect of parathyroidectomy. However, removal of the thyroid gland severely blunted the winter:summer differences.

The percent increase in chloride excretion for TPX (24%) rats was half that recorded for PX animals. But, the data obtained for three TPX animals during the spring months suggest a great enhancing effect of thyroparathyroidectomy on the spring:winter and spring:summer differences.

Thymectomy did not markedly enhance chloride excretion during the winter nor spring months. However, thymectomized animals had chloride excretion rates for the summer months

TABLE 20

EFFECTS OF SURGERY ON SEASONAL VARIATIONS IN CHLORIDE EXCRETION
Meq/Rat/Wk/ 100 Gm Food Intake: Mean ± S.E.

SURGICAL GROUP	# RATS	WINTER(W)	# RATS	SPRING(SP)	# RATS	SUMMER(S)	P VALUES
SHAM	15	37.7 ± 10.7	7	51.5 ± 16.6	10	41.1 ± 6.2	TMX + SHAM :
TMX	13	37.6 ± 13.9	11	57.8 ± 21.1	7	49.2 ± 8.2	W:SP < 0.001 SP:S = 0.05 W:S < 0.001
PX-SHAM (PX)	6	49.2 ± 11.7			3	71.4 ± 33.8	
PX-TMX	4	56.6 ± 14.8			6	79.8 ± 41.3	PX's + TPX's: W:S = 0.05
TPX-SHAM (TPX)	4	49.9 ± 7.8	1	138.7	6	63.0 ± 21.8	
TPX-TMX	5	59.2 ± 12.6	2	109.5 ± 6.1	5	72.8 ± 27.6	

Comparison of Mean (P Values)				
	TPX-TMX : TMX	< 0.01	TMX:SHAM	< 0.05
	TPX: SHAM	< 0.05	TPX : SHAM	< 0.01
	PX-TMX: TMX	< 0.05	TPX-TMX:TMX	= 0.05
	PX : SHAM	< 0.05	PX : SHAM	< 0.05
	TPX's+PX's:TMX+SHAM	< 0.001	TPX's+PX's : TMX+SHAM	< 0.001

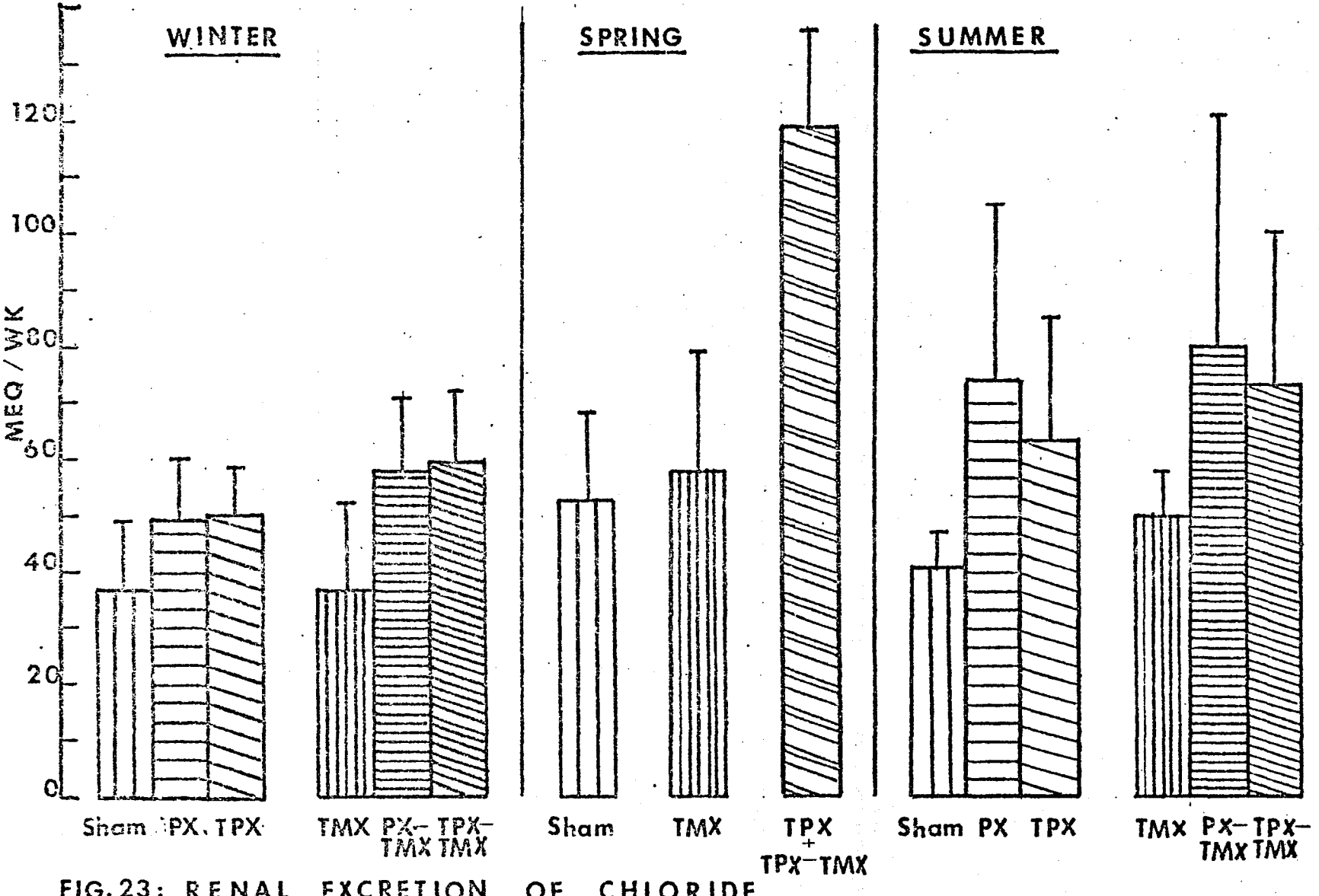

FIG. 23: RENAL EXCRETION OF CHLORIDE

that were significantly higher than those of intact animals. The summer enhancing effect was found also in PX and TPX animals.

C - Effects of vitamin D administration on excretion (Table 21).

The administration of 400 IU D_3 3x weekly for three weeks during the winter increased the chloride excretion in TPX and PX animals (49% increase compared to 29% increase in PX, and 14% in TMX + SHAM animals). Furthermore, thymectomy enhanced vitamin D effect in TPX and PX groups. (Fig. 24)

During the summer, the administration of 400 IU/D_3 increased chloride excretion in SHAM animals ($P<0.01$) and slightly decreased the values of TPX rats. ($P<0.05$) Although this dosage of the vitamin did not appreciably affect the chloride excretion of TMX rats, it did elevate the values for SHAM rats closer to those of TMX rats.

The administration of 800 IU D_3 three times weekly for three weeks, during the winter, increased excretion of chloride in TMX ($P<0.01$) and SHAM ($P<0.03$) animals. (Fig. 25)

Only a few rats were given this dosage of the vitamin in the spring. However, the results indicate little effect of vitamin D_3 on chloride excretion.

The administration of 4000 IU D_3 3x weekly for three weeks increased chloride excretion rates in PX and, more especially in TPX animals (83% increase). (Fig. 26)

TABLE 21

EFFECTS OF VITAMIN D_3 ADMINISTRATION ON CHLORIDE EXCRETION
Meq/Rat/Wk/ 100 Gm Food Intake: Mean ± S.E.

	WINTER						SPRING		
SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk
SHAM	5	47.8 ± 3.1	62.4 ± 31.2	8	21.9 ± 9.4	40.0 ± 5.0▲	1	83.5	94.0
TMX	5	48.7 ± 7.2	47.4 ± 8.4	8	21.9 ± 8.0	38.7 ± 2.8●	1	109.0	102.8
PX-SHAM (PX)	6	49.2 ± 11.7	62.8 ± 30.1						
PX-TMX	4	56.6 ± 14.8	73.9 ± 21.0▲						
TPX-SHAM (TPX)	5	49.9 ± 7.8	71.3 ± 26.1◆				1	138.7	126.8
TPX-TMX	4	59.2 ± 12.6	91.9 ± 5.7 ◆				2	109.5±6.1	127.7±19.0

	SUMMER					
SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 12000IU/Wk
SHAM	6	41.0 ± 6.2	50.8 ± 3.5●	3	120.0 ± 12.6	100.4 ± 15.8
TMX	7	50.1 ± 7.3	51.8 ± 6.0○	1	115.3	101.9
PX-SHAM (PX)	1	56.7	51.2	2	78.7 ± 44.3	107.4 ± 3.8
PX-TMX	1	40.5	43.7	5	87.6 ± 40.9	105.6 ± 35.8○
TPX-SHAM (TPX)	2	52.0 ± 0.7	41.2 ± 9.5○	3	66.9 ± 31.5	119.3 ± 43.6□
TPX-TMX	2	48.1 ± 0.2	39.2 ± 0.1○	3	89.2 ± 22.6	166.3 ± 22.1□

P VALUES
(+D_3 : No D_3)

Individual Groups
▲ < 0.05
● < 0.01

Combined Surgical Groups
○ TMX + SHAM < 0.05
□ TMX + SHAM < 0.01
◆ TPX's + PX's < 0.03

