EFFECTS OF PTH AND cAMP IN THE HAMSTER

THE EFFECTS OF PTH AND cAMP ON RENAL HANDLING OF CALCIUM, MAGNESIUM AND PHOSPHATE IN THE HAMSTER

M.A. BURNATOWSKA

A thesis submitted to the Faculty of Graduate Studies and Research in Partial Fulfilment of the Requirements for the M. Sc. Degree

Department of Physiology
McGill University

March 21, 1977

ABSTRACT

The objective of this study was to further investigate the effects and mechanism of action of parathormone upon the kidney and the role of cAMP as a mediator of its action on calcium and magnesium reabsorption. Hamsters were used in these studies as their renal Ca transport was shown to be highly PTH sensitive. Clearance studies were carried out in acutely thyroparathyroidectomised (TPTX) animals given 0.5% saline ip equal to 5% body weight. Acute TPTX led to a rise in fractional excretion of calcium (FE Ca) (2.8 ± 1.0 to 11.9 ±1.6%, $p<0.001$), FE Mg(5.4 ±1.8 to 18.7±2.5%, $p<0.01$) and was accompanied by a significant drop in plasma Ca, Mg and $FEPO_4$ (41.6± 6.0 to 2,9 ±0.9% $p< 0.02$).

PTH infusion caused a drop in FE Ca(13.0± 2.2 to 1.5 ±0.3% $p <.001$), FE Mg (15.6± 4.0 to 2.3± 1.0% $p<.01$), and a significant rise in plasma Ca and Mg (3.6± 0.2 to 4.1 ± 0.1 mEq/l $p <0.01$ and 1.6± 0.1 to 2.3 ±0.1 mg% $p< 0.001$ respectively). Infusion of cAMP caused a drop in FE Ca (18.9± 2.5 to 9.9 ±1.9% $p <0.01$ and FE Mg (20.4± 1.9 to 13.2 ±2.5 $p <0.01$). Infusion of DBcAMP reduced FE Ca from 17.5 ±2.9 to 6.9 ±0.9($p <0.01$) and FE Mg from 21.5± 3.6 to 8.6 ±1.2%($p< 0.01$). There were no significant effects

of either cAMP or DBcAMP upon total plasma Ca and Mg. The phosphaturic effect of PTH was shown with both cAMP and DBcAMP. Urine flow rate and glomerular filtration rate were not significantly altered in any group. This study therefore presents the first clear evidence that cAMP mediates the renal effects of PTH on calcium and magnesium transport as well as on phosphate transport.

ABERGE

Le but de cette étude a été d'investiguer les effects et le mécanisme d'action de la parathormone (PTH) au niveau du rein ainsi que le rôle de le cAMP comme médiateur de son action sur la réabsorption du calcium et du magnésium. Le transport rénal du calcium étant très sensible à l'action de la PTH chez le hamster, des épreuves de clearance ont été faites chez cette espèce. Les animaux ont été thyroparathyroidectomisés (TPTX) de façon aigue et une solution saline à 0.5%, i.p., à une dose équivalente à 5% du poids corporel a été administrée. La TPTX aigue provoque une élévation de l'excrétion fractionnelle (FE) du calcium (FE Ca 2.8 ± 1.0 à 11.9 ± 1.6%; $p < 0.001$), du magnésium (FE Mg 5.4 ± 1.8 à 18.7 ± 2.5%; $p < 0.01$) accompagnée d'une chute significative du calcium et du magnésium plasmatiques; une chute de l'excrétion fractionnelle du phosphore a été observée (FE PO_4 41.6 ± 6.0 à 2.9 ±0.9; $p < 0.02$).

L'infusion de PTH a causé une chute de: FE Ca (13.0 ± 2.2 à 1.5 ±0.3%, $p < 0.001$), FE Mg (15.6 ± 4.0 à 2.3 ± 1.0% $p < 0.01$), et une élévation significative du calcium et magnésium plasmatiques (3.6 ± 0.2 à 4.1 ± 0.1 mEq/l, $p < 0.01$ et 1.6± 0.1 à 2.3 ±0.1 mg/dl, $p < 0.001$).

