THE EFFECTS OF VITAMIN D METABOLITES ON THE RENAL HANDLING OF CALCIUM, MAGNESIUM AND PHOSPHATE IN THE HAMSTER

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ABSTRACT

The objective of this study was to investigate the role of 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2\ D_3$) in the tubular transport of divalent electrolytes, and its relationship to the renal action of parathyroid hormone (PTH). Hamsters were used in these studies as calcium (Ca) and magnesium (Mg) transport in this species is highly sensitive to PTH. Clearance experiments were performed in the following groups: intact animals (Group I), acutely thyroparathyroidectomized (TPTX) animals (Group II), acute TPTX and continuous infusion of PTH in "low" or "high" doses sufficient to (a) reduce or (b) abolish the hypocalcemic effect of TPTX (Groups III and IV respectively), acute TPTX plus calcium infusion or phosphate (Pi) infusion (Groups V and VI respectively) and TPTX plus dibutyryl cyclic adenosine monophosphate (DBcAMP) infusion (Group VII). Each group was subdivided into two subgroups: an experimental group and a control group. In all animals, a control phase was followed by an experimental phase of either $1,25(OH)_2\ D_3$ infusion (1 unit (U) prime, 0.5 U/hr) to experimental animals or the ethanol vehicle to controls. Glomerular filtration rate (GFR), urine flow rate, plasma calcium (PCa) and plasma magnesium (PMg) were not significantly altered in any of the groups. In Group I and II, the renal handling of electrolytes was not significantly

altered. Group III, treated with $1,25(OH)_2 D_3$, showed an increase in fractional excretion of calcium (FECa) (5.2 ± 1.4 to 13.2 ± 2.2%, $p<0.001$) and FEMg (7.3 ± 1.3 to 17.3 ± 2.2%, $p<0.001$). Administration of $1,25(OH)_2 D_3$ reduced phosphate excretion only in the presence of PTH. The FEPi decreased from 11.9 ± 2.1 to 3.6 ± 0.9%, $p<0.001$ in Group III and 29.2 ± 4.0 to 16.5 ± 1.4%, $p<0.02$ in Group IV. The changes in the control groups were not significant. Infusion of Ca and Pi to TPTX animals in sufficient doses to raise plasma calcium and urinary phosphate levels respectively, did not unmask an hypercalciuric or antiphosphaturic effects of $1,25(OH)_2 D_3$. Similarly, the replacement of PTH with DBcAMP (Group VII) failed to produce the response to $1,25(OH)_2 D_3$. These results suggest that $1,25(OH)_2 D_3$ antagonizes the action of PTH leading to enhancement of tubular reabsorption of Pi and inhibition of calcium and magnesium reabsorption. This effect appears to occur prior to the stimulation of cAMP production. Unlike $1,25(OH)_2 D_3$, the $25(OH) D_3$ metabolite appeared not to influence transport of electrolytes in the kidney, in the presence of a "low" dose of PTH.

Résumé

Dans ce travail, nous étudions le rôle des métabolites de la vitamine D, spécialement le $1,25(OH)_2D_3$, sur le transport tubulaire des ions divalents, ainsi que son interaction avec la parathormone (HPT) au niveau du rein. Nous avons utilisé des hamsters dont le comportement rénal du calcium (Ca^{++}) et du magnesium est très sensible à l'action de la HPT. Nous avons fait des expériences de clearance chez les groupes d'animaux suivants: Groupe (Gr) I, intacts; Gr II, thyroparathyroidectomie aigue (TPTX-A); TPTX-A suivie d'une infusion continue de HPT en "faible" ou "forte" dose pour a) soit réduire soit b) abolir l'effet hypocalcémiant de la TPTX (Gr III et IV respectivement); TPTX suivie d'une infusion de Ca^{++} ou de phosphate (Pi) (Gr V et VI respectivement); TPTX-A suivie d'une infusion de dibutyryl cyclic adenosine monophosphate (DB-cAMP) (Gr VII). Toutes les expériences comportent une période témoin suivie d'une période expérimentale, durant laquelle nous infusons soit $1,25(OH)_2D_3$ (dose initiale 1U suivie de 0.5 U/hre) soit le solvent éthylique (dans le groupe témoin). Le taux de filtration glomérulaire, le débit urinaire, les taux plasmatiques du Ca^{++} et du Mg^{++} ne diffèrent de façon significative dans aucune des groupes. Dans les Gr I et II le comportement rénale des électrolytes ne varie pas de façon significative. Dans le Gr III, traité avec le $1,25(OH)_2D_3$, on note une augmentation de l'excrétion fractionelle (EF) du Ca++

(5.2 ± 1.4 à 13.2 ± 2.2% $p<0.001$) et du Mg^{++} (7.3 ± 1.3 à 17.3 ± 2.2% $p<0.001$). Le $1,25(OH)_2D_3$ ne réduit EFPi qu'en présence de la HPT. Chez les Gr III et IV, on note une diminution de EFPi de 11.9 ± 2.1 à 3.6 ± 0.9% $p<0.001$ et de 29.2 ± 4.0 à 16.5 ± 1.4% $p<0.02$ respectivement. On ne note aucun changement significatif dans les groupes témoins. Lorsque nous infusons une solution contenant du Ca^{++} ou du Pi pour élever les taux plasmatiques du Ca^{++} ou du Pi respectivement, le $1,25(OH)_2D_3$ ne produit pas d'augmentation de l'excrétion du Ca^{++} ou de diminution de l'excrétion du Pi. De plus lorsque nous substituons la HPT par une infusion de DB-cAMP, nous ne notons aucun effet du $1,25(OH)_2D_3$. Nous concluons que le $1,25(OH)_2D_3$ neutralise l'effet de la HPT, augmentant ainsi la réabsorption tubulaire du Pi et diminuant la réabsorption du Ca^{++} et du Mg^{++}. Il semble que cet effet se produise avant que la HPT ne stimule la synthèse de cAMP. Contrairement au $1,25(OH)_2D_3$, le métabolite $25(OH)\ D_3$ ne semble pas affecter le transport rénal des électrolytes, avec HPT.

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1. Effects of vitamin D and PTH on distal tubular calcium reabsorption. R.A.L. Sutton, M. Burnatowska, N.L.M. Wong, J.H. Dirks. Presented at the Int. Symp. on Urolithiasis, Heidelberg, Germany, 1976.

2. Effects of $1,25(OH)_2 D_3$ on Ca, Mg, and Pi excretion in the hamster. M.A. Burnatowska, C.A. Harris, R.A.L. Sutton, J.F. Seely. Clin. Res. 25 (S) p. 705A, 1977.

3. The interaction of $1,25(OH)_2 D_3$ and PTH: Effects on renal handling of Ca, Mg, and Pi in the hamster. M.A. Burnatowska, C.A. Harris, R.A.L. Sutton, J.F. Seely. Presented at the VII Int. Congress of Nephrology, Montreal, 1978.

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ABBREVIATIONS

P	-	plasma concentration
U	-	urinary concentration
V	-	urine flow
FE	-	fractional excretion (UV/P × GFR)
FL	-	filtered load (P or UF × GFR)
P_{UF}	-	ultrafiltrate of plasma
TF	-	tubular fluid
Hct	-	haematocrit
Prot	-	plasma protein
GFR	-	glomerular filtration rate
TPTX	-	thyroparathyroidectomy
PTX	-	parathyroidectomy
PTH	-	parathyroid hormone
PTE	-	parathyroid extract
cAMP	-	cyclic adenosine 3'5' monophosphate
DBcAMP	-	dibutyryl cyclic adenosine 3'5' monophosphate

Cont'd...

ABBREVIATIONS CONT'D

CL Phe S-cAMP	-	8(p-Cl-phenyl-thio) cyclic adenosine 3'5' monophosphate
$25(OH)D_3$	-	25-hydroxycholecalciferol
$1,25(OH)_2D_3$	-	1,25-dihydroxycholecalciferol
mRNA	-	messenger ribonucleic acid
CaBP	-	calcium binding protein
in	-	inulin
UV	-	ultraviolet
n	-	number of animals
SEM	-	standard error of the mean
P	-	significance
NS	-	not significant

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FIGURES:

I. INTRODUCTION AND STATEMENT OF PURPOSE

The role of vitamin D as one of the major physiological regulators of mineral metabolism has been well established. Like parathyroid hormone (PTH) and calcitonin (CT), vitamin D is involved in the regulation of the plasma concentration of calcium and phosphorus. Vitamin D deficiency leads to the formation of rickets in the young and osteomalacia in the adult. The investigations of the biological effects of vitamin D have been largely directed at the evaluation of its action on the skeleton and gastrointestinal tract. As a result, the direct effect of vitamin D on the bone and the gut are well documented, while there have been relatively few studies of the effects of vitamin D on the kidney: Although several studies support the early observation of Harrison and Harrison (1941) suggesting that vitamin D enhances tubular reabsorption of phosphate (Puschett et al. 1971, 1972a, Costanzo et al. 1974, Harris & Seely 1979a), evidence suggesting that vitamin D has a phosphaturic effect has also been presented. (Ney et al. 1968, Bonjour et al. 1977).

Studies regarding the action of vitamin D on the renal transport of calcium are similarly controversial. In the D-depleted state the hormone appears to have an anticalciuric effect (Costanzo et al. 1974, Harris et al. 1976), while both anticalciuric and calciuric effects of vitamin D have been

observed in D repleted state (Puschett et al. 1972a, Litvak et al. 1958). Little evidence has appeared regarding the role of vitamin D in the handling of magnesium by the kidney (Hanna 1961).

The reasons for these rather variable results are not clear, but may be a consequence of changes in the tubular transport of calcium and phosphate independent of a direct renal action of vitamin D. Several recent studies have suggested that there may be an interaction between vitamin D and parathyroid hormone and probably several other polypeptide hormones at the renal tubular level (Popovtzer et al. 1974, Nseir et al. 1978, Popovtzer et al. 1978). In view of these findings therefore, the previously postulated direct anti-phosphaturic and hypocalciuric effects of vitamin D and its metabolites in D replete TPTX dogs (Puschett et al. 1971, 1972a) have to be questioned, as the animals used in these studies were treated with vasopressin to enhance the urinary excretion of phosphate.

The studies performed in animals and humans with intact and functioning parathyroid glands (Harrison and Harrison 1941, Brickman et al. 1974) also have to be reassessed as the enhanced reabsorption of phosphate seen after the administration of vitamin D, could be a result of either a direct action of vitamin D on the secretion of parathyroid hormone (Chertow et al. 1975), or secondary to a suppression of parathyroid hormone secretion resulting from an increase in serum calcium

(Sherwood et al. 1968).

Another possible explanation for the controversial results may be that the endogenous levels of vitamin D render it difficult to demonstrate any further effects of exogenously administered vitamin. The degree of D-depletion in an animal model may also influence the tubular response to vitamin D (Ney et al. 1958).

A number of other factors must also be considered. It is well established that vitamin D and its metabolites 25(OH) D_3 and 1,25(OH)$_2$ D_3 differ significantly in their physiological activities and half lives (Haussler and McCain 1977, Raisz et al. 1972). Thus, the metabolite, its dose and time lag between its administration and the commencement of the experimental observations could be of critical importance when investigating its renal effects. Since during long term studies the extrarenal effects of vitamin D may contribute to the changes in the urinary excretion of the electrolytes, a clear distinction between the results of chronic and acute studies has to be made. The antecedent diet, not always clearly defined, may also be partly responsible for the variability (Bonjour et al. 1977). The enhanced tubular reabsorption of phosphate in dietary phosphate restriction appears to be independent of vitamin D (Brautbar et al. 1979). Finally, a lack of appropriate controls which would account for possible hemodynamic changes and diurnal variations,

also complicate the interpretation of some data (Crawford et al. 1955, Ney et al. 1968).

Thus, whereas in the D-depleted, TPTX state, vitamin D appears to enhance the reabsorption of calcium and phosphate, in a D-replete state the assessment of the significance of the renal action of vitamin D based on the available evidence is rather difficult, and is complicated by the influence of factors not accounted for during these studies.

The present studies were designed, therefore, to further investigate the action of $1,25(OH)_2 D_3$ on the renal transport of calcium, magnesium and phosphate, and to attempt to clarify some of the controversies mentioned above. The effects of $1,25(OH)_2 D_3$ were examined in TPTX, D-replete and D-depleted hamsters. Since in the D-replete state, the anti-phosphaturia of vitamin D has been shown to be a result of the interference of vitamin D with the action of several hormones which are phosphaturic in nature, the possibility of a similar interference of $1,25(OH)_2 D_3$ with the action of PTH on the transport of calcium and magnesium has been examined in this study. To avoid fluctuation in the plasma levels of PTH as a result of either a direct or an indirect action of $1,25(OH)_2 D_3$ on its secretion, studies were conducted in TPTX animals in the presence of various doses of exogenous PTH.

As the PTH-dependent antiphosphaturic effect of vitamin D has been shown to be associated with changes in the urinary excretion of cAMP (Popovtzer et al. 1975), the involvement of the nucleotide in the PTH-dependent action of $1,25(OH)_2 D_3$ on the transport of calcium and magnesium was investigated by replacement of the PTH infusion by DBcAMP.

The hamster has been chosen as the experimental model for the proposed studies for several reasons. It has been demonstrated by Biddulph and co-workers (1973) and recently confirmed in our laboratory that the hamster kidney plays an important role in the marked and rapid changes that occur in plasma calcium after acute TPTX, and is unusually sensitive to exogenous PTH. Similar effects of PTH on the handling of magnesium have been recently reported by our study (Burnatowska et al. 1977). Furthermore, the hamster is the only animal in which a PTH-like effect of cAMP and DBcAMP on the transport of calcium and magnesium has been demonstrated (Burnatowska et al. 1977).

II. REVIEW OF LITERATURE

A. Vitamin D

1. Evidence for Antirachitic Factor – Discovery of Vitamin D.

Although the first well defined description of rickets appeared as early as 1645 (Whistler, quoted by Smerdon, 1950) it was not until the beginning of this century that it was demonstrated to be a deficiency disease. In 1919, Sir E. Mellanby was able to produce rachitic dogs by dietary manipulation. The disease could be prevented or cured by the administration of several fats, cod liver oil being the most effective. He attributed this antirachitic activity to the so called "growth-promoting fats", a source of newly discovered vitamin A, and incorrectly assumed vitamin A to possess antirachitic activity. He was quickly corrected, however, as in 1922, McCollum and co-workers demonstrated the antirachitic factor to be distinct from vitamin A. They observed that whereas the activity of vitamin A could be destroyed by heating and simultaneously oxidizing cod liver oil, the antirachitic activity of the oil was preserved, calcium still being deposited in the bones of young rats treated with this oil. This substance regulating the metabolism of bones was named vitamin D. Almost simultaneously Goldblatt and Soames (1923) reported that rickets could be prevented by ultraviolet (UV) irradiation of food, a finding that was not well understood at the time. Similarly, Steenbock

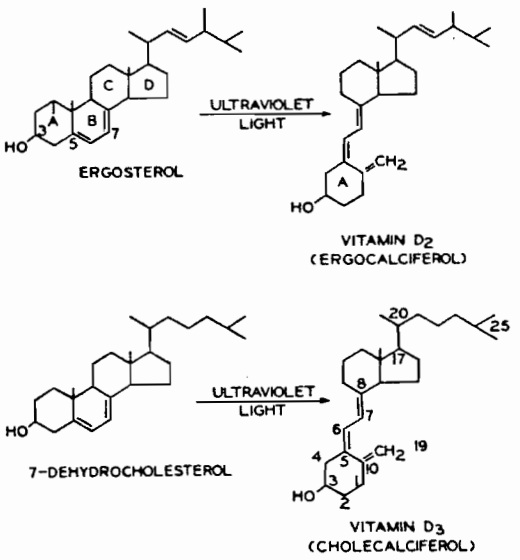


Fig. i. Conversion of ergosterol and 7-dehydrocholesterol to vitamins D_2 and D_3 respectively.
(From DeLuca 1976b).

and Black (1924) demonstrated that exposure of rachitic animals to UV light either from carbon-arc, mercury vapour lamps or the sun was capable of healing rickets, promoting growth and maintaining normal plasma calcium and phosphorus levels. Ultimately, they were able to show that UV light photochemically activates an antirachitic sterol material present both in the diet and the skin (Steenbock and Black 1925). This discovery resulted in the elimination of rickets as a major medical problem and provided information for the isolation and identification of vitamin D.

2. Chemical Characterization of Vitamin D

The above studies led to the isolation and identification of vitamin D_2 (ergocalciferol) by Askew et al. in 1931 and Windaus et al. in 1932. At that time it was generally accepted as the sole vitamin D, and ergosterol (a sterol first derived from the ergot fungus) as its sole precursor (Fig. i, p. 7a). Little work was therefore done regarding other possible sources of vitamin D. In 1934, however, Waddell reported results of several years study which challenged the above hypothesis. He demonstrated that vitamin D could be derived from cholesterol and that this material was not the same as that derived from ergosterol. Vitamin D_3 (cholecalciferol), as well as its precursor 7-dehydrocholesterol were finally isolated and identified by Windaus and co-workers (1936) (Fig. i, p. 7a). Once identified, ergocalciferol and cholecalciferol were considered to be the

active forms of vitamin D, and little was done regarding their fate in the body for over two decades.

3. Metabolism of Vitamin D (Fig. ii, p. 10a)

Although a lag period between the administration of vitamin D and its effects was observed in the early forties, it was not shown until the sixties that the expression of the biological activity of vitamin D requires metabolism to more polar forms. Significant progress in the investigation of the metabolism of vitamin D was made possible only as more satisfactory analytical techniques became available:

i) Chemical synthesis of radioactive vitamin D_3 with high specific activity permitted metabolic experiments with physiological doses.

ii) Development of lipid extraction techniques and new chromatographic systems allowed detection and assay of new metabolites.

iii) Advances in high resolution mass spectroscopy and nuclear magnetic resonance made possible the structural identification of the metabolites.

a) $25(OH)D_3$

In 1966, Lund and DeLuca demonstrated that during the lag time between vitamin D administration and the response in target organs, vitamin D_3 disappeared, and instead a polar metabolite appeared that possessed a very potent vitamin D-like activity. Furthermore, Morrii and co-workers

(1967) observed that this metabolite has much more rapid effects on the intestinal absorption of calcium and bone Ca mobilization than vitamin D_3. These findings led to the isolation and identification of the 25-hydroxymetabolite of vitamin D_3 (25(OH) D_3) by Blunt, DeLuca and Schnoes in 1968 and 25-hydroxymetabolite of vitamin D_2 by Suda and co-workers in 1969. Finally, synthesis of 25(OH) D_3 was accomplished in 1969 by Blunt and DeLuca. Further studies on the metabolism of vitamin D_3 revealed that 25-hydroxylation occurs in the liver (Ponchon et al. 1969). 25 hydroxylase (25-OHase) enzyme has been localized in the liver microsomes and endoplasmic reticulum (Bhattacharyya and DeLuca 1974). Although 25-OHase activity was shown to be under a feedback control of 25(OH)D_3 (Bhattacharyya and DeLuca 1973), the exact mechanism of this regulation is not clear.

b) $\underline{1,25(OH)_2D_3}$

Although 25(OH) D_3 is several fold more active than the parent vitamin D_3 in the prevention and cure of rickets as well as in its stimulation of intestinal absorption of Ca, it is not the most active metabolite of vitamin D. Existence of a more polar metabolite was reported by Haussler et al. in 1968 and Lawson et al. in 1969. Also, Cousins and co-workers in 1970 demonstrated that 25(OH) D_3 was further altered before it would express its biological activity. Eventually, the new metabolite was isolated in pure form from

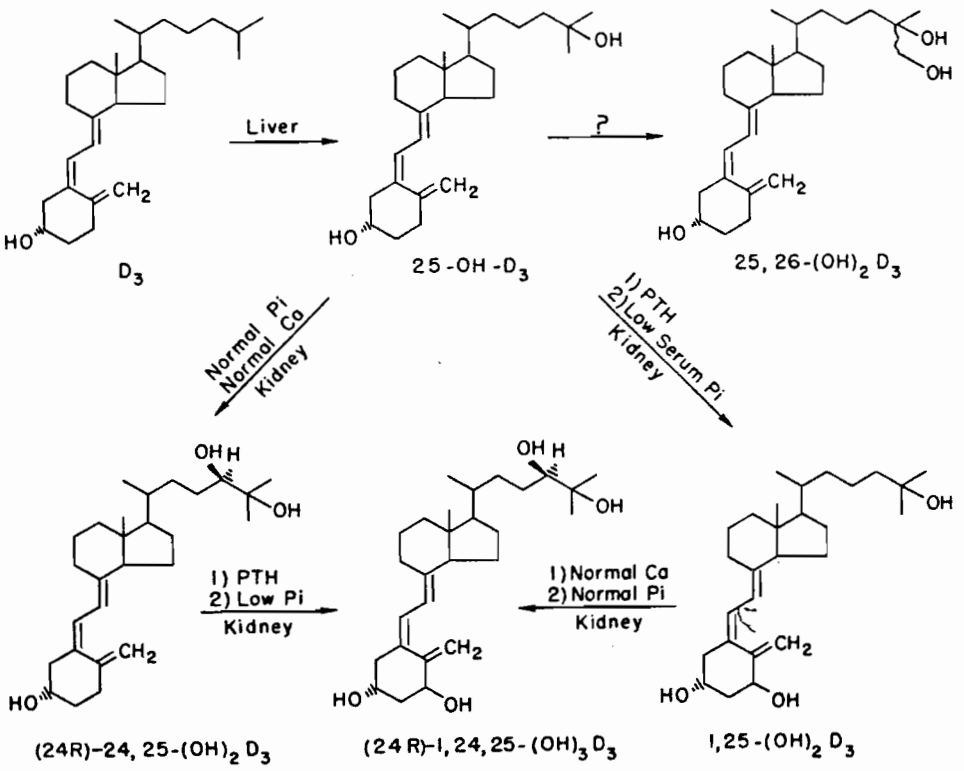


Fig. ii. Summary of vitamin D metabolism. (From DeLuca, 1976a).

chick intestine (Holick et al. 1971a) and identified as $1,25(OH)_2\ D_3$ independently by Holick et al. in 1971b and Lawson et al. also in 1971.

Even prior to the identification of $1,25(OH)_2\ D_3$, Fraser and Kodicek (1970) demonstrated that the kidney is the exclusive site of its synthesis. Nephrectomized (NX) animals failed to produce a metabolite more polar than $25(OH)\ D_3$. The recent study by Brunette et al. (1978b) suggests that the proximal convoluted tubule and the cortical thick loop appear to be the exclusive sites of the synthesis of $1,25(OH)_2\ D_3$ in the kidney. The hydroxylation of $25(OH)\ D_3$ is metabolized by a mitochondrial enzyme 25-hydroxy-vitamin D_3-1 hydroxylase (1-OHase) (Fraser and Kodicek 1970, Gray et al. 1972). This site in the synthesis of $1,25(OH)_2\ D_3$ was shown to be a critical, limiting step and under a feedback control by the end product of the reaction (Tanaka and DeLuca 1974).

c) $\underline{24,25(OH)_2\ D_3}$

In 1971, Boyle and co-workers demonstrated the existence of a metabolite of $25(OH)\ D_3$ other than $1,25(OH)_2\ D_3$. It was eventually isolated and identified as $24,25(OH)_2D_3$ by Holick et al. in 1972. Although the kidney mitochondria appeared to be the only site of synthesis of $24,25(OH)_2\ D_3$ (Boyle et al. 1973), extrarenal 25-hydroxyvitamin D_3-24 hydroxylase has also been noted (Pavlovitch et al. 1973).

Other polar metabolites of $25(OH)_2\ D_3$ have also been identified, but their physiological significance is not known (Suda et al. 1970).

4. Regulation of Metabolism of Vitamin D

As already mentioned, metabolism of 25(OH) D_3 in the kidney can lead to the production of either 1,25(OH)$_2$ D_3 or 24,25(OH)$_2$ D_3 and is modulated according to the calcium and phosphate status of the organism.

a) Role of Calcium and PTH

Boyle et al. in 1971 demonstrated that the accumulation of 1,25(OH)$_2$ D_3 or 24,25(OH)$_2$ D_3 in the blood and target tissues was related to the serum calcium concentration, which they manipulated by means of dietary calcium levels. In hypocalcemia, production of 1,25(OH)$_2$ D_3 was markedly stimulated, whereas in animals that were normocalcemic or hypercalcemic its production was shut down and the synthesis of 24,25(OH)$_2$ D_3 was stimulated.

Because of the relationship that exists between plasma calcium and PTH concentration, a possible role of PTH in the regulation of 1,25(OH)$_2$ D_3 and 24,25(OH)$_2$ D_3 production was investigated (Garabedian et al. 1972). These studies revealed that hypocalcemia is not a direct stimulus for the production of 1,25(OH)$_2$ D_3, but rather expresses its effect indirectly by stimulating the release of PTH. TPTX rats maintained on a low calcium diet lost the ability to produce 1,25(OH)$_2$ D_3 and synthesized 24,25(OH)$_2$ D_3 instead. The ability to produce 1,25(OH)$_2$ D_3 could be restored by the administration of PTH. This feedback regulation of 1,25(OH)$_2$ D_3 synthesis

by PTH appears to occur at the rate-limiting step, as the addition of the hormone to the chick kidney mitochondria preparation in vitro was shown to stimulate 1-hydroxylase activity (Fraser and Kodicek 1973).

b) Role of Phosphate

In animals with normal serum levels of phosphate, removal of the parathyroid glands leads to a fall in the synthesis of $1,25(OH)_2 D_3$ and elevation of $24,25(OH)_2 D_3$ (Garabedian et al. 1972). However, if serum Pi is decreased below 8 mg%, the synthesis of $1,25(OH)_2 D_3$ is stimulated regardless of the parathyroid status (Hughes et al. 1975). Moreover, Tanaka and DeLuca (1973) reported that in phosphate deficiency the production of $1,25(OH)_2 D_3$ is enhanced, while Galante and co-workers (1973) observed that PTX does not lead to a fall in 1-hydroxylase activity in chicks unless plasma phosphate is elevated.

c) Role of $1,25(OH)_2 D_3$

It is possible that $1,25(OH)_2 D_3$ regulates its own synthesis by a feedback mechanism either at the parathyroid gland or the kidney. Evidence suggesting regulation at both sites has been presented. $1,25(OH)_2 D_3$ has been shown to inhibit PTH release (Chertow, 1975, Henry et al. 1977) as well as to decrease the activity of renal 1-hydroxylase (Henry et al. 1974, Colston et al. 1977).

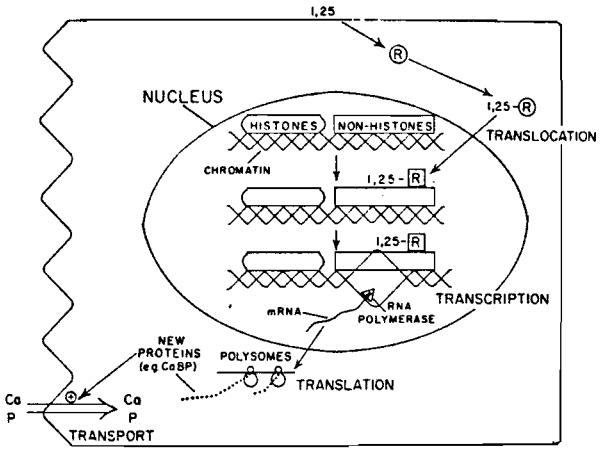


Fig. iii. Proposed molecular mechanism of action of $1,25(OH)_2D_3$ in the intestinal mucosal cell (R represents receptor protein). (From Haussler and McCain 1977).

In summary, the observations that $1,25(OH)_2\ D_3$ is synthesized only in the kidney, that its biosynthesis is regulated by dietary calcium and phosphate levels, by plasma calcium, phosphate, PTH and its own concentration, and that it is the active form of vitamin D in bone and intestine clearly demonstrates the hormonal nature of this metabolite.

5. Mechanism of Action of $1,25(OH)_2\ D_3$

The mechanism by which $1,25(OH)_2\ D_3$ produces its effects in the target tissues is a subject of considerable investigation. The evidence that has accumulated, strongly suggests that the mechanism of action of vitamin D, best documented in the intestine, is similar to that of other steroid hormones. The key steps involved are: a) binding of the hormone to the cytosol receptor protein; b) migration to the nuclear chromatin; c) association with a genome and activation of a selected gene; d) biosynthesis of mRNA; and e) translation of the new mRNA into functional protein(s) capable of altering cellular activity (Fig. iii, p. 13a).

a) Role of Functional Proteins

It has been speculated that if vitamin D were to act via the mechanism of specific protein synthesis it should be possible to block the effect by means of inhibitors. Actinomycin D, an inhibitor of mRNA synthesis has been shown to have an inhibitory effect on the vitamin D (Norman et al. 1965) and $1,25(OH)_2\ D_3$ (Corradino et al. 1973) stimulated increase in the intestinal absorption of calcium in the chick.