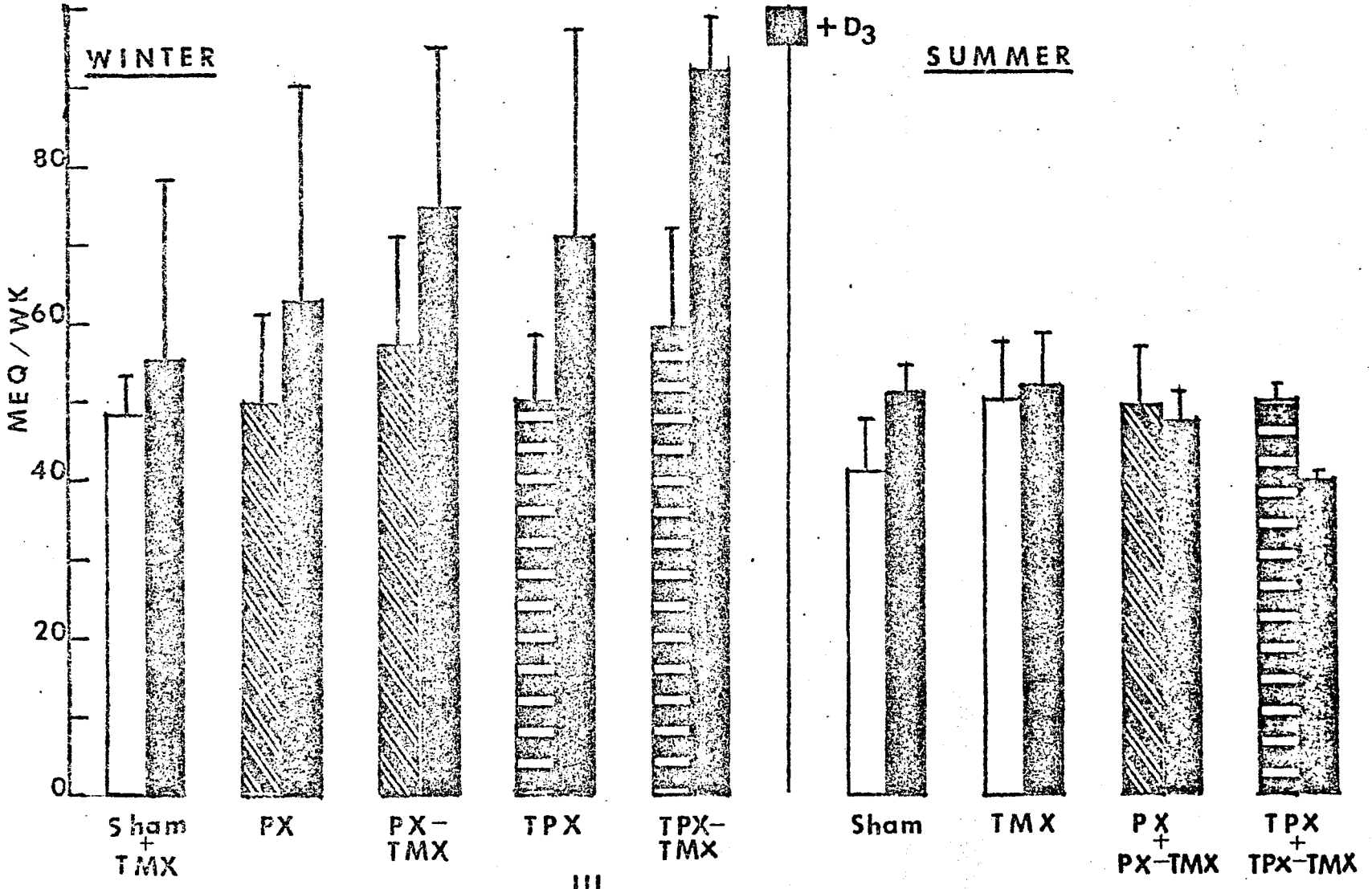

FIG.24 : EFFECTS OF D_3 1200 IU/WK ON CHLORIDE EXCRETION

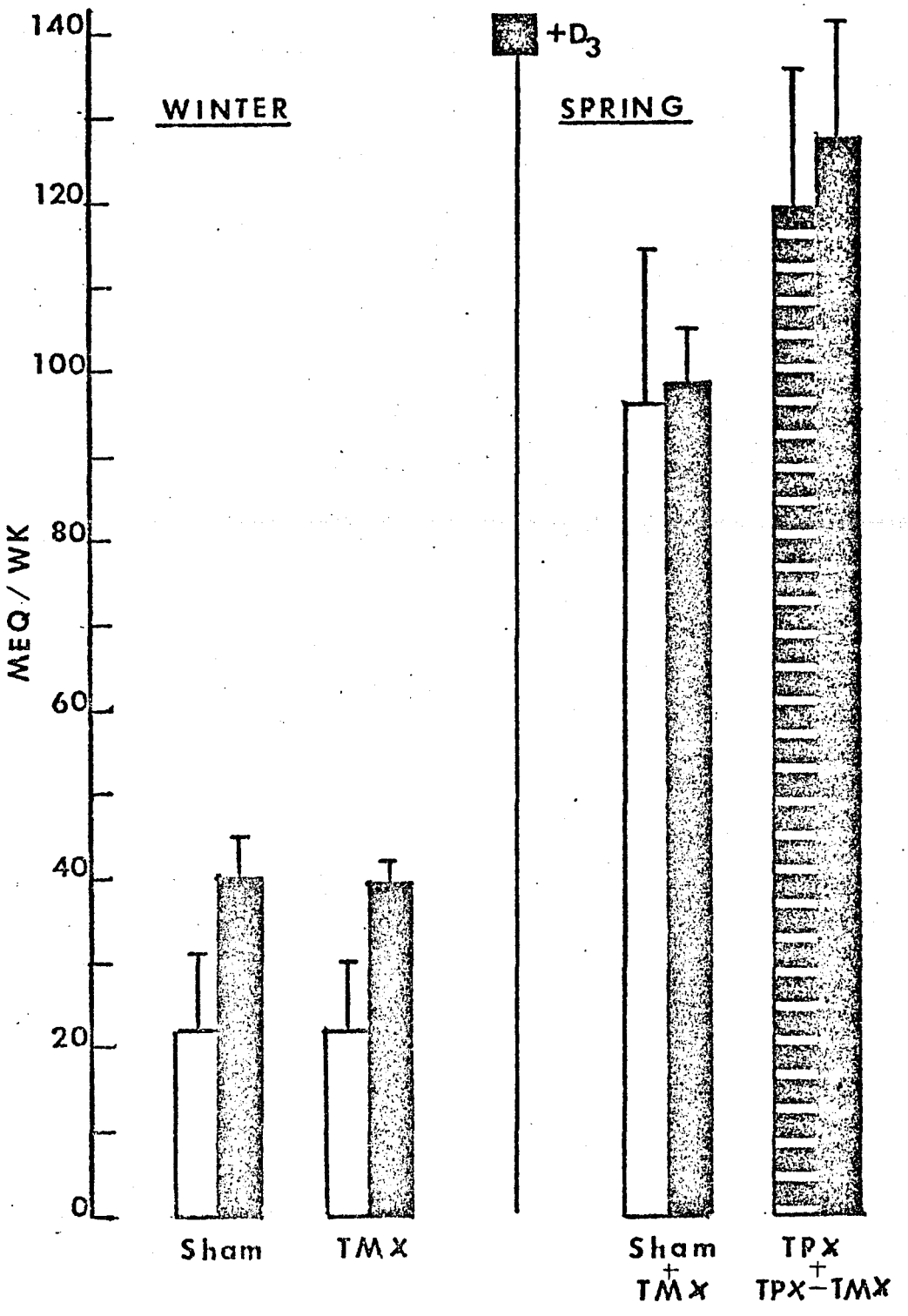

FIG.25 : EFFECT OF D_3 2400 IU/WK ON CHLORIDE EXCRETION

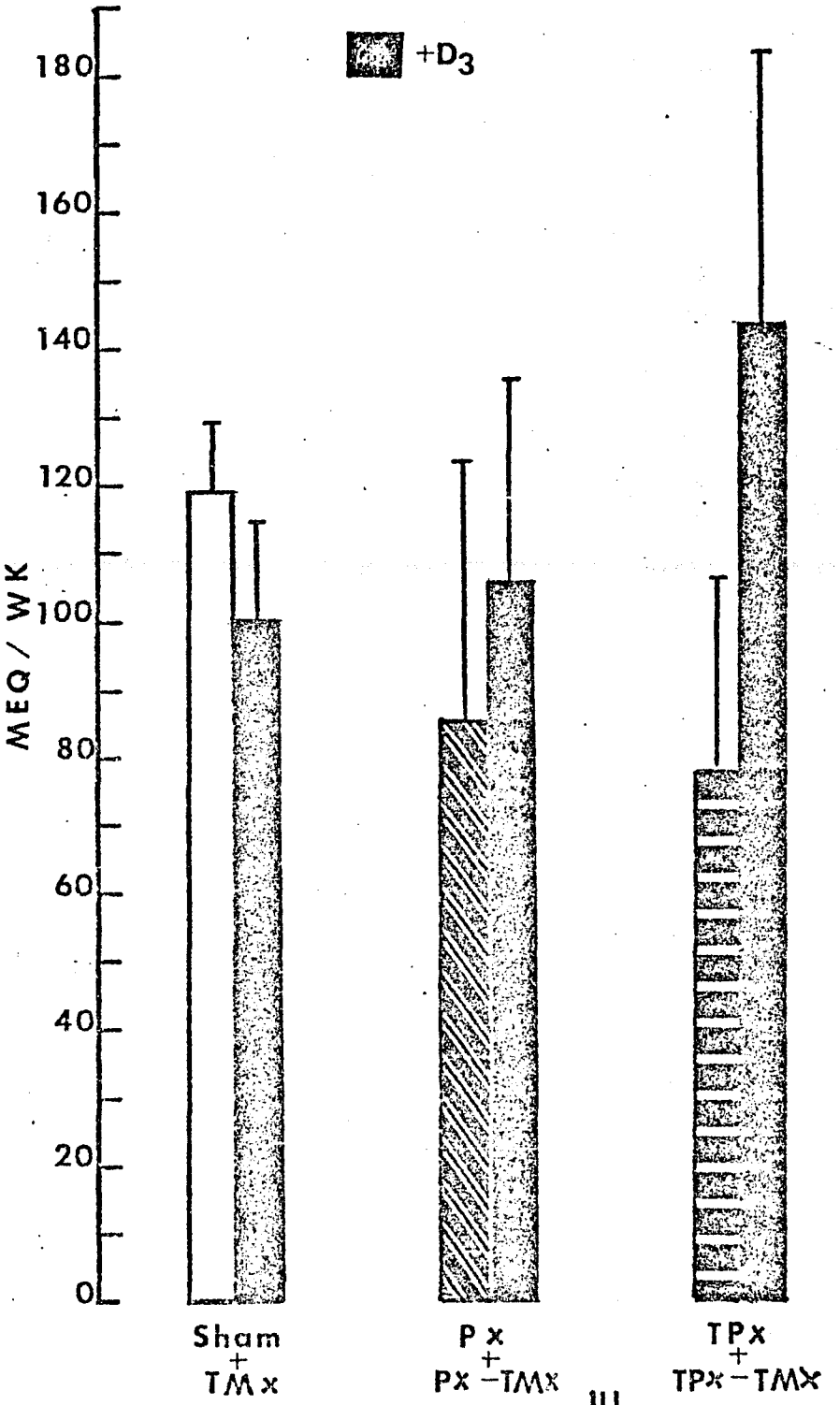

FIG.26 : EFFECT OF D_3 12,000 IU / WK ON CHLORIDE EXCRETION

D - Effect of dietary vitamin deprivation on chloride excretion - Effect of 2400 IU D_3 on D_3-deficient rats. (Table 22)

TMX and SHAM rats fed a D_3-deficient diet during the summer had chloride excretion rates lower (38% lower) than when fed a normal (D-supplemented) diet. The values were even lower than those of winter rats on a normal diet. TMX animals were the most affected by vitamin D deprivation : 64% lower excretion values for TMX compared to 23% for SHAM when deprived of vitamin D_3.

The administration of 2400 IU/wk D_3 to D-deficient rats increased the chloride excretion of TMX and SHAM. (Fig. 9)

Vitamin D-deficient diet had little effect on TMX and SHAM animals during the spring. However, the administration of 2400 IU D_3 to those animals fed the deficient diet lowered the chloride excretion rates to values comparable to those of summer rats on a normal diet.

TABLE 22

EFFECTS OF DIETARY D_3 AND 2400 IU/WK D_3 ADMINISTRATION ON CHLORIDE EXCRETION
Meq/Rat/Wk/ 100 Gm Food Intake: Mean ± S.E.

SPRING

SURGICAL GROUP	# RATS	CONTROL DIET (CD)	D_3-DEFICIENT DIET (DF)	D_3-DEFICIENT DIET + 2400IU/WK D_3 (+D_3)	P VALUES
SHAM	2	54.0 ± 7.4	68.8 ± 13.4	39.0 ± 13.7	TMX + SHAM / DF: +D_3 < 0.01
TMX	3	61.7 ± 19.8	61.9 ± 13.9	31.9 ± 6.8	

SUMMER

SURGICAL GROUP	#RATS	CONTROL DIET (CD)	D_3-DEFICIENT DIET (DF)	D_3-DEFICIENT DIET + 2400IU/WK D_3 (+D_3)	P VALUES
SHAM	6	44.4 ± 6.3	36.0 ± 8.3	42.0 ± 8.3	
TMX	6	48.4 ± 6.5	29.5 ± 4.9	33.7 ± 7.0	CD:DF < 0.03
					TMX + SHAM: DF: +D_3 < 0.05 CD: DF < 0.05

VII - FOOD AND WATER INTAKE - URINE VOLUME

A - Seasonal variations (Table 23, Figs. 27, 28, 29)

Rats with intact thyroid and parathyroid glands (TMX + SHAM) ate more and had higher urine volumes during the summer than during the winter months. No significant changes were observed in their water intakes. Although TPX groups tended to eat less and urinate more during the summer, no apparent trends were observed in PX groups.

B - Effect of surgery (Table 23, Figs. 27, 28, 29)

Parathyroidectomy decreased water intake while not affecting urine volume or food intake during both summer and winter.

Thyroparathyroidectomized animals drank significantly less water than SHAM, TMX and PX animals during both summer and winter. TPX animals also ate less than all other groups (22% less in winter, 32% less in summer). Thyroid removal had little effect on urine volume.

Thymectomy had no significant effect on water and food intake during both summer and winter. However, it increased the urine output especially in the summer. All animals deprived of thymus glands (PX-TMX, TPX-TMX) tended to have higher urine volumes than corresponding controls.

TABLE 23

EFFECTS OF SURGERY ON SEASONAL VARIATIONS IN FOOD AND WATER INTAKE AND URINE VOLUMES

Gm or ml/Rat/Wk: Mean ± S.E.

WINTER

SURGICAL GROUP	SHAM	TMX	PX-SHAM	PX-TMX	TPX-SHAM	TPX-TMX	p VALUES
No. Rats	15	13	6	4	5	4	
FOOD (Gm)	132.1±6.1	129.3±9.7	135.4±15.0	137.3±17.2	101.1±17.1	109.3±20.5	TMX+SHAM:TPX<0.001 PX's:TPX's <0.001
WATER (ml)	237.1±19.2	238.0±25.0	215.7±31.5	217.6±20.6	178.9±34.6	187.6±44.6	TMX+SHAM:PX <0.05 TMX+SHAM:TPX<0.001 PX's:TPX's <0.05
URINE (ml)	54.2±13.7	61.7±14.9	53.7±7.8	65.0±14.2	54.9±17.5	69.9±25.0	PX:PX-TMX <0.001

SUMMER

SURGICAL GROUP	SHAM	TMX	PX-SHAM	PX-TMX	TPX-SHAM	TPX-TMX	p VALUES
No. Rats	17	18	3	6	4	7	
FOOD (Gm)	144.2±11.4	138.8±13.0	125.0±22.5	138.4±10.8	91.4±23.7	97.0±9.8	TMX+SHAM:TPX<0.001 PX's:TPX's <0.001
WATER (ml)	250.6±51.4	244.1±48.3	226.4±41.1	229.0±10.2	175.3±58.0	194.2±21.8	TMX+SHAM:PX <0.05 PX's:TPX's <0.02 TMX+SHAM:TPX<0.01
URINE (ml)	58.4±12.0	68,2±14.3	58.9±11.8	63.0±8.4	62.4±20.5	73.8±19.9	TMX:SHAM <0.05