L'infusion de cAMP a causé une chute de: FE Ca (18.9 ± 2.5 à 9.9 ±1.9%, $p < 0.01$ et FE Mg (20.4 ± 1.9 à 13.2 ± 2.5 $p < 0.01$). L'infusion de DBcAMP a réduite FE Ca de 17.5± 2.9 à 6.9± 0.9 ($p < 0.001$) et FE Mg de 21.5 ± 3.6 à 8.6 ± 1.2% ($p < 0.01$). Aucun

effet significatif de l'cAMP et de la DBcAMP n'a été observé sur le calcium ou le magnésium plasmatique total, L'effet phophaturique de la PTH n'a été démontré qu'avec la cAMP et la DBcAMP.

Le débit urinaire et la filtration glomerulaire sont demeurés stables chez tous les groupes. Cette étude démontre, pour la premiere fois, que la cAMP est le médiateur de la PTH sur le transport rénal du calcium, du magnésium et du phosphore.

ACKNOWLEDGEMENTS

This investigation was carried out in the Renal and Electrolyte Division, Department of Medicine Royal Victoria Hospital and McGill University.

I am indebted to my supervisors Dr. J.H. Dirks and Dr. J.F. Seely and to Doctors C.A. Harris and R.A.L Sutton for their suggestions and encouragement during this study.

Also I wish to express my gratitude to Mrs. A. Redensek for her technical assistance and to Mrs. V. Dottin and Mrs. J. Prue for typing of this thesis.

Some of the work described in this thesis appeared in the following manuscripts:

1. Evidence for Parathyroid Hormone (PTH) enhancement of calcium and magnesium reabsorption in the terminal nephron segment of the Hamster. Harris, C.A., Burnatowska, M., Sutton, R.A.L., Dirks, J.H., Clinical Research 24 p.401A (Abstract) 1976.

2. Effects of PTH and cAMP on renal handling of calcium, magnesium and phosphate in the hamster. Burnatowska, M.A., Harris, C.A., Sutton, R.A.L., Dirks, J.H. Abstract in the Am. Soc . Neph. 9th Annual Meeting, p 2A.

3. Effects of PTH and cAMP on renal handling of calcium, magnesium and phosphate in the hamster. Burnatowska, M.A., Harris, C.A., Sutton, R.A.L., Dirks, J.H. Paper in preparation.

TABLE OF CONTENTS

TABLE OF CONTENTS (CON'D).

ABBREVIATIONS

P	-	plasma concentration
U	-	urinary concentration
V	-	urine flow
FE	-	fractional excretion (UV/P x GFR)
FL	-	filtered load (P or UF x GFR)
P_{UF}	-	ultrafiltrate of plasma
TF	-	tubular fluid
Hct	-	hematocrit
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
n	-	number of animals
SEM	-	standard error of the mean
P	-	probability
NS	-	not significant

LIST OF TABLES AND FIGURES

TABLES:

FIGURES:

CHAPTER I - INTRODUCTION AND STATEMENT OF PURPOSE.

The effects of parathyroid hormone on the renal handling of electrolytes have been a subject of considerable investigation over several decades. The hormone has been shown to increase urinary excretion of phosphate (Greenwald 1925, Ellsworth 1935, Pullman et al. 1960, Strickler et al. 1964, Agus et al. 1971, Amiel et al. 1970, Amiel et al. 1974, Brunette 1974) bicarbonate (Nordin et al. 1960, Hellman et al. 1965, Muldowney et al. 1971) and sodium (Ellsworth 1935, Hellman et al. 1965, Amiel et al. 1970) and to decrease that of calcium (Talmage et al. 1954, Kleeman et al. 1961, Widrow & Levinsky, 1962, Sutton et al. 1976) and magnesium (McIntyre 1963, Massry et al. 1969). The phosphaturic effect of PTH is though to be mediated by cAMP. A rise in urinary cAMP after administration of PTH has been observed in TPTX rats (Chase and Aurbach 1967) as well as in humans (Kaminsky et al. 1970), and PTH sensitive adenyl-cyclase has been localised in the renal cortex (Chase and Aurbach 1968), and more recently to specific portions of the nephron (Chabardes et al. 1975). The interaction of PTH with specific receptors (Sutcliffe et al. 1973, DiBella et al. 1974) is felt to lead to stimulation of adenyl-cyclase at the contraluminal cell membrane (Shlatz et al. 1975), generation of intracellular cAMP, (Aurbach 1972, Melson 1970)

stimulation of protein kinase at the luminal cell membrane, phosphorylation of the membrane (Kinne et al. 1975) and ultimately, activation of ion transport mediating system(s).