No such effect of actinomycin D, however, could be shown in the intestinal preparation from the rat (DeLuca, 1976a).

More insight into the mechanism of action of vitamin D is provided by direct studies. Localization studies revealed that $1,25(OH)_2 D_3$ is bound in the cytoplasm and the nucleus in particular (Tsai et al, 1972, Brumbaugh and Haussler 1974a). Both the cytoplasmic and the nuclear receptors for $1,25(OH)_2 D_3$ were identified (Brumbaugh and Haussler 1974a). The presence of cytoplasmic receptors appears to be a prerequisite for binding of the hormone to the chromatin to occur. They appear to serve as a transport system for the vitamin to the nucleus (Brumbaugh and Haussler 1974a). The binding of $1,25(OH)_2 D_3$ to the nucleus is a rapid, saturable and a highly specific process, that precedes the increase in calcium absorption (Brumbaugh and Haussler 1974b, 1975a, Procsal et al. 1975). Once in the nucleus, $1,25(OH)_2 D_3$ enhances RNA polymerase activity in the gut tissue from rachitic chicks (Zerwekh et al. 1974, 1976) as well as the biosynthesis of RNA both in intestinal (Tsai et al. 1972, Emtage et al. 1974) and kidney tissue (Chen and DeLuca 1973). Furthermore, Spencer et al. (1976) isolated polysomal RNA from D-deficient and $1,25(OH)_2 D_3$ treated chicks and demonstrated that mRNA for Ca binding protein was present only in the polysomes of birds treated with $1,25(OH)_2 D_3$.

In the sixties, presence of a protein with a high affinity for calcium was demonstrated in the intestinal tissue from vitamin D repleted chicks. However, this

protein was absent in rachitic birds (Wasserman et al. 1968). Eventually it was isolated in a pure form from several species and its involvement in the vitamin D-dependant intestinal calcium transport established (Taylor and Wasserman 1972, Wasserman et al. 1974). Furthermore, Corradino et al. (1976) demonstrated that the addition in vitro of purified calcium binding protein (CaBP) to the duodenum from D-deficient animals led to an enhancement in the uptake of calcium.

Other vitamin D induced proteins such as alkaline phosphatase have been identified (Haussler et al. 1970) but their exact role in calcium transport is not clear. It is possible that alkaline phosphatases are more involved in phosphate transport (Moog-Glazier 1972, Kempton et al. 1979) that may be vitamin D dependent. The molecular role of CaBP in the translocation process remains unknown.

b) Role of cAMP

Although available evidence strongly supports a steroid-like mechanism of action of vitamin D, a possible involvement of cAMP cannot be excluded. Neville and Holdworth (1969) reported increased activity in the adenyl cyclase of the intestine from vitamin D deficient chicks treated with vitamin D_3. In line with this observation Corradino et al. (1975 and 1977) observed that the first measurable change in the intestine in vitro studies is an increase in cAMP production within 30 minutes of the introduction of $1,25(OH)_2\ D_3$. The rise in cAMP preceeded the appearance of DNA, RNA, the Ca binding protein (CaBP), and the stimulation of calcium accumulation. The elevation of cAMP concentration of organ cultured

duodena from rachitic chicks treated with $1,25(OH)_2 D_3$ appeared to be a result of increased adenyl cyclase activity and led to an increase in the cyclic AMP dependent protein kinase activity. It is unlikely, however, that cAMP serves as a second messenger for vitamin D, as no new CaBP induction in absence of vitamin D could be seen despite an increase in cAMP levels, as a result of the inhibition of phosphodiesterase (Corradino 1977).

In summary, although cAMP may be involved in the regulation of $1,25(OH)_2 D_3$ activity in the intestine, the mechanism is not known, but the phosphorylation of critical endogenous substance involving a hormone stimulated cAMP dependent protein kinase may be a possibility (Greengard 1978).

6. Physiological Effects of $1,25(OH)_2 D_3$

A deficiency of vitamin D results in the diseases of rickets in the young and osteomalacia in the adult. Both diseases can be characterized as a failure of minerals to deposit in the matrix of bone, as a result of a defective supply of calcium and phosphate from the extracellular fluid to the calcification site. There are two well defined mechanisms that contribute to this function of vitamin D, namely its action on the intestinal calcium absorption and the mobilization of minerals from previously formed bone to the blood. The involvement of vitamin D in the handling of electrolytes by the kidney is a third mechanism, but at present is not well understood.

a) Intestine

The biological effects of $1,25(OH)_2 D_3$, the most potent, rapidly acting metabolite of vitamin D_3 are best defined in the gastrointestinal tract where it stimulates absorption of calcium and phosphate (Harrison and Harrison 1960, Garabedian et al. 1974, Rizzoli et al. 1977, Walling 1977). Vitamin D dependent absorption of calcium in the intestine is an active transport against an electrochemical gradient (DeLuca et al. 1976a). Although the transport of phosphate can passively accompany the translocation of calcium, vitamin D stimulates the active absorption of phosphate independently of that of calcium (Walling 1977a). The expression of vitamin D action in the intestine does not require the presence of PTH (Garabedian et al. 1974). PTH regulation of gut absorption of Ca appears to be indirect via the control of 1-hydroxylase activity in the kidney (Boyle et al. 1972, Ribovich and DeLuca 1976).

b) Bone

Vitamin D mobilizes bone calcium and phosphate into the circulation, so that extracellular fluid levels of these electrolytes are within limits that will allow accretion of new bone mineral (Tanaka and DeLuca 1971). Although it is possible that PTH is involved in this action of vitamin D, the available evidence remains equivocal. Whereas in the organ culture $1,25(OH)_2 D_3$ mediated resorption does not require PTH (Raisz et al. 1972), controversial results

have been obtained from in vivo studies (Garabedian et al. 1974).

c) Parathyroid Gland

Recent studies suggest that $1,25(OH)_2\ D_3$ may have a direct effect on PTH secretion. Chertow and co-workers (1975) demonstrated that injection of $1,25(OH)_2\ D_3$ to rats resulted in a decrease in plasma levels of immuno-reactive PTH after four hours, and prevented the increase in plasma PTH levels after the induction of hypocalcemia. These results are further supported by those of Brumbaugh et al. 1975b) who observed that $1,25(OH)_2\ D_3$ binds specifically to the molecules in cytoplasm and nucleus from parathyroid gland tissue.

d) Kidney

Regulation of the tubular transport of electrolytes by vitamin D will be discussed in Section D, p. 67.

7. Physiological Effects of $24,25(OH)_2\ D_3$

The physiological role of $24,25(OH)_2\ D_3$ is not clear. Although some evidence suggesting its involvement in bone resorption and suppression of PTH release has been presented (Henry et al. 1977), others demonstrated it to be relatively inactive (Boyle et al. 1973, DeLuca 1978). It appears that further hydroxylation to $1,24,25(OH)_3\ D_3$ is required before it can influence intestinal Ca transport or bone Ca mobilization. Thus, the 24-hydroxylation step appears to be the inactivation rather than the activation step in the metabolism of vitamin D.

B. General Characteristics of the Renal Transport of Divalent Electrolytes

Phosphate, calcium and magnesium are essential elements in many diverse physiological and biochemical functions.

Phosphate is required for bone formation and plays an important function as part of such molecules as ATP, DNA and RNA.

Calcium has many important functions, including bone formation, blood clotting, nerve communication, and membrane permeability.

Magnesium is important in biological reactions, including all ATP-ase reactions, genetic information transfer steps (eg. DNA transcription), as well as other diverse processes.

While the ultimate source of these electrolytes for humans and animals is food, the major means of excretion is through the urine. The kidney thus plays an important role in the homeostatic regulation of calcium, magnesium and phosphate concentration in body fluids.

1. Phosphate

a. Reabsorption

Most studies performed at endogenous levels of plasma phosphate show that phosphate clearance is substantially less than GFR (Pitts 1933, Pitts and Alexander 1944,

Thomson et al. 1957, Hellman et al. 1964, Agus et al. 1971, Biddulph et al. 1970) thus, suggesting that excretion is mainly determined by process of filtration and reabsorption. During intravenous infusions of phosphate, urinary levels of phosphate rise rapidly to the point where each increment in the filtered load of phosphate is associated with a similar increment in its urinary excretion, indicating attainment of an upper limit of net tubular phosphate reabsorption, i.e. a transport maximum (Tm) is reached (Pitts and Alexander 1944, Thomson et al. 1957). The Tm phosphate is characterized by a splay, a minimal threshold, i.e., plasma phosphate levels at which phosphate excretion first occurs, and a maximal threshold, i.e. plasma phosphate at which tubular reabsorption of phosphate is fully saturated. The tubule reabsorptive capacity for phosphate is such that a very small increase or a decrease in the plasma phosphate results in changes in the urinary phosphate levels. As changes in GFR have been shown to directly affect the Tm phosphate, the Tm/GFR ratio rather than Tm is often considered to be a more useful expression of the experimental conditions (Bijvoet et al. 1969).

Phosphate Transport Along The Nephron

The ultrafilterability of phosphate at the glomerulus, as established by artificial membrane methods, and recently confirmed by the micropuncture of Bowman's space in Munich-Wistar rats (Harris et al. 1974) is almost complete with the tubule fluid to ultrafiltrate concentration ratio

(TF/UF_{Pi}) close to unity. As the fluid leaves the glomerulus, avid reabsorption of phosphate occurs. In the first third of the proximal nephron TF/UF_{Pi} drops to about 0.6-0.8 in intact animals (Strickler et al. 1964, Amiel et al. 1970, Agus 1971, Puschett 1972b). Similar (Amiel et al. 1970, Wen 1974, Kuntziger et al. 1974, Pastoriza-Munoz et al. 1978) or lower (Maesaka et al. 1973, Knox and Lechene 1975, Harris et al. 1979b) TF/UF_{Pi} ratios have been reported in PTX and TPTX animals. The reason for this variability in the TF/UF_{Pi} ratio in the absence of PTH is not clear, but may be a consequence of the intrinsic tubular adaptation to differences in the antecedent diet (Maesaka et al. 1973, Wen 1974, Mühlbauer et al. 1977). While in the intact animals this ratio remains relatively constant in the remaining segments of the accessible proximal tubule, it tends to drop even further in the absence of parathyroid hormone, indicating that whereas in the presence of PTH proximal phosphate reabsorption is proportional to the reabsorption of tubular fluid, in the absence of the hormone phosphate is reabsorbed at much higher rates.

The proximal tubule reabsorptive capacity for phosphate is not homogeneous, but varies along its length. Employing a stationary microperfusion method, Baumann and co-workers (1975) observed that the reabsorption of phosphate by the early segment was approximately four times higher than that by the distal portion of the proximal convoluted tubule. A tracer microinjection study of Staum et al. (1972) also

showed a gradual decline in phosphate reabsorption along the proximal segments; midway along the proximal tubule more than 95% of the proximally injected tracer was recovered. The last 20-30% of the tubule showed little or no phosphate reabsorption. These observations of different capacities for phosphate reabsorption along the proximal tubule were further confirmed by Brunette et al. (1973), Dennis et al. (1976) and Greger et al. (1977).

The proximal reabsorption of phosphate is dependent at least partially upon the transport of sodium (Agus et al. 1971, Dennis et al. 1976), and is related to the transport of several chemically rather different solutes. Thus, bicarbonate, glucose, alanine and possibly other amino acids inhibit proximal reabsorption of phosphate (Ginsburg 1972, DeFronzo et al. 1976, Dennis and Brasy 1978). Although the mechanism of their interference with the transport of phosphate is not entirely clear, inhibition of the phosphate reabsorption that is associated with an increase in the tubular levels of bicarbonate appears to be a result of the rise in the tubular concentration of the less reabsorbable $HPO_4^=$ ion (Beck et al. 1974), whereas the inhibitory effects of glucose and probably alanine are the result of a competition for a common metabolic energy source (DeFronzo et al. 1976, Dennis and Brasy 1978).

The initial dog clearance (Pitts et al. 1958) and several micropuncture studies in the rat (Strickler

et al. 1964, Gekle et al. 1971a) and dog (Agus et al. 1971, Puschett et al. 1972b, Beck et al. 1973) suggested that phosphate reabsorption takes place exclusively in the proximal tubule. Differences between the fractional deliveries of phosphate to the accessible micropuncture site in the late proximal tubule and to the urine were observed in some of these studies, but were attributed to the reabsorption in the pars recta. Others, however, pointed out that the differences seen in deliveries at the late proximal tubule and the ureteral urine were far in excess of what could be ascribed to reabsorption in the pars recta (Frick et al. 1972, Wen 1974a). Further studies clearly demonstrated that phosphate is reabsorbed beyond the accessible proximal tubule. The original report by Amiel and co-workers (1970) which suggested that phosphate reabsorption occurs in acute PTX rats between the late proximal and the early distal tubule, as well as along the distal convoluted tubule and distally to the late distal puncture site, has been confirmed by several recent micropuncture studies in the rat (LeGrimellec et al. 1973a, Kuntziger et al. 1974, Knox and Lechene 1977, Pastoriza-Munoz et al. 1977), psamommys (De Rouffigmec et al. 1973) hamster (Harris et al. 1979b) and dog (Wong et al. personal communication).

The micropuncture findings suggesting distal phosphate reabsorption are at variance with micro injection (Staum et al. 1972, Brunette et al. 1973) and microperfusion (Dennis et al. 1976, Lang et al. 1977) studies. Employing

these techniques no phosphate transport could be demonstrated beyond the loop of Henle. The reabsorption of electrolytes in the loop is usually attributed to transport in the thick ascending limb. However, recent observations by Rocha (1977) and Shareghi and Agus (1979b) that phosphate is not reabsorbed in this segment casts doubt on this speculation. The reabsorption of phosphate between late proximal and early distal tubule appears to occur not in the loop but in the pars recta (Dennis et al. 1976, Lang et al. 1977).

The reasons for the disparities between the results of micropuncture studies and those of microinjection and microperfusion are not entirely clear. However, it may possibly result from the influence of a number of additional factors that affect the tubular transport of electrolytes. These will be discussed further in Section 4, p. 33. Furthermore, the technical aspects of microinjection and microperfusion studies such as the perfusion and injection rates, concentration of phosphate in the perfusate, contact time and tracer conditions may also alter the tubular handling of phosphate (Poujeol et al. 1977). Finally, Baumann and co-workers (1975) demonstrated different reabsorptive rates for phosphate as determined with radiolabeled and chemical phosphate. In their microperfusion experiemts ^{32}Pi concentration decreased about twice as rapidly as chemical concentration. Thus, it is important to use same method for measuring phosphate concentration in

tubule fluid, ultrafiltrate and urine samples within a given experiment.

In view of these disparities Poujeol and co-workers (1977), re-evaluated the variability of phosphate reabsorption in terminal segments of the rat nephron. They demonstrated that in addition to nephron heterogeneity (Greger et al. 1977, Knox et al. 1977) differences in animal strain were an important factor in determining the capacity of the distal nephron to reabsorb phosphate. Furthermore, Chabardes et al. (1977) demonstrated that the sensitivity of PTH-dependent adenyl cyclase along the nephron varied depending on the species examined. Finally, the antecedent diet of animals used in the various experimental designs can be responsible for the variability in the results, as the tubular transport of Pi adapts to the changes in dietary Pi (Steele 1976a, 1978). Recent micropuncture experiments (Muhlbauer et al. 1977) suggest that the adaption to changes in the intake of phosphate occurs predominantly in the early proximal tubule, but possible changes in the distal transport cannot be excluded, as nephron heterogeneity did not allow for more definite conclusions. These adaptive changes in the transport of Pi appear to be independent of any circulating hormonal substances (Steele 1977a).

In summary, factors capable of influencing phosphate handling have to be considered and accounted for when attempts to localize transport of phosphate along the nephron are made.

b. Secretion

Although secretion of phosphate has been clearly demonstrated in non-mammalian species (Levinsky and Davidson 1957, Wolbach et al. 1970), in mammals it has been difficult to demonstrate. The evidence that has been interpreted as indicative of phosphate secretion demands a careful appraisal. Although Carrasquer et al. (1960) reported secretion of phosphate during P_i loading in dogs, the increase in the clearance of P_i above that of inulin was only transient and otherwise close to unity. Similarly, in phosphate loaded dogs Handler et al. (1962) were unable to show clearance of phosphate to exceed that of inulin. Furthermore, administration of glucose, PTH and saline infusions, manipulations known to increase excretion of phosphate also failed to show secretion of phosphate. In recent studies by Boudry et al. (1975), the clearance of phosphate slightly exceeded that of inulin in conscious but not anaesthetized rats at high plasma phosphate concentrations. The net phosphate addition was suggested to occur in the terminal part of the nephron, as the fractional delivery of phosphate to the superficial distal puncture site was less that in the ureteral urine. However, similar observations of greater delivery of phosphate in the urine compared to that at the superficial distal tubule reported by Knox et al. (1977) were clearly demonstrated to be a result of increased delivery from deep nephrons and not due to addition in the

terminal nephron. Finally, during proximal tubule microperfusion studies, a fall in the specific radioactivity of the perfused radioactive phosphate solution was taken as evidence of net phosphate addition in this segment (Boudry et al. 1975). However, the possibility of phosphate back influx as a result of leakage was not investigated in this study. In similar studies by Greger et al. (1977) in which a marker for contamination was included, proximal tubular transport of phosphate was shown to be a unidirectional reabsorptive process without a significant secretory component. Several micropuncture (Strickler et al. 1964, Amiel et al. 1970), microperfusion (Dennis et al. 1976) and microinjection (Staum et al. 1972, Brunette et al. 1973) studies designed to elucidate the site(s) and magnitude of phosphate transport along the nephron also failed to demonstrate the existence of a secretory component. Thus, although a small backflux of phosphate in the proximal tubule has been demonstrated, it is probably negligible with regard to the regulation of net phosphate reabsorption (Schneider and McLane 1977). It is also possible, as suggested by a recent stationary split droplet study in intact rats, that the influx of phosphate is a result of emptying of the cellular pool rather than transcellular back influx (Shirley et al. 1976). Intracellular phosphate concentration is greater than that in the tubule fluid and the interior of the tubular cell is electronegative with respect to the tubular fluid, so that an electrochemical

gradient exists, encouraging P_i diffusion from the cells .

Further studies are required, however, before secretion of phosphate can be considered as an important factor in its renal handling.

2. Calcium

In plasma, calcium exists in three forms: bound to protein, as a free ion and complexed to other anions. Only the free and the complexed ions which under normal non-diuretic conditions comprise about 63% of the total calcium (Harris et al. 1974) are freely filterable at the glomerulus.

Micropuncture studies have shown that of the filtered load of calcium about 35% remains at the late proximal puncture site, approximately 20% is reabsorbed in the loop of Henle, 10% along the distal convoluted tubule and a small residual portion in the collecting system (Lassiter et al. 1963, Edwards et al. 1974, Agus et al. 1973). Thus, the total reabsorption of calcium along the nephron normally exceeds 98% of the load filtered by the glomerulus.

Transport of Calcium in the Nephron

In the proximal tubule, the potential difference between the lumen and the cell interior is about 60 mV, interior negative, and the concentration of free ionic calcium in the cell cytosol is several hundred fold lower than extracellular fluid. The entry of calcium into the cell is

therefore down a steep electrochemical gradient and probably passive, whereas its extrusion at the basolateral membrane occurs against an electrochemical gradient, and must be an active process. The energy source may be Ca activated ATPase, or Na-K-activated ATPase which by causing active Na extrusion allows countertransport of Ca against passive Na entry (Ullrich 1976).

A TF/UF calcium ratio of just above unity is established in the first portion of the tubule and proximal reabsorption accounts for 60 to 65% of the filtered load of calcium (Lassiter et al. 1963, Duarte and Watson 1967, Agus et al. 1973, Le Grimellec et al. 1973a, De Rouffignac et al. 1973, Harris et al. 1974). Under most experimental conditions, calcium reabsorption in the proximal tubule parallels that of sodium (Lassiter et al. 1963, Edwards et al. 1974, Costanzo and Windhager 1978).

Whereas no calcium transport could be demonstrated in either thin descending or thin ascending limb of the loop of Henle, avid reabsorption of this cation has been shown to occur in the thick ascending segment (Rocha et al. 1977). The mechanism responsible for this transport appears to be quite different from that in the proximal tubule. It has been demonstrated that sodium transport in this segment is secondary to the active reabsorption of chloride which creates a positive intraluminal potential difference (Rocha and Kokko 1973). As sodium and calcium transport was observed to be parallel in the loop (Sutton 1976), it is likely that calcium reabsorption in the ascending limb is also passive, as a result of a

positive intraluminal potential difference. An active calcium transport in the ascending limb of the loop has been recently suggested (Rocha et al. 1977) but not confirmed (Shareghi and Stoner 1978). The observation that furosemide, which is known to block chloride transport in the ascending limb, abolishes the normal concentration gradient in the distal tubule for sodium and calcium, is consistent with the notion that the reabsorption of both ions is secondary to active chloride transport (Edwards et al. 1973, Shareghi and Stoner 1978).

The removal of calcium along the distal tubule appears to be an active process against high concentration gradients. Distal TF/UF calcium ratios as low as 0.3 have been reported by several investigators (Le Grimellec et al. 1973b, Edwards et al. 1974, De Rouffignac et al. 1973, Agus et al. 1977, Harris et al. 1979b, Costanzo and Windhager 1978). In this segment reabsorption of calcium appears to be independent of that of sodium, at least under some experimental conditions (Costanzo and Weiner 1976, Costanzo and Windhager 1978, Sutton et al. 1976). Whereas the bulk of the filtered calcium is reabsorbed in the proximal tubule, distal reabsorption accounts for less than 10% of the filtered load but appears to be the major site responsible for the regulation of urinary calcium excretion.

3. Magnesium

At the glomerulus 70-80% of the total plasma magnesium is freely filterable as established by in vitro techniques and by direct micropuncture of surface glomeruli in the Munich-Wistar rats (Brunette et al. 1971, 1975) Le Grimellec et al. 1975).

Although magnesium appears to be reabsorbed along the nephron in a manner roughly similar to that of calcium, quantitative distribution of its reabsorption has been shown to vary in different species examined (Brunette et al. 1969, Wen et al. 1970, Murayama et al. 1972, Le Grimellec et al. 1973b, De Rouffignac et al. 1973, Harris et al. 1979b). Thus, the early micropuncture studies in the dog revealed late proximal tubule TF/UF Mg between 1.0 and 1.05, indicating reabsorption in proportion to that of sodium and water (Brunette et al. 1969, Wen et al. 1970). Although further reabsorption occurred beyond the proximal segment as distal TF/UF Mg fell to 0.5 (Wen et al. 1970), proximal reabsorption accounted for over 60% of the filtered load. Very little importance could be attached to the reabsorption of magnesium in the collecting duct. Recently, however, Quamme et al. (1978) reported a higher proximal tubule TF/UF magnesium ratio in this species. A TF/UF magnesium ratio of 1.23 was reported, and proximal tubule reabsorption accounted for only 29% of the filtered load of magnesium. The reasons for these differences remain to be settled.

In rodents, tubular fluid to ultrafiltrate magnesium ratio is much higher and rises along the proximal tubule reaching a mean of about 1.65 in the rat (Brunette et al. 1974, Le Grimellec et al. 1973a) and 1.52 in psamommys (De Rouffignac et al. 1973), suggesting that the proximal epithelium is poorly permeable to magnesium. Proximal tubule microinjection of ^{28}Mg study of Brunette et al. (1971) also supports these observations. In these species net proximal reabsorption of magnesium accounts for only about 20-25% of the filtered load.

The micropuncture studies of late proximal and early distal tubule (Le Grimellec et al. 1973a,b) and of early distal tubule and the tip of the loop of Henle (De Rouffignac et al. 1973, Brunette et al. 1974) demonstrated extensive reabsorption of magnesium along the ascending limb of the loop of Henle, accounting for 60-70% of the filtered load. The mechanism of magnesium reabsorption in the loop is not clear. Recent perfusion studies in the isolated rabbit cortical thick ascending limb suggest, however, that the reabsorption of magnesium in this segment like that of calcium is highly voltage-dependent and can be abolished with furosemide (Shareghi and Agus 1979b). Under normal conditions, relatively little magnesium reabsorption occurs beyond the loop of Henle. The distal convoluted tubule reabsorption accounts for less than 10% of the filtered load (Brunette et al. 1971, Murayama et al. 1972, Quamme et al. 1978), and no magnesium reabsorption

could be shown in the collecting duct, when investigated in young rats (Brunette et al. 1978a). Tubular transport of magnesium has been shown to exhibit a limit in net reabsorption or Tm, similar to that demonstrated for phosphate (Massry et al. 1969).

Although the existence of a secretory component in the transport of magnesium has been suggested by a few studies (Wen et al. 1970, Brunette et al. 1975) others were unable to show magnesium secretion in similarly magnesium-loaded animals (Massry et al. 1969, Samiy 1960, Brunette et al. 1971). It is also possible, as suggested by micropuncture studies in psammomys (De Rouffignac et al. 1973) and recently in the rat (Brunette et al. 1978a) that addition of magnesium does occur along the loop (De Rouffignac et al. 1973) or possibly in the pars recta or the early non accessible descending limb (Brunette et al. 1978a), but that this reflects a backflux of magnesium rather than an active secretion. It is also possible that concentration of magnesium in the loop is due to reabsorption of water without magnesium, rather than net addition of magnesium (Brunette et al. 1978a).

In summary, the available evidence demonstrates that renal handling of magnesium is principally a filtration-reabsorption process.

4. Factors Influencing Renal Transport of Electrolytes

The renal transport of divalent electrolytes is under the influence of several hormonal and non-hormonal factors,

a) Hormonal Influence

Hormones that have been shown to have a direct tubular effect on the transport of calcium, phosphate and possibly magnesium include; parathyroid hormone (PTH), calcitonin (CT), vitamin D, vasopressin (VP), growth hormone (GH), glucagon and insulin. Whereas a more detailed review of the involvement of PTH and vitamin D in the regulation of the renal handling of these electrolytes will be presented in Sections C and D respectively, the effects of the other hormones will be discussed here in general terms only.

Calcitonin

Although the renal action of calcitonin has been studied extensively, its physiological importance is still rather poorly defined. The phosphaturic effect of CT in the intact animals (Kenny and Heiskell 1965) is difficult to interpret as it may be a secondary result of its action to lower serum calcium which in turn may stimulate the secretion of parathyroid hormone. Studies in TPTX animals are not conclusive either, as phosphaturia has been observed by some investigators (Robinson et al. 1962, Williams et al. 1972, Popovtzer et al 1977) but not others (Biddulph et al. 1969). Its action on renal calcium handling is even more controversial. At present, it appears that calcitonin depresses tubular reabsorption of calcium, and previously divergent results can be reconciled if the dual effect of the hormone on bone and kidney is taken into consideration (Barlet 1972). Furthermore, changes in PTH status, as a result of a calcitonin-induced drop in plasma calcium may

also obscure a direct calciuric effect. However, identification of calcitonin specific receptors, and calcitonin sensitive adenyl-cyclase in the kidney tissue (Heershe et al. 1974, Marx et al. 1973, Chabardes et al. 1976), strongly suggests a direct tubular effect.

Growth Hormone

Chronic administration of GH appears to exert an effect on the renal handling of calcium and magnesium which is opposite to that of PTH. Thus, its long term administration results in increased urinary excretion of calcium and magnesium (Ikkos et al. 1959). Unlike PTH, however, it has been shown to have a phosphate-retaining effect (Corvilain and Abramov 1962). However, the acute administration of growth hormone failed to affect clearance of calcium and phosphate in either intact or TPTX dogs (Westby et al. 1977).

Glucagon

When administered in pharmacological doses glucagon increased urinary excretion of calcium, magnesium and phosphate (Pullman et al. 1967). At physiological concentrations, however, it does not seem to affect tubular reabsorption of these electrolytes (Saudek et al. 1973).