P VALUES (TMX+SHAM) FOOD: Winter: Summer < 0.001
URINE: Winter: Summer < 0.05

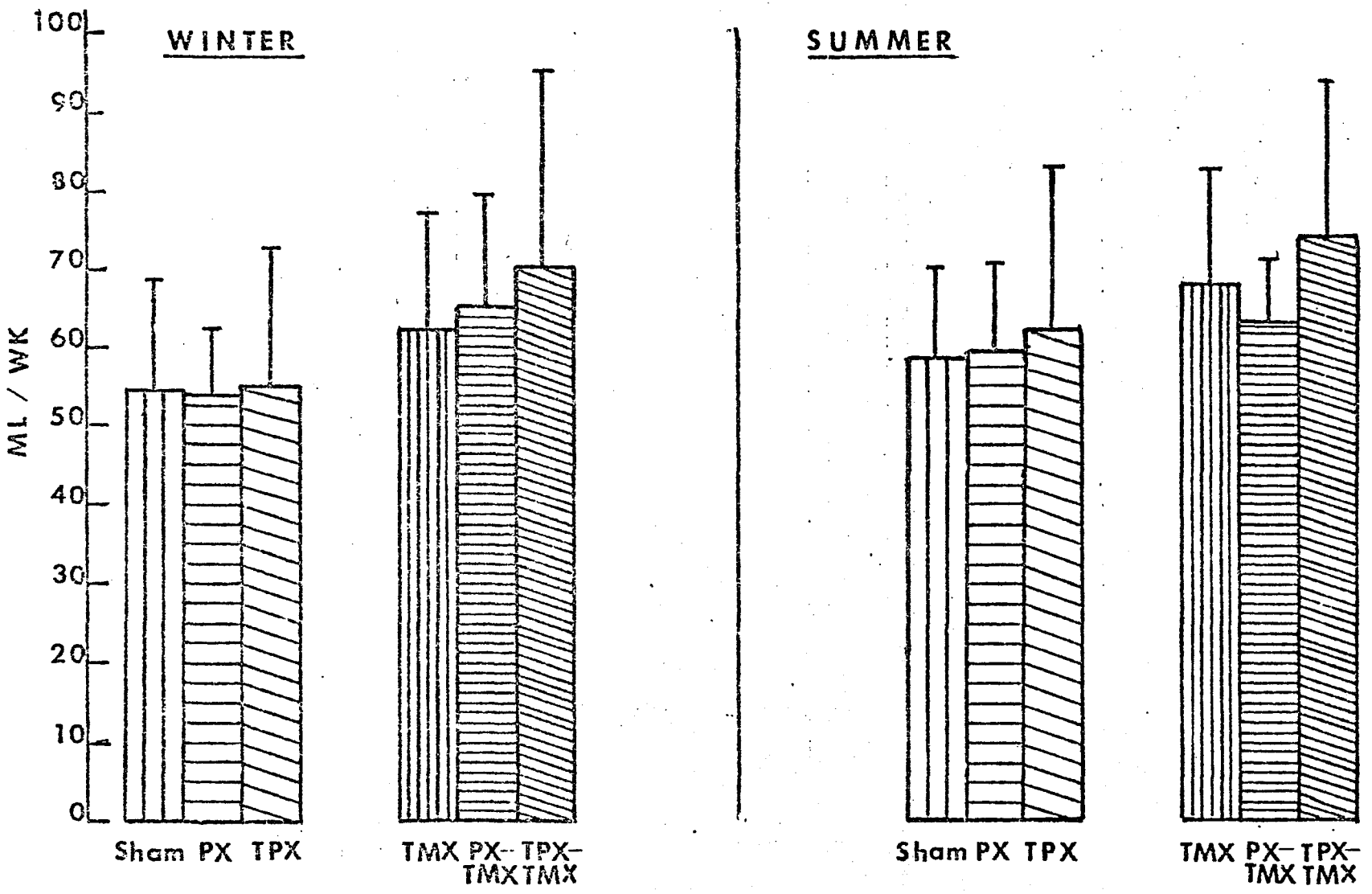

FIG.27 : SEASONAL VARIATION IN URINE VOLUME

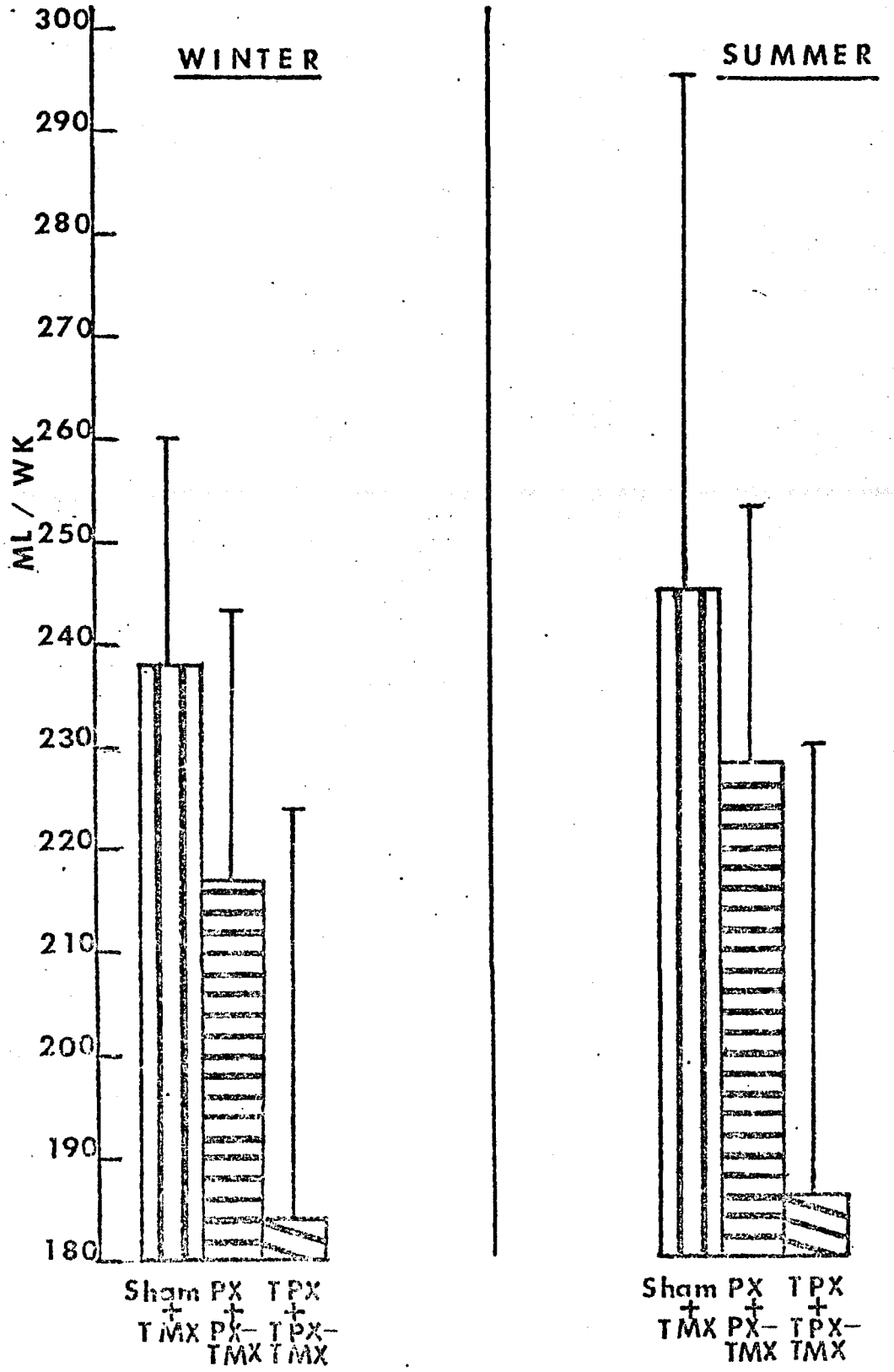

FIG.20 : SEASONAL VARIATION IN WATER INTAKE

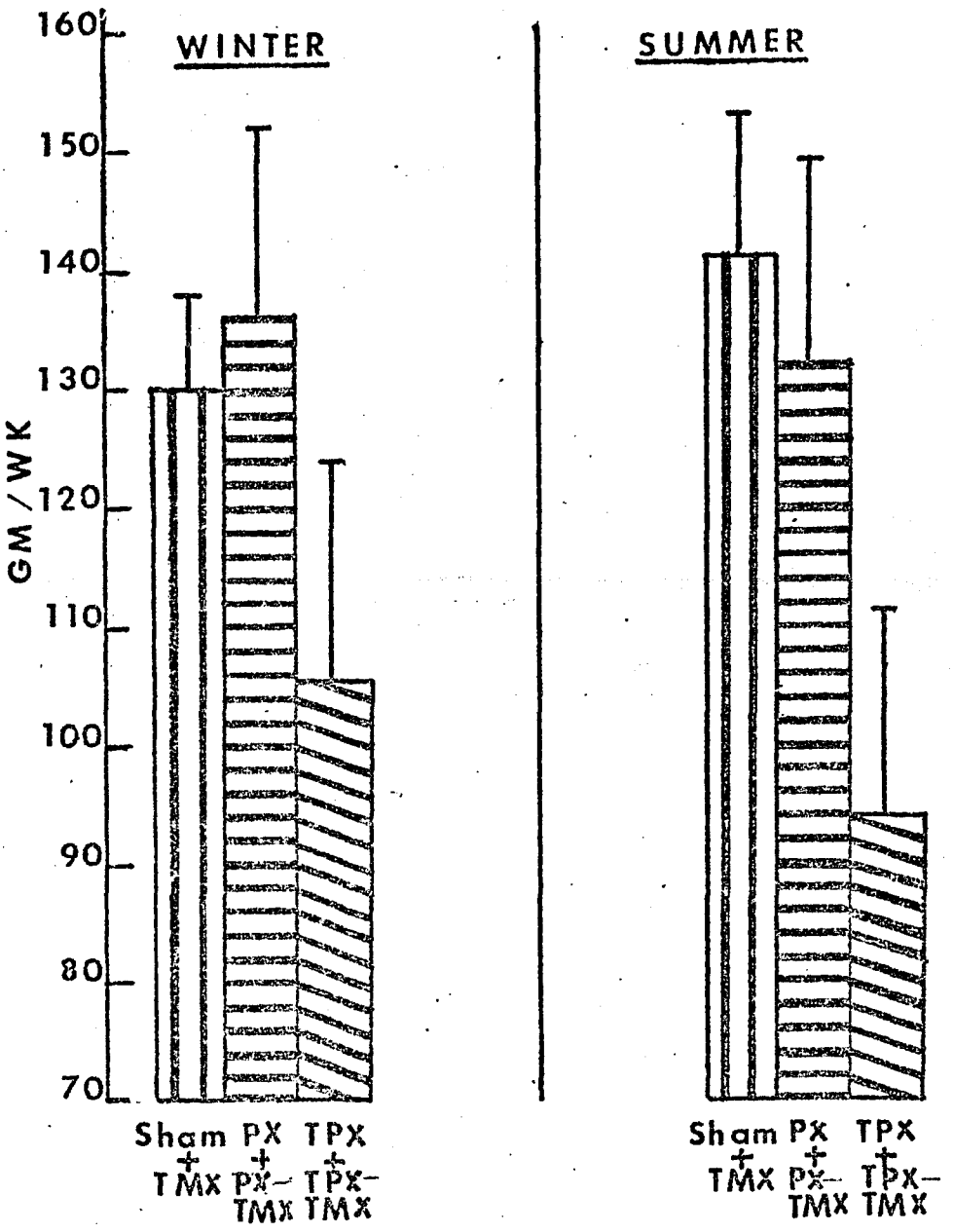

FIG. 29 : SEASONAL VARIATION IN FOOD INTAKE

C - Effect of vitamin D (Tables 24, 25, 26)

- Effects of 1,200 IU/wk

The administration of 400 IU D_3 three times weekly during the winter months, had no effect on water and food intake but increased the urine volumes of all groups, most especially of TMX and SHAM animals.

The administration of similar doses in the summer did not appreciably alter the urine volumes but significantly decreased water and food intake of SHAM animals.

- Effects of 2400 IU/wk D_3

The administration of 800 IU 3x weekly during the winter significantly increased urine volumes and food intake, without apparent change in water intake, of TMX and SHAM rats. The few animals treated with this dose in the spring-summer months showed similar patterns of responses.

- Effects of 12,000 IU/wk D_3

The administration of this high vitamin D dose during the summer months decreased food intake in all groups. There was also a dramatic rise in urine volumes (87%) in all rats, with also increased water intake (24%).

- Effects of vitamin D-deficient diet. (Table 27)

TMX and SHAM animals fed the deficient diet during spring and summer months ate and drank less, and had lower urine volumes than animals fed a normal diet.

The administration of 2400 IU/wk D_3 to D-deficient rats significantly decreased urine output of TMX animals. No apparent effect on water and food intake was observed.

TABLE 24

EFFECTS OF VITAMIN D_3 ADMINISTRATION ON FOOD INTAKE

Gm/Rat/Wk: Mean ± S.E.

WINTER

SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk
SHAM	5	133.3±2.9	133.2±3.7	8	143.8±15.6	154.0±4.9 ●
TMX	5	133.0±8.3	129.3±11.3	8	143.0±16.2	153.7±5.8
PX-SHAM	6	125.4±15.0	133.8±10.7			
PX-TMX	4	137.4±17.2	129.6±15.5			
TPX-SHAM	5	101.1±17.1	94.9±26.7			
TPX-TMX	4	109.3±20.5	105.9±15.7			

SUMMER

SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk	# RATS	Before D_3	After D_3 12,000IU/wk
SHAM	6	146.2±10.3	130.6±6.6 ■	1	146.2	148.7	3	147.2±6.3	132.6±6.4 ●
TMX	7	139.1±16.5	136.4±8.9 ■	1	148.0	158.2	1	132.4	115.6
PX-SHAM	1	127.2	125.7				2	134.0±23.1	118.3±15.4
PX-TMX	1	107.2	125.1				5	140.7±10.4	126.4±11.2 ●
TPX-SHAM	2	111.2±23.5	105.9±13.6	1	76.3	79.8	3	96.4±9.0	81.8±11.8
TPX-TMX	2	97.8±17.1	102.5±12.0	2	102.4±6.0	110.0±12.5	3	92.9±3.9	68.6±7.1 Δ

P Values ($+D_3$: No D_3)

Individual Surgical Groups

■ < 0.02

Δ < 0.01

Combined Surgical Groups

● TMX + SHAM < 0.05

TABLE 25

EFFECTS OF VITAMIN D_3 ADMINISTRATION ON URINE VOLUMES
ml/Rat/Wk: Mean ± S.E.