Although cAMP has been shown to mimic the phosphaturic effect of PTH in vivo (Agus et al. 1973, Kuntziger et al. 1974) and to stimulate bone calcium resorption in vitro (Raisz 1969), several investigators have failed to show any consistent effect on renal calcium and magnesium handling. (Buttlen and Jard 1972, Kuntziger et al. 1974).

The present studies therefore, were undertaken to further elucidate the role of cAMP as a mediator of various PTH effects. The experiments were performed on hamster, whose renal calcium transport has been shown to be unusually sensitive to PTH (Biddulph et al. 1972, Biddulph et al. 1974).

CHAPTER II - HISTORICAL REVIEW OF THE LITERATURE.

A. General Nature of Renal Tubular Transport of Phosphate Calcium and Magnesium.

1. Phosphate

The kidney plays a key role in the homeostatic regulation of inorganic phosphate in body fluids. In general, the view that phosphate is largely freely filtered at the glomerulus of which a portion is reabsorbed, and the excess excreted in the urine, agrees well with the experimental evidence. Most studies performed at endogenous levels of plasma phosphate show that phosphate clearance is substantially less than GFR (Pitts 1933, Pitts 1944, Thomson 1957, Hellman 1964), thus suggesting that excretion is mainly determined by the processes of filtration and reabsorption. During intravenous infusion of phosphate, urinary phosphate rises rapidly to the point where each increment in the filtered load is associated with a similar increment in the urinary excretion, indicating attainment of an upper limit of net tubular phosphate reabsorption, i.e. a transport maximum or Tm is reached (Pitts 1944, Thomson 1957). The classical characteristics of a substance that exhibits Tm phenomenon, e.g. glucose, have also been demonstrated for the transport of phosphate. Thus, splay, minimal threshold, i.e. plasma phosphate levels at which phosphate excretion

first occurs, maximal threshold, i.e. plasma levels at which tubular reabsorptive mechanism is fully saturated, and a theoretical mean threshold (Tm/GFR) can be shown during phosphate loading studies. The Tm/GFR is often considered as a more useful expression of experimental conditions in many studies, as it takes into consideration variations in GFR previously shown to directly affect the Tm phosphate (Bijvoet et al. 1969). Unlike glucose however, which under normal conditions is never excreted in the urine, as its Tm is set very high, the reabsorptive capacity for phosphate is set at such a value, that a slight increase or a decrease in plasma phosphate concentration results in a change in the rate of excretion (Thomson et al. 1957). Also, unlike glucose, phosphate reabsorptive capacity is variable and under the influence of body stores of ions and several hormonal factors.

Sites of Phosphate Transport.

The ultrafilterability of phosphate at the glomerulus as established by the artificial membrane methods and recently confirmed by micropuncture of Bowman's space in Munich-Wistar rats, is almost complete, with the ratio of (TF/P)PO_4 close to unity (Harris et al. 1974).

Early investigations suggested that the proximal tubule was almost entirely responsible for phosphate reabsorption (Pitts 1933, Strickler 1964). Introduction of more sophisticated methods unmasked the existence of a distal mechanism of phosphate reabsorption (Amiel 1970, Beck 1973, Knox & Lechene 1975). It became apparent from these studies, that a significant portion of phosphate can be reabsorbed distally, and can equal about 30-40% of the filtered load. The amount of phosphate reabsorbed distally appears to depend on its delivery from the proximal tubule(Amiel 1970). Variability in the intrinsic capacity for phosphate reabsorption along the proximal segment, normally responsible for 60% to 70% of the filtered load, has been reported by micro-perfusion and tracer injection studies: ^{32}P microinjection showed maximum phosphate reabsorption to occur in the first third of the proximal tubule (Staum et al. 1972). Greater reabsorption of phosphate in the first portion of the proximal tubule was also reported by Brunette (1973). Thus, in normal subjects, about 30% of the filtered phosphate leaves the proximal tubule, and 10-25% of it can be reabsorbed distally in the loop of Henle, distal convoluted tubule and the collecting system while 5-10% is excreted (Agus et al. 1972, Amiel et al. 1969).