Insulin

The role of insulin in the regulation of the tubular transport of divalent electrolytes has not been studied extensively. Recently, DeFronzo and co-workers (1975, 1976) have presented evidence that effects previously

thought to be due to glucose administration are rather attributable to the increase in endogenous insulin secretion which occurs in response to glucose loading. These conclusions were based on clearance experiments in both humans (De Fronzo et al. 1975) and dogs (De Fronzo et al. 1976) in which steady state hyperinsulinemia was produced while maintaining blood glucose concentration at euglycemic levels by simultaneous glucose infusion. These experiments led to a decrease in excretion of sodium, potassium, phosphate and free water and an increase in the urinary calcium excretion. Furthermore, micropuncture results in the dog suggested that whereas phosphate transport was enhanced in the proximal tubule, that of sodium and water was reduced significantly. Thus, the decreased phosphate excretion may be attributed to an increase in proximal reabsorption, and the reduction in net sodium excretion must reflect an increase in the reabsorption beyond the proximal tubule. Although the increase in calcium excretion can be attributed to the reduction in proximal reabsorption, additional depression in distal nephron cannot be excluded.

Vasopressin

At concentrations normally found in plasma vasopressin acts chiefly on the renal concentrating mechanism via changes in distal nephron permeability to water (Handler and Orloff 1973). In pharmacological doses, however, it has been shown to exert a diuretic effect associated with natriuresis, chloruresis, phosphaturia (Kurtzman et al. 1975), calciuria

(Thorn 1960) and possibly magnesiuria (reported in Forsling 1976). Neither the site nor mechanism of vasopressin induced changes in the tubular transport of these electrolytes are clear. Attempts by Kurtzman et al. (1975) to elucidate the mechanism of action showed that it was not a result of the hypertensive effect of vasopressin, as the changes in solute excretion were still seen when the pressor effects were blocked with sodium nitro-prusside. Changes in the glomerular filtration rate and the renal blood flow could not, therefore, be involved. Renal hemodynamic changes were also ruled out following measurements of cortical renal blood flow with microspheres. Thus, the most likely cause for the changes in the urinary excretion of these electrolytes was an effect of vasopressin on their tubular transport. Kurtzman et al. (1975) were unable to demonstrate any effect of vasopressin on the transport of sodium, phosphate, glucose or bicarbonate in the proximal tubule. They concluded, therefore, that the diuresis and an increase in the urinary excretion of sodium and phosphate, but not glucose or bicarbonate, were a result of the action of vasopressin in the nephron at a site distal to the proximal tubule. The physiological significance of the effects of the pharmacologic doses of vasopressin on the transport of electrolytes remains equivocal.

Adrenal Cortical Hormones

Acute administration of mineralocorticoids enhances distal sodium reabsorption and appears not to have a significant influence on calcium, magnesium and phosphate excretion in either man or dog (Massry et al. 1967b, Lemann et al 1970b). During long term administration of mineralocorticoids, however, sodium excretion returns to the baseline and that of calcium progressively increases (Massry et al. 1968). The changes in the urinary excretion of calcium are unlikely to be a result of a direct action of mineralocorticoids on the tubular calcium transport, as they can be prevented by restriction of sodium intake. The following sequence of events appears to be responsible for these effects: The initial sodium retention that occurs during the prolonged mineralocorticoids administration leads to extracellular fluid volume expansion (ECFVE) which leads to escape from hormonal effect on sodium retention. Thus, ECFVE causes a decrease in proximal tubule reabsorption of electrolytes and water and enhances delivery of these ions to the distal segment, where mineralocorticoids promote sodium reabsorption without a direct effect on calcium or magnesium (Suki et al. 1968).

b) Nonhormonal Factors

The nonhormonal factors that regulate renal handling of calcium, magnesium and phosphate include; extracellular fluid volume expansion, changes in

renal hemodynamics, changes in GFR, diuretics, hypercalcemia, and hypermagnesemia, phosphate loading and depletion, acid-base status, glucose, fasting, diet and diurnal variations.

Extracellular Fluid Volume Expansion(ECFVE)

Volume expansion with saline results in a significant increase in urinary calcium and magnesium excretion in parallel with the increase in the excretion of sodium, despite no change or a drop in the glomerular filtration rate (Massry et al. 1967a). Although micropuncture studies (Duarte and Watson 1967) demonstrated that volume expansion produced by saline infusion decreases the proximal tubule reabsorption of calcium and sodium, the changes in urinary calcium, magnesium and sodium excretion appear to be a result of a decrease in distal reabsorption, as the expansion of the extracellular fluid with a hypertonic albumin induced a natriuresis but not hypercalciuria despite a decrease in their proximal reabsorption (Davis and Murdough 1970).

Saline infusion induced phosphaturia can be clearly demonstrated in the intact animal , but is much reduced in the PTX state (Frick 1969, Beck and Goldberg 1974). Studies on the mechanism of the phosphaturic effect of ECFVE with saline and its blunting by PTX demonstrated that saline inhibits proximal phosphate reabsorption in the intact and TPTX state in a similar manner. In the presence of PTH, however, distally delivered phosphate is excreted whereas in the absence of the hormone phosphate delivered distally is

avidly reabsorbed (Frick 1972, Beck et al. 1974, Lechene et al. 1977).

Renal Hemodynamics

The role of renal vasodilators and increased perfusion pressure on the renal handling of calcium, magnesium, sodium and phosphate has been investigated. Gonda et al. (1969), Ahumada and Massry (1971) and Thompson et al. (1971) found that infusion of acetylcholine or bradykinin into the renal artery with or without systemic administration of angiotensin augments urinary excretion of these electrolytes. These changes in the urinary levels of calcium, magnesium and phosphate probably reflect inhibition of proximal tubule fluid reabsorption, as high correlation between calcium and sodium clearance was observed under these hemodynamic changes.

Glomerular Filtration Rate

Changes in the filtered load of calcium, magnesium or phosphate as a result of changes in GFR alone do not cause large changes in their excretion. The adjustments in their transport along the nephron appear to be a result of the existance of a glomerulo-tubular balance for these electrolytes (Massry and Kleeman 1972b).

Diuretics

The primary effect of diuretics appears to be on the reabsorption of sodium, chloride and bicarbonate. An increase in the urinary excretion of phosphate is usually

associated with these changes. The magnitude of the phosphaturia varies with different diuretics and seems to depend on their site of action along the nephron. Parathyroid hormone status also appears to be an important factor in determining the magnitude of the diuretic-induced phosphaturia, as in its absence the proximally rejected phosphate can be reabsorbed distally (Eknoyan 1970, Beck et al. 1973, Goldberg et al. 1973).

The direction and the magnitude of the action of diuretics on the tubular reabsorption of calcium and magnesium also depends on the site of action of a given diuretic. The osmotic diuretics, mercurials, ethacrynic acid and furosemide have a hypercalciuric and hypermagnesiuric effect (Seely and Dirks 1969, Edwards et al. 1973). However, although thiazides inhibit proximal tubule fluid, sodium and calcium reabsorption, in the distal tubule they enhance calcium reabsorption and further inhibit that of sodium. (Edwards et al. 1973, Costanzo and Weiner 1974, Costanzo and Windhager 1978).

Hypercalcemia and Hypermagnesemia

An increase in the filtered load of either calcium or magnesium produced by the elevation of their concentration in the plasma results in marked increments in their urinary excretion, (Le Grimellec et al. 1973b, Brunette et al. 1974, Edwards et al. 1974) in contrast to small changes observed after augmenting the filtered load by an increase in

GFR (Massry and Kleeman 1972b). Although hypercalcemia may alter transport of magnesium and phosphate indirectly via changes in the parathyroid hormone status, its direct inhibitory effect on the transport of magnesium (Coburn et al. 1970b) and the enhancement of phosphate reabsorption (Lavender et al. 1963), has been demonstrated. Similarly, the infusion of magnesium leads to an increase in calcium and a decrease in phosphate excretion, both in the presence (Le Grimellec et al. 1973b) and absence (Massry et al. 1970a, Clark et al. 1968) of parathyroid hormone.

Phosphate Loading and Depletion

Changes in plasma phosphate by varying the filtered load, affect its urinary excretion rate in a manner that depends on the closeness of plasma phosphate to the Tm/GFR. The hypocalciuria and hypomagnesiuria usually associated with phosphate infusion, although partly attributed to a decrease in serum ionized calcium and an increase in PTH levels (Le Grimellec et al. 1974) is also a result of a direct tubular effect of phosphate on the reabsorption of calcium and can be seen in TPTX animals (Coburn et al. 1971).

During phosphate depletion, a marked increase in the urinary excretion of calcium and magnesium occurs (Coburn et al. 1970a, Kreusser et al. 1978). The magnitude of the hypercalciuria and hypermagnesiuria appear

to be related to the severity of the phosphate depletion and the degree of hypophosphatemia (Kreusser et al. 1978).

Acid-Base Status

Although in chronic studies on the effects of acid-base balance on the renal handling of electrolytes difficulties may arise in distinguishing between direct renal effects and secondarily induced changes in bone metabolism and parathyroid function, Lemann and co-workers (1967) showed that hypercalciuria produced by NH_4Cl feeding in man resulted from reduced tubular calcium reabsorption. It persisted despite a decrease in the filtered level, and was independent of parathyroid hormone status. An impairment of calcium reabsorption by chronic, but not acute metabolic acidosis was also demonstrated in the dog (Sutton et al. 1975). Micropuncture study results attributed these effects to the changes in the transport of calcium beyond the proximal tubule puncture site. Furthermore, metabolic alkalosis ($NaHCO_3$ administration) selectively enhanced calcium reabsorption at the site(s) beyond the proximal tubule, by mechanism independent of parathyroid status.

The effects of metabolic acidosis and alkalosis on the tubular transport of phosphate were also investigated. Although Pitts and Alexander (1944) reported that changes in acid-base balance did not alter Tm phosphate

in dogs, more recent studies demonstrated that the administration of bicarbonate is usually associated with an increase in the excretion of phosphate (Puschett et al. 1969, Zielinowski et al. 1979). Although the mechanism of bicarbonate-induced phosphaturia is not clear, it has been postulated that the alkalinization of tubule fluid shifts the equilibrium of the $HPO_4^{=}$ / $H_2PO_4^{-}$ ratio and increases the concentration of the less transportable $HPO_4^{=}$ ion. Microperfusion studies of Beck et al. (1974, 1975) further support this hypothesis. These investigators demonstrated that the efflux of ^{32}Pi was much higher from an acidic solution than from an alkaline one.

Glucose

It has been demonstrated that glucose loading leads to an increase in the urinary excretion of calcium, magnesium (Lemann et al. 1970a), and phosphate (Pitts and Alexander 1944, Corman et al. 1978). Whereas the mechanism of glucose -induced hypercalciuria and hypermagnesiuria is not clear, the phosphaturic effect of glucose appears to be a result of a competitive action between phosphate and glucose for a common energy requiring transport in the proximal tubule. This conclusion is further substantiated by the observation that phlorizin, a known inhibitor of glucose reabsorption has been shown to reduce phosphate excretion and reverse glucose-induced phosphaturia (Pitts and Alexander 1944, Corman 1978).

Fasting

Fasting results in significant losses of calcium, magnesium, sodium and phosphate in the urine. This decrease in tubular reabsorption appears to be a result of ketoacidosis that usuall accompanies starvation (Drenic 1972).

Diurnal Variations

Diurnal variations in the excretion of divalent electrolytes have also been reported. Urinary calcium excretion peaks at the middle of the day and appears to be related to the dietary intake, while that of magnesium peaks at dawn (Min et al. 1966). Phosphate excretion reaches minimal values in the morning and peaks in the evening (Mudge et al. 1973).

C. Parathyroid Hormone

1. Chemistry

The early recognition of the role of the parathyroid glands in calcium and phosphate homeostasis led to intensive investigation into methods of extraction, purification and chemistry of the hormone. Twenty-four years elapsed, however, between the isolation of the first biologically potent extract from parathyroid glands by Collip (1925) and the preparation of highly purified hormone by Aurbach (1959). It took another decade before composition of the parathyroid hormone became apparent. The amino acid composition of human, bovine and porcine PTH is very similar, comprising 84 residues with a total molecular weight of 9,500 (Aurbach et al. 1972, Brewer et al. 1972). The biological activity of the hormone resides in the initial 30 residues of the amino terminal (Keutmann et al. 1972) and synthetic peptides comprising the first 34 amino acids are biologically active (Potts 1971). PTH is synthesized as a prohormone (proPTH) of 109 amino acids and a molecular weight of about 12,000 (Habner et al. 1972), which has to undergo at least two specific cleavages before being fully activated. The first cleavage occurs in the parathyroid cells where proPTH is converted to the 84 residue peptide (Chu et al. 1973). This fraction, after secretion into the circulation, undergoes

further cleavage, probably in the liver, into a large biologically inactive fragment (M.W. 7,500), and a small biologically active N terminal fragment (Fischer 1972). The half life of PTH, like many polypeptide hormones in the circulation is relatively short. It has been measured for both the exogenous and endogenous hormone and shown to be about twenty minutes (Melick et al. 1965).

2. Synthesis and Degradation

The synthesis and secretion of PTH is regulated by the extracellular concentration of ionized calcium. (Sherwood et al. 1968, Hamilton et al. 1971, Tragovnik et al. 1971). Some evidence for the involvement of magnesium in the regulation of the levels of circulating PTH also exists (Buckle et al. 1968, Massry et al. 1970b). No such relation of PTH levels to the plasma phosphate has been shown (Sherwood et al. 1968), and phosphate regulation of the secretion of PTH has been shown to be indirect, via the changes in plasma calcium levels.

PTH is inactivated by enzymatic degradation in the kidney and the liver, since both nephrectomy and hepatectomy result in a prolonged half-life of the hormone (Fang et al. 1972).

3. Physiological Effects of Parathyroid Hormone

The physiological importance of parathyroid hormone in calcium and phosphate metabolism was established

in the first quarter of this century. The first landmark in understanding parathyroid function was the work of MacCallum and Voegtlin (1909) who observed that removal of the parathyroid glands from dogs caused a drop in plasma calcium followed by tetany, which could be temporarily relieved by infusion of calcium chloride. The second was the success of Collip (1925) who was the first to prepare parathyroid extracts, and to show the role of PTH in the regulation of plasma calcium and bone resorption. Greenwald and Gross (1925) recognized the important phosphaturic effect of PTH upon the kidney. From those observations developed two schools of thought on the mechanism of PTH action. The Collip and Thompson School, proposed that the primary effect of PTH was on bone, leading to its dissolution and subsequent rise in plasma calcium levels. The renal effects were thought to occur secondary to bone resorption (Thompson and Collip 1932). The Albright School believed that PTH acted directly on the kidney to regulate electrolyte levels in body fluids and that the bone changes were secondary. The observed rise in plasma calcium was interpreted as an indirect effect to keep the product of the plasma concentrations of calcium and phosphate ions constant. Unfortunately, neither group could present experimental evidence in direct confirmation of their particular theory. The initial interest in the physiology of parathyroid glands, calcium and phosphate

metabolism abated for several years, owing apparently to lack of better hormone preparation and the difficulty in finding a convenient, economical assay method. Only twenty years later, as more purified parathyroid extracts became available could these initial interpretations be modified and PTH was shown to have four separate,major actions:

i) Increase in urinary phosphate excretion (Greenwald 1925, Pullman 1960).

ii) Decrease in urinary calcium excretion, preceding any change in plasma calcium (Talmage et al. 1955, Kleeman et al. 1961).

iii) Acceleration of metabolic destruction of bone (Barnicot 1948, Chang 1951, Gaillard 1961).

iv) Increased calcium absorption from the intestine (Rasmussen 1959b, Cramer 1961).

a) Bone

Parathyroid hormone is a major physiological regulator of bone resorption. The transfer of calcium to extracellular fluid from the bone has been suggested to be the result of two rather diverse effects of PTH; a rapid mechanism that regulates the pumping system which compensates for a continuous leakage of calcium into bone, and a slower effect that depends upon the action of PTH on bone remodelling. (Talmage 1967).

b) Gut

Although it has generally been believed that PTH increases intestinal absorption of calcium (Robinson 1968), this has not been proven. Accumulating evidence suggests that the involvement of PTH in intestinal calcium absorption is only indirect, via the stimulation of the production of $1,25(OH)_2 D_3$ by the kidney (Boyle et al. 1972).

c) Kidney

1) Phosphate

The role of PTH in renal phosphate handling has been recognized since the discovery by Greenwald (1925) that parathyroidectomy causes a rapid fall in the rate of urinary phosphate excretion and conversely that injection of parathyroid extract increases urinary phosphate excretion. Although phosphaturia was among the earlier detectable responses to PTH, it was undecided for several years whether it was due to a direct effect of "hypercalcemic hormone", a separate "phosphaturic hormone", or a non-hormonal component of parathyroid extract. Some evidence for a separate "phosphaturic fragment" was presented by Stewart and Bowen (1952), who showed that the treatment of parathyroid extract with formaldehyde abolishes the hypercalcemic response, but not the phosphaturic one. No separate fragment was isolated, however, and the phosphaturic effect could be reproduced with a highly purified PTH (Aurbach 1959) as well as with

synthetic N-terminal fragments of PTH (Potts 1971).

In the earlier literature, considerable controversy also existed as to whether PTH had any direct effect on tubular phosphate transport. The crude parathyroid extracts used in early investigations were highly contaminated with proteins known to produce renal vasodilation leading to a rise in GFR, and in the filtered load of phosphate. This problem was resolved with the development of more purified preparations of PTH (Aurbach 1959). The phosphaturic effect of the hormone was then shown to be independent of changes in GFR indicating a direct effect on the renal tubular transport of phosphate. Two mechanisms of action of parathyroid hormone were therefore considered and investigated:

i) An increase in tubular phosphate secretion.

ii) A decrease in phosphate reabsorption.

The evidence that the phosphaturic effect of PTH is a result of enhanced tubular secretion is controversial and inconclusive, as the existence of such a component of phosphate handling mechanism in mammals is still in serious question (see p. 26). Present evidence favours inhibition of tubular reabsorption of phosphate by PTH, rather than enhanced secretion. The early clearance studies (Pullman et al. 1960, Bartter et al. 1961) clearly demonstrated

PTH to have a phosphaturic effect independent of the hemodynamic changes.

Action of PTH Along the Nephron

Once the phosphaturic effect of PTH had been firmly established, attempts to localize this action along the nephron were undertaken. The early stop flow studies (Samiy et al. 1965) indicated that inhibition of the reabsorption of phosphate is a result of a proximal action of PTH. Several micropuncture experiments have been devoted to elucidating the sites of action of PTH. The proximal tubule micropuncture studies in dog (Agus et al. 1971) and rat (Gekle et al. 1971a) also implied that the PTH-induced phosphaturia was a result of the proximal action of the hormone. The mechanism of PTH-induced phosphaturia is not clear. Agus and co-workers (1971) suggested it to be a consequence of a direct inhibitory action of PTH on proximal sodium reabsorption rather than that of phosphate. In these studies the urinary sodium excretion was shown to be unchanged, indicating further distal reabsorption of sodium while excretion of phosphate was increased. Later studies in dog demonstrated, however, a dissociation of the effect of PTH on sodium and phosphate transport along the proximal tubule (Wen et al. 1974). However, a fraction of proximal handling of Pi appears to be closely linked to that of sodium (Agus 1973, Dennis et al. 1978).

It is believed, however, that although PTH inhibits proximal phosphate reabsorption, its major effect in regulating phosphate excretion is its action to reduce distal reabsorption. Distal tubule sensitivity to PTH was first suggested by Amiel et al. (1970). In these studies the segmental reabsorption of phosphate was compared in the intact and PTX rat. In the absence of PTH a significant enhancement of tubule Pi reabsorption was observed in segments beyond the late proximal tubule, namely in the loop of Henle, distal tubule and beyond the distal puncture site.

Distal nephron phosphate transport and its sensitivity to PTH may also be unmasked by such manoeuvers as extracellular fluid volume expansion and administration of diuretics. Micropuncture studies of volume expansion indicated that in the presence of PTH most of the phosphate delivered distally is excreted (Frick 1972, Puschett et al. 1972b, Beck and Goldberg 1974). In TPTX animals, whereas the proximal tubule response to saline infusion was similar to that in the intact state, avid phosphate reabsorption occurred distally and little increase in phosphate excretion occurred in the urine (Beck and Goldberg 1974). Many diuretics are also phosphaturic in nature to a varied degree. In general, the most effective are the ones that have carbonic anhydrase inhibitory activity (Goldberg et al. 1973). Thus, the administration of

acetazolamide to intact animals results in a significant phosphaturia, as the proximally rejected phosphate escapes distal reabsorption. In the TPTX animals, however, the large phosphate load leaving the proximal tubule is reabsorbed distally and little if any phosphaturia occurs (Beck and Goldberg 1973).

Although microinjection (Brunette et al. 1971) and microperfusion (Dennis et al. 1976) studies failed to show any action of PTH beyond the loop of Henle, these are in disagreement with the observations mentioned above, and several recent distal tubule micropuncture studies. Thus, Knox and Lechene (1975), Lechene et al. 1977, Harris et al.(1979b) and Pastoriza-Munoz et al. (1978) demonstrated that in addition to having an effect in the proximal tubule, PTH inhibits reabsorption of phosphate between the late proximal and the early distal puncture site, in the distal convoluted tubule and beyond the late distal puncture site, probably the collecting duct. These sites of action are also in line with the identified sites of the presence of PTH sensitive adenyl cyclase (Chabardes et al. 1975)(discussed in Section C, 4, iii., p. 61).

2) The Effects of PTH on Renal Calcium Handling

The direct effect of PTH on renal calcium handling was rather difficult to demonstrate since it is a sum of several forces acting in opposite directions.

Its effects on bone resorption and intestinal absorption lead to an increase in plasma calcium concentration and a consequent rise in the filtered load which may increase urinary calcium excretion therefore masking a direct effect on tubular calcium reabsorption. However, it has now been well established that parathyroid hormone enhances tubular reabsorption of calcium; several clearance studies in TPTX rat (Talmage et al. 1955) TPTX hamster (Biddulph et al. 1970), dog (Widrow and Levinsky 1962) and man (Kleeman et al. 1961) demonstrated that the administration of either PTH or PTE led to a decreased urinary excretion of calcium at either constant or elevated filtered loads. Furthermore, in the hypoparathyroid state in man a high clearance of calcium relative to plasma calcium concentrations was observed whereas the reverse has been shown in hyperparathyroid patients (Nordin et al. 1969).

Sites of Action of PTH

The localization of the anticalciuric effect of PTH within the renal tubule is not completely understood. Evidence concerning an effect of PTH on proximal calcium transport is equivocal. Whereas Frick and co-workers (1965) and Sutton (1976) failed to show any action of the hormone in the proximal tubule, an enhancement of proximal calcium reabsorption by PTH has been postulated by Kuntziger et al. (1974) and recently by Harris et al. (1979b). In

contrast to these studies others (Agus et al. 1973) have reported an increased delivery of calcium out of the proximal tubule. This inhibition of the proximal reabsorption of calcium was closely correlated to that of sodium and may reflect a nonspecific effect of PTH on proximal tubule fluid reabsorption.

Unlike the equivocal evidence for the proximal effect of PTH, there is clearcut evidence of a distal effect of this hormone on calcium handling. Enhancement of distal reabsorption of calcium first suggested by stop-flow studies (Widrow and Livinsky 1965) has been confirmed by several micropuncture (Agus et al. 1973, Sutton et al. 1976, Harris et al. 1979b) and microperfusion (Greger et al. 1978, Shareghi and Stoner 1978) studies. Although Sutton et al. (1976) demonstrated that PTH enhances calcium transport at a site prior to the superficial distal punctore site as well as in the terminal nephron, beyond the accessible distal convoluted tubule, only randon distal samples were taken in this study, and the precise localization of these effects was not possible. In a study of Harris et al. (1979b) in TPTX hamsters early and late segments of the distal tubule and the late proximal tubule were punctured. The results tend to confirm the existence of an effect of PTH on calcium between the late proximal and early distal tubule, and in the terminal nephron. Recent perfusion studies of the isolated rabbit cortical tubule (Bourdeau and Burg, 1979) further localize the site of PTH dependent Ca reabsorption in the loop of the thick ascending limb. A lack of an effect along distal convoluted tubule observed in the study of Harris was

attributed to the fact that at low calcium deliveries, the distal convoluted tubule absorbs calcium considerably even in the absence of PTH.

3) Effects of PTH on Renal Magnesium Handling

The effect of PTH on the renal transport of magnesium is even less well defined than its effects on that of calcium or phosphate. The early clearance experiments gave equivocal results. MacIntyre and co-workers (1963) were the first to clearly demonstrate an enhancement of the tubular reabsorption of Mg in PTX rats given PTH. These observations were later confirmed by Massry et al. (1969) and Burnatowska et al.(1977). The only micropuncture study that has examined the effect of PTH on the transport of magnesium along the nephron is that of Harris et al. (1978) in the acutely TPTX hamster. These results indicate that PTH enhances magnesium reabsorption in the proximal tubule, between accessible late proximal and early distal tubule, i.e. a site where the bulk of magnesium reabsorption appears to occur, and at some point distal to the late site of puncture. Further studies are required to evaluate the quantitative effect of the hormone within each segment.

4) Mechanism of Action of PTH

The concept has been developed that many hormones act by way of a double messenger system. Such

hormones are regarded as a first messenger which travel from their cells of origin to the cells of target tissue where they stimulate formation of a second messenger. Although it is possible that several second messengers exist, so far only cyclic 3', 5' - adenosine monophosphate (cAMP) has been identified as such a messenger (Rall and Sutherland 1958) and implicated in the action of many polypeptide hormones, endocrine and exocrine secretion and neuro-transmitter release (reviewed by Robinson, Butcher and Sutherland 1968). Calcium ion has also been suggested to be an integrator of metabolic events in cells (Rasmussen and Tenenhouse 1968). In this thesis, cAMP is only a regulator of the permeability of cellular membranes to Ca or of the binding of this ion to membranes, and changes in the intracellular Ca ion concentration are responsible for the physiological response to given stimuli.

However, at present, the available evidence suggests that the mechanism of action of PTH on kidney depends upon the following sequence of events; interaction of the hormone with specific receptors on the cell surface of the renal tubular epithelium - activation of adenyl-cyclase as the result of the interaction with receptors: generation of intracellular cAMP, activation of protein kinase within the brush border of renal cells: phosphorylation of an enzyme(s) or a membrane component; and, as a consequence, activation of a system mediating transport of ions. (fig. iv., p. 58a).

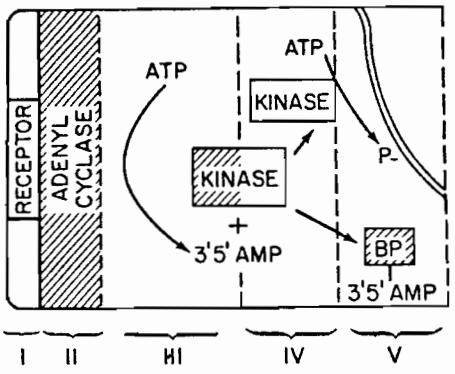


Fig. iv. Scheme proposed for sequence of metabolic events of the mechanism of action of parathyroid hormone on bone and kidney: I, binding of the hormone to receptor site; II, activation of adenylate cyclase; III, increased intracellular concentration of cyclic 3', 5'-AMP; IV, binding of 3', 5'-AMP to receptor protein causing its dissociation from kinase and consequent activation of kinase enzyme; V, increased phosphorylation of substrate protein. This entire sequence of events may take place within the plasma membrane of the cell. BP, binding protein.
(From Aurbach et al. 1976).

i) cAMP Generation in the Kidney

Changes in the urinary levels of cAMP can be regarded as a physiological consequence of the above system. Presence of cAMP in urine was first demonstrated in humans (Butcher & Sutherland 1962), but no physiological importance was then attached to it. Chase and Aurbach (1967), were the first to postulate its possible role in mediating the action of parathyroid hormone. They showed that urinary cAMP levels were lower in the TPTX animals as compared to the intact. Intravenous injection of PTH into TPTX rats induced an immediate and marked rise in urinary cAMP, which either preceded or coincided with the phosphaturic effect of the hormone. Similar changes in the cAMP levels in the urine after PTH administration have been reported in humans (Kaminsky et al. 1970).

These changes in the urinary cAMP levels appear to reflect cyclic nucleotide elaborated from the renal cells in response to the hormone rather than a rise in its clearance from plasma: In the study of Kaminsky et al. (1970) it was possible to segregate cAMP appearing in the urine according to nephrogenous or systemic origin. Only the nephrogenous part was under the control of PTH. Using radio-active cAMP, Chase and Aurbach (1967) and Butlen and Jard (1972) also showed that the rise in the renal output of the nucleotide after the administration of PTH reflected the changes in renal synthesis and excretion, and not its clearance. Furthermore,

an increase in the intracellular concentration of the nucleotide in the renal tissue has been detected after injection of the hormone in vivo (Rasmussen and Tenenhouse 1968), after addition of the hormone to isolated renal tubules (Aurbach 1972b) or to isolated intact cell preparations from the renal cortex (Melson and Aurbach, 1970).