		WINTER			
SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk
SHAM	5	63.9 ± 16.8	99.7 ± 37.8●	8	55.9 ± 14.1	63.9 ± 12.5□
TMX	5	72.1 ± 20.2	95.8 ± 28.8●	8	59.9 ± 7.4	67.0 ± 7.2 ▽
PX-SHAM	6	53.4 ± 7.0	58.3 ± 7.0 □			
PX-TMX	4	65.0 ± 14.2	77.8 ± 28.9●			
TPX-SHAM	5	54.9 ± 17.5	60.5 ± 24.6■			
TPX-TMX	4	69.9 ± 25.0	74.8 ± 10.1			

		SUMMER						
SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk	# RATS	Before D_3	After D_3 12,000IU/Wk
SHAM	6	63.0 ± 15.0	69.2 ± 9.3	1	56.0	70.7	3	68.8 ± 11.6	113.1 ± 14.1■
TMX	7	68.5 ± 10.7	69.1 ± 8.8	1	90.3	91.3	1	73.3	115.0
PX-SHAM	1	62.7	72.3				2	57.7 ± 16.5	110.3 ± 29.0▽
PX-TMX	1	61.3	77.0				5	63.0 ± 9.4	117.5 ± 19.4▽
TPX-SHAM	2	62.2 ± 14.4	61.5 ± 21.9	1	93.0	111.7	3	60.4 ± 19.6	147.4 ± 9.2 ▽
TPX-TMX	2	48.4 ± 8.0	67.5 ± 20.5	2	91.2 ± 6.9	93.2 ± 3.5	3	79.1 ± 11.8	141.4 ± 10.0▽

P Values ($+D_3$: No D_3)

Individual Surgical Groups
□ < 0.05
▽ < 0.001

Combined Surgical Groups
● TMX + SHAM < 0.05
■ TMX + SHAM < 0.01

TABLE 26

EFFECTS OF VITAMIN D_3 ADMINISTRATION ON WATER INTAKE
ml/Rat/Wk: Mean ± S.E.

	WINTER					
SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk
SHAM	5	224.0±18.8	242.2±48.5	8	257.2±19.1	274.8±30.2
TMX	5	232.6±30.3	227.0±38.0	8	257.5±19.0	263.3±22.7
PX-SHAM	6	215.7±31.5	207.3±27.2			
PX-TMX	4	217.6±20.6	227.3±27.2			
TPX-SHAM	5	178.9±34.6	167.5±50.9			
TPX-TMX	4	187.6±44.6	188.4±30.9			

	SUMMER								
SURGICAL GROUP	# RATS	Before D_3	After D_3 1200IU/Wk	# RATS	Before D_3	After D_3 2400IU/Wk	# RATS	Before D_3	After D_3 12,000IU/Wk
SHAM	6	300.0±44.7	276.0±20.0 ●	1	186.0	195.7	3	234.1±10.1	273.1±22.8 ≙
TMX	7	230.7±64.9	254.2±64.9	1	236.7	239.7	1	246.3	272.2
PX-SHAM	1	230.7	284.3				2	219.7±55.8	267.6±27.4 ♦
PX-TMX	1	240.0	266.3				5	228.6±11.4	274.5±27.0 ♦
TPX-SHAM	2	239.9±49.7	212.5±41.7	1	153.3	191.3	3	165.8±36.5	256.2±37.5 ♦
TPX-TMX	2	211.8±36.1	226.8±63.8	2	192.6±5.2	208.0±11.3	3	183.5±16.7	231.7± 5.3 ♦

P Values ($+D_3$: No D_3)

Individual Groups:
● < 0.05
♦ < 0.01

Combined Groups
≙ TMX+SHAM : < 0.05

TABLE 27

EFFECTS OF DIETARY D_3 AND 2400 IU/Wk D_3 ADMINISTRATION ON FOOD AND WATER INTAKE AND URINE VOLUMES

Gm or ml/rat/wk: Mean ± S.E. (SUMMER)

SURGICAL GROUP (# RATS)		CONTROL DIET (CD)	D_3-DEFICIENT DIET (DF)	D3-DEFICIENT DIET + 2400IU/Wk D_3 (+D_3)	P VALUES
SHAM (8)	FOOD (Gm)	144.1 ± 10.0	112.8 ± 9.6	115.2 ± 11.9	CD:DF < 0.001
	WATER (ml)	253.0 ± 21.0	206.3 ± 39.2	199.0 ± 40.8	CD:DF < 0.01
	URINE (ml)	57,8 ± 11.0	69.9 ± 34.9	70.6 ± 33.6	
TMX (9)	FOOD (Gm)	139.1 ± 13.2	115.5 ± 13.8	114.7 ± 20.5	CD:DF < 0/01
	WATER (ml)	254.7 ± 38.7	191.6 ± 42.4	195.4 ± 33.2	CD:DF < 0.01
	URINE (ml)	66.4 ± 16.6	59.0 ± 20.1	48.1 ± 19.0	CD:DF < 0.05 DF:+D_3< 0.05

VIII - BODY WEIGHTS, WET ORGAN WEIGHTS AND HEMATOCRITS

Body weights, wet organ weights and hematocrits for all groups are summarized in Table 28. The thoracic cavity was carefully examined and any animal (TMX, PX-TMX, TPX-TMX) showing thymus tissue was discarded.

The conditions of the experiments do not allow adequate comparison of mean weights of the various organs: too much variation exists in the initial and final body weights and most importantly there is no D_3-untreated (control) group. Although no conclusions can be drawn as to the effects of vitamin D, the following need to be mentioned:

a - Thyroparathyroidectomy impaired somatic growth in all groups studied.

b- The influences of D_3 on renal excretion do not seem to be related to changes in kidney size.

TABLE 28

I- BODY WEIGHTS, WET ORGAN WEIGHTS, AND HEMATOCRITS OF RATS TREATED WITH D_3 1200 IU/WK FOR 3 WEEKS (WINTER)★

Surgery: No. of rats	SHAM ❁ 5	TMX ❁ □ 5	PX-SHAM ❁ 6	PX-TMX ❁ 4	TPX-SHAM ❁ ⊙ 5	TPX-TMX ● 4
Initial body weight (g)	264 ± 76	268 ± 70	200 ± 14	199 ± 15	191 ± 41	214 ± 22
Final body weight (g)	336 ± 55	332 ± 51	355 ± 44	338 ± 51	240 ± 82	272 ± 16
Heart (mg)	925 ± 157	893 ± 222	994 ± 92	930 ± 32	636 ± 192	655 ± 87
Kidneys (mg)	2413 ± 270	2265 ± 432	2370 ± 188	2300 ± 362	1403 ± 855	1681 ± 399
Adrenals (mg)	46 ± 9	49 ± 13	43 ± 8	42 ± 8	35 ± 14	36 ± 22
Seminal vesicles (mg) (empty)	279 ± 75	372 ± 200	381 ± 78	367 ± 94	231 ± 103	306 ± 76
Testes (mg)	2562 ± 741	2808 ± 491	3010 ± 286	3316 ± 357	1807 ± 999	2448 ± 976
Ventral prostate (mg)	391 ± 179	298 ± 181	355 ± 137	373 ± 48	227 ± 172	276 ± 133
Coagulating glands (mg)	132 ± 20	79 ± 28	143 ± 34	125 ± 57	76 ± 40	80 ± 15
Thymus (mg)	234 ± 105	---	403 ± 117	---	267 ± 161	---
Hematocrits (%)	43 ± 2	45 ± 1	49 ± 2	50 ± 1	46 ± 2	46 ± 3

★Means values and S.E.
❁Aortas dilated with loss of elasticity in most animals
⊙Necrosis of heart in one animal
□White spots on kidneys in one animal

TABLE 28 (continued)

II- BODY WEIGHTS, WET ORGAN WEIGHTS, AND HEMATOCRITS OF RATS TREATED WITH D_3 2400 IU/WK FOR 3 WEEKS (WINTER)☆

Surgery :	SHAM	TMX ⊙ ▪	SHAM ★ ▪	TMX ★ ⊙ ▪
No. of rats	4	4	4	4
Initial body weight (g)	202 ± 37	181 ± 22	175 ± 37	188 ± 31
Final body weight (g)	368 ± 31	328 ± 8	331 ± 24	330 ± 8
Heart (mg)	970 ± 40	947 ± 68	938 ± 102	990 ± 69
Kidneys (mg)	2230 ± 126	1967 ± 162	1918 ± 285	2111 ± 245
Adrenals (mg)	50 ± 10	42 ± 12	38 ± 7	46 ± 15
Seminal vesicles (mg) (empty)	206 ± 30	333 ± 30	239 ± 58	251 ± 80
Testes (mg)	2678 ± 206	2546 ± 135	2569 ± 301	2817 ± 191
Ventral Prostate (mg)	370 ± 91	352 ± 64	337 ± 50	424 ± 123
Coagulating glands (mg)	130 ± 18	149 ± 12	98 ± 44	113 ± 44
Thymus (mg)	330 ± 88	---	314 ± 43	---
Hematocrits (%)	45 ± 4	43 ± 2	46 ± 3	49 ± 1

☆Means values and S.E.
★Autopsy performed 3 weeks after the animals had been withdrawn from the vitamin treatment
● Severe kidney damage in one animal
▪ White spots on stomach and intestine of two rats
⊙Dilated, stiffened aorta in most animals

TABLE 28 (continued)

III- BODY WEIGHTS, WET ORGAN WEIGHTS, AND HEMATOCRITS OF RATS TREATED WITH D_3 1200 IU/WK FOR 3 WEEKS (SUMMER)☆

Surgery No. of rats	SHAM ○ ▫ 6 ○	TMX ○ ▫ 7 ○	PX-SHAM ○ ▲ 1	PX-TMX ○ ▲ 1	TPX-SHAM ▫ ▲ 2	TPX-TMX ○ ▲ 2
Initial body weight (g)	155 ± 25	143 ± 6	192	174	192 ± 22	200 ± 1
Final body weight (g)	300 ± 22	288 ± 10	300	260	228 ± 11	232 ± 27
Heart (mg)	739 ± 104	762 ± 50	637	798	552 ± 53	560 ± 50
Kidneys (mg)	1882 ± 350	1716 ± 204	1635	1081	1195 ± 64	1405 ± 290
Adrenals (mg)	36 ± 8	39 ± 6	26	53	18 ± 4	32 ± 12
Seminal vesicles (mg) (empty)	287 ± 130	206 ± 86	124	200	248 ± 39	296 ± 112
Testes (mg)	2402 ± 245	2345 ± 236	2224	2112	2196 ± 160	2435 ± 35
Ventral prostate (mg)	241 ± 106	251 ± 51	178	210	231 ± 18	302 ± 103
Coagulating glands (mg)	141 ± 39	108 ± 30	69	80	123 ± 18	112 ± 29
Thymus (mg)	412 ± 118	---	400	---	378 ± 66	---
Hematocrits (%)	50 ± 1	49 ± 1	38	42	42 ± 3	39 ± 0

☆ Means values and S.E.
○ Severe kidney damage, calcifying necrosis
▫ Some kidney atrophy
○ Aorta dilated, stiffened
▲ white specks on kidneys, stomach, intestines

TABLE 28 (continued)

IV- BODY WEIGHTS, WET ORGAN WEIGHTS, AND HEMATOCRITS OF RATS TREATED WITH D_3 2400 IU/WK FOR 3 WEEKS (SUMMER)*

Surgery:	SHAM	TMX	TPX-SHAM ●	TPX-TMX ▽	D_3-DEFICIENT DIET SHAM ● ▽ ○	D_3-DEFICIENT DIET TMX
No. of rats	1	1	1	2	8	9
Initial body weight (g)	175	191	177	172 ± 16	212 ± 26	219 ± 31
Final body weight (g)	416	393	208	273 ± 37	336 ± 33	344 ± 35
Heart (mg)	1038	1068	597	620 ± 6	958 ± 96	992 ± 138
Kidneys (mg)	2763	2573	1398	1198 ± 168	2166 ± 306	2197 ± 315
Adrenals (mg)	20	61	27	30 ± 12	49 ± 10	51 ± 12
Seminal vesicles (mg) (empty)	380	527	389	410 ± 35	230 ± 29	261 ± 28
Testes (mg)	3009	3099	3093	3002 ± 352	2846 ± 245	2874 ± 181
Ventral prostate (mg)	299	549	361	694 ± 116	327 ± 91	311 ± 89
Coagulating glands (mg)	82	94	111	142 ± 28	109 ± 29	116 ± 21
Thymus (mg)	524	---	159	---	289 ± 103	---
Hematocrits (%)	51	46	42	50 ± 0	52 ± 1	52 ± 3

* Means values and S.E.
● Aorta dilated and stiffened in most animals
▽ White spots or streaks on stomach of one animal
○ Severe kidney damage in one rat

TABLE 28 (continued)

V- BODY WEIGHTS, WET ORGAN WEIGHTS, AND HEMATOCRITS OF RATS TREATED WITH D_3 12,000 IU/WK FOR 3 WEEKS (SUMMER)☆

Surgery:	SHAM ◊	TMX ●	PX-SHAM ● ■	PX-TMX	TPX-SHAM	TPX-TMX ● ◊ ■
No. of rats	3	2	2	5	3	4
Initial body weight (g)	210 ± 25	214 ± 20	238 ± 12	230 ± 14	231 ± 33	214 ± 22
Final body weight (g)	324 ± 32	296 ± 6	323 ± 24	338 ± 26	209 ± 18	211 ± 45
Heart (mg)	959 ± 128	922 ± 36	876 ± 13	861 ± 78	479 ± 65	541 ± 34
Kidneys (mg)	2262 ± 443	2451 ± 569	3022 ± 942	2488 ± 201	1512 ± 288	1669 ± 254
Adrenals (mg)	44 ± 18	68 ± 8	55 ± 4	49 ± 8	37 ± 8	43 ± 10
Seminal vesicles (mg) (empty)	172 ± 58	268 ± 43	322 ± 57	266 ± 65	137 ± 72	237 ± 49
Testes (mg)	2695 ± 294	2898 ± 122	2790 ± 273	2991 ± 76	2251 ± 36	2739 ± 392
Ventral prostate (mg)	319 ± 85	395 ± 91	288 ± 74	408 ± 110	244 ± 180	318 ± 203
Coagulating glands (mg)	103 ± 14	88 ± 12	90 ± 2	115 ± 30	83 ± 44	98 ± 36
Thymus (mg)	256 ± 45	---	272 ± 30	---	146 ± 7	---
Hematocrits (%)	48 ± 2	52 ± 2	48 ± 7	48 ± 5	44 ± 4	48 ± 3

☆ Means values and S.E.
● Dilated aorta
◊ white streaks on stomach and kidneys
■ severe kidney and stomach damage: calcification

DISCUSSION

I - Calcium (Ca) and Phosphorus (P) metabolism

Vitamin D_3 is synthesized from endogenous precursors when there is adequate exposure to natural sunlight (69, 121). More of the vitamin, and of its major metabolite, 25-hydroxy D_3, are produced and stored during the "summer" than during the darker months. 25-hydroxy-D_3 serves as the precursor of other regulators (especially the highly potent hormone, 1,25-$(OH)_2$-D_3) but it also acts directly when present in sufficiently high concentrations. Since influences are exerted on numerous biological functions, it is likely that seasonal variations in the amounts of vitamin D-related secosteroids contribute substantially to the maintenance of circumannual rhythms of electrolyte metabolism.