Possible Tubular Secretion of Phosphate.

Whether tubular secretion of phosphate exists or plays a role in the mechanism of phosphate excretion in mammals is still a subject of controversy. Secretion of phosphate has been clearly demonstrated in amphibia (Walker & Hudson 1937), aglomerular fish (Marshall et al. 1933), glomerular fish (Wolbach et al. 1970) and the chicken (Levinsky et al. 1957). The evidence that has been interpreted as indicative of phosphate secretion by a mammalian kidney however demands a careful appraisal. Phosphate secretion by the dog kidney has been reported by Barclay et al. (1949) and Carrasquer et al. (1960). These studies are not conclusive however, since similar results of other workers have been interpreted as evidence against secretion as several points that exceeded the unity clearance ratio were considered to be within the range of experimental error associated with clearance determination (Pitts 1933). Attempts to show phosphate secretion in humans also gave inconclusive results (Webster et al. 1967). In a series of experiments employing a variety of manipulations known to increase phosphate excretion (infusion of glucose, PTH, PAH, Mannitol, and phosphate or sodium loading) phosphate secretion could not be demonstrated (Handler 1962). Similarly, others using different methods (stop-flow, arterial injection

of labelled phosphate, micropuncture) were also unable to demonstrate the presence of a secretory mechanism in the renal transport of phosphate in either the proximal or the distal segment (Samiy et al. 1965, Strickler et al. 1964, Amiel et al. 1970, Agus et al. 1973). Thus, in contrast to fish, amphibia, and birds, in which phosphate secretion has been clearly demonstrated, no conclusive demonstration has been reported in mammals, and although secretion remains a distinct possibility, accumulating evidence suggests phosphate excretion to be the result of a balance between its filtration and reabsorption.

2. Calcium.

The handling of calcium by the kidney has been a subject of considerable investigation. Under normal nondiuretic conditions micropuncture studies indicate calcium reabsorption to occur throughout the nephron, paralleling that of sodium (Lassiter et al. 1963, Edwards et al. 1971, Murayama et al. 1972). In order to study the renal handling of calcium however, some assessment of its filterability at the glomerulus has to be made. In plasma, calcium exists in three forms, as a free ion, bound to protein, and complexed with various anions. Only the calcium in the ionic form is important physiologically and whereas both free and

complexed are freely filterable. Like phosphate, Ca concentration in the glomerular filtrate has been approximated by artificial membrane filtration methods, and recently confirmed by micropuncture studies. Measured Ca concentration in fluid obtained from Bowman's space gave values equal to approximately 63% of plasma values which agrees well with the generally accepted value of 60% ultrafilterability (Harris et al. 1974).

Passive reabsorption of Ca in the proximal tubule, as reflected by tubular fluid to ultrafiltrate ratio (TF/UF) Ca of about 1.0 established in the first portion of the tubule, accounts for 50 to 55% of the filtered load of calcium (Lassiter et al. 1963, Edwards et al. 1974, de Rouffignac et al. 1973). Transport of calcium beyond the proximal segment is an active process against high concentration gradients. Distal tubule (TF/UF) Ca ratios of 0.3 to 0.5 have been reported by several investigators (Edwards et al 1973, de Kouffignac et al. 1973, Agus et al. 1975, Le Grimellec 1973). Determination of quantitative distribution of calcium reabsorption along the distal nephron showed the loop of Henle to be responsible for the reabsorption of 20 to 30% of the filtered load of calcium, the distal convoluted tubule for 10 to 15%, and collecting duct for 5 to 8%.

Tubular transport of calcium is under the influence of both hormonal and nonhormonal factors and the accumulating evidence suggests that while the bulk of Ca is reabsorbed proximally in parallel with Na these physiological adjustments of calcium excretion are mediated in the distal segment, where independent transport of Ca and Na probably exists.