The importance of cAMP in the mechanism of action of PTH is further strengthened by studies of patients with pseudohypoparathyroidism, a condition representing an end organ unresponsiveness to PTH, despite normal or elevated levels of circulating PTH. In these patients, a lack of phosphaturia after PTH administration is paralleled by a lack of rise in the cAMP levels in the urine (Chase et al. 1969).

ii) Hormone Receptors in the Kidney

Since many other hormones also alter the metabolism of cAMP, the question of specificity arises, and considerable importance has been attached to the anatomical localization of the above effects. Specific receptors for parathyroid hormone have been identified in the renal cortex (Sutcliffe et al. 1973, DiBella et al. 1974) using ^{125}I labeled PTH. Biologically inactive PTH, as well as other hormones such as vasopressin, glucagon and epinephrine failed to inhibit binding of the active hormone.

iii) Adenyl Cyclase

The binding of the hormones to membrane receptors is a process closely linked to, but separable from, activation of adenyl-cyclase in the receptor tissue for these hormones. Accumulating evidence suggests that although interaction of many amine and polypeptide hormones leads to the activation of this system and a subsequent rise in the intracellular cAMP production, the receptors for these hormones are highly specific and probably located on distinct cell types (Aurbach and Heath 1974). Thus, PTH stimulates adenyl-cyclase predominantly in the renal cortex (Chase et al. 1968), and recently it has been shown to be preferentially distributed on the contraluminal plasma membrane of the cortical epithelial cells (Shlatz et al. 1975). Furthermore, PTH-sensitive adenyl-cyclase has been identified in the proximal tubule, pars recta, thick ascending limb of Henle, distal convoluted tubule, and cortical collecting duct (first branching portion) (Chabardes et al. 1975). These are also the known sites of PTH-induced alterations in the transport of electrolytes.

Vasopressin, another hormone whose renal effects are mediated by cAMP, stimulates adenyl-cyclase of the renal medulla only (Chase et al. 1968), in keeping therefore with its main effect on the permeability to water in the collecting system (Handler and Orloff,1973).

Calcitonin, which induces an increase in the urinary excretion of Ca and Pi (Kenny and Heiskell 1965, Barlet 1972), is also believed to act via the adenyl-cyclase

cAMP system (Heersche 1974). CT-sensitive adenyl-cyclase has been identified (Marx et al. 1973) and localized to the cortical and medullary thick ascending limb of the loop of Henle and to the distal convoluted tubule (Chabardes et al. 1976). Its effect has also been shown to be specific and distinct from that of PTH, as an additive effect is observed in vitro when the hormones are administered together (Marx et al. 1972). Other hormones which regulate functions by activating adenyl-cyclase in their target tissues (e.g. corticotropin and glucagon acting on the adrenal and liver respectively) were found not to bind to the PTH-specific receptors of the renal tissue (Sutcliffe et al. 1973), or to influence urinary levels of cAMP (Chase and Aurbach 1967).

iv) Protein Kinase

In cells responding to hormones through the activation of adenyl-cyclase and intermediation of cAMP, cyclic nucleotides cause activation of protein kinase(s) (Forte et al.1972). The renal tissue studies showed that it is the activation of the brush border protein kinase by the elevated intracellular concentration of cAMP that is responsible for the preferential phosphorylation of the luminal membrane (Kinne et al. 1975), and ultimately, for the PTH-mediated alterations in the tubular transport of solutes.

v) Involvement of cAMP in Ion Transport

To further strengthen the hypothesis of cAMP involvement in the action of PTH, in vitro and in vivo studies involving the nucleotide have been performed in an

attempt to reproduce the known effects of PTH.

In Vitro Studies

It has been shown that exogenous cAMP does not always mimic the effect of PTH, possibly because most cells are relatively impermeable to phosphorylated compounds in general and cAMP is also subject to rapid hydrolysis by phosphodiesterase into the inert 5'AMP metabolite. Butyrated derivatives have often been used to replace cAMP as they have been shown to have higher cell membrane permeability as compared to the parent nucleotide (Posternak et al. 1962, Henion et al. 1967). Furthermore, Heershe et al. (1971) showed DBcAMP to be more phosphodiesterase resistant, while the results of Aurbach et al. (1972b) suggest that it acts as an inhibitor of the phosphodiesterase, causing thereby an accumulation of endogenous cAMP.

The concentration of exogenously administered nucleotide also appears to be of vital importance. Raisz et al. (1969) showed that whereas PTH had a marked effect on bone resorption, no such effect could be shown with cAMP in doses up to 3×10^{-3} M. DBcAMP, however, stimulated resorption at doses of 5×10^{-5} M to 7×10^{-4} M, the response being similar to that obtained with the low doses of PTH. At concentrations above 10^{-3} M the effect was lost.

Although DBcAMP appears to be more potent than cAMP itself, the question as to whether the biological effect of DBcAMP is due to augmentation of intracellular

concentration of cAMP, or to a direct action on the protein kinase remains to be answered.

In Vivo Studies

Effects of cAMP on the Transport of Phosphate

The physiological effects of cyclic nucleotides have also been investigated. The phosphaturic action of PTH on the kidney has been consistently reproduced with both cAMP and DBcAMP (Rasmussen et al. 1968a, Russell et al. 1968, Agus et al. 1971, Kuntziger et al. 1974, Burnatowska et al. 1977). In dogs, either systemic or renal artery infusion of DBcAMP led to a rise in urinary phosphate excretion, indicating a direct tubular effect. Replacement of the active nucleotide with 5'cAMP, its inert metabolite, failed to inhibit phosphate reabsorption. Furthermore, micropuncture results suggested that the proximal tubule was a predominant site of the PTH-like effects of DBcAMP; a drop in phosphate reabsorption was accompanied by a drop in reabsorption of sodium, thus supporting the view that phosphaturia resulted from the inhibition of the proximal sodium reabsorption (Agus et al. 1971). A similar inhibition of proximal tubule fluid reabsorption by DBcAMP has also been observed in isolated perfused proximal tubules (Hamburger et al. 1974). Studies in TPTX rats revealed the existence of cAMP-sensitive PO_4 transport beyond the proximal tubule (Kuntziger et al. 1974). The effects of cAMP were

localized to the terminal nephron, at the sites thought to be involved in the effect of PTH on the transport of phosphate (Sutton et al. 1976, Pastoriza-Munoz 1978, Harris 1979b) and where PTH-sensitive adenyl-cyclase (Chabardes et al. 1975) had been identified.

Effects of cAMP on the Transport of Calcium

Whereas DBcAMP, when administered to either acutely or chronically TPTX rats, caused a rise in plasma calcium similar to that shown with PTH no such effect could be seen with cAMP (Wells and Lloyd 1969). In these studies, theophylline, a known cyclic neucleotide phosphodiesterase inhibitor which causes a rise in the intracellular cyclic AMP concentrations, also produced a significant increase in serum calcium. When administered with DBcAMP, the effect was additive. On the contrary, imidazole, a potent activator of the phosphodiesterase, antagonized the hypercalcemic effect of both PTH and DBcAMP.

Despite a hypercalcemic effect of DBcAMP, the initial clearance and micropuncture studies (Rasmussen 1968a, Agus 1971, Kuntziger 1974) failed to demonstrate a PTH-like effect of cAMP or DBcAMP on the urinary excretion of either calcium or magnesium. Recent studies in the TPTX hamster, however, strongly suggest the involvement of cAMP in the PTH stimulated reabsorption of calcium and magnesium,

as a significant decrease in the fractional excretion of these electrolytes was observed after the administration of either cAMP or DBcAMP (Burnatowska et al. 1977). Some evidence has been presented suggesting that cAMP enhances calcium reabsorption in the thick ascending limb of Henle's loop (Bourdeau and Burg 1979) and in the distal convoluted tubule (Costanzo et al. 1978b), the known sites of PTH-sensitive calcium transport. However, further studies are required before PTH-like effects of cyclic nucleotides on the transport of electrolytes along the nephron become fully elucidated.

D. The Effects of Vitamin D and Its Metabolites on the Kidney

1. Effects of Vitamin D on the Tubular Transport of Phosphate

Despite numerous studies on the renal effects of vitamin D, its action on the transport of phosphate is not clearly defined. Evidence suggesting both a phosphaturic and an antiphosphaturic effect has been presented.

a) Evidence Supporting the Antiphosphaturic Effect of Vitamin D

In 1941, Harrison and Harrison reported that the administration of 20,000 U of vitamin D_2 over three days to either D depleted or D repleted dogs led to a decrease in urinary excretion of phosphate. These results have been questioned, however, as the animals had intact parathyroid glands, and it is possible that the observed effect could be due to secondary hypoparathyroidism, the primary effect of vitamin D being to increase plasma Ca, thus inhibiting the release of PTH. Ultimately, the drop in the circulating levels of PTH could be directly responsible for the enhancement of the tubular transport of phosphate. Costanzo and co-workers (1974) demonstrated, however, that while vitamin D deficient TPTX rats receiving vitamin D excreted urine free of phosphate regardless of the filtered loads, rats that were D deficient excreted phosphate even at low filtered loads.

Studies in vitamin D repleted animals also support an antiphosphaturic effect of vitamin D and its metabolites. It is not clear however whether the presence of PTH is required for the effect. Puschett and co-workers (1971, 1972a) demonstrated a direct tubular effect on vitamin D_3 as well as 25(OH) D_3 and 1,25(OH)$_2$ D_3 in the absence of PTH, whereas Popovtzer et al. (1974) reported that the antiphosphaturic effect of either 25(OH) D_3 or 1,25(OH)$_2$ D_3 was seen only in the presence of PTH. In the latter study neither the correction of the hypocalcemia of PTX nor elevation of the baseline phosphate excretion by either volume expansion or infusion of phosphate restored the antiphosphaturic effect of vitamin D metabolites in the absence of parathyroid hormone. In the former study in TPTX dogs, the baseline phosphate excretion was elevated to about 20% of the filtered load by concomitant volume expansion and administration of vasopressin. It is possible, therefore, that the administration of vasopressin may have influenced the renal action of vitamin D. The question as to whether vasopressin is involved in the observed effects of vitamin D has been examined recently. The experiments were repeated in similarly treated TPTX dogs under condition of volume expansion alone or in combination with vasopressin administration (Nseir et al. 1978), as well as in vitamin D depleted, TPTX rats (Puschett et al. 1978). In both species the administration of 1,25(OH)$_2$ D_3 failed to produce the antiphosphaturic response unless vasopressin was present. Furthermore, they demonstrated that in vitamin

D replete TPTX dogs (Puschett et al. 1971) as well as in D deficient TPTX rats (Puschett et al. 1975, 1978), PTH and vitamin D_3 at certain dose levels have antagonistic effects on the urinary excretion of phosphate.

Recently, Popovtzer and co-workers extended their previous studies on the interaction of vitamin D and PTH to the renal action of other hormones. They demonstrated that in D repleted TPTX rats 25(OH) D_3 interfered with the phosphaturic action of both calcitonin (Popovtzer et al. 1977) and glucagon (Popovtzer et al. 1978), within 60 minutes of their administration.

Neither the exact site of the action of vitamin D nor the site of interaction with that of PTH or other hormones on the tubular transport of phosphate is known. Gekle et al. (1971b) demonstrated that administration of vitamin D_3 enhanced proximal tubule reabsorption of phosphate in D depleted intact or TPTX as well as in rachitic, phosphate infused rats. The data are not conclusive however as a decrease in the response occurred within 180-240 minutes after the commencement of vitamin infusion and the tubular reabsorption returned to the pre-treatment levels. Furthermore, urinary phosphate was not measured, thus making impossible any speculations regarding the effect of vitamin D on the distal tubular transport of phosphate. The only other micropuncture study regarding the action of vitamin D_3 along the nephron is that of Wong and co-workers in D repleted dogs (pers. com.). In

this study, an enhancement of Pi reabsorption beyond the proximal tubule was observed in the intact but not TPTX dogs given 25(OH) D_3. Further studies are required to localize the exact site of action of vitamin D_3 on the tubular reabsorption of Pi.

In summary, although it is possible that vitamin D enhances tubular reabsorption of phosphate in the absence of PTH, the evidence presented above suggests that its action might be an indirect and nonspecific interference with direct tubular effects of polypeptide hormones.

b) Evidence Suggesting a Phosphaturic Effect of Vitamin D

Along with the evidence pointing to the enhancement of the tubular reabsorption of phosphate as a result of the action of vitamin D, reports suggesting an opposite effect have also accumulated. In 1968, Ney and co-workers demonstrated that in vitamin D depleted TPTX dogs the administration of large doses of vitamin D_2 (100,000 U/day, I.V.), led to an increase in the urinary excretion of phosphate within 24 hours. The phosphaturic effect persisted for the six days of study, as the tubular reabsorption of phosphate was reduced from 99 to 85% with no change in GFR. The absence of an effect up to two hours after intravenous administration of 60,000 U of D_3 may be explained by the time required for conversion of the parent vitamin to more polar metabolites.

Similarly, a rise in the urinary excretion of phosphate for a given filtered load has been observed in vitamin D repleted TPTX rats (Crawford et al. 1955). A criticism pertinent to both studies is that neither one included an appropriate control group.

Recently, $1,25(OH)_2 D_3$ was shown to have a phosphaturic effect in chronically TPTX rats fed a high phosphate diet. However, in TPTX rats fed a low phosphate diet and sham rats given a diet with either high or low content of phosphate, the administration of $1,25(OH)_2 D_3$ had no effect (Bonjour et al. 1977). It is thus possible that the phosphaturic effect of vitamin D is dependent on the antecedent diet and might be a result of extrarenal actions of the hormone.

2. Effects of Vitamin D on Tubular Calcium Transport

The possibility that vitamin D might also influence the tubular transport of calcium has been examined by several investigators again with rather conflicting results. Evidence suggesting that vitamin D has a hypercalciuric as well as a hypocalciuric effect, has been presented.

a) Evidence Suggesting a Hypocalciuric Effect of Vitamin D

Data from studies in D depletion suggest that vitamin D deficiency impairs the tubular reabsorption of calcium and that the administration of either vitamin D or its

metabolites can reverse the effect. In 1960, Gran and co-workers reported that treatment of rachitic dogs with vitamin D led to a fall in urinary calcium despite an increase in plasma calcium concentration. Subsequently, Ney et al. (1968) reported that in D depleted TPTX dogs infusion of 60,000 U/hour of vitamin D_3 for two hours also led to a drop in urinary calcium excretion. However, chronic administration of vitamin D_2 (100,000 U/day) did not further enhance calcium reabsorption. On the contrary, a gradual increase in the urinary calcium to pre-treatment levels was observed. However, the latter effect might be due to an extrarenal action of vitamin D_3. More recently, Costanzo and co-workers (1974) showed that fractional excretion of calcium (FECa) was lower in vitamin D repleted than D depleted TPTX rats. A significant enhancement of calcium above sodium reabsorption was observed in the D repleted group, thus suggesting a distal effect of vitamin D. These observations are further supported by the study of Harris et al. (1976) also in D depleted TPTX rats. In this study, the acute administration of $1,25(OH)_2\ D_3$ led to a significant drop in the FECa while FENa was significantly increased. However, Steele et al. (1975) reported that in similarly D depleted TPTX rats, the hypocalciuric effect of $1,25(OH)_2\ D_3$ could be seen only 14 hours after the administration of the hormone. The enhancement of tubular calcium reabsorption was not associated with any change in the transport

of sodium, but was associated with an increase in plasma Pi not seen in D depleted animals. Thus, it is possible that in this study the lower urinary calcium in the D repleted group compared to the D depleted animals might be only a secondary effect to the increased plasma phosphate, as that suggested by a recent study of Brautbar et al. (1979) also in D deficient rats.

Studies in the D repleted state are complex. Puschett et al. (1971,1972) demonstrated that in D repleted TPTX dogs, $25(OH)_2\ D_3$ and $1,25(OH)_2\ D_3$ enhanced tubular reabsorption of calcium. This effect was nonselective as a decrease in urinary excretion of calcium was accompanied by a similar fall in urinary sodium levels. Furthermore, these animals were given vasopressin and recent studies of the same investigators suggest that vasopressin is required in order for the hypocalciuric effect of either $25(OH)\ D_3$ or $1,25(OH)_2\ D_3$ to be seen (Nseir et al. 1978).

It would appear that while vitamin D_3 enhances tubular reabsorption of Ca in D depleted states, in D repletion the presence of vasopressin is required for the anticalciuric effect to be seen.

b) Evidence Suggesting a Hypercalciuric Effect of Vitamin D.

The evidence suggesting that vitamin D increases urinary calcium excretion comes predominantly from clinical investigations. In 1958, Litvak et al. demonstrated

that large doses of vitamin D or Dihydrotachysterol cause hypercalciuria in hypoparathyroid patients. Similarly, in the intact patients, it has been shown that the administration of vitamin D caused hypercalciuria with no changes in total plasma calcium (Brickman and co-workers 1974). Several other studies in hypoparathyroid, normal, and rachitic hyperparathyroid patients also suggest that vitamin D acts to increase the urinary excretion of calcium. This evidence, however, does not exclude the possibility that the increase in urinary calcium excretion occurred as a result of an increase in the filtered load of this cation, an increase in its intestinal reabsorption or maybe a suppression of PTH secretion. A recent balance study in TPTX rats on the effects of $1,25(OH)_2 D_3$ on calcium metabolism tends to confirm these suggestions. Although the administration of $1,25(OH)_2 D_3$ resulted in an increase in urinary calcium excretion, it did not appear to be a result of a direct tubular action of the hormone, as it could be accounted for by an increased gut calcium absorption. (Rizzoli et al. 1977).

c) Evidence Suggesting That Vitamin D Has No Renal Effect

Contrary to all references above, Bernstein et al. (1963) failed to show any effect of vitamin D on calcium handling by the kidney in two hypoparathyroid patients. Also, no effect of $1,25(OH)_2 D_3$ on the renal calcium transport could be seen when investigated in conscious, TPTX rats (Hughi et al. 1979).

3. Factors Responsible for the Conflicting Results

The reasons for the disparity in these studies are not apparent. However, several factors may be responsible for the conflicting results.

i) In subjects with intact parathyroid glands, it is possible that hypercalciuria as well as antiphosphaturia occurred as a result of a suppression of PTH secretion due to either an immeasurable rise in the ionized plasma Ca, or perhaps to a direct effect of vitamin D on the parathyroid glands.

ii) During chronic administration of vitamin D, its effects on other target organs have to be accounted for before any renal effect can be suggested.

iii) In the studies in the D repleted state, the endogenous levels of vitamin D might render it difficult to observe any further effects of exogenous hormone.

iv) The degree of D depletion might be important in establishing the level of Pi and Ca excretion by the kidney.

v) Studies during vitamin D depletion are usually associated with Pi depletion. Pi depletion is often induced in D deficiency to improve the ability of these animals to tolerate a parathyroidectomy. It is possible, therefore, that changes in plasma phosphate and possibly intracellular Pi might be responsible for the observed effect, independently of the action of vitamin D.

vi) The dose of vitamin D or PTH might be an important factor in determining the renal response to vitamin D. Puschett has reported that 25(OH) D_3 and PTH have synergistic or antagonistic effects on the excretion of Pi, depending on the doses used. In D deficient TPTX rats, the administration of 1 U 25(OH) D_3 for six hours induced an antihosphaturic response only in the presence of 0.2 U of PTH/hour, while neither agent alone affected the urinary excretion of phosphate. On the other hand, 2 U PTH/hour only caused a phosphaturia when given with 1 U of vitamin D (Puschett et al. 1975).

vii) Variations in hormonal and non hormonal factors shown to influence tubular handling of electrolytes (discussed p. 33) can also produce changes in the renal handling of calcium and phosphate independent of vitamin D when not accounted for.

viii) The antecedent diet may affect the response to vitamin D. The fact that the kidney responds to variations in the dietary intake of Pi by changing its tubular capacity to transport Pi, is well documented (Steele 1976a, 1976b). Recently, Bonjour et al. (1977) demonstrated that the phosphaturic response to 1,25(OH)$_2$ D_3 of chronically TPTX rats depends on their phosphate status, whereas Brautbar et al. (1979) suggested that the enhanced tubular reabsorption of phosphate during dietary phosphate restriction is independent of the action of vitamin D.

ix. Finally, it is also possible that vitamin D has a bidirectional effect on the handling of phosphate. Thus, $1,25(OH)_2 D_3$ can enhance phosphate excretion in the phosphate loaded or hyperphosphatemic state, whereas it can reduce urinary phosphate excretion in a hypophosphatemic state. Such effect would account for the observed effect of $1,25(OH)_2 D_3$ to raise serum Pi in hypophosphatemic rats and to lower it in hyperphosphatemic rats (Garabedian et al. 1976). The mechanism of this bidirectional effect of vitamin D on plasma phosphate is not clear.

4. Mechanism of Action of Vitamin D in the Kidney - Role of cAMP

The synthesis of new mRNA as well as functional calcium binding protein shown to occur in the kidney in response to the administration of vitamin D (Chen and DeLuca 1973, Morrisey and Rath 1974, Harrison et al. 1977), suggests the existence of a steroid-like mechanism of action of vitamin D analogous to that in the intestine (Discussed on page 13). However, changes in the activity of the renal adenyl-cyclase-cAMP system observed after the administration of vitamin D or its metabolites suggest a possible involvement of cAMP in the vitamin D dependent transcellular transport of electrolytes; Forte and co-workers (1976) demonstrated that in vitamin D deficient rats, renal PTH-sensitive adenyl-cyclase was reduced. Contrary to this observation, however, Popovtzer and Robinette (1975) reported that $25(OH) D_3$ inhibited the PTH induced rise in the urinary cAMP. Also, the antiphosphaturic effect of vitamin D was shown to be associated with the inhibition of calcitonin and glucagon (Popovtzer et al. 1977, 1978), but not vasopressin (Nseir et al. 1978) induced urinary cAMP. The

reasons for these disparities are not clear. Recent studies of Kaukuta et al. (1977) demonstrated, however, that a low plasma calcium rather than the absence of vitamin D was responsible for a decrease in the renal tissue adenyl-cyclase activity. Furthermore, although the antiphosphaturic effect of 25(OH)D_3 observed in the presence of polypeptide hormone was abolished when the infusion of the hormone was replaced with DBcAMP, the administration of calcium had a similar effect (Puschett et al. 1974). Therefore, it is difficult to determine the role of cAMP and calcium in the action of vitamin D, as both substances may effect calcium levels, and calcium ion itself may act as a second messenger (see p. 58). It is thus possible, that vitamin D-induced changes in cAMP levels, reflect not a direct involvement of the nucleotide in its mechanism of action, but rather an interaction with the renal action of other hormones. The available evidence renders it difficult to localize the possible step of the interaction between vitamin D and PTH, calcitonin, vasopressin, and glucagon in the kidney. It is unlikely, however, that vitamin D exerts its blocking effect by competing with these hormones for the binding site. The renal tissue receptors for polypeptide hormones are highly specific and probably located on distinct cell types (Aurbach and Heath 1974). Parathyroid hormone binds specifically to the receptors in the renal cortex, while specific receptors for vasopressin have been localized in the renal medulla (Chase and Aurbach 1968). Receptors for calcitonin, although not clearly defined, have been shown to be specific and independent of those for PTH (Heersche et al. 1974, Chabardes et al. 1976). Moreover,

the structural dissimilarity between sterol and peptide hormones makes the competition for the same site even more unlikely. It is more probable that vitamin D interferes with the activation of the adenyl-cyclase cAMP system, which although closely linked, is a separate process from the binding of the hormone to the receptor. The results of the investigation on the interaction of vitamin D with parathyroid hormone, DBcAMP, calcitonin and glucagon would support this hypothesis. The absence of a similar change in urinary cAMP levels in the presence of vasopressin points to a step beyond cAMP production as a site of the interference. Thus, although vitamin D metabolites might interfere with the action of polypeptide hormones prior to cAMP production, interaction at a more distal site, possibly at the level of cAMP dependent protein kinase activity leading to changes in the phosphorylated proteins as suggested recently for other steroid hormones (Greengard 1978) cannot be excluded.

5. Summary

The available evidence suggests that in vitamin D deficient animals the administration of vitamin D or its metabolites enhances renal tubular reabsorption of calcium and phosphate.

In vitamin D repleted states the results are less consistent. While in the dog, vitamin D appears to enhance calcium reabsorption at least in the presence of

vasopressin, it appears to have a hypercalciuric effect in man under a variety of conditions.

The antiphosphaturic effect of vitamin D observed in D replete animals appears to be nonspecific and a result of the interference of vitamin D with the renal action of several polypeptide hormones and possibly other hormones that also influence tubular transport of phosphate.

Virtually nothing is known regarding the involvement of vitamin D in the tubular transport of magnesium.

The tubular mechanism by which vitamin D exerts these divergent effects remains unknown. The available evidence is equivocal and points to functional proteins as well as cAMP as possible mediators of the action of vitamin D.

Further studies are required to fully elucidate not only the effect of vitamin D on the tubular transport of electrolytes, but also the mechanism of this action and the possible involvement of the adenyl-cyclase-cAMP system.

III. HAMSTER — A MODEL FOR THE PROPOSED STUDY

In designing the present experiments to further elucidate the action of vitamin D on the renal transport of electrolytes and the involvement of PTH in this action, the golden hamster appeared to be a good model, particularly with regard to the effects on Ca and Mg handling. Biddulph and co-workers (1969, 1970) demonstrated that the hamster kidney plays a dominant role in the marked and rapid changes that occur in plasma calcium after TPTX, and is unusually sensitive to exogenous PTH. They showed that a fall in plasma Ca with a concomitant increase in urinary calcium after acute TPTX could be prevented by infusion of PTH.

Urinary Ca was shown to be highly influenced by the levels of dietary calcium, plasma Ca remaining constant, further underlining the importance of the kidney in the minute to minute regulation of plasma calcium (Biddulph et al. 1973).

A phosphaturic effect of PTH was also seen in the TPTX hamsters. Unlike in other species, where PTH causes a fall in plasma phosphate, in the hamster, PTH failed to lower serum phosphate (Biddulph et al. 1969). These observations have also been confirmed in our laboratory. Furthermore, in addition to regulating PCa, the hamster kidney was also shown to play an important part in the PTH dependent changes in plasma and urinary magnesium (Burnatowska et al. 1977).

Finally, unlike other species, where only the phosphaturic effect of PTH could be mimicked with cAMP, we were able using

this model to demonstrate a PTH-like effect of cAMP and DBcAMP on the tubular reabsorption of Ca and Mg as well as Pi (Burnatowska et al. 1977).

PROTOCOL

- MALE GOLDEN HAMSTERS 100-130 G B.W.
- FREE ACCESS TO FOOD AND WATER BEFORE EXPERIMENT
- INACTIN ANESTHESIA 18 MG/100 G B.W.
- REPLACEMENT OF SURGICAL LOSSES WITH 1% B.W. OF 0·9 % NaCl

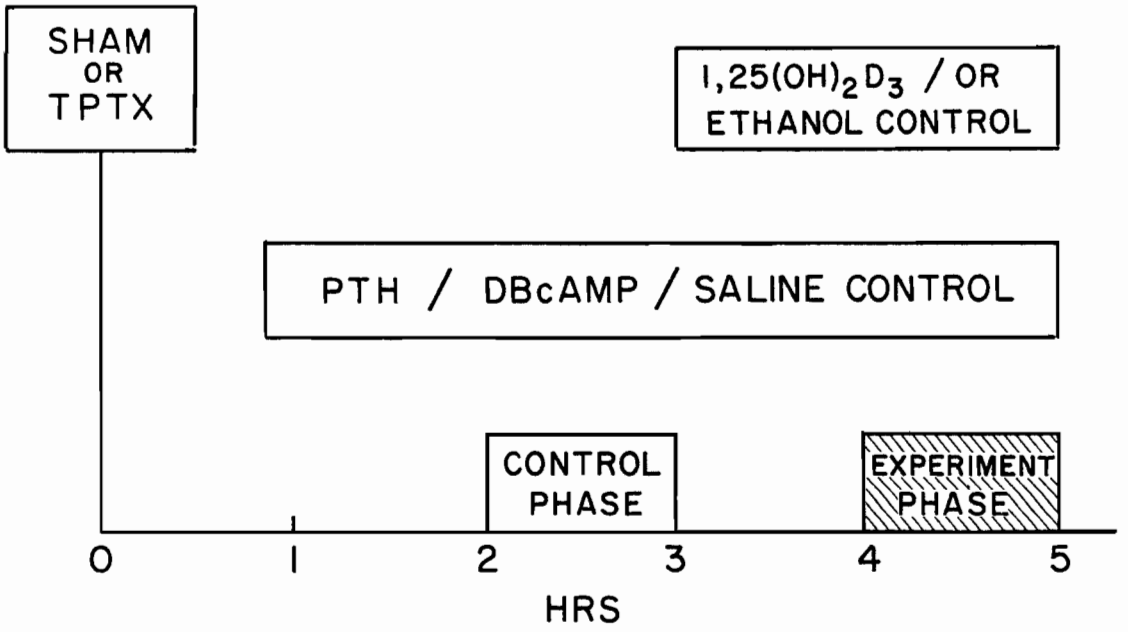


Fig. 1. Experimental Protocol.