1,25-$(OH)_2$-D_3 is the major physiological stimulant for the intestinal absorption of dietary calcium and phosphorus (69, 102, 174). It thereby contributes to maintenance of plasma concentrations of calcium and of inorganic phosphate and it provides the minerals required for the calcification of bone and other hard tissues. Vitamin D metabolites have also been implicated in the regulation of biosynthesis of the collagenous matrix of the bone (217), and in the renewal of bone cell populations. They may contribute more directly to the processes of mineralization (27, 179, 222, 226). Additionally, they seem to be involved more generally in the control of calcium exchanges between cells and extracellular fluids.

Relatively high concentrations of 1,25-$(OH)_2$-D_3 facilitate bone resorption (174) and the transfer of calcium and phosphorus from bone to the blood plasma. There are indications that the various vitamin D metabolites differ in their relative bone mineralizing versus bone resorbing potencies (226).

There is strong evidence that vitamin D metabolites also act directly on the kidney to adjust P reabsorption rates to changing needs (104, 236, 286).

By elevating plasma calcium concentrations, vitamin D metabolites indirectly reduce the secretion of parathyroid hormone (219, 244). 1,25-$(OH)_2$-D_3 and 24,25-$(OH)_2$-D_3 may also interact directly with receptors in the parathyroid glands (121, 129). However, they exert essential "permissive influences" that facilitate the effects of PTH on bone.

Parathyroid hormone promotes the transfer of calcium and phosphorus from bone and other tissues (137, 229) to the blood plasma, and it thereby elevates plasma calcium concentrations. It also promotes renal conservation of calcium (106), and it indirectly enhances absorption of dietary mineral by stimulating the conversion of 25-OH-D_3 to 1,25-$(OH)_2$-D_3 (69, 94, 121).

PTH markedly increases the renal excretion of inorganic phosphate (106, 209). As a consequence, it lowers plasma P concentrations and it thereby increases the fraction of calcium ion. However, the influences of PTH on production of 1,25-$(OH)_2$-D_3 accelerate the delivery of dietary phosphate to the bloodstream.

PTH is essential for maintaining normal bone remodeling and growth (209, 225, 247, 315). It promotes proliferation of osteoprogenitor cells and the modulation of osteoblasts to osteocytes.

Plasma concentrations of Ca^{++} will tend to rise when high levels of PTH favor bone resorption over bone formation, promote the transfer of minerals to the blood plasma, stimulate renal absorption of calcium and enhance absorption of dietary minerals. They will tend to fall when new bone formation is favored over bone resorption.

Urinary calcium excretion is augmented mostly at times when a larger quantity of the ion is filtered by the renal glomeruli. Thus, although PTH acts directly on the kidneys to promote calcium conservation, it usually increases calcium excretion because of its influences on the blood calcium concentrations. Pharmacological doses of vitamin D can also enhance calciuresis, since they promote resorption. However, physiological levels of vitamin D metabolites tend to decrease calcium excretion by shunting the mineral to sequestration sites in bone and other tissues, as well as by enhancing its reabsorption in the kidney.

Concentrations of vitamin D metabolites that favor bone formation over bone resorption reduce renal excretion of phosphate (even though they enhance dietary absorption) and they also increase renal tubular reabsorption of the mineral. In this way, they oppose the phosphaturic actions of PTH.

The rats of this study were exposed to longer periods

of natural daylight during the "summer" than during the "winter" months. The lower serum and urinary calcium values, and the lower rates of phosphate excretion are all consistent with increased synthesis of vitamin D metabolites and increased rates of bone formation relative to bone resorption.

Role of parathyroid hormone

There are several indications that PTH secretion is reduced during the summer months:

1) less phosphate (relative intake) is excreted;
2) serum calcium concentrations are lower;
3) parathyroidectomy is less effective for decreasing phosphate excretion than it is in the winter;
4) parathyroidectomy significantly elevates P serum in the winter but has inconsistent effects in the summer.
5) while parathyroidectomy reduces serum calcium levels in both summer and winter, the effects can be reversed by thyroidectomy only in the summer.

The data are in agreement with the hypercalcemic and hypophosphatemic effects of PTH reported by others. In the winter, higher levels of PTH could account for the higher levels of serum calcium, primarily because of enhancement of bone resorption, but also through inhibition of renal phosphate conservation. In the summer, PTH is probably more effective at the kidney level, where it promotes calcium reabsorption. The phosphaturic action of PTH, apparent in the winter, is probably counteracted by the opposing effects of higher vitamin D concentrations in the summer. Calciferols additio-

nally suppress PTH secretion (46).

Role of thyroid gland

The thyroid gland secretes iodinated hormones (T_4 and T_3) and calcitonin. The iodinated hormones exert numerous influences on mineral metabolism (75, 106, 140) and on bone physiology, but most effects of deficiency take time to develop. It is likely that many of the differences between the PX and TPX animals of this study are related to loss by the latter of the more rapidly acting peptide (CT) hormone. However, iodinated thyroid hormones do exert influences on electrolyte metabolism, and deficiency effects have not been ruled out.

CT inhibits bone resorption (96, 187, 275) and thereby slows the rate of transfer of minerals to the bloodstream. It also tends under some conditions, to increase the renal excretion of calcium and of phosphorus (76). While it can lower plasma calcium concentrations that are high, the administration of even fairly large doses usually has little effect on the blood levels of normocalcemic individuals. In intact animals, any tendency for CT to lower plasma calcium may be countered by compensatory increases in PTH.

In these studies, parathyroidectomy lowered the serum calcium concentrations during the winter (when vitamin D synthesis is diminished). At this time, thyroidectomy elicited no obvious influences. TPX animals had serum Ca concentrations and urinary calcium excretions virtually identical with those

of PX rats. By contrast, TPX rats had markedly higher serum calcium concentrations than PX rats during the summer.

The findings suggest that the ability of one or more thyroid factors to influence plasma calcium concentrations involves important interactions with vitamin D metabolites.

The higher rates of calcium excretion by TPX (as compared with PX) rats are reasonably attributed to the higher blood levels of calcium and therefore increased glomerular filtration of the mineral.

Removal of the thyroid gland had little or no net influences on the serum phosphate concentrations, or on phosphate excretion during the winter. However, TPX animals excreted even more phosphate than SHAM operated ones during the summer; and excretions were very much greater than those of PX rats. Thus, maximum effects of thyroid deprivation were elicited when D_3 synthesis was high and no opposing influences could be exerted by parathyroid hormones.

CT promotes hypophosphatemia and hypocalcemia in several ways that include both inhibition of bone resorption and inhibition of renal reabsorption (223, 253, 254, 293). CT can either increase or decrease excretions of Ca and P, depending on the relative magnitudes of its effects on bone and kidney which are determined by the state of bone remodeling. In normal individuals, the effects on bone are believed to dominate (296). The renal influences are not essential for the hypocalcemia and hypophosphatemia. Furthermore, although the hypophosphatemic effect of CT has been elicited following its administration in combination with PTH, CT neither increases

nor decreases PTH-induced phosphaturia.

However, under conditions of decreased bone resorption, e.g. in PX animals, or under conditions of higher rates of bone calcification (high D_3), the effects of CT on the kidney become more important. Consistent with this is the abolition of CT effects on plasma P concentrations by nephrectomy (254).

The data of this present study are consistent with a concept of multiple sites of CT actions that affect calcium as well as phosphate excretion.

Role of thymus gland

The thymus gland may confer some protection against excessive loss of minerals into the urine. During the winter months (when all surgical groups excrete more calcium), the losses for thymectomized groups were all greater than those for corresponding sham-thymectomized ones. The effects of thymectomy in the winter seem to be greatest in animals without thyroid glands. The excretion of phosphate during the winter is also greater in TMX than SHAM rats, and it is further enhanced in the TMX-TPX than just the TPX groups.

The data on calcium excretion during the summer are consistent with a thyroid-dependent increase in mineral retention, since all animals with thyroid glands excreted less calcium in the summer, whereas TPX groups did not. Both thymectomy and parathyroidectomy reduced the summer:winter differences in Ca excretion.

In view of the present findings, the following hypothesis is formulated: The thymus gland promotes calcium (and phospho-

rus) retention by stimulating bone calcification (or inhibiting bone resorption). Such action is anti-PTH to some extent and similar to that of CT. Such an effect of the thymus on bone mineralization would lead to a reduction in serum calcium (hypocalcemic effect). The latter would stimulate PTH secretion and eventually 1,25-$(OH)_2$-D_3 formation. Together, PTH and D_3 metabolites would then promote intestinal Ca and P transport, bone demineralization, renal tubular Ca reabsorption and elevation of serum calcium concentrations.

The above hypothesis takes into account the observed effects of thymectomy (in this study) in decreasing serum calcium and increasing Ca excretion more substantially when PTH and CT are present. Further support for the hypothesis found in the literature, includes the following:

1) A factor from the thymus lowers blood calcium (170). (However, the present hypothesis thinks of this effect as transient)
2) The thymus exerts influences that are antagonistic to those of PTH (170).
3) The thymus stimulates bone growth (22, 309).
4) The "thymus factor" is calcitonin produced by "C" cells that have migrated to the thymus (99).

Role of vitamin D

A - Role of endogenous vitamin D in calcium (Ca) metabolism

The seasonal variations in Ca urinary excretion and serum levels, suggest that some vitamin D effects on calcium metabolism can be associated with (are dependent upon) while

others can be dissociated from (are independent of) PTH actions. Vitamin D decreases calcium excretion in the summer when there is increased synthesis of endogenous D_3 as well as decreased secretion of PTH. Both D_3 and PTH would promote Ca reabsorption in the kidney independently and together. But the effect of either one of these agents (at physiological concentrations) on bone calcium is dependent on the other. The vitamin D effects on Ca excretion are seen in both the absence and presence of PTH, but they are lessened rather than abolished by parathyroidectomy.

However, parathyroidectomy enhances the serum calcium depression seen in the summer, suggesting that PTH is an important factor regulating blood calcium. In other words, the decrease in serum calcium seen in the summer is not solely dependent upon higher D concentrations but is also related to lower PTH levels. Complete removal of PTH worsens the hypocalcemic condition. In addition, higher endogenous D_3 would probably be favoring calcification. It is also possible that high D levels stimulate CT secretion, directly or indirectly. This could further contribute to the hypocalcemia and hypocalciuria. Frankel and Yasumura (91, 92) demonstrated that D_3 can cause depletion of the thyroid content of calcitonin in rats. Prolonged D_3 administration also stimulates calcitonin synthesis. Additional evidence for influences of D_3 on calcitonin secretion come from the effects of thyroparathyroidectomy on both calcium excretion and serum calcium, observed in the present study: seasonal variations were not marked

if present at all, in TPX animals. This strongly suggests an important involvement of thyroid gland in vitamin D action, at least in the summer. Finally, thymectomy enhanced seasonal variations in calcium excretions in TPX animals. The latter is consistent with some antagonistic influence of the thymus on vitamin D and/or PTH action.

B - Role of endogenous vitamin D on phosphate (P) metabolism

As pointed out previously, higher endogenous D levels seem to be responsible for the decreased P excretion observed in summer animals as compared with winter ones. Vitamin D could theoretically elevate serum P in two ways: by enhancing intestinal absorption (via PTH-dependent and PTH-independent mechanisms) and by increasing renal conservation of the mineral. However, such actions are overpowered by strong stimulation of ion uptake by tissues such as bone. During the winter, PTH effects on urinary excretion should become more evident, since D_3 levels are low.

By comparison with the variability in excretion, the blood phosphate levels seem to be under rigorous control and to resist seasonal influences. It is likely that calcitonin contributes to such stability, since it (as well as PTH) can promote development of hypophosphatemia. The findings on animals thyroidectomized during the summer months, and others involving D_3 administration to TPX rats, support the concept that the thyroid gland participates in responses to calciferols.

The above considerations support a role for endogenous

vitamin D_3 in preventing phosphate diuresis, such role being more important than maintaining normal blood phosphate level. As discussed later, vitamin D_3 was found to promote Cl excretion. It is quite possible that D action on renal P handling are related to or may even be secondary consequence of the effects on chloride.

C - Effects of administration of 1200 IU/week D_3

Effects on calcium

The administration of 1200 IU D_3 weekly during the winter increased calcium excretion in all groups, more especially in rats with intact parathyroid and thyroid glands. By contrast, there was little influence on serum calcium concentrations. The administration of similar doses during the summer decreased calcium excretion in all groups including PX and TPX rats; and there was a concomittant rise in serum calcium concentrations.