3. Magnesium

The available evidence suggests that magnesium is reabsorbed along the nephron (Murdough et al. 1960, Samiy et al. 1960) in a manner roughly similar to that of calcium under a variety of conditions. Like Ca, a fraction of Mg which is protein bound is not filterable at the glomerulus. Available evidence suggests that about 70% of Mg is freely filterable at the glomerulus (Brunette et al. 1971). However, unlike calcium quantitative distribution of Mg reabsorption along the nephron has been shown to vary in different species examined. Thus, micropuncture studies in the dog revealed (TF/UF) Mg of 1.05 in the late proximal tubule, indicating that Mg reabsorption occurs in proportion to sodium and H_2O reabsorption, and that the proximal tubule reabsorbs about 60% of the filtered load (Brunette et al.1969). Distal tubule(TF/UF) Mg of 0.5 suggests the

existence of an active transport mechanism in the loop of Henle or early distal segment. Little importance could be attached to its transport in the collecting duct (Wen et al. 1970).

In rodents, however, late proximal tubule (TF/UF) Mg reaches a value of about 2.0. In these species the proximal tubule thus, accounts for the reabsorption of only 25% of the filtered load of magnesium. The Loop of Henle appears to be a main site of Mg transport since as much as 60% of the filtered Mg can be absorbed in this segment. Only a small portion is handled by the distal convoluted tubule and the collecting duct (Murayama et al. 1972). Thus, at present, the loop of Henle appears to be a main site of magnesium handling in all species studied and the difference in the proximal transport of Mg in dogs and rodents remains to be solved.

Tm for Magnesium.

Suggestive evidence for the existence of limited capacity of magnesium reabsorption has been presented and Tm magnesium was shown to exhibit characteristics of Tm phosphate (Massry et al. 1969).

Possible secretion of Magnesium.

The possibility of net tubular secretion of magnesium has also been suggested. The evidence favouring secretion comes from studies of Averill and Heaton (1960), who could consistently show clearance of magnesium to exceed its filtered loads in rats, whose plasma magnesium levels were raised well above normal. Others, however, in similarly magnesium loaded dogs were unable to show secretion (Samiy et al. 1960, Murdough 1960, Massry 1969). Microperfusion studies in the rat, with intracapillary microinjection of magnesium, also failed to support existence of a secretory mechanism (Brunette et al. 1971). Strong evidence against secretion comes from studies in chickens, where infusion of magnesium into one renal portal system failed to unmask transport of magnesium from the peritubular capillaries into the lumen (Robinson 1962). Thus, at present the evidence in favour of magnesium secretion is equivocal and in need of further elucidation.

B. Factors influencing the Renal Transport of Divalent Electrolytes.

The renal tubular handling of the divalent electrolytes Ca, Mg, PO_4 is under the influence of body stores and several hormonal and nonhormonal factors. Accumulating evidence

suggests that stimuli that influence a distal transport system are the most important in the regulation of divalent electrolyte transport, and their final output in the urine.

Several hormones have been shown to have a direct effect on nephron transport systems. Administration of either parathyroid hormone or vitamin D and its metabolites, enhances calcium reabsorption (Talmage et al. 1954, Puschett et al. 1972) while calcitonin and growth hormone have the opposite effect (Kenny et al. 1965, Ikkos et al. 1959). Whereas PTH and calcitonin have been shown to be phosphaturic in nature, (Greenwald et al. 1925, Kenny et al. 1965) available evidence suggests that vitamin D and growth hormone have a phosphate retaining effect (Puschett et al. 1972, Ikkos et al. 1959). Adrenal cortical hormones appear not to have a direct effect on either calcium or phosphate transport, and those observed seem to be secondary to extracellular fluid volume expansion.

Of the various hormones mentioned however, the one that is most clearly important in the regulation of renal excretion of phosphate and calcium is parathyroid hormone. A potentially important role exists for vitamin D and growth hormone, but information is insufficient to warrant any conclusions regarding these agents.

1. Role of Parathyroid Hormone

i. 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. 34 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 the composition of parathormone 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 (Brewer et al. 1972, Aurbach 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 synthetised as a prohormone (proPTH) of 109 amino acids and molecular weight of about 12,000 (Habener et al. 1972), which has to undergo at least one specific cleavage before being fully activated. This 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, in the periphery into a large biologically inactive fragment (M.W. 7500), 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).

ii. 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, Oldham et al.1971, Tragovnik et al. 1971, Massry et al. 1969). Some evidence for the involvement of magnesium in the regulation of the levels of circulating PTH also exists (Buckle et al. 1968). No such relation of PTH levels to plasma phosphate has been shown (Sherwood et al. 1968), and phosphate regulation of secretion of PTH has been shown to be indirect, via 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).

iii. Effects of Parathyroid Hormone on the Renal Handling of Electrolytes.