IV. METHODS

1. Animal Preparation

Standard clearance experiments were performed on male, golden hamsters (Mesocricetus auratus), 100-130 g. body weight, allowed free access to food and water. Animals were anesthetized intra-peritoneally (ip) with Inactin (Promonta, Hamburg, Germany), 18 mg/100 g body weight and tracheotomized. Acute thyroparathyroidectomy (TPTX) was performed by cauterization in all but the intact group. Polyethylene catheters (PE50) were inserted into the jugular vein for infusion of inulin, and into the carotid artery for blood sampling. Urine was collected from a catheter inserted into the bladder. Animals were placed on a thermostatically controlled heated table to maintain body temperature, (monitored by a thermoprobe) at 37°C. Surgical fluid losses were replaced by intravenous (i.v.) administration of 1% body weight of 0.9% saline after completion of surgery (Fig 1, p. 83a).

2. Experimental Protocol

a) Vitamin D Replete Studies

Animals were divided into groups as shown in Table I; each group was subdivided into two subgroups: a control group (A) and an experimental group (B). Furthermore, each subgroup comprised of two phases, control (C) and experimental (E). The protocol employed was identical in all TPTX

animals except for different drugs infused either throughout the experiment or in the second phase only. Whereas the control groups (A) received ethanol vehicle only in the experimental phase (E), the experimental groups (B) received $1,25(OH)_2 D_3$.

Clearance periods were commenced two hours post TPTX and consisted of two 60 minute phases (control and experimental) separated by a 60 minute interval. The control (C) and experimental (E) phases each consisted of two 30 minute urine collection periods. Urine was collected under oil in finely graduated tubes. A blood sample was taken at the end of each phase for determination of hematocrit, protein, inulin, and electrolyte concentration. The red blood cells of all but the intact group animals were reinfused after being suspended in 0.9% saline solution containing 2 mEq/L $CaCl_2$ and 6% bovine serum albumin. Throughout the experiments all animals were infused with 3.5% inulin in 0.9% saline at 0.029 ml/min. The infusion of inulin began at least an hour before commencement of the first phase to assure the equilibration of inulin concentration in the extracellular fluid compartment.

b) Vitamin D Deficient Studies

Vitamin D deficiency was produced by feeding animals a diet lacking vitamin D for at least five weeks. In addition to being vitamin D deficient, these animals were also phosphate depleted as a result of a low phosphate and high calcium content of the diet. Animals were housed in the absence

of daylight or fluorescent lighting. Food and water was allowed ad libitum up to the time of experiments. Clearance experiments were performed in a manner identical to that in group II.

3. Materials

a) $1,25(OH)_2 D_3$

The administration of $1,25(OH)_2 D_3$ (Hoffman-LaRoche) was begun at the completion of the control phase at a dose of 1 unit (65.5 pM) prime followed by 0.5 U/hr.

The control animals were given ethanol alone in the second phase (5 µl prime followed by 5 µl/hr) as this served as a vehicle for $1,25(OH)_2 D_3$ in the experimental animals (Fig. 1).

b) $25(OH) D_3$

$25(OH) D_3$ (Hoffman-LaRoche) was administered at a dose of 5 U prime followed by 2.5 U/hr to TPTX, PTH infused (2 U/hr-569 U/mg) hamsters (Group VIIIB). The control (Group VIIIA), was infused with PTH throughout the experiment and was given ethanol vehicle (5 µl prime followed by 5 µl/hr) in the second phase.

c) Parathyroid Hormone

In order to study the interaction of vitamin D and PTH, Groups III and IV were infused throughout the study with purified bovine PTH (Wilson Laboratory) beginning at the

completion of the surgery. Group III animals were infused with PTH in doses sufficient to reduce but not prevent the hypocalcemia resulting from TPTX. The results include an initial group of hamsters infused with 2 U/hr. of purified PTH (Wilson: 569 U/mg) and a subsequent group infused with a different batch of purified PTH (336 U/mg) at 0.8 U/hr. (Table V). These two doses produced similar levels of plasma calcium (4.0 ± 0.5 and 3.9 ± 0.4 mEq/L respectively). The reason for the disparity in dose levels required to produce the same effect appeared to be a difference in degree of purification in the two batches of PTH. Additional studies have indicated that this may be due to the fact that the more purified PTH may bind to the glassware used in the infusion apparatus, whereas the less purified hormone contains more protein which serves as a carrier and thus reduces binding to the glassware and tubing. **Infusion** of 1 unit of highly purified PTH to TPTX animals brought PCa to 3.7 mEq/L (n=3) as compared to 4.4 mEq/L (n=3) when infused in 0.2 g% albumin. Group IV animals were infused with PTH in doses which restored normocalcemia in the TPTX animals. Again, lower doses (2-3 U/hr.) of the less purified than of the more purified hormone (3.3-5 U/hr.) were required to produce this effect (Table VI).

e) $CaCl_2$ Infusion

Group V animals were infused throughout with 75 mEq/L $CaCl_2$ added to the standard infusions in order to prevent the hypocalcemia of TPTX.

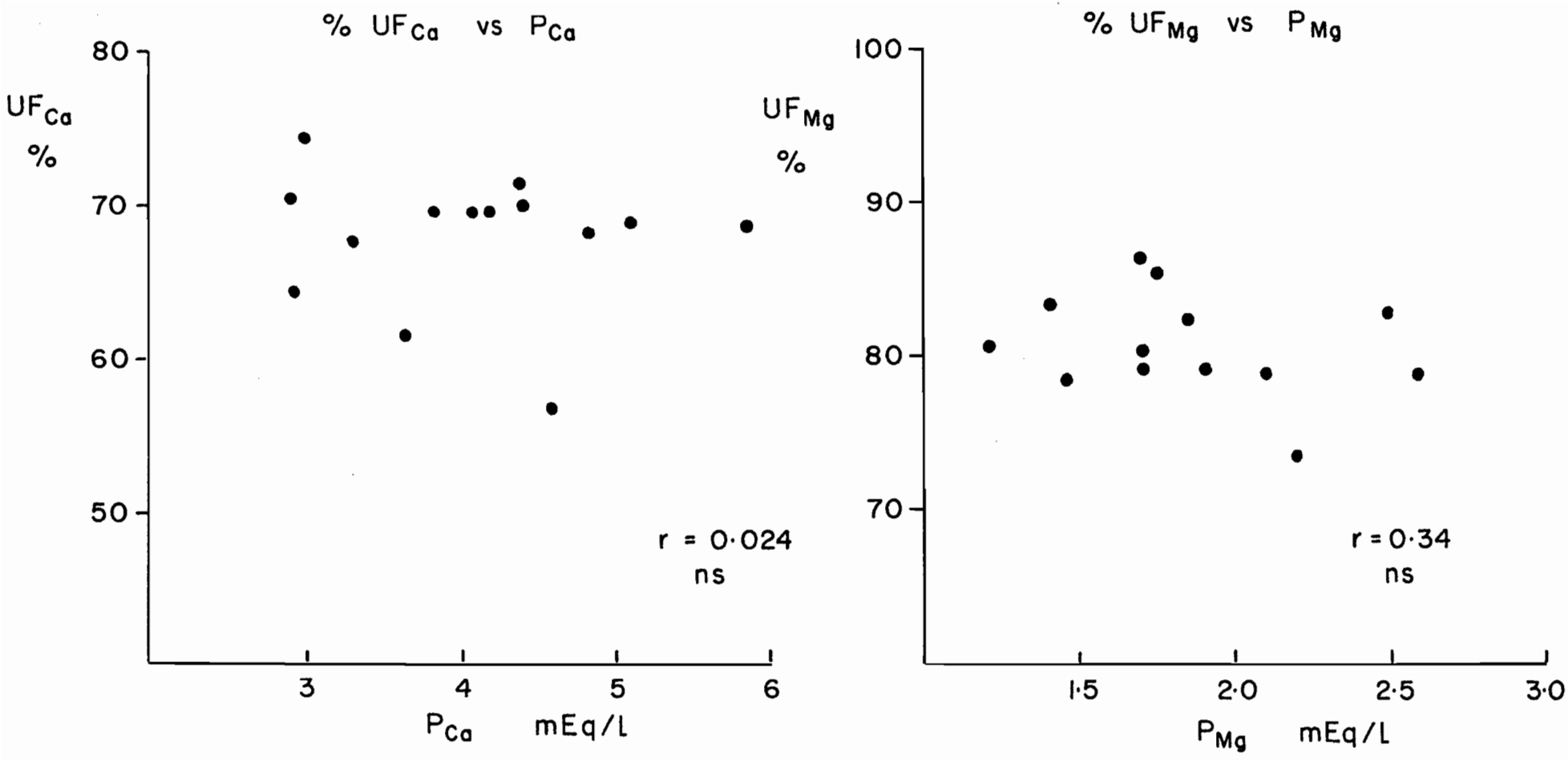


Fig. 2. Calcium and magnesium ultrafiltration $\left(\left(\frac{UF}{P}\right)_x \times 100\%\right)$ as a function of plasma calcium and magnesium.

f) Phosphate Infusion

To elevate baseline phosphate excretion in the TPTX hamsters, Group VI animals were infused throughout with 50 mM phosphate (Na_2HPO_4: NaH_2PO_4 = 4:1) which elevated mean plasma phosphate concentration to 2.2 ± 0.4 mM.

g) DBcAMP

To investigate a possible step of interactions between PTH and vitamin D, the infusion of the hormone was replaced with that of DBcAMP at 0.6 mg/hr (1.2×10^{-6} M/hr). DBcAMP was used in this study since it appeared to be more effective then cAMP and its 8p-chlorophenylthio analog in mimicking a PTH-like effect on the renal handling of divalent electrolytes (Table XI).

4. Chemical Methods

In all groups plasma proteins were precipitated with trichloroacetic acid prior to analysis of plasma inulin and phosphate concentrations. Plasma and urine concentrations of inulin were determined by the anthrone method of Führ, Kaczmarczyk and Krüttgen (1955), calcium and magnesium by atomic absorption spectrophotometry (Perkin Elmer 303), phosphate by the Chen method (1956) and sodium by flame photometry. Determination of fractional excretion of calcium and magnesium was based on mean ultrafilterable values ± standard error (SE) of 66.9% (± 5.9, n=16) and 79.3% (± 1.2, n=16) respectively (Fig.2). These numbers were derived from ultrafiltration of

pooled blood from a) TPTX animals, b) TPTX animals infused with different doses of PTH, or c) intact hamsters using artificial membranes (Aminco) with pH adjusted to 7.4 with CO_2. Neither the percent ultrafilterable calcium nor magnesium varied significantly over the range of plasma concentrations obtained in these studies.

Microhematocrit Reader (Model L-550A, Phillips-Drucker) was used to determine hematocrit levels. Plasma protein was measured with a refractometer (American Optical Company, Series PR-A).

5. Methods of Analysis

Significance of the difference between the means in the two phases was obtained by a paired Student t test. Unpaired t test was used for comparison of different groups of animals.

Following standard formulae were used in the calculation of the results:

$$GFR = (U/P) \text{ in } V$$

$$FE_x = \frac{(U/P)x}{(U/P)_{in}} \times 100\% \quad \text{or} \quad \frac{(U/UF)x}{(U/P)_{in}} \times 100\%$$

$$FL_x = P_x \text{ or } UF_x \times GFR$$

V. RESULTS

A. Vitamin D Replete Studies

1. Comparison of Intact and TPTX Hamsters

The effects of acute TPTX are summarized in Table II. These results were obtained by comparison of the plasma and urinary levels of electrolytes in the control phase of the intact hamsters of Group I (pooled A and B) with the control phase of the TPTX animals of Group II (pooled A and B). No significant differences in GFR and urine flow rate were observed between the two groups of animals. In the TPTX hamsters total plasma calcium was lower than in the intact animals (2.8 vs. 4.7 mEq/L, $p<0.001$), while fractional calcium excretion (FE Ca) was higher (19.8 vs. 2.4%, $p<0.001$). Similarly, plasma magnesium was lower in the TPTX group (1.2 vs. 1.7 mEq/L, $p<0.001$), while fractional urinary magnesium excretion was higher (21.2 vs. 4.7%, $p<0.001$). The fractional phosphate excretion of 22.8% in the intact group was significantly different from 4.9% in the TPTX hamsters ($p<0.001$). Although plasma phosphate tended to be higher in the TPTX group, it was not significantly different from that in the intact group (1.3 vs. 1.1 mM). Fractional sodium excretion was not significantly different between the two groups.

2. Effects of $1,25(OH)_2 D_3$ in the Intact Animals

The effects of $1,25(OH)_2 D_3$ in the presence of endogenous PTH are summarized in Table III. There was no significant difference in either the glomerular filtration rate (GFR) or urine flow between the two phases of either the control or the experimental group. A significant drop in the hematocrit and plasma protein is probably attributable to the fact that no attempt was made in either of these two groups to replace the blood taken for analysis. While neither the control nor the experimental group showed any significant changes in either total plasma calcium (PCa), or the filtered load of Ca (FLCa), a small but significant increase in the fractional excretion of calcium (FECa) was observed in the control, but not in the experimental group. The changes in the handling of magnesium were similar to those in calcium. It is possible, however, that the increase in FECa and FEMg in the control group may be a result of hemodynamic changes, as it was accompanied by a small increase in FENa and urine flow rate, whereas these parameters tended to drop in the experimental group.

The infusion of $1,25(OH)_2 D_3$ led to a significant reduction in the FEPi from 38.3 to 15.9% ($p<0.01$) while the FEPi of the control group was not significantly changed (13.8 to 15.4%). The changes in plasma Pi and FLPi were not significant in either group. The reason for the difference in baseline excretion of phosphate between the two groups is not clear as both groups were handled in an identical fashion.

In animals with intact parathyroid glands, the administration of vitamin D or its metabolites influences the secretion of parathyroid hormone (Chertow et al. 1975, Canterbury et al. 1977). Previous studies on the renal effects of vitamin D conducted in animals with intact parathyroid glands (Harrison and Harrison 1941) have often been criticized as it is possible that the observed changes in the transport of electrolytes were a result of changes in the levels of circulating PTH, rather than a direct tubular effect of vitamin D. Recent study of Brautbar et al. (1979) tends to confirm these spectulations. Thus, to avoid possible changes in the plasma levels of parathyroid hormone further studies were conducted in the TPTX hamsters in the presence of known amounts of exogenous PTH.

3. Effects of $1,25(OH)_2D_3$ in TPTX Animals in the Presence or Absence of Exogenous PTH

a) Comparison of Different Batches of PTH

Because different batches of parathyroid hormone were used throughout the study, their effectiveness in restoring plasma calcium concentration as well as the urinary excretion of calcium and phosphate in acutely TPTX animals was compared. The results are summarized in Tables V and VI. To reduce the hypocalcemic effect of TPTX to about 3.9 to 4.0 mEq/L, infusion of 2 U/hr of the 569 U/mg batch and 0.8 U/hr of the 336 U/mg batch of the hormone were required. At these doses

the FEPi was elevated to 11.7 and 11.1% by each batch respectively. These results were therefore pooled, and the amount of PTH used termed as "low dose" (Table V). The "high dose" was the amount of PTH that completely prevented the hypocalcemia of TPTX, and further elevated urinary phosphate. At least 3.3 U/hr of the more purified (569 U/mg) and 2-3 U/hr of the less purified (336 U/mg) hormone were required to elevate plasma calcium to 4.4 mEq/L. At these doses of PTH administration FEPi rose to 29.9 and 32.1% respectively (Table VI). The changes in the handling of magnesium reflected those in calcium with the various doses of PTH, except for a small but significant difference in PMg, that was observed with different batches of PTH when administered at high doses. The reason for this difference is not clear, as it occurred in the absence of a significant difference in plasma calcium and FEPi; the parameters which were the measure of the effectiveness of PTH.

b) Hemodynamic Changes

Although GFR and urine flow rate varied between the groups, the changes between the control and experimental phase within each group were not significant. The reason for a fall in hematocrit and protein in some groups but not in others is not clear as in all groups the blood taken for analysis was replaced with the red blood cells suspended in 6 g% albumin solution (Groups II - IX).

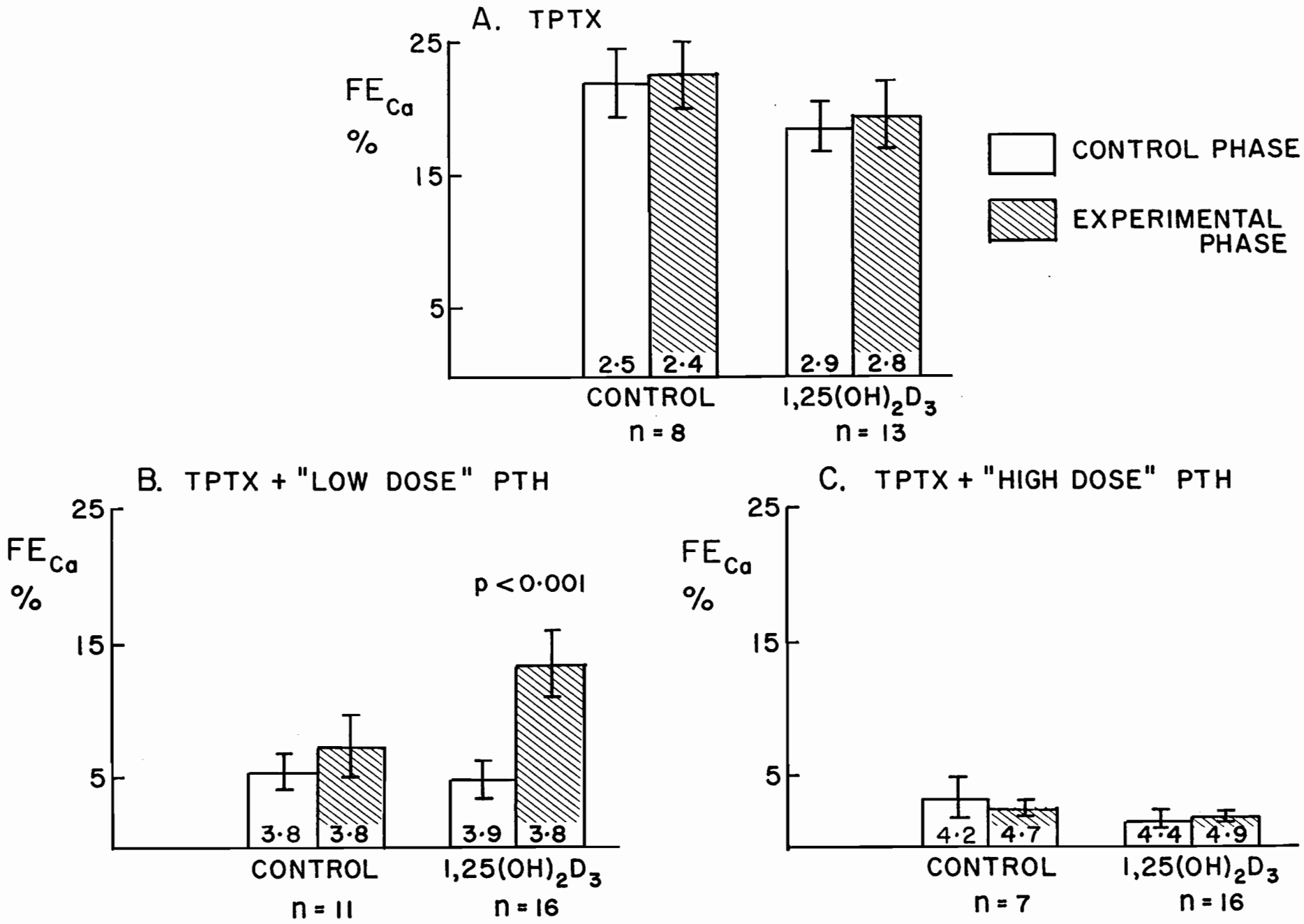

A. TPTX
25
15
5
FE_{Ca}
%
2·5
2·4
2·9
2·8
CONTROL
n = 8
1,25(OH)$_2$D$_3$
n = 13
CONTROL PHASE
EXPERIMENTAL PHASE
B. TPTX + "LOW DOSE" PTH
p < 0·001
3·8
3·8
3·9
3·8
CONTROL
n = 11
1,25(OH)$_2$D$_3$
n = 16
C. TPTX + "HIGH DOSE" PTH
4·2
4·7
4·4
4·9
CONTROL
n = 7
1,25(OH)$_2$D$_3$
n = 16

Fig. 3. Effects of $1,25(OH)_2D_3$ on fractional calcium excretion (FECa) in the hamster: n=number of animals per group. Figures inside the bars represent mean plasma calcium in mEq/l.

c) Effects of $1,25(OH)_2 D_3$ on Ca Handling

1) TPTX

In the TPTX animals, neither vehicle nor $1,25(OH)_2 D_3$ caused a significant change in FECa (22.0 to 22.6% and 18.5 to 19.4% respecively). Plasma Ca and FLCa remained unchanged throughout the study in both groups as well (Table IV, Fig. 3).

2) TPTX and Low Dose PTH

The effects of $1.25(OH)_2 D_3$ on the transport of Ca in the presence of various doses of PTH are summarized in Figure 3 and Tables VII and VIII. The dose of PTH used in Group III was such that it reduced but did not prevent the hypocalcemic and antiphosphaturic effect of TPTX. While the administration of the vehicle had no significant effect on PCa (3.8 to 3.8 mEq/L), FLCa (2.3 to 2.6 μEq/min.) and FECa (5.6 to 7.5%), the administration of $1,25(OH)_2 D_3$ resulted in a significant increase in FECa from 5.2 to 13.2% ($p<0.001$) with no effect on either PCa (3.9 to 3.8 mEq/L) or FLCa (2.6 to 2.7 μEq/min).

3) TPTX and High Dose PTH

The effects of $1,25(OH)_2 D_3$ on the Ca handling in the presence of higher doses of PTH are summarized in Table VIII, Fig. 3. This dose of PTH further enhanced tubular reabsorption of Ca. $1,25(OH)_2 D_3$ had no significant

EFFECTS OF $1,25(OH)_2D_3$ IN THE TPTX CALCIUM INFUSED HAMSTER

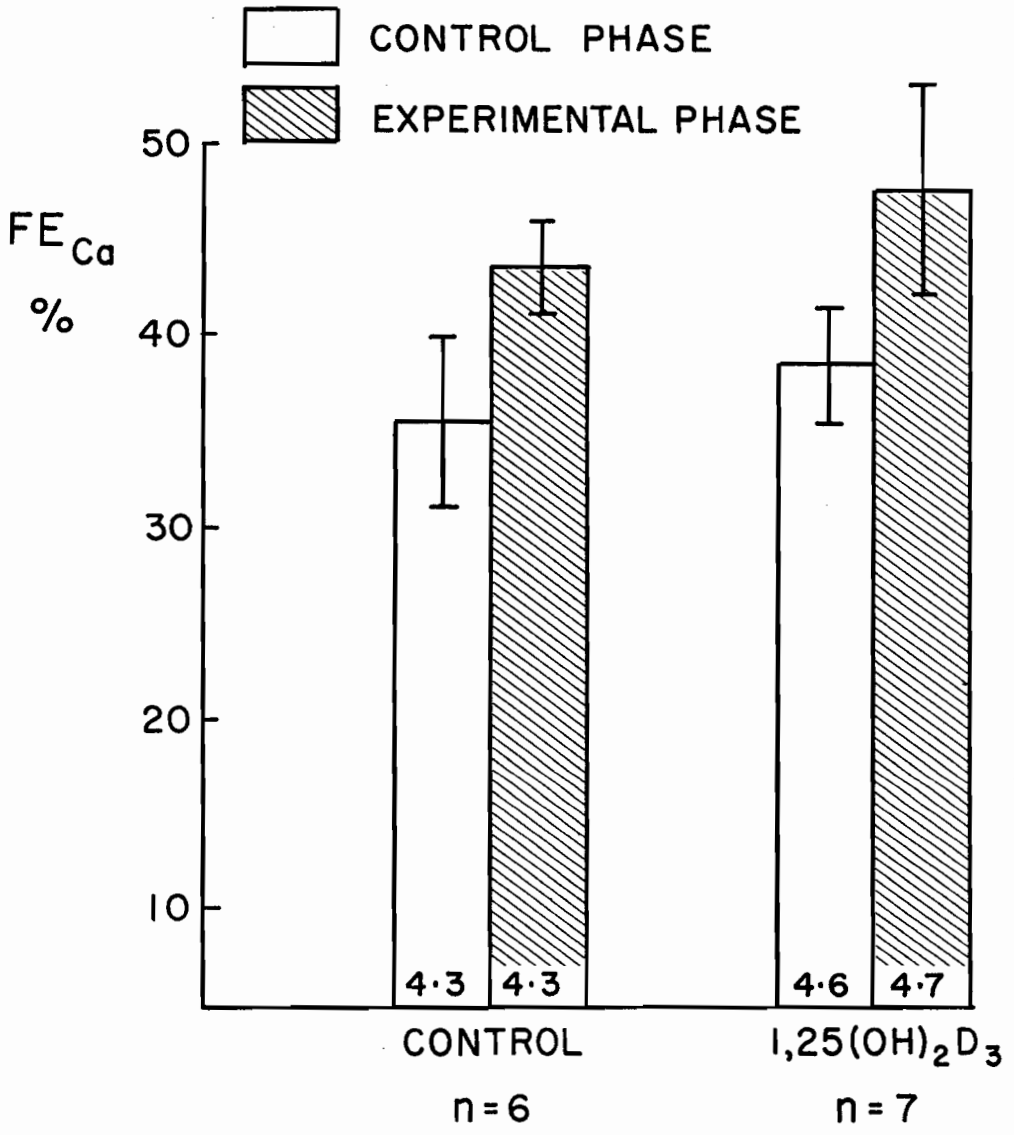


Fig. 4. Effects of $1,25(OH)_2D_3$ on fractional calcium excretion in the TPTX hamster infused with 75 mEq/l calcium chloride at 0.029 ml/min. n=number of animals per group. Figures inside each bar indicate mean plasma calcium in mEq/l.

effect on PCa, FLCa or FECa. PCa continued to increase in both the control and the experimental group (4.2 to 4.7, $p<0.01$ and 4.4 to 4.9 mEq/L, $p<0.001$ respectively). The changes in the filtered load of calcium were not significant however (control 3.01 to 2.52 μEq/L, experimental 3.13 to 3.62 μEq/min).

4) TPTX and $CaCl_2$

The results of the investigation of the role of PCa in the calciuric response to $1,25(OH)_2 D_3$ are shown in Figure 4 and Table IX. The infusion of $CaCl_2$ throughout the study prevented a fall in plasma Ca following TPTX. Despite normal Ca levels, however, $1,25(OH)_2 D_3$ failed to affect tubular transport of Ca in the absence of PTH. The changes in the FECa in both the control (35.3 to 43.8%) and the experimental (39.5 to 47.8%) groups were not significant.

d) Effects of $1,25(OH)_2 D_3$ on the Transport of Magnesium

The changes in the transport of magnesium following the administration of $1,25(OH)_2 D_3$ were similar to those changes in the transport of calcium.