The findings are consistent with some improvement of absorption of dietary calcium during the winter. However, during the summer months, since the reduction in calcium excretion was associated with an elevation of serum Ca concentrations, it is evident that vitamin D provoked changes other than those related to intestinal absorption. The data support influences of D_3 on increasing intracellular storage of calcium and/or activation of bone mineralization.

That vitamin D is exerting divergent effects on serum calcium handling at different times of the year can be explain-

ed on the basis of the various metabolites involved in the mode of action of the vitamin. It is generally believed that when plasma calcium concentrations are high (such as in winter) substantial amounts of exogenous D_3 are converted to 24,25-$(OH)_2$-D_3. This metabolite can increase intestinal calcium absorption, possibly following 1-hydroxylation, but it has little effect on bone and kidney. The findings of the present experiments are consistent with 24,25-$(OH)_2$-D_3 increasing dietary absorption of calcium during the winter and therefore increasing the amount of Ca filtered by the glomerulus. This could explain the greater urinary loss of calcium. The data demonstrate that the vitamin is more effective in animals with intact parathyroid and thyroid glands. It is likely that PTH, because of its bone resorbing activity, contributes to the increased load of calcium at the kidney, and that this effect is superimposed on its stimulation of 24,25-$(OH)_2$-D_3. Therefore, PTH effects on calcium excretion are additive to those of 24,25-$(OH)_2$-D_3. Furthermore, it has been reported that injections of D_3 in D-sufficient humans increase calcium excretion before any changes are observed in plasma calcium or PTH levels (33). Only later is PTH secretion inhibited (46).

During the summer months, when serum calcium is low, (PTH level is reduced), more vitamin D_3 is converted to 1,25-$(OH)_2$-D_3 (124). The 1-hydroxylation reaction is known to be influenced by PTH (124). Therefore, in animals with intact parathyroid glands (TMX, SHAM), the observed effects of vitamin D could be explained by mechanisms involving PTH actions on bone

and kidney. However, one point of interest is that, although PTH stimulates 1,25-$(OH)_2$-D_3 formation (when serum Ca is low), PTH secretion is eventually inhibited (feed-back) by the metabolite following its increased synthesis (46). In animals deprived of parathyroid glands, (PX, TPX), D_3 must be acting via different mechanisms. Possibly, exogenous D_3 is converted to 25-OH-D_3 or 24,25-$(OH)_2$-D_3 which would improve intestinal calcium transport and favor bone mineralization (241), therefore explaining the changes in serum and urine concentrations. The data on PX and TMX animals do support actions of D_3 independent of PTH or thyroid gland. Reynolds et al (217) reported that cultures of bone from TPX mice respond to additions of 1,25-$(OH)_2$-D_3 in moderate doses, suggesting the existence of additional mechanisms of bone mineralization (other than those involving PTH or CT). More recently, Treschsel et al (300) demonstrated the existence of PTH-independent regulation of plasma 1,25-$(OH)_2$-D_3 in rats. Whether exogenous D_3 is converted into 1,25-$(OH)_2$-D_3 or to 24,25-$(OH)_2$-D_3 during the summer months, the end result in serum calcium and calcium excretion would be the same.

Effects on phosphate

The administration of 1200 IU D_3 weekly significantly increased phosphate excretion in PX animals during the winter. Although the change was not statistically significant in the other surgical groups, it is important to notice the trend towards an increase in excretion in TMX and SHAM, but a decrease in TPX rats. These findings suggest the involvement of

the thyroid gland in the observed stimulation of P excretion by vitamin D_3. Similarly, during the summer, D_3 increased P excretion in all groups except in TPX animals which again showed a slight decrease.

This dose had little influence on serum P concentrations during the winter, but it elevated the serum values of SHAM animals during the summer. The same dose diminished calcium excretion and elevated serum calcium values during the summer. Therefore, it is apparent that vitamin D_3 is exerting its influences on calcium and phosphorus metabolism via different mechanisms. The thyroid gland seems to be essential for normal renal handling of phosphate. It has been reported that the phosphatemic effect of D_3 is independent of the effect exerted on urinary phosphate excretion (286). This seems to be exemplified in summer SHAM rats with increased P excretion rates and increased serum P concentrations.

The role of thyroid (CT) in D action will be considered in the following section.

D - Effects of administration of 2400 IU/wk D_3

Effects on calcium

The administration of 2400 IU/wk D_3 had similar effects during the winter and summer months. It is possible that this higher dosage in winter animals (compared to 1200 IU/wk) depressed PTH secretion to levels closer to those of summer animals. The vitamin decreased calcium excretion and increased serum calcium values in the animals studied (TMX, SHAM, TPX's).

This further supports the concept of a direct influence of vitamin D, independent of PTH or thyroid gland, as already suggested by the responses elicited by injections of 1200 IU D_3 in PX and TPX animals. Since the elevation in serum concentrations is associated with a reduction in excretion, it is most likely that vitamin D is directly promoting renal calcium reabsorption. This is probably attributable to increased intracellular calcium storage and/or bone calcification. Direct effects of D_3 on kidney tissue have been reported in the literature (56, 104, 236, 286). Moreover, D_3-induced calcium binding protein has been identified in chick renal tubules (301, 302), but its relationship to cytosol Ca content has not been established.

Effects on phosphate

The administration of 2400 IU/wk of D_3 during the February-March months significantly increased the phosphate excretion (without affecting the serum P values) of TMX and SHAM animals. Similar doses during the summer reduced the amount of phosphate excreted, but again without affecting the serum concentrations of TMX, SHAM and TPX animals. These responses further suggest the dissociation of renal and phosphatemic effects of D_3. It is important to point out the different responses elicited by 1200 IU/wk versus 2400 IU/wk on P excretion during the summer. The small dose increased the excretion rates in intact animals whereas the higher dose reduced the rates. However, in both cases, TPX animals showed a reduction in excretion. The phosphaturia observed following

1200 IU injections was attributed earlier, at least in part, to D_3 stimulation of calcitonin secretion. It is possible that the higher dose (2400 IU), superimposed upon higher endogenous D_3 concentrations during the summer, counteracted the effect of calcitonin in TMX and SHAM animals, while D_3 (as expected) prevented P diuresis.

The findings strongly suggest the importance of a thyroid factor, possibly calcitonin, in realization of vitamin D actions. The phosphaturia observed following D administration seems to be mediated via stimulation of CT secretion (92, 311).

E - Effects of administration of 12,000 IU/wk D_3

Effects on calcium

The administration of 12,000 IU D_3 weekly during the summer increased calcium excretion and serum calcium levels of all animals, with and without parathyroid and thyroid glands. Such responses are similar to those reported in literature during hypervitaminosis or following administration of pharmacological doses. Such high vitamin D levels do not require PTH to promote bone resorption. The vitamin in this case, is believed to be mostly converted to 25-OH-D_3, nullifying possible negative feed-back effects on the 25-hydroxylase system (69). High concentrations of 25-OH-D_3, besides stimulating intestinal calcium transport, could enhance bone demineralization, thereby increasing serum calcium content and urinary calcium. 25-(OH)-D_3 may compete for 1,25-$(OH)_2$-D_3 receptors of intestine and bone, bring excessive amounts

of calcium into the system, and thereby promote development of metastatic calcification (69). Very recent evidence shows that hypervitaminosis D does not increase resorption of bone, but rather that it inhibits mineralization by impairing the uptake of Ca by bone. Ultimately, dietary calcium and calcium derived from bone resorption are spilled into the urine (147).

Effects on phosphate

The administration of 12,000 IU D_3 weekly during the summer increased P excretion in all rats. However, the increase was diminished by thyroparathyroidectomy. Again, as before, there is a suggestion of thyroid involvement in vitamin D action.

Serum P concentrations were reduced following the injections with no apparent differences among the surgical groups.

The changes in serum and urine concentrations do point to phosphate depletion elicited by very high doses of vitamin D. Pharmacological doses of D_3 are known to be phosphaturic (215, 311).

F - Effects of dietary D_3-deprivation - Effects of administration of 2400 IU/wk to D-depleted rats.

Effects on calcium

TMX and SHAM rats kept on the D-deficient diet during the summer showed no significant change in calcium excretion when compared with animals maintained on a normal (D-supplemented) diet. Serum calcium values remained fairly constant

except in the TMX group (which showed a rise in serum levels). The D-deficient diet probably increased PTH secretion (which is otherwise low during the summer). This should explain the ability of SHAM rats to maintain serum calcium. On the other hand, excessive elevation of serum calcium concentration would generally be counteracted by release of CT. By contrast, TMX animals, deprived of a hypocalcemic factor (as demonstrated earlier) should (and did) show higher serum calcium concentrations. The hypercalcemic effects of PTH that result from accelerated bone demineralization are believed to be vitamin D-dependent. The animals eating the D-deficient diet may have possessed sufficient endogenous vitamin to mediate the response. Absolute vitamin D deficiency states are difficult to achieve particularly in a short time (226). The animals of this study were fed the deficient diet for only three weeks and they were exposed to sunlight. Dietary D_3 is indispensable only when animals are deprived of adequate light (172). Thus, the rats of this study were probably D-depleted rather than D-deficient. The effects of PTH on renal conservation of calcium are believed to be independent of the vitamin D status (16, 226).

An additional factor that can contribute to the maintenance of blood calcium levels is the high calcium content of the diet (1.2 mg%). It is known that high-calcium diets can provide sufficient mineral for passive transport across the intestinal wall (226).

Because of the lesser amounts of available vitamin D, a decrease in bone mineralization would be expected in our

summer animals. It is well known that D_3 is needed to promote bone remodelling, and that severe D-deficiency leads to appearance of rickets and other abnormalities (69). It had been proposed that D-deficient rats do not have an active 24-hydroxylase enzyme (69, 124) in the kidney (although some is present in intestine, cartilage etc...) Therefore they are unable to make adequate amounts of $24,25\text{-}(OH)_2\text{-}D_3$. The latter metabolite is believed by many to be the major one promoting bone mineralization (124). It does not seem to share with 1,25HCC the ability to reduce the rate of synthesis of bone collagen. However, it is believed to inhibit the secretion of PTH (27).

All of the above would contribute to the maintenance of urinary and serum Ca within normal ranges. As aforementioned the removal of an antagonist to PTH, would further raise the plasma Ca values of TMX rats.

Unfortunately, the effects of the vitamin D_3 deficient diet were not studied in parathyroidectomized or thyroparathyroidectomized animals. One would expect much greater disturbances in calcium metabolism under those conditions.

The administration of 2400 IU D_3 to D-depleted rats led to decreased calcium excretion. Similar effects have been reported by Nicolaysen and Eeg-Larsen (226). In the present study, the concentrations of urinary calcium were actually below the levels that could be detected with the methods employed (0.5 mg/100 ml), suggesting that the D-repleted animals were keeping their calcium, probably via

enhanced renal reabsorption. As expected, there was a rise in serum calcium concentration, in fact to values above those considered normal (13.5 mg%). Again, the response to D was even more pronounced in TMX rats. The findings are consistent with vitamin D improving intestinal calcium transport, promoting bone mineralization and enhancing renal calcium conservation. Goldberg et al (106) have reported that small doses of D_3 administered to D-deficient, but intact (with PTH and CT) animals reduce calcium excretion by enhancing the deposition of bone minerals. In addition, 25-OH-D_3 was found to be the most potent metabolite for promoting renal Ca (and P reabsorption and bone calcification when given to D-deficient men (27, 53).

Effects on phosphate

There was a tremendous increase in P excretion (greater than 300% increase) when rats previously fed a normal diet were switched to a vitamin D-deficient diet. The findings strongly support the concept of a role for vitamin D in preventing phosphate diuresis. As already discussed in the calcium section, decreased bone mineralization and enhanced PTH secretion probably contributed to the phosphaturia.

By contrast, the serum phosphate concentrations did not show any significant decrease. This is surprising, considering the excessive amounts of phosphate being excreted. The phosphate content of the diet (0.86%) would be expected to provide enough mineral to maintain the plasma levels. Furthermore, since there is little or no 24,25-$(OH)_2$-D_3 to

inhibit its secretion, PTH probably increases demineralization and thereby provides phosphate for transfer to the blood plasma. Calcitonin could be essential for counteracting the excessive resorbing activity of PTH and therefore for maintaining the phosphate (and calcium) content of serum within normal range. Calcitonin, as previously described, acts at two sites to promote hypophosphatemia (and hypocalcemia). In cases of enhanced bone turnover, the anti-resorbing activity of CT is apparent. Therefore, the defect in D-depleted animals is probably not primarily at the level of bone remodeling in the intact animals (with parathyroid and thyroid glands) but at the level of the kidney: D-deficient animals are unable to efficiently reabsorb P in the renal tubules. The defect in P handling should be the most obvious, for both PTH and CT are phosphaturic agents and their secretions are stimulated under conditions of D-deficiency (when anti-phosphaturic actions of D_3 are elevated). D_3-deficient animals do not suffer defective renal calcium conservation. They are able to efficiently reabsorb calcium from the glomerular filtrate.

Similar results have been reported by Costanzo et al (62): D_3-deficient rats have defective P reabsorption even when the blood calcium concentration is in the normal range.

The administration of 2400 IU/wk D_3 to D-depleted animals (TMX and SHAM) decreased the phosphate excretion rates. This supports the notion that vitamin D is anti-phosphaturic. D-repleted rats had lower serum P concentrations than D-depleted ones. It is quite likely that the vitamin D injections

promoted sequestration of the available phosphate for bone mineralization. The same injections increased serum Ca concentrations; but it is well known that the increase in blood Ca content usually precedes that of serum P. Under the present experimental conditions, a decrease in serum P was observed, probably because the concentrations were measured at a time of active mineralization. Calcitonin secretion (stimulated by PTH or D_3) is a possible contributor to the low serum P values, since that peptide inhibits release of phosphate from bone. However, Costanzo et al (62) found that when D_3 was administered to D-deficient rats, there was decreased urinary phosphate and calcium excretion, and such responses were more pronounced in TPX animals. This supports the notion of a direct effect of D_3 on the renal handling of phosphate and calcium (independent of PTH and of the thyroid gland) in D-deficiency. In a few cases, Costanzo et al (62) found that exogenous D_3 increased the excretion of phosphate in parathyroidectomized and thyroparathyroidectomized animals They attributed this effect to residual PTH (but it could also be related to residual calcitonin).