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, to show the role of PTH in plasma calcium and bone resorption regulation. 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, and the initial intensive 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, economic 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:

1. Increase in urinary phosphate excretion(Greenwald 1925, Pullman 1960).
2. Decrease in urinary calcium excretion, preceding any change in plasma calcium (Talmage et al. 1954, Kleeman et al. 1961).
3. Acceleration of metabolic destruction of bone (Barnicot 1948, Chang 1951, Gaillard 1961).
4. Increased calcium absorption from the intestine (Talmage & Elliot 1958, Rasmussen 1959, Cramer 1961).

a) Effect of PTH on Renal Phosphate Transport.

The role of the parathyroids in renal phosphate metabolism

has been recognised since the discovery by Greenwald (1925) that parathyroidectomy causes a rapid fall in the rate of urinary phosphate excretion and conversely, 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 the "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 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 transport of phosphate. The crude parathyroid extracts used in early investigation 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 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 the phosphate. Two mechanisms of action of parathyroid were therefore considered and investigated:

1. An increase in tubular secretion of phosphate.
2. 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. 6). Present evidence favours inhibition of tubular reabsorption of phosphate after PTH, rather than enhanced secretion (Handler 1962, Agus 1973, Murayama 1972).

Evidence of Tubular Reabsorption of Phosphate

Clearance Studies

The original observations of Greenwald and Gross (1925) have been confirmed and extended over the past few decades by several investigators. Pullman et al. (1960) employed unilateral infusion of highly purified PTH into the renal artery of dogs and showed a unilateral phosphaturic response, with no changes in renal hemodynamics or plasma phosphate. Similarly, Bartter (1961) showed that both in dog and man, infusion of parathyroid extract diminishes

phosphate reabsorption with no effect on filtration rate. Tm for phosphate reabsorption was readily established in man as well as in dog and absolute phosphate reabsorption was constant over a wide range of filtered load. Furthermore, in TPTX dogs, a rise in urinary phosphate after PTH infusion was observed with no change in the level of phosphate filtered, while stop flow study results suggested that in the absence of PTH most of the reabsorption occurred proximally and parathormone administration inhibited the bulk of this proximal phosphate reabsorption (Samiy et al. 1965).

Micropuncture studies - Site(s) of PTH action

Introduction of micropuncture techniques facilitated the study of electrolyte handling along the tubule. It was first used to investigate the mechanism of PTH - induced phosphaturia by Strickler et al. (1964) in normal and phosphate loaded animals. The results provided further confirmation of previous stop flow studies, as phosphate reabsorption was shown to occur almost entirely in the proximal tubule of rat. Agus et al. (1972) suggested that the inhibition of proximal phosphate reabsorption was the consequence of a direct action of PTH on proximal sodium reabsorption, rather than that on phosphate; whereas

urinary sodium excretion was shown to be unchanged, indicating further distal sodium reabsorption, phosphate excretion was increased. However, dissociation of the proximal effects of PTH on sodium and phosphate in the dog has been reported by Wen (1974). At present, it is known that at least a portion of the proximal phosphate handling is closely linked to that of Na (Agus et al. 1973).

Distal phosphate reabsorption was suggested by the results of Wen's study, but the phosphaturic effect of PTH was attributed to the proximal tubule. Clear evidence for inhibition of distal phosphate reabsorption by PTH comes from studies of Amiel, Kuntziger and Richet (1969) in normal and in both acutely and chronically TPTX rats. Acute and chronic TPTX animals reabsorb a greater fraction of phosphate delivered to the distal tubule in the terminal nephron as compared with normal. Further studies in the rat (Kuntziger and Amiel 1970) suggested the loop of Henle as another possible site of the action of PTH. This is in agreement with the observations of Brunette (1974) who also showed the contribution of the loop of Henle to the phosphaturic effect of PTH. Micropuncture studies on the TPTX hamster (Harris et al. 1976)

point not only to a drop in the proximal phosphate reabsorption after PTH administration, but also in the loop of Henle and beyond the distal tubular puncture site, indicating an effect within the collecting system. The role of the collecting system in the phosphate handling was also noted by Amiel (1970). Finally, studies of Knox and Lechene (1975) confirm observations of others, that in addition to the proximal action, inhibition of distal phosphate reabsorption is an important factor in the phosphaturic effect of PTH.