1) TPTX

In the TPTX hamsters neither $1,25(OH)_2 D_3$ nor the vehicle had any significant effects on FEMg (21.5 to 20.3% and 21.0 to 16.6% respectively), PMg (1.3 to 1.4 and 1.2 to 1.3 mEq/L respectively), or FLMg (0.79

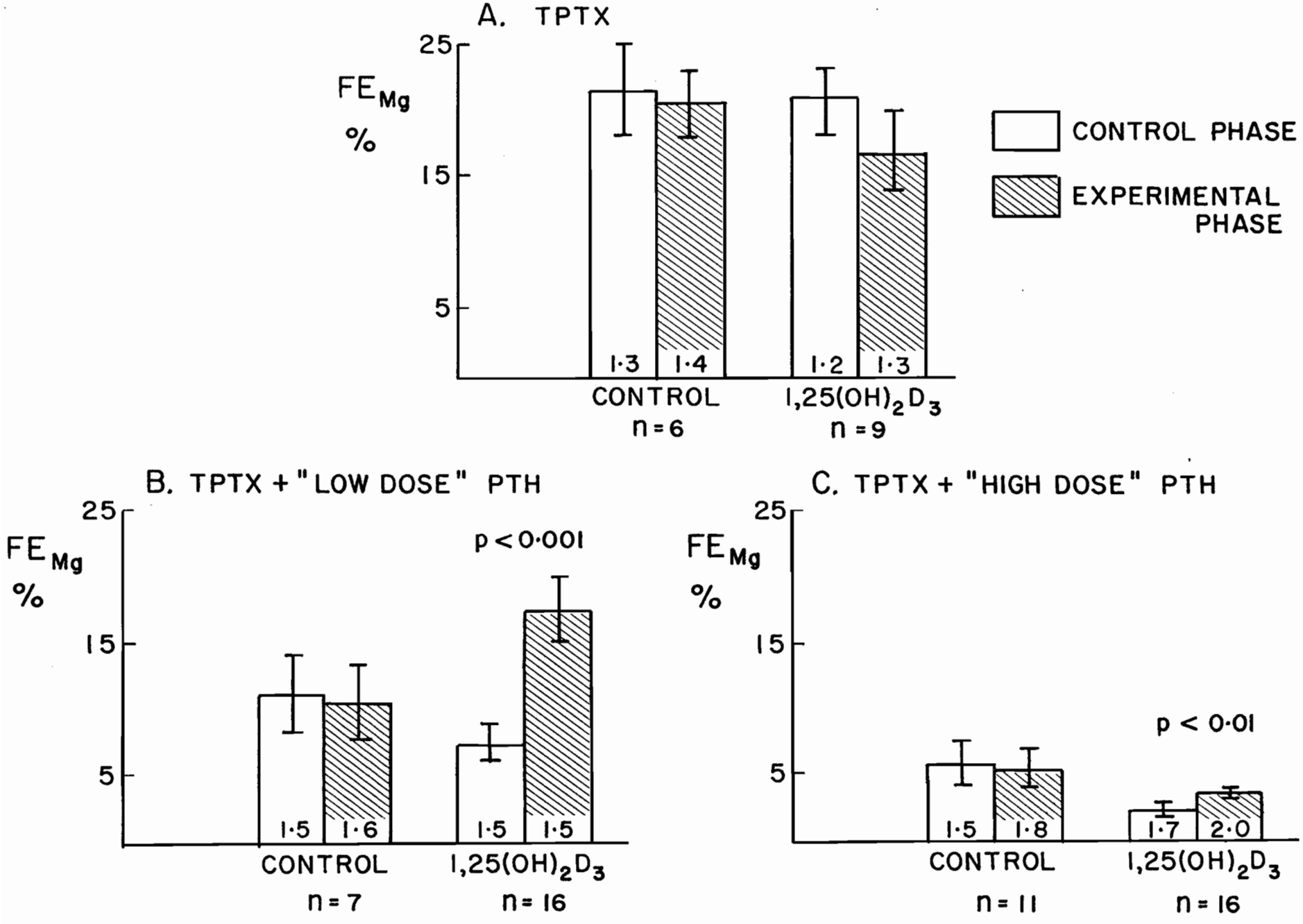

A. TPTX
25
15
5
FE Mg
%
1·3
1·4
1·2
1·3
CONTROL
n = 6
1,25(OH)2D3
n = 9
CONTROL PHASE
EXPERIMENTAL PHASE
B. TPTX + "LOW DOSE" PTH
p < 0·001
1·5
1·6
1·5
1·5
CONTROL
n = 7
1,25(OH)2D3
n = 16
C. TPTX + "HIGH DOSE" PTH
p < 0·01
1·5
1·8
1·7
2·0
CONTROL
n = 11
1,25(OH)2D3
n = 16

Fig. 5. Effects of $1,25(OH)_2D_3$ on fractional magnesium excretion (FEMg) in the hamster. n=number of animals in each group. The numbers inside the bars represent mean plasma magnesium in mEq/l.

to 0.73 and 0.62 to 0.69 μEq/min) (Table IV and Figure 5).

2) TPTX; Low Dose PTH

In the presence of lower doses of PTH, the administration of $1,25(OH)_2 D_3$ led to a significant increase in FEMg from 7.3 to 17.3% ($p<0.001$) while the infusion of vehicle had no effect on FEMg (11.1 to 10.6%). Neither the administration of the vehicle nor $1,25(OH)_2 D_3$ had any significant effect on plasma magnesium (1.5 to 1.6 mEq/L and 1.5 to 1.5 mEq/L respectively) and FLMg (1.34 to 1.38 μEq/min and 1.21 to 1.18 μEq/min respectively) (Table VII and Figure 5).

3) TPTX; High Dose PTH

In the presence of higher doses of PTH, PMg tended to increase throughout the experiment in both the control (1.5 to 1.8 mEq/L, $p<0.001$) and the experimental (1.7 to 2.0 mEq/L, $p<0.001$) groups, while the FLMg did not change significantly. A small but significant increase in the FEMg occurred in the $1.25(OH)_2 D_3$ treated group (2.2 to 5.5%, $p<0.01$) but not in the control group (6.0 to 5.0%) (Table VIII and Figure 5).

e) Effects of $1,25(OH)_2 D_3$ on the Transport of Phosphate

1) TPTX

TPTX greatly reduced FEPi and no further enhancement of Pi reabsorption could be seen following the administration of $1,25(OH)_2 D_3$ (8.5 to 5.5%) or the vehicle

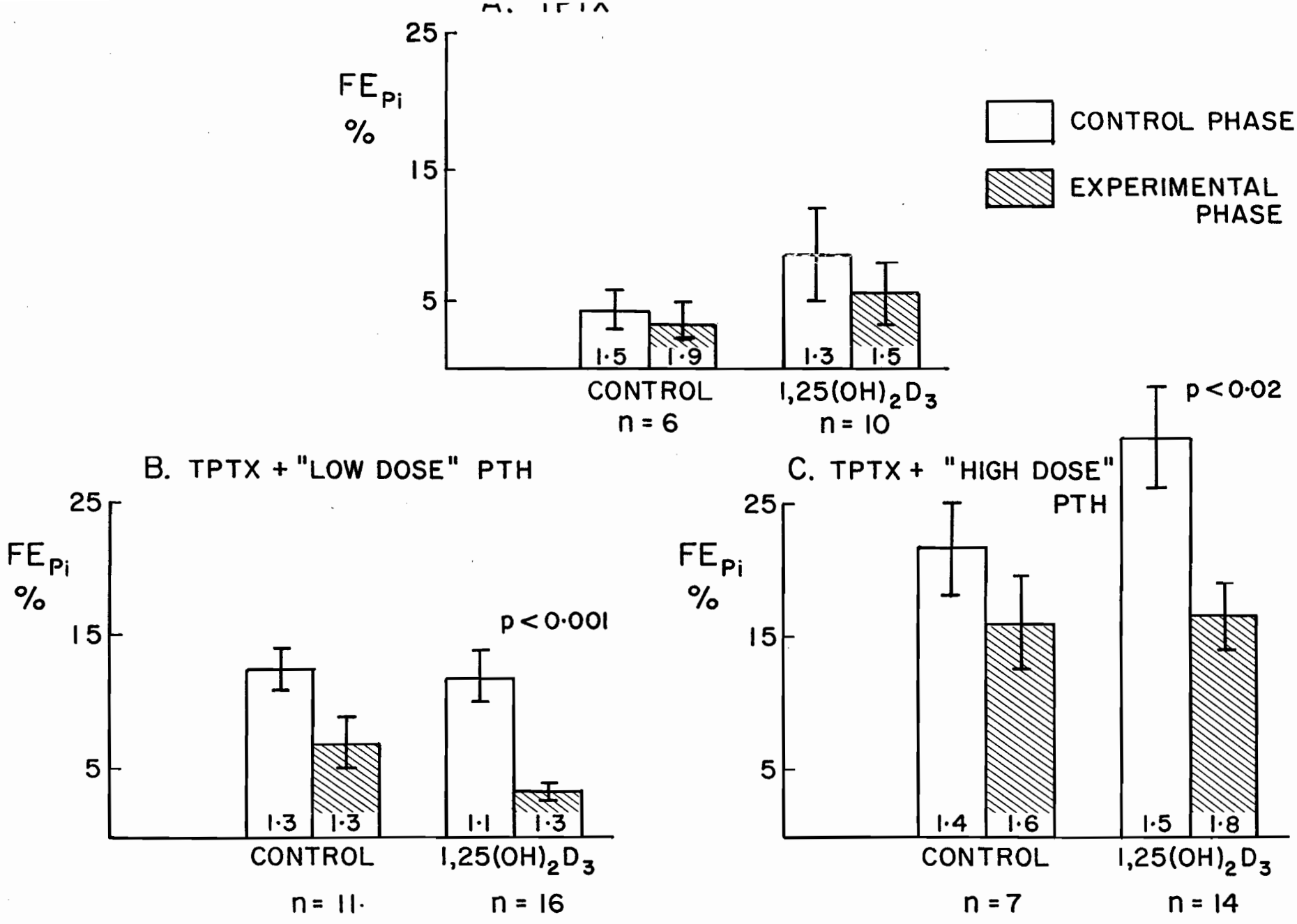

A. TPTX
25
15
5
FE_{Pi} %
CONTROL PHASE
EXPERIMENTAL PHASE
1·5
1·9
1·3
1·5
CONTROL
n = 6
1,25(OH)$_2$D$_3$
n = 10
B. TPTX + "LOW DOSE" PTH
p < 0·001
1·3
1·3
1·1
1·3
CONTROL
n = 11
1,25(OH)$_2$D$_3$
n = 16
C. TPTX + "HIGH DOSE" PTH
p < 0·02
1·4
1·6
1·5
1·8
CONTROL
n = 7
1,25(OH)$_2$D$_3$
n = 14

Fig. 6. Effects of $1,25(OH)_2D_3$ on fractional phosphate excretion in the hamster. n=number of animals in each group. The numbers inside the bars represent mean plasma phosphate in mM.

(4.4 to 3.5%). A significant increase in the plasma Pi (1.5 to 1.9 mM, $p<0.05$), but not FLPi (1.3 to 2.1 μM/min,) was observed in the control group. Changes in the vitamin D treated group were not significant (1.3 to 1.5 mM and 1.0 to 1.1 μM/min, respectively) (Table IV).

2) TPTX and Low Dose PTH

In the presence of low dose PTH, $1,25(OH)_2 D_3$ had an antiphosphaturic effect, as FEPi fell from 11.9 to 3.6% ($p<0.001$) while the changes in the FEPi of the control group were not significant (12.7 to 7.1%). This fall in the FEPi of the experimental group was associated with a small but significant increase in PPi (1.1 to 1.3 mM, $p<0.05$) while there was no significant change in the PPi of the control group (1.3 to 1.3 mM) (Table VII, Figure 6).

3) TPTX and High Dose PTH

Although higher doses of PTH further increased the baseline phosphate excretion, the antiphosphaturic effect of $1,25(OH)_2 D_3$ could still be seen. A fall in the FEPi after $1,25(OH)_2 D_3$ from 29.2 to 16.5% ($p<0.02$) was associated with an increase in plasma Pi from 1.5 to 1.8 mM ($p<0.01$), while the changes in the FEPi and PPi of the control group were not significant (21.9 to 15.8% and 1.4 to 1.6 mM respectively) (Table VIII, Fig. 6).

EFFECTS OF $1,25(OH)_2D_3$ IN THE TPTX, PHOSPHATE INFUSED HAMSTER

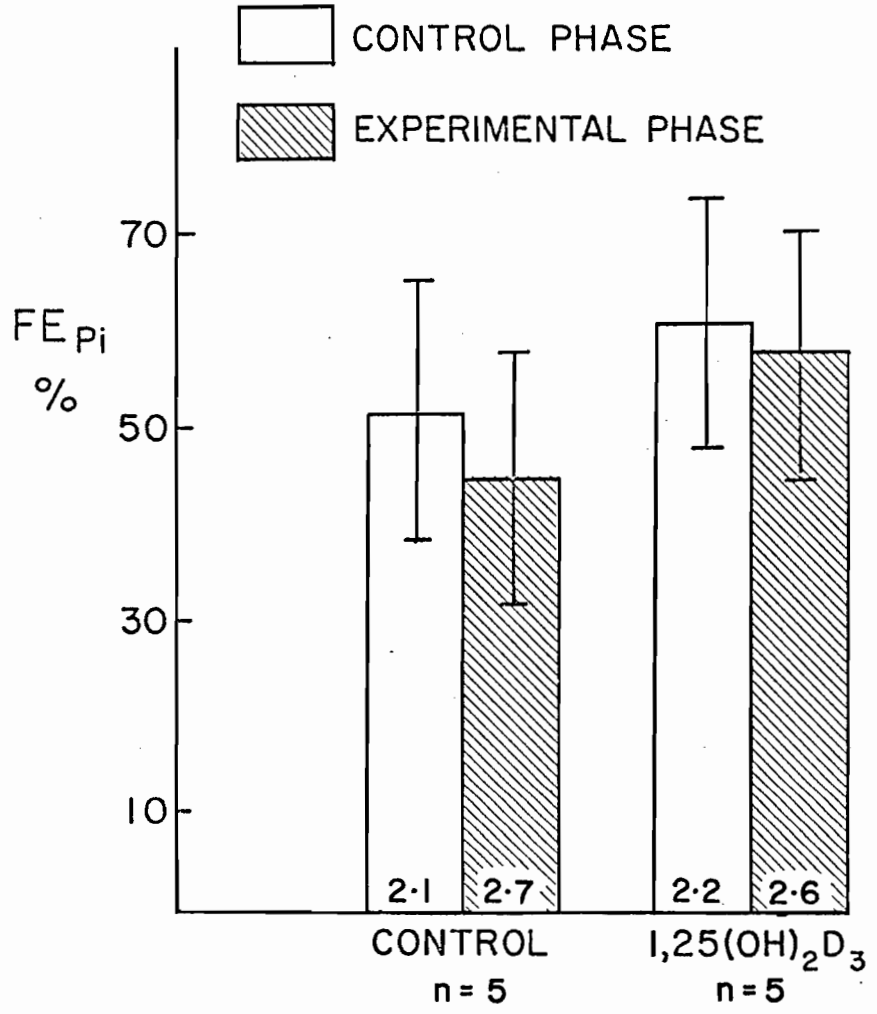


Fig. 7. Effects of $1,25(OH)_2D_3$ on fractional phosphate excretion in the TPTX hamster infused with 50 mM phosphate (NaH_2PO_4: Na_2HPO_4=1:4). n=number of animals per group. Figures inside the bars indicate mean plasma phosphate in mM.

4) TPTX and Phosphate Infusion

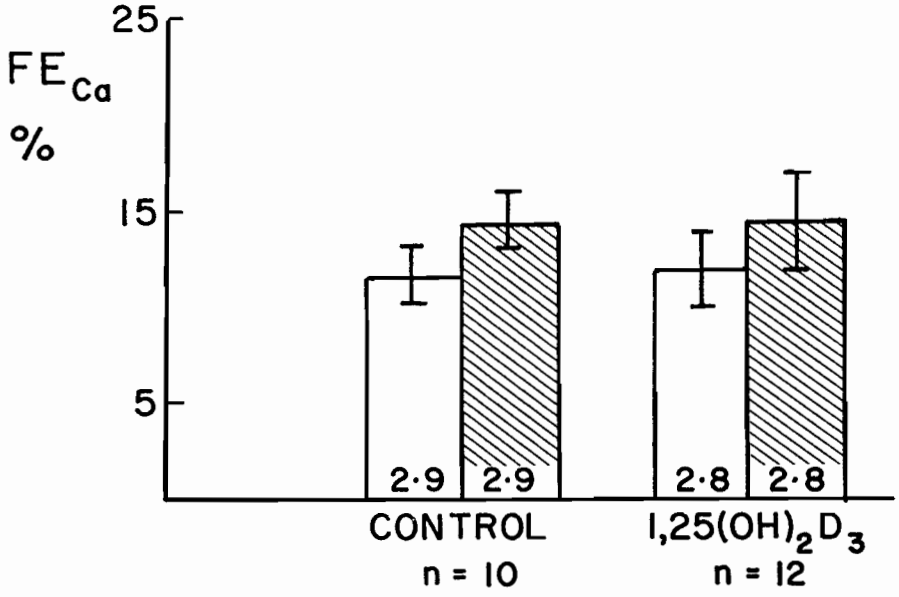
Figure 7 and Table X summarize the effects of $1,25(OH)_2 D_3$ in TPTX hamsters in which the base-line phosphate excretion was elevated by a constant infusion of 50 mM Pi. Although this infusion elevated PPi as well as FEPi, the antiphosphaturic effect of $1,25(OH)_2 D_3$ could not be demonstrated (FEPi; 60.7 to 58.1% after $1,25(OH)_2 D_3$ and 51.2 to 44.9% in the control group).

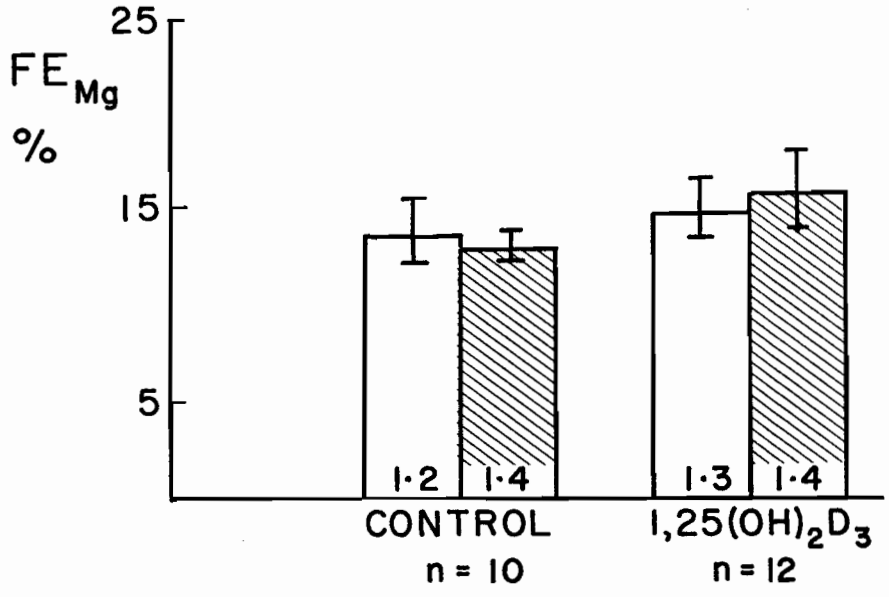
4. Effects of $1,25(OH)_2 D_3$ in TPTX, DBcAMP Infused Hamsters

a) Comparison of the Effects of cAMP, DBcAMP and ClPheS-cAMP in the TPTX Hamster

The effectiveness of cyclic AMP, dibutyryl cAMP, (DBcAMP), and 8(p-chloro-phenyl-thio)cAMP (ClPheScAMP) in reproducing renal effects of parathyroid hormone was tested. The results are summarized in Table XI. Whereas all three analogs appeared to be equally effective in reducing fractional excretion of calcium (cAMP 18.9 to 9.9%, $p<0.01$; DBcAMP 17.5 to 6.9%, $p<0.01$; ClPheScAMP 29.4 to 14.1%, $p<0.02$) and magnesium (cAMP: 20.4 to 13.2%, $p<0.01$, DBcAMP: 21.5 to 8.6% $p<0.01$; ClPheScAMP: 28.3 to 12.1%,$p<0.01$), DBcAMP appeared to be most effective in reproducing the phosphaturic effect of PTH. The administration of DBcAMP increased the FEPi from 2.0 to 12.7%, ($p<0.02$), while cyclic AMP caused a small but significant increase in the fractional phosphate excretion

EFFECTS OF $1,25(OH)_2D_3$ IN THE TPTX HAMSTER GIVEN DBcAMP ($1{\cdot}2 \times 10^{-6}$ M/hr)





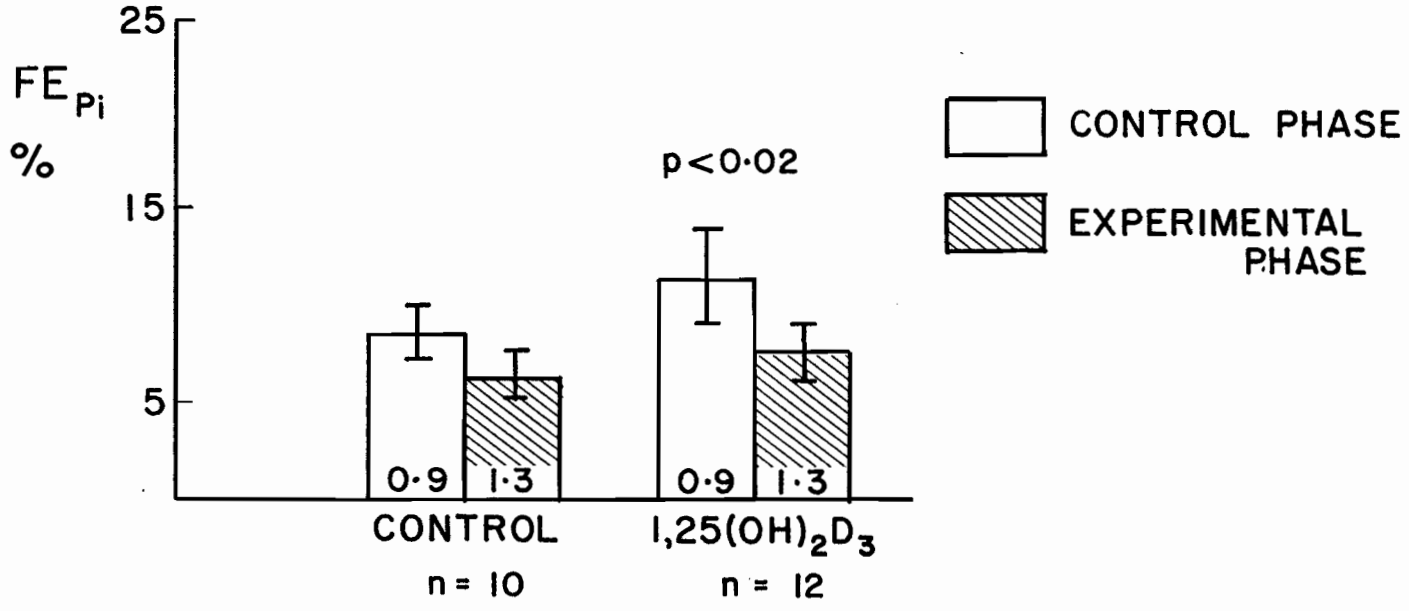


Fig. 8. Effects of $1,25(OH)_2D_3$ on fractional excretion of calcium (FECa), magnesium (FEMg) and phosphate (FEPi) in the TPTX hamsters given DBcAMP (1.2×10^{-6} moles(M)/hr). n-number of animals per group. Numbers inside the bars represent mean plasma calcium (mEq/l), magnesium (mEq/l) and phosphate (mM).

Fig. 8. Effects of $1,25(OH)_2D_3$ on fractional excretion of calcium (FECa), magnesium (FEMg) and phosphate (FEPi) in the TPTX hamsters given DBcAMP (1.2×10^{-6}M/hr). n=number of animals per group. Numbers inside the bars represent mean plasma calcium (mEq/l), magnesium (mEq/l) and phosphate (mM).

from 0.1 to 3.2%, ($p<0.02$), while a small change in the urinary excretion of phosphate after the administration of ClPheScAMP was not significant (FEPi; 1.7to 3.6%). Despite a phosphaturic effect of cAMP and DBcAMP an increase in PPi was also demonstrated in the presence of both nucleotides.

b) Effects of $1,25(OH)_2 D_3$ in the TPTX Hamster Given DBcAMP

The infusion of DBcAMP (1.2×10^{-6} M/hr) throughout the experiment resulted in a decrease in the base-line FECa and FEMg and an increase in FEPi, as compared to the TPTX animals (Group II), but did not affect the tubular reabsorption of these electrolytes to a degree observed with PTH (Group III and IV) (Table XII).

The administration of $1,25(OH)_2 D_3$ to these animals had no significant effect on PCa (2.8 ± 0.2 vs. 2.8 ± 0.2 mEq/L), FLCa (1.84 vs. 1.77 µEq/min) and FECa (12.0 ± 2.1 vs. 14.7 ± 2.1%). The changes in PCa, FLCa and FECa of the control group were not significant either (2.9 to 2.9 mEq/L, 1.77 to 1.96 µEq/min, and 11.7 to 14.4% respectively). Similarly, $1.25(OH)_2 D_3$ had no effect on either PMg (1.3 to 1.4 mEq/L) FLMg (0.88 to 0.93 µEq/min) or FEMg (15.1 to 16.0%). Although FEMg and FLMg of the control group was not significantly changed (13.9 to 13.0% and 0.86to 1.05 µEq/min respectively), a small but significant increase in PMg occurred (1.2 to 1.4 mEq/L, $p<0.02$) in this group.

Plasma phosphate increased significantly in both the control (0.9 to 1.3 mM, $p<0.05$) and the experimental (0.9 to 1.3 mM, $p<0.05$) groups. However, only in the control group was the increase in the FLPi significant. FEPi tended to fall in both groups to the same degree, but only after the administration of $1,25(OH)_2 D_3$ was the change statistically significant (control 8.8 to 6.2%, experimental 11.7 to 7.7%, $p<0.02$). In neither group was the change in FENa significant (Table XII).

5. Effects of $25(OH)_2 D_3$ in TPTX Hamsters in the Presence of Low Dose PTH

Table XIII summarizes the effects of $25(OH)_2 D_3$ in the presence of a low dose of parathyroid hormone.

a) Calcium

Neither the administration of the vehicle nor $25(OH)_2 D_3$ appeared to have any significant effect on the renal handling of calcium. Although a significant fall in the filtered load of Ca occurred in the experimental (3.25 to 2.50 μEq/min, $p<0.05$), probably as a result of a drop in GFR (1.35 to 1.00 ml/min, $p<0.05$), but not the control (2.88 to 2.36 μEq/min) group, the changes in plasma Ca (control; 3.9 to 4.2 mEq/L, experimental; 3.8 to 4.0 mEq/L), and FECa (control; 5.3 to 6.6%, experimental; 4.6 to 8.6%) were not significant.

b) Magnesium

Whereas neither the vehicle nor $25(OH)_2 D_3$ had any effect on the plasma Mg (1.5 to 1.7 mEq/L in both groups) or its filtered load (1.17 to 1.02 µEq/min and 1.39 to 1.18 µEq/min respectively) a significant increase in FEMg was observed after the administration of $25(OH)D_3$ (5.6 to 10.1%, $p<0.05$), but not after the vehicle (7.6 to 7.4%).

c) Phosphate

The changes in the filtered load of Pi in the control group (1.31 to 1.12 µM/min) and the experimental group (1.45 to 1.22 µM/min) were not significant. Plasma phosphate was observed to increase in both groups, but in neither group was this change significant (1.1 to 1.3 mM, and 1.1 to 1.2 mM respectively). The FEPi tended to fall in both groups, but in neither one was this change statistically significant (control 13.1 to 6.2%, experimental 12.6 to 9.2%).