The findings of Costanzo et al do not exclude roles for PTH and CT in the phosphatemic actions of vitamin D, but tend to further emphasize the dissociation between the influences of D_3 on blood and those on the kidneys.

Influences of thymus on vitamin D actions

Thymus involvement in vitamin D action is apparent in the observed summer:winter differences as well as in the

responses to administered D_3. Although the effects of thymectomy seemed to vary with the time of D_3 administration, they indicate a role for the thymus in promoting bone formation. The important point to consider is the dual action of vitamin D on bone minerals, i.e. its bone demineralization as well as its bone mineralization activities.

A - In the presence of PTH, the thymus gland antagonizes the vitamin D effect on calcium and phosphorus metabolism. Under those conditions, with low to moderate doses of D_3, bone resorbing activity is enhanced (via 1,25-$(OH)_2$-D_3). The anti-resorbing influence of thymus is then more apparent.

B - In the absence of PTH, or when PTH secretion is depressed, the thymus gland enhances vitamin D effects on bone mineralization. Under those conditions, there is a lesser rate of bone resorbing activity, with accelerated bone calcification. Therefore, the effects of vitamin D parallel those proposed for the thymus.

II - Urinary Sodium (Na) Excretion

A - Seasonal variations

The seasonal variations in excretion patterns for sodium were of smaller magnitude than those for the other ions. However, a marked decrease was observed in spring, with values lower than those for both winter and summer rats. This is the reverse of the pattern for chloride excretion, in which the highest values were found in the spring.

These findings could result from the dual effects of "decreased PTH in spring" and "elevated-spring endogenous D." The overall influences of PTH have been reported to culminate in enhancement of sodium excretion, whereas there are cases (discussed later) in which D injections promoted Na reabsorption.

B - Sodium excretion and relationship with other ions

Sodium and calcium: Several investigators have observed an interdependence in the renal handling of calcium and sodium. Micropuncture studies indicate that such Na-Ca interrelationships are localized to the early portions of the nephron, i.e. the proximal tubule (37, 106, 159, 197, 310) and the loop of Henle (106). However, even within the ascending (but not descending) loop of Henle, the transport of the ions is independent (134, 156).

Sodium and phosphate

In the proximal tubule, phosphate and sodium reabsorption are closely linked (3). By contrast, transport of P

seems to be totally dissociated from that of Na in the distal tubule (106).

The effect of PTH on P reabsorption has to be attributed, for the most part, to inhibition of Na reabsorption by PTH in the proximal tubule (106).

C - Role of parathyroid glands

In the present study, parathyroidectomy decreased sodium as well as phosphate excretion in both summer and winter. The concomitant increase in calcium excretion in the same animals supports the concept that the net effects of PTH on sodium and calcium excretion are dissociated.

PTH is known to inhibit proximal sodium reabsorption. However, net sodium excretion may not be affected because of the counteracting effects of mineralocorticoids at the distal portions of nephron (291). The results reported herein are consistent with possible adrenocortical influences following parathyroidectomy that lead to a reduction in Na excretion.

D - Role of thyroid gland

Calcitonin increases the urinary excretion of numerous solutes, including sodium. It is a potent natriuretic in rats and in man (4, 140) when given in either physiological or pharmacological amounts. Moreover, the increment in sodium excretion is linearly related to the log dose of the hormone. The effect seems to be exerted at the level of proximal tubule and to be independent of both PTH and of the

mineralocorticoids (140). No significant correlation has been found between calciuric and natriuretic effects of the hormone.

In view of the preceding, one would expect thyroidectomy to decrease sodium excretion with TPX animals showing the lowest excretion values (compared to PX, for instance). However, in the present study, thyroidectomy blunted the effects of parathyroidectomy on Na excretion; and TPX animals had values similar to those of intact rats.

The natriuretic effects of CT were observed following the administration of exogenous peptide. In many cases, preparations with prolonged actions were utilized. The failure to see a reduction in sodium excretion following thyroidectomy in the present study could be attributed to a variety of factors, e.g. relatively minor influences of endogenous hormone on sodium excretion, the presence of lower concentrations of the peptide, or the influences of potent regulators such as the mineralocorticoids that exert opposing actions.

Furthermore, thyroidectomy depletes the animals of thyroid hormones other than calcitonin. Whereas plasma levels of Na (and K) and their urinary excretions are not greatly affected by thyrotoxicosis, it is reported that thyroid-deficient animals have impaired sodium metabolism (140); and hypothyroid rats suffer defective Na conservation. Several hypotheses concerning the roles of thyroid hormones have been proposed but none seem to be totally adequate. Since an increase in Na excretion is seen in thyroid-deficient rats,

it has been proposed that the defect is in the Na-K-ATPase system of kidney responsible for active Na transport (75). Thyroid hormones are known to stimulate active Na transport; and some correlation has been found between decreased ATPase activity and decreased Na transport in hypothyroid rats. Thyroid hormones may therefore establish a proper balance between retention and excretion.

It should also be pointed out that in addition to the mineralocorticoids, calcitonin and other factors, renal prostaglandins have been proposed as possible "natriuretic hormones." PGE and PGA have been reported to exert anti-hypertensive effects by promoting vasodilation, and the excretion of sodium, water and K^+ (166). However, PGA findings have been for the most part discounted.

E - Role of thymus gland

Thymectomy tended to substantially increase sodium excretion during the summer in otherwise intact rats. A smaller increment was observed during the winter. However, thymectomy also enhanced the effects of parathyroidectomy: PX-TMX rats had lower excretion values than PX rats. Evidently, thymus removal results in some impairment of regulatory mechanisms present in the intact animal. Such a conclusion had been previously proposed by several investigators (133, 185). In one of those studies (185), heparin, administered at time of year when Na excretion is normally low, was found to increase sodium, as well as H_2O, $PO_4^{\equiv}$ excretion in TMX but not

in intact rats drinking tap water. However, it increased excretion of Na, K, Cl and water in both TMX and SHAM when the animals drank sodium chloride solutions. It was suggested that heparin is a "normalizer," capable of decreasing or increasing excretion of the above ions to maintain proper balance. Removal of thymus would impair such control. The thymus gland has been reported to produce heparin-like, sulfated mucopolysaccharides (49, 66, 81). In addition, prostaglandins have been found to be synthesized in T-cells and to play important regulatory roles in immune responses (312). Current evidence suggests that most prostaglandins, exert their actions in the immediate vicinity of their production or release, acting as local hormones (190). Prostacyclin effects on the kidney may, however, depend on actions exerted at more distal sites.

F - Role of vitamin D

Effects of 1,200 IU/wk D_3

This dose of D_3 increased Na excretion in rats with intact thyroid glands during the winter. Its effects were greatest in SHAM groups. While PX animals showed a slight increase in excretion, TPX animals tended to decrease their Na output following the vitamin administration. During the summer, D_3 injections increased the excretion rates in all groups, again most significantly in SHAM. These findings suggest some involvement of thyroid gland in vitamin D action, at least when low levels of the vitamin are present.

Effects of 2,400 IU/wk D_3

There was increased sodium excreted in SHAM and TMX following D_3 administration, during the winter months. As with the lower dose (1200 IU/wk), TPX animals showed a decrease in excretion rates when given D_3 in the spring.

Effects of 12,000 IU/wk D_3

The administration of this dose increased the excretion of Na in TPX groups, as well as (although to lesser magnitude) in TMX and SHAM RATS. This dose similarly increased the excretion rates of Ca, P, Cl (and K) in all animals. Such influences of D_3 are probably related to hypervitaminosis.

It is also observed, (although significance of such point is unclear) that as the dose of administered exogenous D is increased, the magnitude of the responses of TMX and SHAM animals decline whereas that of the PX and TPX groups shows the reverse pattern.

Effects of dietary-D_3 deprivation - Effect of 2400 IU/wk D_3 administered to D-deficient rats

Although the D-deficient diet did not significantly influence sodium (or chloride) excretion in the few animals studied during the spring, TMX and SHAM rats fed this diet during the summer showed a significant decrease in Na and Cl excretion rates. However, summer animals had higher excretion values prior to the D_3-deficient treatment, and the spring: summer differences were abolished when the animals were maintained on the D-deficient diet. A similar pattern has been observed for chloride excretion: D-deficient diet did

not affect chloride excretion in the spring, but it reduced it in the summer.

The administration of 2,400 IU/wk D_3 to D-deficient rats reduced the sodium excretion during the spring and summer. This suggests a direct influence of vitamin D on sodium handling.

From the data obtained following the administration of low to moderate doses of vitamin D, it seems that D_3 promotes sodium retention. Puschett et al (236, 237) have reported enhancing effects of D_3 on sodium reabsorption. At physiological doses, D_3 decreased sodium excretion when injected into TPX dogs. It also decreased P and Ca excretion (a finding consistent with observations made in this study). It is suggested that 25-(OH)-D_3 is the metabolite involved. More recently Costanzo et al (62) described effects of exogenous D_3 in rats. When given to D-repleted rats, exogenous D resulted in a hypervitaminosis effect. However, when D_3 was given to D-deficient rats, it elicited responses showing a positive, although not linear, relationship between Ca and Na reabsorption. D_3 increased the renal reabsorption of P, Ca, Na in D-deficient animals and was most effective in TPX rats. The present study supports these findings: except in high dosage (12,000 IU), D_3 was found to decrease the excretion of Na (and of P, Ca) in TPX animals on a normal diet, and to also decrease the excretion values of TMX and SHAM on a D-deficient diet.

The increase in Na^+ excretion in responses to D_3

observed in the present study in TMX and SHAM given low to moderate doses (1200, 2400 IU) probably involves stimulation of thyroid gland. (No increase was observed in TPX rats).

Costanzo et al (62) suggested that D_3 influences on Na may be exerted at the distal portion of the nephron, for in some cases, D_3 changed Ca excretion relative to Na excretion without changing P excretion.

Thymus gland and vitamin D

Exogenous D affects were enhanced by the presence of thymus gland. When D_3 increased Na excretion, SHAM animals responded more vigorously than TMX animals. Similarly, when D_3 decreased excretion, the decrease was also more significant in SHAM. The thymus therefore appears to be a modulator of vitamin D action.

III - Urinary Potassium (K) Excretion

Seasonal variations

The seasonal variations in excretion patterns for potassium were minimal. As for sodium excretion, the only significant change occurred in the spring. At that time of the year, the values obtained were lower than those for winter and summer animals.

Role of parathyroid hormone

Removal of the parathyoid glands had little effect on potassium excretion during the winter and summer. PTH is not known to be a regulator of K metabolism and whatever little influence it may have would be expected to be overshadowed by the powerful actions of mineralocorticoids. PTH inhibits bicarbonate reabsorption in proximal (and distal) tubule of the kidney (106, 210), as well as sodium transport in the proximal tubule. Since transport of K^+ is closely related to that of sodium and very much affected by the state of HCO_3^- reabsorption (296), it is quite possible that PTH tends to promote K^+ retention to some extent. In a recent study (17) hyperaldosteronism was shown to be associated with hyperkaluria and hypokalemia; but the defect was attributed to primary hyperparathyroidism.

Role of thyroid gland

TPX animals had higher excretion rates than PX animals but values similar to those of intact rats (TMX, SHAM). The data do not indicate any clear function for either calcitonin or for PTH on renal K^+ handling.

Reports on CT regulation of K^+ excretion are conflicting. Kaliuresis has been described in rats (4, 24) - but only slight or inconsistent effects have been found in humans (5, 223). Bijvoet et al (24) attributed the kaliuresis resulting from prolonged administration of calcitonin to probable increases of aldosterone secretion secondary to the preceding natriuretic effect of the hormone.

Role of thymus gland

Removal of thymus gland had some effect in the summer. TMX animals had higher excretion rates than the SHAM group. No other appreciable influences of surgery were observed. However, it should be mentioned that TMX rats also had higher values for Na, Cl excretions and urine output than SHAM during the summer. It is possible that thymectomy has a general effect on electrolyte balance, and that it deprives the animals of the "normalizer" discussed earlier.

Role of vitamin D

The administration of exogenous vitamin D elicited neither dramatic nor consistent responses. 1200 IU and 2400 IU injected weekly increased the excretion rates in TMX and SHAM during winter and spring. During the summer months, TMX rats did not respond to 1200 IU (whereas SHAM did): the vitamin did not elicit apparent changes in K excretion (nor in Na). PX and TPX groups showed little change in their excretion rates.

The administration of 12,000 IU D_3 weekly increased the excretion values for Na, Ca, P, Cl, in all surgical groups,

but it enhanced K^+ excretion only in PX groups.

However, TMX and SHAM maintained on a D-deficient diet had a dramatic reduction (50 to 56%) in K^+ excretion during both spring and summer. No reduction in Na excretion was observed in the spring under the same conditions. 2400 IU administered to D-deficient TMX and SHAM, although it further decreased the excretion rates in the spring, had no apparent effect in the summer. The vitamin D studies do not show any clear pattern of vitamin D influence on K^+ excretion. Any variations observed could be related to the actions of other factors stimulated by vitamin D administration or to the influences of the vitamin on other ions such as sodium.