The distal capacity for phosphate reabsorption and its sensitivity to PTH can be unmasked by saline loading or administration of diuretics. Saline-induced phosphaturia has been studied in recent years, and it has been suggested that this is a consequence of PTH release since saline infusion into TPTX animals produced no phosphaturia (Frick et al. 1969, Massry 1969). Micropuncture studies of volume expansion indicate that in presence of PTH most of the phosphate delivered distally is excreted (Puschett et al. 1972). After TPTX, proximal tubule response to saline infusion is similar to that of intact animals, but despite large loads delivered distally, little phosphate appears in the urine (Beck 1973, Frick 1969).

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). Following infusion of Acetazolamide into intact animals, proximally rejected phosphate escapes distal reabsorption. In TPTX animals similarly treated, the large phosphate load leaving the proximal tubule is reabsorbed distally, and little if any phosphaturia occurs (Beck et al. 1973). Thus, these studies reveal the existance of high capacity reabsorptive mechanism in the distal tubule for PO_4. The combination of distal load and extent of inhibition by PTH determines the magnitude of phosphaturia under a variety of experimental conditions.

b. The effects of PTH on Renal Calcium Handling

The net effect of PTH on renal calcium handling is rather difficult to predict, since it is the sum of several forces acting in opposite directions. Its effects on bone resorption and intestinal reabsorption lead to increased plasma calcium concentration, and a consequent rise in filtered calcium load which may increase urinary calcium excretion, therefore masking a direct effect on tubular calcium reabsorption. The evidence for a direct

effect to enhance tubular calcium reabsorption is considerable. Talmage, Kraintz and Buchanan (1955) demonstrated in rats that TPTX causes increased renal calcium excretion at the same time lowering plasma calcium;parathyroid extract injection restored both variables to normal. Widrow and Levinsky (1962) showed a drop in calcium excretion during PTH infusion in dog, despite a rise or no change in filtered load of calcium. Kleeman and co-workers (1961) made similar observations in man, showing that PTH specifically decreased renal calcium clearance. Eisenberg (1965) gave PTH to hypoparathyroid patients continuously infused with calcium and showed the hypocalciuric effects to be independent of changes in plasma calcium. Similar observations of high calcium clearance relative to plasma calcium in hypoparathyroidism have been reported by others both in man and laboratory animals (Nordin & Peacock 1969, Biddulph et al. 1970). Also, low calcium clearance relative to plasma calcium levels has been shown in hyperparathyroid patients. (Nordin et al. 1969).

Sites of the Action of PTH on Renal Calcium Transport Along the Nephron

The site at which parathyroid hormone enhances calcium reabsorption has not yet been clearly identified. The previously mentioned stop-flow study of Widrow and Levinsky

in the dog, suggested the distal segment of nephron as a possible site of this effect. Micropuncture and microperfusion attempts have been made to further elucidate localisation of PTH effects on tubular calcium transport. Frick et al. (1965) showed proximal reabsorption of calcium was unaffected by PTH, suggesting a more distal site of action. Micropuncture studies in the dog demonstrated that PTH actually has a dual effect on calcium transport: in the proximal tubule absolute and fractional reabsorption of calcium and sodium were inhibited; urinary calcium excretion was reduced despite increased sodium excretion indicating a disproportionate enhancement of calcium reabsorption in the distal nephron (Agus et al. 1973).

The above observations of a selective enhancement of calcium reabsorption at a distal site, are in agreement with those recently reported by Sutton et al.(1976). They showed that PTH increases rejection of sodium and calcium proportionately in the late proximal tubule in both intact and TPTX dogs. Increased delivery of both ions to the distal tubule after PTH administration was therefore observed. However, the ratio of fractional excretion of Ca to Na decreased, suggesting selective enhancement of calcium reabsorption before the superficial distal puncture

site. Further drop in Ca/Na ratio in the final urine in both groups points to a similar action of PTH on calcium reabsorption beyond the accessible distal convoluted tubule. Recent micropuncture studies in the hamster, whose renal calcium transport is highly sensitive to PTH strongly supports these observations, as PTH effects to enhance calcium reabsorption could be localised both to the ascending limb of the loop of Henle and to the terminal nephron segment, (Harris et al. 1976).