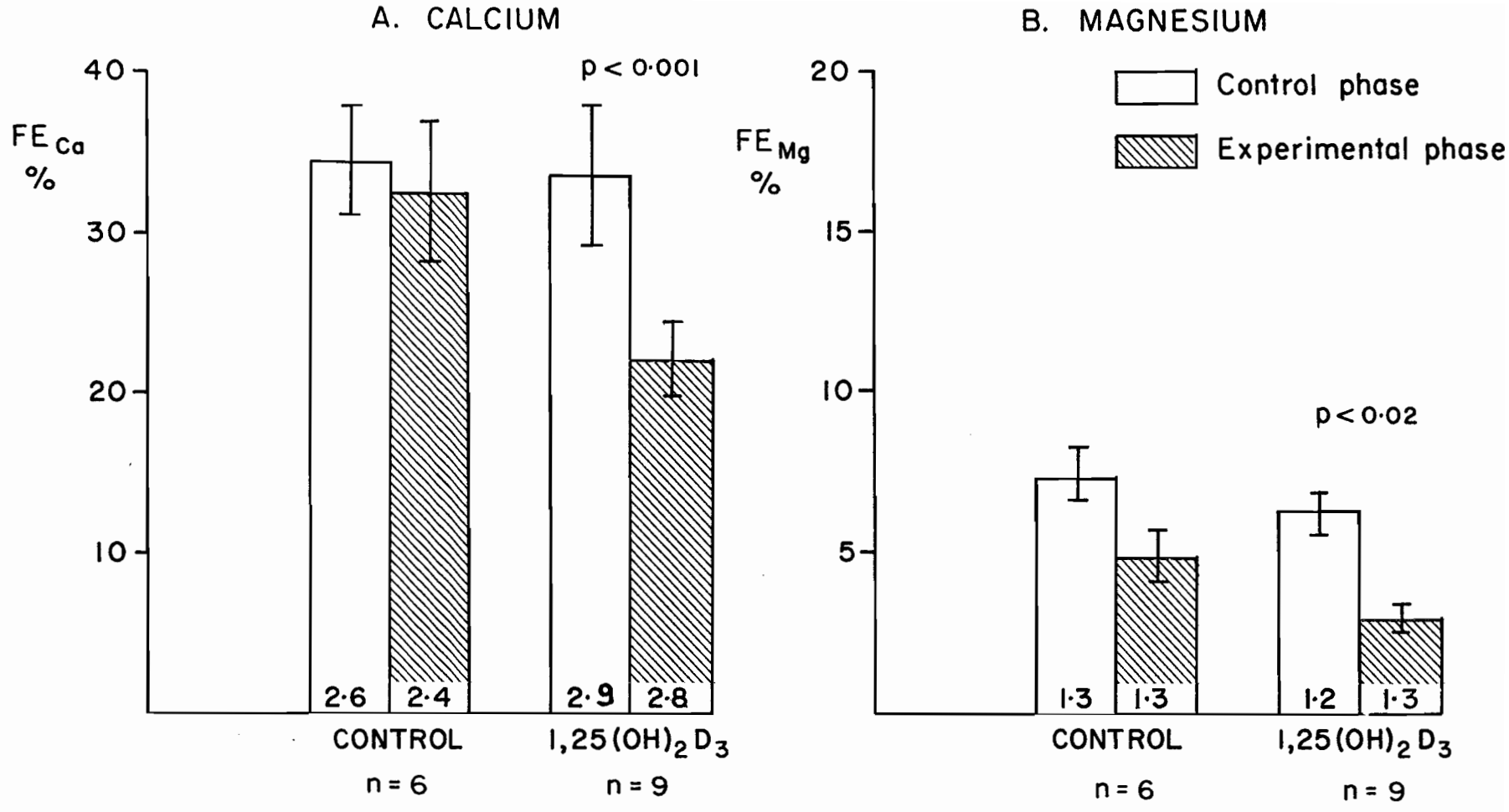

A. CALCIUM
B. MAGNESIUM
Control phase
Experimental phase
FE_{Ca} %
FE_{Mg} %
40
30
20
10
20
15
10
5
p < 0·001
p < 0·02
2·6
2·4
2·9
2·8
1·3
1·3
1·2
1·3
CONTROL
1,25(OH)$_2$D$_3$
n = 6
n = 9

Fig. 9. Effects of $1,25(OH)_2D_3$ in vitamin D deficient, TPTX hamsters on A-fractional calcium excretion, B-fractional magnesium excretion. n=number of animals in each group.

B. Vitamin D Deficient Study

1. Effects of $1,25(OH)_2 D_3$ in D Deficient TPTX Hamsters

The effects of $1,25(OH)_2 D_3$ in vitamin D deficient TPTX hamsters are summarized in Table XIV. Although there was no significant change in either GFR or urine flow between the two phases of each group, a fall in hematocrit and plasma protein in the control but not in the experimental group was significant.

The administration of the vehicle had no significant effect on either plasma Ca (2.6 vs. 2.4 mEq/L) or FECa (34.7 vs. 32.4%), while $1,25(OH)_2 D_3$ reduced FECa significantly (33.3 to 22.1%, $p<0.01$), but not PCa (2.9 to 2.8 mEq/L) (Fig. 9). The low baseline fractional magnesium excretion in both groups as compared to that in non-D deficient TPTX hamsters (Group II) was probably a result of low magnesium content in the diet. The changes in the handling of magnesium after $1,25(OH)_2 D_3$ were similar to those in calcium. While neither plasma Mg nor FEMg of the control group changed significantly (1.3 to 1.3 mEq/L and 7.4 to 4.7% respectively), the administration of $1,25(OH)_2 D_3$ significantly reduced FEMg from 6.5 to 2.9%, ($p<0.02$) while plasma Mg remained unchanged (1.2 to 1.3 mEq/L).

Plasma and urinary levels of phosphate were below levels of detection by the available technique, as in addition to being vitamin D depleted, the animals were Pi depleted as well.

VI. DISCUSSION

Despite numerous studies on the renal effects of vitamin D and its metabolites, its action on the transport of divalent electrolytes is not clearly defined. Evidence suggesting that vitamin D enhances as well as inhibits tubular reabsorption of calcium and phosphate has been presented (Harrison and Harrison 1941, Litvak et al. 1958, Puschett et al. 1972, Costanzo et al. 1974, Harris et al. 1976, Bonjour et al. 1977). The present studies were performed with the objective of clarifying the action of $1,25(OH)_2 D_3$ on the renal transport of calcium, magnesium, and phosphate, and to further elucidate the role of parathyroid hormone in these effects and the mechanism of interaction between the action of PTH and vitamin D.

Previous studies in this laboratory confirmed the initial observations made by Biddulph and co-workers (1970, 1973) regarding the high sensitivity of renal calcium transport to parathyroid hormone in the hamster and the importance of the renal effects of the hormone in the maintenance of plasma calcium within a normal range. Furthermore, the hypocalciuria and hypomagnesiuria of PTH, could be reproduced with either cAMP or DBcAMP in this species (Burnatowska et al. 1977) but not others (Agus et al. 1971, Kuntziger et al. 1974). The hamster, therefore, appeared to be a good model for the proposed investigations. Studies were carried out in the

presence and absence of parathyroid hormone. The dose of $1,25(OH)_2\ D_3$ used was constant in all experiments, whereas the amount of PTH administered was varied.

Physiological Dose of $1,25(OH)_2\ D_3$

The physiological dose of $1,25(OH)_2\ D_3$ has not been established. The available evidence suggests, however, that the dose of 2 units (U) (130) pM) used in this study is probably within the physiological range. In humans, plasma levels of $1,25(OH)_2\ D_3$ have been measured in several laboratories. Haussler and Baylink (1976b) reported levels of 3.3 ng/dl which are similar to 2.9 ng/dl measured by Eisman (1976). In rats fed vitamin D_3 the basal level of serum $1,25(OH)_2\ D_3$ was 17 ng/100 ml (0.41 nM), while levels of 100 ng/100 ml (2.5 nM) of 1,25 dihydroxy-vitamin D_3 were observed in rats that were fed a calcium deficient diet (reported in Chertow et al. 1975). Dose response studies also suggest that the amount of $1,25(OH)_2\ D_3$ used in this study is within the physiological range. Raisz and co-workers (1972) found that an in vitro resorptive response to $1,25(OH)_2\ D_3$ was detectable at about 0.1 nM and that there was a log dose response between 0.1 nM and 10 nM. Finally, Chertow et al. (1975) demonstrated that 130 pM of $1,25(OH)_2\ D_3$ decreased rat plasma levels of PTH in vivo, while doses of 1 to 100 nM of the vitamin were effective in reducing hormone secretion by the parathyroid gland

tissue in vitro. Thus, although it is difficult to be certain of what constitutes a physiological dose of $1,25(OH)_2 D_3$, the dose of 2 units used in this study is probably within the physiological range.

Physiological Dose of Parathyroid Hormone

Circulating parathyroid hormone is heterogeneous and consists of intact hormone and fragments of varying activity and rate of metabolic clearance. The ability to detect PTH levels varies from laboratory to laboratory, as antisera used by different investigators are directed against various immunologic components (Schneider and Sherwood 1974). Despite these complications, however, a good inverse relation exists between the concentrations of calcium and parathyroid hormone in the plasma (Sherwood et al. 1968). It would appear therefore that doses of the hormone that restore normocalcemia to a parathyroidectomized subject are probably within a physiological range.

Parathyroid hormone extract (PTE)-induced reversal of the hypocalcemia and hypercalciuria resulting from parathyroidectomy was investigated in the hamster by Biddulph and Gallimore (1970, 1973). A linear fall in urinary calcium accompanied by a rise in plasma calcium was observed over a dose range of 0.8 to 10 units of PTE. The latter dose was the minimum dose required to reduce calcium excretion levels to those observed in the intact animals. Recently, we were able

to reproduce these effects with a comparable dose (10 U) of highly purified PTH (Burnatowska et al. 1977). In the present study, we also used highly purified PTH. However, several batches of the hormone with variable degrees of purification were used. The degree of purification of the hormone appeared to be an important factor in determining its potency in restoring plasma calcium to a desired level. At comparable doses the less purified batch of the hormone appeared to be more effective than the more purified one in restoring plasma calcium levels (Tables V and VI). However, addition of 0.2% bovine serum albumin to the more purified hormone infusate increased its potency (see Methods p. 86). The effects of the infusion medium upon the physiological response to parathyroid hormone were investigated previously. The prevention of the surface absorption of the hormone by protein has been reported (Rasmussen 1959a, Munson et al. 1961). However, these studies demonstrated that only the potency of a more purified hormone but not that of a crude extract was enhanced by the addition of proteins. It would appear, therefore, that in the present studies the more purified hormone binds to the infusion apparatus, whereas the less purified contains more protein which serves as a carrier for the hormone, thus preventing binding to the apparatus.

Comparison of Intact and TPTX Hamsters

Parathyroid glands are essential for life; their removal results in death due to hypocalcemia if appropriate therapy is not applied. In addition to regulating calcium excretion, PTH is also known to reduce magnesium excretion and to produce phosphaturia in several species (MacIntyre et al. 1963, Pullman et al. 1960, Talmage et al. 1955, Greenwald and Gross 1925). Our study in the hamster further supports these observations. Acute TPTX resulted in a rapid fall in plasma calcium and magnesium concentration accompanied by an increase in the urinary excretion of these electrolytes. The urinary excretion of phosphate was significantly lower in the TPTX group, whereas the difference in plasma phosphate concentration between the two groups was not significant. Although the reason for the lack of an increase in plasma phosphate in this species in the absence of PTH is not clear, it has also been observed by other investigators (Biddulph et al. 1969).

There is some evidence that PTH has a natriuretic effect (Agus et al. 1971). However, removal of parathyroid glands in the hamster appeared to have no significant effect on the urinary excretion of sodium.

Effects of $1,25(OH)_2 D_3$ in the Intact Hamster

Our results from the studies on the renal effects of $1,25(OH)_2 D_3$ in animals with intact parathyroid glands are rather difficult to interpret. Although a fall

in the fractional excretion of phosphate after the administration of $1,25(OH)_2 D_3$ but not the vehicle suggests that vitamin D has an antiphosphaturic effect in the presence of endogenous PTH, a difference in the baseline FEPi complicates the interpretation of these results. The reason for this difference is not clear as both groups of animals were treated similarly. Whereas the hemodynamic changes may account for the increase in FECa and FEMg observed in the control group, as they were associated with an increase in the urine flow rate and FENa, it is also possible that changes in the circulating levels of PTH might be responsible for the difference in the urinary excretion of calcium and magnesium between the control and the experimental group. Several lines of evidence support the concept that $1,25(OH)_2 D_3$ acts on parathyroid glands; 1) Selective localization of $1,25(OH)_2 D_3$ in chick parathyroid-glands has been demonstrated by Henry and Norman (1975), 2) Cytoplasmic and nuclear binding of $1,25(OH)_2 D_3$ in chick parathyroid glands was also reported (Brumbaugh et al. 1975b). 3) Calcium binding protein similar to that identified in the intestine to be vitamin D dependent has also been found in the porcine parathyroid glands (Oldham et al. 1974), 4) Direct influence of $1,25(OH)_2 D_3$ on PTH secretion has been demonstrated. However, whereas Chertow et al. (1975) reported that in rats $1,25(OH)_2 D_3$ inhibited PTH secretion in vivo and in vitro when administered at a dose of 130 pMol (54 ng) and

1 nM respectively, Canterbury et al. (1978) observed that the administration of 250 ng of $1,25(OH)_2 D_3$ doubled the concentration of PTH when measured in the thyroid venous efflux, but not that in the systemic blood. The reason for these differences are not clear. Theoretically $1,25(OH)_2 D_3$ should suppress PTH as a result of a negative feedback mechanism. Otherwise, both hormones would perpetuate each others secretion or activation and lead to a hypercalcemic state. Although some arguments have been presented in an attempt to explain these differences (Canterbury et al. 1978), it is possible that the stimulating effect of $1,25(OH)_2 D_3$ on parathyroid hormone secretion has no physiological relevance as it occurred only when administered in pharmacologic doses. Further studies are required to resolve this conflict.

In view of these findings, therefore, and the rather contradictory results from the studies of the effects of vitamin D on the kidney in the presence of endogenous PTH (Harrison and Harrison 1944, Brickman et al. 1974) further studies were carried out in the TPTX hamsters in which plasma calcium and urinary phosphate levels were altered by the administration of appropriate doses of PTH or infusion of these electrolytes.

The experimental protocol in these studies was such so that the differences between the individual animals, as well as changes independent of the action of PTH

and $1,25(OH)_2 D_3$ could be accounted for. Thus, whereas each animal served as its own control (control phase vs. the experimental phase), each group was divided into two subgroups; a control group A and the experimental group B, to avoid possible influence of such factors as the hemodynamic changes or diurnal variations.

Effects of $1,25(OH)_2 D_3$ in the Presence and Absence of Exogenous PTH

Calcium

The results of the present study regarding the role of $1,25(OH)_2 D_3$ in the tubular transport of calcium are in conflict with previous work. We have shown that in the hamster $1,25(OH)_2 D_3$ increases calcium excretion in the presence of low dose PTH, but not in the TPTX hamsters infused with higher doses of PTH or in the absence of the hormone. As already defined, a "low dose" of PTH was the amount of the hormone that reduced but did not prevent hypocalcemia of TPTX, while "high dose" of the hormone restored the normocalcemia in TPTX animals. To examine the possibility that severe hypocalcemia rather than the absence of PTH could be responsible for the lack of the calciuric response to $1,25(OH)_2 D_3$ in the TPTX hamsters, a group of animals was infused with calcium. However, despite normocalcemia sustained throughout the experiment the infusion of calcium failed to restore the

calciuric response to $1,25(OH)_2 D_3$ seen in the presence of parathyroid hormone. Although Puschett and co-workers (1971, 1972) demonstrated that $25(OH) D_3$ and $1,25(OH)_2 D_3$ have an anticalciuric effect in D replete TPTX dogs, this effect was unselective, as the fall in fractional excretion of calcium was accompanied by a significant fall in the fractional excretion of sodium. Furthermore, vasopressin was administered continuously in these animals. Recent studies of Nseir and co-workers (1978) suggest that the presence of vasopressin in TPTX animals is an absolute requirement for the above effects of vitamin D to be seen. Vasopressin, when administered in pharamcological doses inhibits tubular reabsorption of calcium, magnesium, phosphate, and sodium (Thorn 1960, Wen 1974, Forsling 1976). Thus, it is possible that in addition to having a direct tubular effect on the transport of calcium as suggested by the studies in D deficient animals (Costanzo et al. 1974, Harris et al. 1976), vitamin D may also have an inhibitory effect on the calciuric action of vasopressin (Puschett's study) and anticalciuric action of PTH (this study). It is also possible that this inhibitory action is nonspecific and also affects other polypeptide hormones acting in the kidney.

Magnesium

Little evidence exists regarding the involvement of vitamin D in the tubular transport of magnesium. Although an increase in the urinary excretion of magnesium

was observed after the administration of vitamin D to D deficient TPTX rats by Hanna (1961) these studies are not conclusive as they lack appropriate controls. Furthermore, these were long term experiments and it is possible that some extrarenal mechanisms may be responsible for the observed effect.

Our data suggest that vitamin D influences the renal handling of magnesium in a manner similar to that of calcium. Only in the presence of PTH could the hypermagnesiuric effect of $1,25(OH)_2 D_3$ be demonstrated. The reason for the changes in plasma magnesium that occurred in some groups but not others is not clear. They are unlikely, however, to be responsible for the effects attributed to $1,25(OH)_2 D_3$. Although significant, these were rather small changes, and appeared to occur at random. Furthermore, similar changes in other groups were not statistically significant.

Phosphate

The results of this study suggest that $1,25(OH)_2 D_3$ has an antiphosphaturic effect in the presence of PTH. In the absence of the hormone, regardless of the plasma phosphate levels or the baseline phosphate excretion, no such effect was seen. These results support those of Popovtzer and co-workers (1974) who showed that a decrease in urinary phosphate excretion occurred after the administration

of 25(OH) D_3 and 1,25$(OH)_2$ D_3 only in the presence of PTH. In their studies, neither the correction of the hypocalcemia resulting from TPTX nor the elevation of baseline phosphate excretion by either volume expansion or infusion of phosphate could restore the antiphosphaturia of vitamin D in the absence of PTH. In line with these observations are those of Puschett et al. (1971) who demonstrated that in TPTX dogs, 25(OH) D_3 can reverse the phosphaturic effect of lower (10-30 U/hr) but not higher (50-50 U/hr) doses of PTH.

Furthermore, vitamin D has been reported to interfere with the influence of PTH on the reabsorption of bicarbonate (Siegfried et al. 1977).

Demonstration of an interaction between parathyroid hormone and vitamin D metabolites led to the extension of these studies to other hormones that influence tubular reabsorption of phosphate. Studies of Nseir et al. (1978) suggest that vasopressin may play a role similar to that suggested for parathyroid hormone in the antiphosphaturic effect of either 25(OH) D_3 or 1,25$(OH)_2$ D_3. Only in the presence of vasopressin could the antiphosphaturic action of both metabolites be demonstrated. Similarly, 25(OH) D_3 was demonstrated to interfere with phosphaturic action of calcitonin (Popovtzer et al. 1977) and glucagon (Popovtzer et al. 1978), when tested in D repleted, TPTX rats.

In view of these findings, therefore, the previously postulated direct tubular effect of the metabolites of vitamin D in the D repleted state has to be questioned, as the dogs used in these studies were treated with vasopressin to elevate urinary excretion of phosphate (Puschett et al. 1971).

In summary, it would appear that in vitamin D replete animals 25(OH) D_3 and 1,25(OH)$_2$ D_3 influence tubular transport of phosphate indirectly. Both metabolites probably exert their renal action by inhibiting the direct actions of PTH, vasopressin, calcitonin, glucagon, and possibly other hormones that influence tubular handling of electrolytes.

Mechanism of Action of Vitamin D in the Kidney

The mechanism of the renal action of vitamin D and its metabolites is poorly defined. Whereas synthesis of new mRNA and calcium binding protein (CaBP) has been shown to occur in the kidney in response to the administration of vitamin D (Chen et al. 1973, Morrisey et al. 1974), it has not been localized to particular nephron segments. However, CaBP has been found to be several times more concentrated in the kidney cortex than in the medulla (Sands et al. 1971). This finding, although it may not have any bearing on the effect of vitamin D, is not inconsistent with the suggested site(s) of action of vitamin D.

A possible role of cAMP in the vitamin D dependent transcellular transport of electrolytes must also

be considered, as data suggesting the involvement of the nucleotide at least in the intestinal action of vitamin D have been presented (Corradino et al. 1977). It is possible, however, that the observed changes in the level of cAMP after the administration of vitamin D reflect, not a direct involvement of the nucleotide in the mechanism of action of vitamin D but rather its interaction with parathyroid hormone or other hormones that express their activity via cAMP. Several in vivo studies tend to support this hypothesis. Popovtzer and co-workers (1974, 1977, 1978) demonstrated that in the rat the antiphosphaturic effect of 25(OH) D_3 was associated with a decrease in parathyroid hormone (1974) calcitonin (1977) and glucagon (1978) induced increases in urinary cAMP. In line with these observations are those of Puschett et al. (1974) who showed that the administration of DBcAMP to TPTX rats inhibited the antiphosphaturic effect of 25(OH) D_3. It is possible, however, that changes in the intracellular concentration of calcium rather than DBcAMP could be directly responsible for the observed effect, as the infusion of calcium had a similar effect.

Unlike the PTH, calcitonin, and glucagon antiphosphaturic effect of 25(OH) D_3, vasopressin-dependent antiphosphaturia of 25(OH) D_3 and 1,25(OH)$_2$ D_3 was not associated with any significant change in the urinary levels

of cAMP (Nseir et al. 1978). These studies would therefore suggest that while vitamin D inhibited the action of PTH, calcitonin and glucagon at a site prior to the cAMP production, the action of vasopressin was inhibited at a site distal to the stimulation of adenyl-cyclase-cAMP system. It is also possible that species difference may account for these differences; the former studies were conducted in rats, whereas the latter was done in dogs.

The purpose of this part of our study was to further elucidate the role of cAMP in the renal action of vitamin D, and possible site of interaction with PTH. The butyrated derivative of cAMP was used to replace PTH for several reasons; it was shown to be more permeable in certain tissues (Posternak et al. 1962, Henion et al. 1967), more resistant to phosphodiesterases, and possibly in itself an inhibitor of phosphodiesterase which is responsible for the breakdown of cAMP to the physiologically inert 5'AMP (Heersche et al. 1971). Furthermore, previous studies in this laboratory (Burnatowska et al. 1977) suggested that DBcAMP was more effective than cAMP in mimicking the effect of PTH in the hamster. The butyrated derivative decreased the fractional calcium and magnesium excretion by 66% and 60% respectively, whereas decreases after cAMP were 48% and 36% respectively. Also, DBcAMP appeared to be more effective than ClPheS-cAMP in reproducing the phosphaturia of PTH, at the dose tested (Table

XI). ClPheScAMP, a new analog of cAMP, shown to be many times more effective than DBcAMP in stimulating renal tissue protein kinase activity in vitro (Hall et al. 1977), failed to induce phosphaturia in TPTX hamsters. It is possible, however, that the amount of the analog used is of critical importance in eliciting the physiological response (Wells and Lloyd 1969).

In the present study, continuous infusion of DBcAMP to TPTX hamsters reduced urinary excretion of calcium and magnesium and elevated that of phosphate. However, as in the previous study (Burnatowska et al. 1977) the increase in the total plasma calcium and magnesium observed during parathyroid hormone infusion was not observed with DBcAMP. A rather small increase in plasma magnesium was observed in both the control and the experimental groups, but only in the control group was this change statistically significant. There are several possible reasons for the failure to show a PTH-like effect of cAMP or its derivatives on plasma calcium and magnesium. First, changes in ionized or ultrafilterable calcium (not measured for individual animals) may not be reflected by changes in total plasma calcium; second, the tubular effects on calcium and magnesium are less than with PTH; third, the arbitrary dose used may have been too high or too low, fourth, PTH may cause an increase in plasma calcium and magnesium by some renal or extrarenal mechanism not mediated by cAMP. A fifth possibility is that PTH may increase plasma

calcium and magnesium concentration by activation of adenyl-cyclase system, while other effects of cAMP may tend to decrease the plasma levels of these electrolytes. Certainly cAMP is known to mediate the action of many other hormones, including calcitonin which generally acts in an opposite direction to PTH on calcium metabolism (Heath and Aurbach 1974).

The administration of $1.25(OH)_2 D_3$ in the presence of DBcAMP failed to suppress PTH-like effects of the nucleotide on either calcium or magnesium transport.

Although a small but significant drop in FEPi occurred in the experimental group, it is not clear whether it was a result of the renal action of $1,25(OH)_2 D_3$. A similar, though not significant, change in the FEPi was observed in the control group. Furthermore, the initial plasma phosphate in these animals was lower than in the previous groups, and continued to increase throughout the day. It is possible therefore that low plasma phosphate and possibly the intracellular phosphate levels in this group influenced tubular reabsorption of phosphate independently of the action of DBcAMP or of $1,25(OH)_2 D_3$. The enhancement of phosphate reabsorption in the presence of PTH has been demonstrated to occur in animals that were kept on a phosphate-restricted diet, despite a normal response of adenyl-cyclase-cAMP system to the hormone (Steele 1976). These studies would have to be repeated, therefore,

in animals in which plasma phosphate concentration was elevated, before any definite conclusions regarding the site of interference of $1,25(OH)_2 D_3$ with the phosphaturic effect of PTH can be drawn. Thus, at least with respect to calcium and magnesium, this study suggests that vitamin D may interfere with the action of PTH prior to cAMP production.

Effects of $25(OH) D_3$

Although changes in the renal handling of calcium and phosphate after the administration of $25(OH) D_3$ in the presence of low dose PTH were not significant, a possible effect of this metabolite similar to that observed after $1,25(OH)_2 D_3$ cannot be excluded. It is possible that longer time is required for these effects of $25(OH) D_3$ to be seen to allow for its further metabolism before being biologically active. A small but significant change in the FEMg in the vitamin D treated group, but not in the control group, would support this hypothesis.

Vitamin D Depleted Studies

One drawback to the studies discussed above is that the animals were not D depleted, which may have obscured any intrinsic effect of $1,25(OH)_2 D_3$ on the transport of calcium and phosphate in the absence of PTH. Previous studies in D depleted TPTX rats suggested that vitamin D enhances tubular reabsorption of calcium (Costanzo et al. 1974, Harris

et al. 1976). The effect appeared to be a specific one as the observed changes in the urinary calcium excretion were independent of changes in sodium handling.

The present studies in TPTX, D deficient hamsters, although preliminary, are in agreement with the above experiments. Whereas in D replete, TPTX hamsters no effect of $1,25(OH)_2 D_3$ on calcium and magnesium handling could be seen, in animals that were maintained on a vitamin D deficient diet for about five weeks, the administration of $1,25(OH)_2 D_3$ led to a significant fall in the fractional excretion of calcium and magnesium. Changes in the FECa and FEMg in the control group were not significant.

Costanzo and co-workers (1974) demonstrated that urinary excretion of phosphate in vitamin D deplete rats exceeded that of D replete animals for the filtered load of Pi. In our study, the urinary and plasma phosphate levels were below the level of detection by the available methods.

Thus, the results of this study at least with regard to calcium, further support the notion that in D replete state the effects of physiological doses of $1,25(OH)_2 D_3$ may be obscured by the endogenous levels of vitamin D.

VII. SUMMARY - CONTRIBUTION TO ORIGINAL KNOWLEDGE

The physiological role of vitamin D in the renal handling of electrolytes, unlike that in the gut and the bone, is poorly defined. The objective of the present study, therefore, was to investigate renal actions of $1,25(OH)_2 D_3$ metabolite of vitamin D and their relation to the action of parathyroid hormone (PTH).

We have demonstrated that in a vitamin D replete state, acute administration of $1,25(OH)_2 D_3$ results in the enhancement of the reabsorption of phosphate only in the presence of PTH. In the absence of the hormone, regardless of plasma phoshate levels, $1,25(OH)_2 D_3$ failed to affect phosphate handling. It had a calciuric effect in the presence of PTH at doses that reduced but did not prevent the hypocalcemia of TPTX. In the absence of the hormone, despite sustained normocalcemia, it did not alter the excretion of calcium significantly. Changes in magnesium handling in response to $1,25(OH)_2 D_3$ reflected those in calcium.

In vitamin D deficiency, however, $1,25(OH)_2 D_3$ enhanced reabsorption of calcium and magnesium in the absence of PTH.

It is possible, therefore, that in addition to having a direct tubular effect masked in D repletion, $1,25(OH)_2 D_3$ also has an indirect inhibitory effect on the renal action of PTH or polypeptide hormones in general. Whereas the interference of vitamin D with the phosphaturic action of PTH and other

hormones has previously been demonstrated, this is the first study that suggests a similar interference of $1,25(OH)_2 D_3$ with the action of parathyroid hormone on the reabsorption of calcium and magnesium. Furthermore, the results of the studies involving cyclic necleotides suggest that this interaction of $1,25(OH)_2 D_3$ and PTH occurs prior to cAMP production.

VIII. PROPOSED STUDIES ON THE RENAL ACTION OF VITAMIN D

Whereas the present study answered some of the questions regarding the action of vitamin D on the tubular transport of electrolytes, several questions have been raised and can form the basis for further studies.

Thus, it would be of interest to investigate the site of the observed interaction between the action of $1,25(OH)_2\ D_3$ and that of PTH on the transport of phosphate, calcium and magnesium along the nephron.