IV - Urinary Chloride (Cl) Excretion

Seasonal variation

The excretion values for chloride were found to be higher during the summer than during the winter months. The seasonal variation was observed in all the groups studied (TMX, SHAM, PX and TPX's). Therefore, something other than PTH or a thyroid factor is enhancing chloride excretion in the summer. Since its endogenous synthesis is increased in the summer, vitamin D might be responsible for the observed changes in chloride excretion. Indeed, the effects of exogenous D administration seemed to support this possibility and they will be discussed in a later section.

Although the values for summer were generally higher than those for the winter months, the excretion rates for TMX, and SHAM were highest during the spring. Only three TPX rats were observed during the spring. These too, had higher values than either winter or summer TPX animals. Such a large increase in excretion could be related to lesser secretion of PTH with the advent of lighter days, and to increased vitamin D synthesis. The animals could be more sensitive to vitamin D in the spring, since they are observed following a period in which D synthesis is low. A cyclic nature of remodelling has been described and some parts of skeleton seem to remodel more frequently than others. Seasonal variations include greater osteoid tissue formation in the late winter and spring than during late summer and autumn. These changes could be attributed to vitamin D (1). Certain

aspects of bone remodelling could be more sensitive to D in spring. Similarly, a D effect on chloride excretion could be more powerful at that time of year. During the summer months, the animals are probably able to adjust better to chronically high concentrations of vitamin D. Studies by two laboratories showed lowest plasma concentrations of vitamin D in December and January and the highest values in June-July (289) or September (211). But, McLaughlin et al (211) found the greatest variability in March (March:Sept. $P<0.001$, March:Dec. $P<0.05$). In some persons, the concentrations of 25-OH-D_3 (the major circulating vitamin D metabolite) reached peak values during that month, after which they fell slowly to rise again during the summer.

Seasonal variations in the response of toad urinary bladders to antinatriferic activity have been reported (38). Similar to the findings of McLaughlin et al, data obtained during the month of March were strikingly different from those of any other month. While the antinatriferic activity in plasma ultrafiltrates of diuretic dogs was quite high in most months, it fell sharply in March to rise again in April. The mechanisms are unknown.

Role of parathyroid glands

In the present study, parathyroidectomy increased chloride excretion during all time periods studied. The effects were of greater magnitude during the winter, when plasma PTH levels are generally higher. The findings, plus observations by others that hyperparathyroidism is often

associated with hyperchloremic acidosis (242), are consistent with the concept that PTH promotes the renal conservation of chloride.

Role of thyroid gland

Removal of the thyroid gland did not significantly alter the excretion of chloride during either the winter or the summer. (TPX animals had excretion rates similar to those of PX animals). Both PX and TPX groups, however, had higher values than rats with intact thyroid and parathyroid glands.

Removal of the thyroid gland severely blunted the winter: summer differences. This points to some involvement of the thyroid gland in the observed seasonal variations in chloride excretion. A thyroid factor (calcitonin?) could be mediating the effects of vitamin D in the summer (see vitamin D section on chloride).

Several laboratories have reported influences of calcitonin on the renal handling of chloride (140). Acute administration of several calcitonin preparations cause chloruresis in rats (4) and man (5, 24). Therefore, thyroid hormones seem to oppose some effects of PTH. One could expect PX animals to gradually have higher excretion values than TPX animals, for chronically parathyroidectomized animals can progressively show stimulated calcitonin secretion (292). Under such conditions (low resorbing activity) the renal effects of CT would be more apparent (140). Although such expectation was not fulfilled in the winter experiments, it was suggested in the summer (summer values for chloride: PX-

77 Meq, TPX-67 Meq). Some other factor, probably a calciferol, is also affecting chloride excretion.

Role of thymus gland

Removal of the thymus gland increased the excretion of chloride during the summer months, more significantly so in rats with intact thyroid and parathyroid glands. Since the enhancing effect of thymectomy on chloride excretion was statistically significant only in otherwise intact summer animals, it is possible that thymus gland is not a prime regulator of chloride metabolism. However, it is quite likely that parathyroidectomy removes some essential condition for thymic action. The thymus does seem to mimic PTH influences on chloride retention, and to antagonize the effects of endogenous D.

Role of vitamin D

The possible effects of endogenous calciferols on chloride excretion were discussed earlier. The higher rates of chloride excretion in spring and summer months were attributed to higher rates of endogenous D synthesis. A lesser rate of PTH secretion at those times cannot be the sole factor responsible for the observed seasonal variations, for higher values were observed in the intact animals (PTH present) as well as in animals deprived of parathyroid glands. That the seasonal variation was blunted by thyroid removal suggests a role for thyroid.

The effects of exogenously administered vitamin D

provide further support for the above conclusions.

A - Effects of 1,200 IU/wk D_3

The administration of this dose greatly increased the excretion of TPX and PX rats during the winter. The lack of response in rats with intact parathyroid and thyroid glands is consistent with greater antagonism by higher levels of PTH during the winter months. In addition, the stimulation of chloride excretion seen in the animals with no PTH and/or CT strongly suggests a direct action of vitamin D on chloride metabolism, independent of the above named hormones, at least in the winter.

The same dose administered in the summer brought up the values for SHAM animals close to those for TMX ones. However, it slightly decreased the excretion of chloride in TPX animals. Only two PX animals were given the vitamin. They did not seem to show any noticeable response. The higher rate of chloride excretion in summer (due probably to high D) had previously been shown to somehow be dependent on thyroid gland (see Seasonal variation). The effect of the low dose of D_3 in TPX animals seems to confirm this hypothesis. The involvement of the thyroid gland in vitamin D action in the summer has also been strongly suggested by the phosphate study discussed earlier.

B - Effects of 2,400 IU/wk D_3

The administration of this moderate dose of D_3 during the winter months increased chloride excretions of TMX and SHAM animals. It seems that at that level, vitamin D was able to counteract the influences of PTH on promoting chloride retention, probably by directly depressing PTH secretion. An effect on calcitonin secretion is also possible.

The few animals given this dose during the spring showed little response to the administered vitamin D. These animals, however, had very high rates of excretion prior to D treatment. This was attributed earlier to increased sensitivity to endogenous D at that time of year.

C - Effects of 12,000 IU/wk D_3

The administration of a high dose of vitamin D during the summer greatly increased excretion values of rats deprived of parathyroid and/or thyroid glands (TPX and PX). It did not affect the excretion of intact rats (TMX, SHAM) studied at the same time. However, for some unknown reason, those rats had very high excretion rates prior to D treatment. This is similar to the previously mentioned observation concerning the lack of effect of 2,400 IU/wk D_3 in spring rats.

The effects of this high dose of vitamin D_3 seem to be pharmacological, since they are accompanied by great increases in calcium, phosphorus and sodium excretions.

D - Effects of dietary vitamin D_3 deprivation - Effects of 2,400 IU/wk D_3 in D_3-deficient animals

The effects of a D-deficient diet in the summer clearly show the influence of vitamin D in promoting chloride excretion. Animals (TMX, SHAM) maintained on the D-deficient diet had Cl excretion values much lower than those of controls fed a normal diet (64% reduction in TMX, 23% in SHAM). In fact, D-deficient summer rats had excretion values similar to those of low D-treated winter rats on a normal diet.

When 2,400 IU D_3 was administered weekly to the D-deficient rats, the excretion rates went up, again strongly supporting the concept of a chloriuretic influence of vitamin D.

The excretion of chloride has been linked to that of sodium. The two ions seem to be reabsorbed together in certain portions of the kidney such as distal tubule. However, studies with diuretics show that chloride transport can be dissociated from sodium transport in ascending portion of the loop of Henle (40). In this study, the vitamin D-deficient diet reduced the excretion of both ions. When D_3 was subsequently administered, the excretion of chloride increased while that of sodium decreased. This is consistent with vitamin D exerting direct actions on kidney tubules leading the inhibition of chloride reabsorption, which is partly independent of changes in sodium excretion. The ascending limb of Henle's loop is a possible target site for the vitamin. Vitamin D-deficient animals tend to retain more chloride than D-

sufficient ones, for they lack the renal effect of D. In addition, the absence of D probably enhances PTH secretion. Hyperchloremic acidosis has been reported to occur in cases of vitamin D deficiency (52). Moreover, it has been shown that rats with systemic metabolic acidosis have a reduced ability to convert 25-OH-D_3 to 1,25-$(OH)_2$-D_3 when given ammonium chloride, and as a result, these animals cannot lower their blood chloride as compared to healthy ones (323). The evidences from the literature and the data from the present experiments demonstrate the direct involvement of vitamin D in chloride metabolism.

Dietary D-deprivation did not alter the chloride excretion of spring rats (TMX, SHAM). However, the injections of 2,400 IU/wk D_3 for three weeks unexpectedly lowered the excretion rates to values closer to those of summer rats. In fact, "spring deficient-rats" supplemented with D_3 had almost same values (37.8 Meq) as "summer deficient-rats" supplemented with D_3 (36.7 Meq). The reason for this is unclear.

The above data from spring experiments, seem to confirm the earlier postulate concerning the greater sensitivity of spring animals to "sudden" increases of endogenous vitamin D. The spring months appear to be a transition or adjustment period. The greater sensitivity to endogenous D in the spring may protect the animals against the effects of dietary D-deprivation (or increased endogenous synthesis may compensate

for lack of D_3 in diet).

Influences of thymus in D action

Thymic influences on chloride excretion seem to be antagonistic to those of calcitonin and vitamin D. The data point to promotion of Cl retention by thymus gland, an action similar to that of PTH. The mechanism is unknown: it is affected by parathyroidectomy but is similar in PX and TPX animals. An interesting suggestion is that thymic effect is anti-calcitonin. Such observations go against the proposed identification of the thymic factor with calcitonin, produced by C-cells that have migrated into the thymus.

V - Food and Water Intakes - Urine Volumes

Animals with intact parathyroid and thyroid glands had greater food intakes and urinary outputs during the summer months. The changes in urine volumes cannot be explained on the basis of water intake, since the values for the latter are similar for both winter and summer animals.

The administration of low and moderate doses of vitamin D increased urinary volumes significantly during the winter, but only slightly during the summer months. No correlation can be made between the observed increase and the changes in water intake (which were minimal) following the vitamin treatment. The findings suggest influences of vitamin D on H_2O reabsorption in the kidney via effects on calcium concentrations, or other antagonism of ADH (118a).

The administration of higher doses of vitamin D (12,000 IU/wk) however resulted in a 24% increase of water intake and 87% increase in urine volume during the summer months. This dosage of D_3 also produced hypercalciuria, hyperphosphaturia and hyperchloruresis. The data are consistent with some D_3 (or Ca^{++}) interference with ADH (118a).

Vitamin D deficient animals (TMX, SHAM) had reduced water intake, but the urine output was lower only in TMX rats. The administration of 2400 IU/wk D_3 further decreased the urine volumes of D-deficient TMX's without affecting the water intake.

The effects of low and moderate doses of vitamin D on food intake were not consistent, and no clear pattern can

be drawn relating the changes in food intake to those of electrolyte excretion. However, the changes resulting from the administration of 12,000 IU/wk D_3 clearly demonstrate the effects of hypervitaminosis. The hypercalciuria and hyperphosphaturia (among other effects) were accompanied by reduced food intake. This observation would be consistent with the possibility that the great electrolyte content of urine is coming from sources other than dietary, most probably from resorbing bone. However, it does not exclude the possibility of increased intestinal absorption without rise in food intake.

SUMMARY AND CONCLUSIONS

A - Role of the thymus gland in regulation of the metabolism of calcium, inorganic phosphate and related electrolytes.

The thymus gland seems to confer protection against the excessive loss of minerals into the urine, especially during times of the year when the excretion values are high. It enhances the retention of Ca and of P and may thereby facilitate bone mineralization or retard demineralization.

Although it is not a prime regulator of chloride metabolism, the thymus shares with PTH the ability to promote chloride retention. Its overall effects on sodium and potassium balance seem to be of a "normalizing" nature, since the magnitudes of seasonal variations in excretion are exaggerated by thymectomy.

B - Vitamin D, thymus gland and seasonal interactions in the regulation of some aspects of electrolyte excretion

In the present study, the effects of administration of low, moderate and large doses of vitamin D on Ca, P, Na and H_2O balance were similar to ones reported by other investigators. However, seasonal variations in the responses to low and moderate dosages were observed, and evidence was obtained for an important role of calciferols in the regulation of Cl excretion.

Animals of all surgical groups excreted more chloride, but less inorganic P, Na, K, Ca during April-August (when

endogenous D_3 is highest) than during November-March. The seasonal variations in chloride excretion may be directly related to the higher levels of the calciferols, since animals receiving exogenous D_3 developed chloruresis while those on D-deficient diets retained excessive amounts of the ions.

A role for the thymus gland in the regulation of responses to calciferols is suggested by the findings that (1) influences of thymectomy on electrolyte metabolism are greatest during the spring and summer months, and (2) thymus deprivation affected responses to D_3 administration.

C - Some interrelationships among thymus, parathyroid and thyroid glands

While certain of the effects of thymectomy were best demonstrated in animals with intact thyroid and parathyroid glands, e.g. those on SHAM, those on TPX and PX point to some influences of the thymus that are antagonistic to those of PTH.

Some effects of low and moderate doses of vitamin D were demonstrated in the parathyroidectomized as well as in the SHAM and TMX groups. The observations are consistent with the concept that calciferols exert both PTH-dependent and PTH-independent actions.

The phosphatemic action of D_3 was found to be dissociable from its phosphaturic one. Thyroidectomy affected the phosphaturic and natriuretic effects of D_3 administration in a manner different from the influences exerted by thymus gland

removal.

In conclusion, the study demonstrates substantial influences of calciferols on chloride metabolism and influences of the thymus gland on electrolyte metabolism that differ from those attributed to either PTH or calcitonin.

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