Further studies are also required on the mechanism of this interaction between PTH and $1,25(OH)_2\ D_3$ in the kidney. It has been demonstrated that $25(OH)\ D_3$ is capable of blunting not only the phosphaturic effect of PTH but also of inhibiting the increased excretion of cAMP. Similar studies could be done to investigate whether such changes in the urinary excretion of cAMP are also associated with the inhibition of hypocalciuric and hypomagnesiuric effect of PTH after the administration of $1,25(OH)_2\ D_3$.

In the present study, whereas $1,25(OH)_2\ D_3$ inhibited the phosphaturic effect of various doses of PTH, it only suppressed the effect of a low dose of PTH on the transport of calcium. The hypermagnesiuric effect of $1,25(OH)_2\ D_3$ could be seen at both doses of PTH. However, it was greatly reduced in the presence of a higher dose of PTH. It is possible, therefore, that the reduced or absent effect of $1,25(OH)_2\ D_3$ on the

tubular transport of calcium and magnesium at higher levels of PTH may be a result of $1,25(OH)_2 D_3$ levels being too low. Thus, the studies with higher doses of $1,25(OH)_2 D_3$ may help to resolve this problem.

Finally, the studies regarding the effects of $1,25(OH)_2 D_3$ on the transport of divalent electrolytes in vitamin D deficient, TPTX state would have to be repeated at the micropuncture level to localize the site of a direct action of vitamin D along the nephron.

TABLE I. EXPERIMENTAL PROTOCOL

GROUP		n	PARATHYROID STATUS	EXPERIMENTAL PHASE
I	A	8	INTACT	ETHANOL
	B	8		$1,25(OH)_2D_3$
II	A	8	TPTX	ETHANOL
	B	13		$1,25(OH)_2D_3$
III	A	11	TPTX + LOW DOSE PTH	ETHANOL
	B	16	(2U/hr of 569U/mg + 0.8U of 336U/mg)	$1,25(OH)_2D_3$
IV	A	7	TPTX + HIGH DOSE PTH	ETHANOL
	B	16	(3.3U/hr of 569U/mg + 2U of 336U/mg)	$1,25(OH)_2D_3$
V	A	6	TPTX + $CaCl_2$	ETHANOL
	B	7	(75 mEq/l at 0.029 ml/min)	$1,25(OH)_2D_3$
VI	A	4	TPTX + PHOSPHATE	ETHANOL
	B	5	(50mM Na_2HPO_4: NaH_2PO_3=4:1 at 0.029 ml/min)	$1,25(OH)_2D_3$

Cont'd...

TABLE I CONT'D

GROUP		n	PARATHYROID STATUS	EXPERIMENTAL PHASE
VII	A	10	TPTX + DBcAMP (0.6 mg/hr)	ETHANOL
	B	12		$1,25(OH)_2D_3$
VIII	A	7	TPTX + LOW DOSE PTH	ETHANOL
	B	10		$25(OH)D_3$
IX	A	6	VITAMIN D DEFICIENT	ETHANOL
	B	9	TPTX	$1,25(OH)_2D_3$

n: number of animals; TPTX: thyroparathyroidectomized; A: control group; B: experimental group.

TABLE II. COMPARISON OF INTACT AND TPTX HAMSTERS

	INTACT n=16	TPTX n=17	p<
GFR ml/min	0.86 ± 0.11	0.78 ± 0.05	N.S.
V µl/min	3.4 ± 0.5	4.4 ± 0.4	N.S.
PCa mEq/l	4.7 ± 0.1	2.8 ± 0.1	0.001
PMg mEq/l	1.7 ± 0.1	1.2 ± 0.1	0.001
PPi mM	1.1 ± 0.2	1.3 ± 0.2	N.S.
PNa mEq/l	140.8 ± 2.2	139.1 ± 1.6	N.S.
FECa %	2.4 ± 0.4	19.8 ± 1.7	0.001
FEMg %	4.7 ± 0.7	21.2 ± 1.8	0.001
FEPi %	22.8 ± 4.0	4.9 ± 1.3	0.001
FENa %	1.05 ± 0.20	0.82 ± 0.20	N.S.

values are means ±S.E.; n: total number of hamsters.

abbreviations: GFR: glomerular filtration rate; V: urine flow rate; P: plasma; FE: fractional excretion

N.S.: not significant.

TABLE III. EFFECTS OF $1,25(OH)_2D_3$ IN THE INTACT HAMSTER

	GROUP IA INTACT + ETHANOL			GROUP IB INTACT + $1,25(OH)_2D_3$		N=8
	C	E	P<	C	E	P
Hct %	49.4 ± 1.0	48.3 ± 1.2	N.S.	53.6 ± 1.1	50.0 ± 1.0	0.02
Prt g %	4.4 ± 0.1	4.0 ± 0.2	0.02	4.6 ± 0.1	4.0 ± 0.1	0.01
GFR ml/min	0.93 ± 0.18	0.87 ± 0.16	N.S.	0.72 ± 0.14	0.72 ± 0.11	N.S.
V µl/min	2.9 ± 0.5	3.9 ± 1.0	N.S.	3.9 ± 0.9	2.6 ± 0.4	N.S.
P Ca mEq/l	4.6 ± 0.2	4.5 ± 0.2	N.S.	4.8 ± 0.3	4.8 ± 0.2	N.S.
FL Ca µEq/min	2.63 ± 0.45	2.41 ± 0.39	N.S.	2.23 ± 0.47	2.11 ± 0.24	N.S.
FE Ca %	2.2 ± 0.3	4.0 ± 0.7	0.02	2.6 ± 0.8	2.7 ± 0.5	N.S.
PMg mEq/l	1.8 ± 0.2	2.0 ± 0.2	N.S.	1.9 ± 0.1	1.9 ± 0.1	N.S.
FL Mg µEq/min	1.13 ± 0.19	1.09 ± 0.17	N.S.	1.26 ± 0.28	1.16 ± 0.11	N.S.
FEMg %	4.6 ± 0.8	10.8 ± 2.0	0.01	4.8 ± 1.4	7.1 ± 1.0	N.S.
PPi mM	1.1 ± 0.2	1.2 ± 0.2	N.S.	1.1 ± 0.1	1.1 ± 0.2	N.S.
FL Pi µM/min	1.04 ± 0.20	1.00 ± 0.09	N.S.	0.72 ± 0.17	0.72 ± 0.07	N.S.
FE Pi %	13.8 ± 4.4	15.4 ± 7.0	N.S.	38.3 ± 5.5	15.9 ± 3.5	0.01
FE Na	0.9 ± 0.2	1.4 ± 0.4	N.S.	1.3 ± 0.2	0.8 ± 0.2	N.S.

C: control phase; E: experimental phase; Hct: haematocrit; Prt: plasma protein; FL: calulated filtered load; other abbreviations as in Table II.

TABLE IV. EFFECTS OF $1,25(OH)_2D_3$ IN THE TPTX HAMSTER

	GROUP IIA TPTX + ETHANOL C	GROUP IIA TPTX + ETHANOL E	(n=8) P<	GROUP IIB TPTX + $1,25(OH)_2D_3$ C	GROUP IIB TPTX + $1,25(OH)_2D_3$ E	(n=13) P<
Hct %	51.8 ± 0.6	50.4 ± 0.4	N.S.	53.1 ± 0.7	52.3 ± 0.7	0.02
Prt g%	4.4 ± 0.2	4.0 ± 0.3	0.02	4.5 ± 0.2	4.1 ± 0.2	0.001
GFR ml/min	0.94 ± 0.11	0.88 ± 0.16	N.S.	0.77 ± 0.07	0.79 ± 0.09	N.S.
V µl/min	5.4 ± 0.7	4.0 ± 0.8	N.S.	3.7 ± 0.5	3.3 ± 0.2	N.S.
P Ca mEq/l	2.5 ± 0.1	2.4 ± 0.1	N.S.	2.9 ± 0.1	2.8 ± 0.1	N.S.
FL Ca µEq/min	1.52 ± 0.14	1.36 ± 0.18	N.S.	1.45 ± 0.14	1.38 ± 0.14	N.S.
FE Ca %	22.0 ± 2.8	22.6 ± 3.3	N.S.	18.5 ± 2.1	19.4 ± 2.6	N.S.
P Mg mEq/l	1.3 ± 0.1	1.4 ± 0.1	N.S.	1.2 ± 0.1	1.3 ± 0.1	N.S.
FL Mg µEq/min	0.79 ± 0.07	0.73 ± 0.10	N.S.	0.62 ± 0.08	0.69 ± 0.10	N.S.
FE Mg %	21.5 ± 3.3	20.3 ± 2.4	N.S.	21.0 ± 1.9	16.6 ± 2.9	N.S.
PP_i mM	1.5 ± 0.3	1.9 ± 0.4	0.05	1.3 ± 0.2	1.5 ± 0.1	N.S.
FL Pi µM/min	1.33 ± 0.29	2.07 ± 0.45	N.S.	0.98 ± 0.18	1.13 ± 0.19	N.S.
FE Pi %	4.4 ± 1.7	3.5 ± 1.8	N.S.	8.5 ± 3.1	5.5 ± 2.7	N.S.

abbreviations as in Table III.

TABLE V. COMPARISON OF THE EFFECTS OF DIFFERENT BATCHES OF PTH—"LOW DOSE"

	TPTX + PTH (UNITS/HR)		
	2U of 569U/mg	0.8U of 336U/mg	p<
GFR ml/min	1.2 ± 0.3	1.1 ± 0.1	N.S.
V µl/min	4.4 ± 1.1	4.5 ± 0.5	N.S.
PCa mEq/l	4.0 ± 0.5	3.9 ± 0.4	N.S.
PMg mEq/l	1.4 ± 0.1	1.4 ± 0.9	N.S.
PPi mM	1.2 ± 0.2	1.1 ± 0.1	N.S.
FECa %	2.3 ± 0.3	6.4 ± 2.0	N.S.
FEMg %	6.0 ± 1.5	9.1 ± 2.3	N.S.
FEPi %	11.7 ± 3.3	11.1 ± 2.7	N.S.

abbreviation as in Table III.

TABLE VI. COMPARISON OF THE EFFECTS OF DIFFERENT BATCHES OF PTH—"HIGH DOSE"

	TPTX + PTH (UNITS/HR)		
	3.3-5U/hr	2-3U/hr	
	569U/mg	336U/mg	p<
GFR ml/min	1.2 ± 0.2	1.1 ± 0.1	N.S.
V µl/min	3.9 ± 0.5	3.5 ± 0.1	N.S.
PCa mEq/l	4.4 ± 0.3	4.4 ± 0.1	N.S.
PMg mEq/l	1.6 ± 0.1	1.8 ± 0.1	<0.01
PPi mM	0.9 ± 0.2	1.0 ± 0.1	N.S.
FECa %	1.8 ± 0.3	1.5 ± 0.4	N.S.
FEMg %	2.7 ± 0.5	1.8 ± 0.6	N.S.
FEPi %	29.9 ± 6.4	32.1 ± 8.4	N.S.

abbreviations as in Table III.

TABLE VII. EFFECTS OF 1,25(OH)$_2$D$_3$ IN THE TPTX HAMSTER IN PRESENCE OF LOW DOSE PTH

	GROUP IIIA TPTX + PTH(LOW DOSE) + ETHANOL (n=11)			GROUP IIIB TPTX + PTH(LOW DOSE) + 1,25(OH)$_2$D$_3$ (n=16)		
	C	E	P<	C	E	P<
Hct %	53.1 ± 1.2	52.1 ± 2.0	N.S.	53.7 ± 0.8	52.7 ± 0.9	0.02
Prt g%	4.3 ± 0.2	4.3 ± 0.2	N.S.	4.6 ± 0.2	4.2 ± 0.1	0.01
GFR ml/min	1.00 ± 0.10	1.09 ± 0.09	N.S.	1.06 ± 0.09	1.07 ± 0.12	N.S.
V µl/min	4.0 ± 0.5	3.9 ± 0.4	N.S.	4.5 ± 0.6	4.7 ± 0.5	N.S
P Ca mEq/l	3.8 ± 0.2	3.8 ± 0.2	N.S.	3.9 ± 0.2	3.8 ± 0.2	N.S.
FL Ca µEq/min	2.32 ± 0.25	2.57 ± 0.16	N.S.	2.62 ± 0.25	2.66 ± 0.39	N.S.
FE Ca %	5.6 ± 1.3	7.5 ± 2.1	N.S.	5.2 ± 1.4	13.2 ± 2.2	0.001
P Mg mEq/l	1.5 ± 0.1	1.6 ± 0.1	N.S.	1.5 ± 0.1	1.5 ± 0.1	N.S.
FL Mg µEq/min	1.34 ± 0.26	1.38 ± 0.11	N.S.	1.21 ± 0.10	1.18 ± 0.13	N.S.
FE Mg %	11.1 ± 3.1	10.6 ± 2.7	N.S.	7.3 ± 1.3	17.3 ± 2.2	0.001
PP_i mM	1.3 ± 0.2	1.3 ± 0.2	N.S.	1.1 ± 0.1	1.3 ± 0.1	0.05
FL P_i µM/min	1.39 ± 0.22	1.30 ± 0.10	N.S.	1.15 ± 0.11	1.23 ± 0.11	N.S.
FE P_i %	12.7 ± 1.9	7.1 ± 2.1	N.S.	11.9 ± 2.1	3.6 ± 0.9	0.001
FE Na %	1.1 ± 0.1	0.8 ± 0.2	N.S.	0.9 ± 0.2	0.9 ± 0.2	N.S.

abbreviations as in Table III.

TABLE VIII. EFFECTS OF $1,25(OH)_2D_3$ IN PRESENCE OF HIGH DOSE PTH

	GROUP IVA TPTX + HIGH DOSE PTH + ETHANOL (n=7)			GROUP IVB TPTX + HIGH DOSE PTH + $1,25(OH)_2D_3$ (n=16)		
	C	E	P<	C	E	P<
Hct %	53.6 ± 0.7	50.9 ± 1.1	0.01	52.7 ± 0.8	51.5 ± 0.6	0.02
Prt g%	4.3 ± 0.1	3.9 ± 0.2	0.05	4.3 ± 0.1	3.9 ± 0.1	0.001
GFR ml/min	1.10 ± 0.16	0.84 ± 0.08	N.S.	1.17 ± 0.07	1.09 ± 0.08	N.S.
V µl/min	6.4 ± 1.5	4.6 ± 0.9	N.S.	3.9 ± 0.3	4.4 ± 0.5	N.S.
P Ca mEq/l	4.2 ± 0.1	4.7 ± 0.2	0.01	4.4 ± 0.1	4.9 ± 0.2	0.001
FL Ca µEq/min	3.01 ± 0.60	2.52 ± 0.41	N.S.	3.13 ± 0.14	3.62 ± 0.25	N.S.
FE Ca %	3.1 ± 0.8	2.8 ± 0.8	N.S.	1.7 ± 0.2	2.7 ± 0.6	N.S.
P Mg mEq/l	1.5 ± 0.1	1.8 ± 0.1	0.001	1.7 ± 0.1	1.95 ± 0.1	0.001
FL Mg µEq/min	1.14 ± 0.20	0.91 ± 0.09	N.S.	1.39 ± 0.06	1.50 ± 0.10	N.S.
FE Mg %	6.0 ± 1.8	5.6 ± 1.9	N.S.	2.2 ± 0.4	5.5 ± 1.0	0.01
PP_i mM	1.4 ± 0.2	1.6 ± 0.1	N.S.	1.5 ± 0.2	1.8 ± 0.2	0.01
FL Pi µM/min	1.65 ± 0.40	1.15 ± 0.05	N.S.	1.74 ± 0.04	1.96 ± 0.02	N.S.
FE Pi %	21.9 ± 4.2	15.8 ± 3.5	N.S.	29.2 ± 4.0	16.5 ± 2.5	0.02
FE Na %	1.6 ± 0.4	1.4 ± 0.4	N.S.	1.1 ± 0.1	1.2 ± 0.2	N.S.

abbreviations as in Table III.

TABLE IX. EFFECTS OF $1,25(OH)_2D_3$ IN THE TPTX, CALCIUM INFUSED HAMSTER

	GROUP VA TPTX + $CaCl_2$ + ETHANOL			GROUP VB TPTX + $CaCl_2$ + $1,25(OH)_2D_3$		
	C	E	P< (n=6)	C	E	P (n=7)
Hct %	53.0 ± 0.8	51.6 ± 1.2	N.S.	51.8 ± 0.4	50.4 ± 1.6	N.S.
Prt g%	4.5 ± 0.2	4.0 ± 0.1	<0.02	4.7 ± 0.1	3.9 ± 0.1	<0.01
GFR ml/min	1.1 ± 0.1	1.1 ± 0.1	N.S.	1.0 ± 0.1	1.0 ± 0.1	N.S.
V µl/min	6.6 ± 1.6	6.9 ± 1.0	N.S.	5.2 ± 0.6	8.3 ± 2.0	N.S.
PCa mEq/l	4.3 ± 0.4	4.3 ± 0.3	N.S.	4.6 ± 0.3	4.7 ± 0.3	N.S.
FL Ca µEq/min	3.1 ± 0.5	3.1 ± 0.4	N.S.	2.9 ± 0.3	3.1 ± 0.3	N.S.
FE Ca %	35.3 ± 4.5	43.8 ± 2.3	N.S.	39.5 ± 3.0	47.8 ± 5.2	N.S.
PMg mEq/l	1.2 ± 0.1	1.2 ± 0.1	N.S.	1.1 ± 0.02	1.2 ± 0.03	<0.02
FL Mg µEq/min	0.9 ± 0.1	0.9 ± 0.1	N.S.	0.8 ± 0.1	0.8 ± 0.1	N.S.
FE Mg %	22.2 ± 3.3	21.0 ± 3.7	N.S.	27.2 ± 2.2	22.2 ± 3.8	N.S.
FE Na %	1.1 ± 0.3	1.3 ± 0.4	N.S.	1.0 ± 0.1	1.7 ± 0.3	N.S.

abbreviations as in Table III.

TABLE X. EFFECTS OF 1,25(OH)$_2$D$_3$ IN THE TPTX PHOSPHATE INFUSED HAMSTER

	GROUP VIA TPTX + 50mM PHOSPHATE (n=4)			GROUP VIB TPTX + 50mM PHOSPHATE + 1,25(OH)$_2$D$_3$ (n=5)		
	C	E	P	C	E	P
Hct %	52.0 ± 1.1	53.0 ± 1.2	N.S.	52.3 ± 0.9	50.3 ± 1.3	N.S.
Prt g%	4.7 ± 0.2	4.2 ± 0.2	<0.02	4.2 ± 0.2	3.9 ± 0.2	N.S.
GFR ml/min	0.8 ± 0.2	0.8 ± 0.1	N.S.	0.7 ± 0.1	0.8 ± 0.2	N.S.
V µl/min	4.0 ± 0.1	4.4 ± 1.2	N.S.	3.5 ± 0.6	5.1 ± 1.3	N.S.
P Ca mEq/l	2.6 ± 0.2	2.6 ± 0.2	N.S.	2.6 ± 0.2	2.5 ± 0.2	N.S.
PPi mM	2.1 ± 0.5	2.7 ± 0.7	N.S.	2.2 ± 0.4	2.6 ± 0.5	N.S.
FLPi µM/min	1.9 ± 0.5	2.1 ± 0.9	N.S.	1.7 ± 0.5	2.5 ± 0.9	N.S.
FEPi %	51.2 ± 20.1	44.9 ± 13.1	N.S.	60.7 ± 12.9	58.1 ± 12.7	N.S.

abbreviations as in Table III.

TABLE XI. COMPARISON OF THE EFFECTS OF cAMP, DBcAMP AND Cl-Phe-S-cAMP IN THE TPTX HAMSTER*

	TPTX ± cAMP		n=8	TPTX ± DBcAMP		n=9
	C	E	P	C	E	P
GFR ml/min	0.62 ± 0.12	0.75 ± 0.14	N.S.	0.50 ± 0.06	0.64 ± 0.04	N.S.
V ul/min	1.9 ± 0.2	2.1 ± 0.2	N.S.	3.1 ± 0.6	2.7 ± 0.3	N.S.
PCa mEq/l	3.0 ± 0.1	2.8 ± 0.2	N.S.	3.5 ± 0.1	3.3 ± 0.1	0.02
FECa %	18.9 ± 2.5	9.9 ± 1.9	0.01	17.5 ± 2.9	6.9 ± 1.6	0.01
PMg mEq/l	1.6 ± 0.2	1.7 ± 0.2	N.S.	1.2 ± 0.1	1.3 ± 0.3	N.S.
FEMg %	20.4 ± 1.9	13.2 ± 2.5	0.01	21.5 ± 3.6	8.6 ± 1.2	0.01
PPi mM	1.6 ± 0.2	2.3 ± 0.3	0.02	1.4 ± 0.2	1.6 ± 0.2	0.02
FEPi %	0.1 ± 0.1	3.2 ± 0.9	0.02	2.0 ± 0.1	12.7 ± 2.6	0.01

CONT'D...

TABLE XI CONT'D

	TPTX ± Cl-Phe-S-cAMP		n=5
	C	E	P
GFR ml/min	0.78 ± 0.05	0.82 ± 0.11	N.S.
V ul/min	4.0 ± 0.7	6.6 ± 2.1	N.S.
PCa mEq/l	2.9 ± 0.1	2.9 ± 0.2	N.S.
FECa %	29.4 ± 3.4	14.1 ± 2.5	0.02
PMg mEq/l	1.3 ± 0.1	1.5 ± 0.5	N.S.
FEMg %	28.3 ± 4.7	12.1 ± 1.6	0.01
PPi mM	0.8 ± 0.1	1.1 ± 0.1	N.S.
FEPi %	1.7 ± 0.1	3.6 ± 0.2	N.S.

*Part of the results on the effects of cAMP and DBcAMP have been published in A.J.P. 233: 514-518 (1977).

abbreviations as in Table III.

TABLE XII. EFFECTS OF $1,25(OH)_2D_3$ IN THE TPTX, DBcAMP INFUSED HAMSTER

	GROUP VIIA TPTX + DBcAMP (n=10)			GROUP VIIB TPTX + DBcAMP + $1,25(OH)_2D_3$ (n=12)		
	C	E	P<	C	E	P<
Hct %	54.9 ± 0.8	53.9 ± 1.0	N.S.	50.1 ± 2.1	50.0 ± 2.1	N.S.
Prt g%	4.6 ± 0.2	4.2 ± 0.2	N.S.	4.1 ± 0.1	4.0 ± 0.2	N.S.
GFR ml/min	0.97 ± 0.05	1.04 ± 0.13	N.S.	1.04 ± 0.08	1.01 ± 0.09	N.S.
V µl/min	6.3 ± 1.7	4.0 ± 0.5	N.S.	6.4 ± 1.1	4.1 ± 0.5	N.S.
PCa mEq/l	2.9 ± 0.2	2.9 ± 0.2	N.S.	2.8 ± 0.2	2.8 ± 0.2	N.S.
FLCa µEq/min	1.77 ± 0.14	1.96 ± 0.32	N.S.	1.84 ± 0.17	1.77 ± 0.19	N.S.
FECa %	11.7 ± 1.4	14.4 ± 1.8	N.S.	12.0 ± 2.1	14.7 ± 2.1	N.S.
PMg mEq/l	1.2 ± 0.1	1.4 ± 0.1	0.02	1.3 ± 0.1	1.4 ± 0.1	N.S.
FLMg µEq/min	0.86 ± 0.06	1.05 ± 0.14	N.S.	0.88 ± 0.07	0.93 ± 0.08	N.S.
FEmg %	13.9 ± 1.7	13.0 ± 0.7	N.S.	15.1 ± 1.7	16.0 ± 2.3	N.S.
PPi mM	0.9 ± 0.1	1.3 ± 0.1	0.05	0.9 ± 0.1	1.3 ± 0.2	0.05
FLPi µM/min	0.86 ± 0.06	1.21 ± 0.07	0.01	0.99 ± 0.11	1.22 ± 0.13	N.S.
FEPi %	8.8 ± 1.5	6.2 ± 1.4	N.S.	11.7 ± 2.3	7.7 ± 1.6	0.02
PNa mEq/l	145.5 ± 5.3	142.7 ± 5.4	N.S.	143.0 ± 2.5	145.7 ± 6.7	N.S.
FENa %	0.8 ± 0.2	0.8 ± 0.3	N.S.	0.9 ± 0.3	0.7 ± 0.2	N.S.

abbreviations as in Table III.

TABLE XIII. EFFECTS OF 25(OH)D_3 IN PRESENCE OF LOW DOSE PTH

GROUP VIII

	TPTX + PTH (LOW DOSE) + ETHANOL (n=7)			TPTX + PTH (LOW DOSE) + 25(OH)D_3 (n=10)		
	C	E	P	C	E	P
Hct %	51.5 ± 1.7	50.3 ± 1.9	N.S.	52.7 ± 1.1	52.9 ± 1.5	N.S.
Prt g%	4.5 ± 0.2	3.9 ± 0.2	0.001	4.4 ± 0.2	4.0 ± 0.2	0.001
GFR ml/min	1.20 ± 0.20	0.84 ± 0.11	N.S.	1.35 ± 0.1	1.00 ± 0.1	0.05
V µl/min	6.3 ± 1.2	3.8 ± 0.6	0.05	4.8 ± 0.7	4.8 ± 0.5	N.S.
PCa mEq/l	3.9 ± 0.2	4.2 ± 0.3	N.S.	3.8 ± 0.2	4.0 ± 0.2	N.S.
FLCa µEq/min	2.88 ± 0.47	2.36 ± 0.29	N.S.	3.25 ± 0.27	2.50 ± 0.25	0.05
FECa %	5.3 ± 1.1	6.6 ± 2.6	N.S.	4.6 ± 1.1	8.6 ± 2.2	N.S.
PMg mEq/l	1.5 ± 0.1	1.7 ± 0.1	N.S.	1.5 ± 0.1	1.7 ± 0.1	N.S.
FLMg µEq/min	1.17 ± 0.15	1.02 ± 0.11	N.S.	1.39 ± 0.10	1.18 ± 0.12	N.S.
FEMg %	7.6 ± 1.4	7.4 ± 2.2	N.S.	5.6 ± 1.1	10.1 ± 2.0	0.05
PPi mM	1.1 ± 0.1	1.3 ± 0.1	N.S.	1.1 ± 0.1	1.2 ± 0.2	N.S.
FLPi µM/min	1.31 ± 0.24	1.12 ± 0.16	N.S.	1.45 ± 0.14	1.22 ± 0.12	N.S.
FEPi %	13.1 ± 3.1	6.2 ± 2.2	N.S.	12.6 ± 2.6	9.2 ± 2.1	N.S.
FENa %	1.4 ± 0.3	1.0 ± 0.2	N.S.	0.8 ± 0.1	1.1 ± 0.2	N.S.

abbreviations as in Table III.

TABLE XIV. EFFECTS OF $1,25(OH)_2D_3$ IN THE VITAMIN D DEFICIENT TPTX HAMSTER

(GROUP IX)	TPTX + ETHANOL			TPTX + $1,25(OH)_2D_3$		
	C	E	P< (n=6)	C	E	P (n=9)
Hct %	51.6 ± 0.9	49.0 ± 0.8	0.05	52.8 ± 1.3	51.0 ± 0.9	N.S.
Prt g%	5.2 ± 0.2	4.8 ± 0.2	0.01	5.1 ± 0.4	4.9 ± 0.2	N.S.
GFR ml/min	0.84 ± 0.10	0.77 ± 0.09	N.S.	0.50 ± 0.08	0.59 ± 0.10	N.S.
V µl/min	2.5 ± 0.4	2.4 ± 0.6	N.S.	2.2 ± 0.7	2.1 ± 0.2	N.S.
PCa mEq/l	2.6 ± 0.1	2.4 ± 0.2	N.S.	2.9 ± 0.2	2.8 ± 0.2	N.S.
FLCa µEq/l	1.38 ± 0.20	1.13 ± 0.2	0.05	0.90 ± 0.14	1.03 ± 0.20	N.S.
FECa %	34.7 ± 3.3	32.4 ± 4.3	N.S.	33.3 ± 4.6	22.1 ± 2.7	0.01
PMg mEq/l	1.3 ± 0.02	1.3 ± 0.1	N.S.	1.2 ± 0.1	1.3 ± 0.1	N.S.
FLMg µEq/l	0.74 ± 0.09	0.68 ± 0.08	N.S.	0.39 ± 0.07	0.48 ± 0.9	N.S.
FEMg %	7.4 ± 1.8	4.7 ± 1.7	N.S.	6.5 ± 1.3	2.9 ± 0.4	0.02

abbreviations as in Table III.

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