Thus, precise localisation of the effect of PTH on renal calcium transport remains uncertain. Present evidence points to the loop of Henle and more distal segments inaccessible to micropuncture as the sites of hormone action.

c. The 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 calcium or phosphate. Experimental evidence for the relation between calcium and magnesium metabolism dates back to the work of Mendel and Benedict in 1909. The recognition of the role of PTH in calcium homeostasis led to early investigations into PTH effects on plasma magnesium. Although a rise in plasma magnesium in dogs

injected with parathyroid extract was reported by Greenberg & Mackay (1932), no effect of PTH on plasma Mg could be seen in hyperparathyroidism (Bulger & Gausmann 1933). The subsequent studies were as equivocal as the earlier ones. Despite a drop in plasma Mg after PTX in dogs an increase rather than a drop in urinary magnesium was observed after PTH administration (Heaton 1960). However, PTX in rats (MacIntyre et al. 1963) led to a drop in plasma Mg as well as a fall in Mg and Ca excretion during PTH administration. Calcium retention correlated well with the dose of PTH. Experimental magnesium deficiency led to hypercalcemia, hypophosphatemia and a rise in urinary phosphate. This controversy as to the effects of PTH on urinary magnesium was later resolved by Coburn and Massry (1969). Investigation of the effects of calcium infusion on renal handling of magnesium with normal and reduced GFR, showed that calcium infusion caused a rise in urinary magnesium even if filtered load of magnesium dropped, concluding that they share common pathways for reabsorption. This study would also explain Heaton's results, since the animals he used were hypercalcemic. In later studies Massry and co-workers (1970) have shown that the effect of PTH on renal magnesium transport is independent from that of calcium, and that PTE causes a decrease in the fractional

excretion of magnesium despite an increased filtered load thus confirming findings of MacIntyre et al. In hypoparathyroid patients a drop in urinary magnesium associated with a drop in urinary calcium after intramuscular PTH injection was observed (Bethune 1968).

Little, if any, evidence for the precise localisation of PTH enhancement of magnesium reabsorption in the nephron exists. Micropuncture studies of Brunette (1969) point to the loop of Henle as a possible site, while the evidence from Harris et al. (1976) study in the hamster points to both loop of Henle and the terminal nephron as the sites of PTH action on the transport of magnesium. Thus although the effect of parathyroid hormone on renal magnesium handling in various segments of the nephron seems to parallel that on calcium, the evidence is equivocal and awaits further investigation.

2. The Role of cAMP in the Mechanism of Action of PTH on the Renal Transport of Electrolytes.

It is currently felt that many hormones act by way of a double messenger system. Hormones are regarded as a first messenger which travel from their cells of origin to the cells of target tissue where they then stimulate formation of a second messenger. At present, the only second messenger identified is cAMP (Sutherland

& Rahl 1958), and its implication in the action of many peptide hormones, endocrine and exocrine secretion, as well as neurotransmitter release, strongly favours the proposed mechanism (Robinson & Butcher 1962).

An increasing body of evidence supports the hypothesis that cAMP is also a key mediator in the action of PTH, and that this action of the hormone on the 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.

1 cAMP Generation in the Kidney

Changes in the urinary levels of cAMP can be regarded as a physiological consequence of a direct activation 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 & 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 effects on the urinary cAMP levels reflect cyclic nucleotide elaborated from the renal cells in response to the hormone and not 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. Chase & Aurbach (1967) and Buttlen and Jard (1972) using radioactive cAMP also showed that the rise in the renal output of the nucleotide after PTH reflected renal synthesis & excretion, and was not a result of a rise in its clearance. Furthermore, an increase in the intracellular concentration of the nucleotide in the kidney cells has been detected after injection of the hormone in vivo (Rasmussen & Tenenhouse 1968), after addition of the hormone to isolated

renal tubules (Aurbach 1972) or to isolated intact cell preparations from the renal cortex (Melson 1970).

The importance of cAMP is further strengthened by the findings in 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 paralled by a lack of rise in the cAMP levels in the urine (Chase 1972).

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 localisation of these effects. Specific receptors for parathyroid hormone have been identified in the renal cortex by Sutcliffe et al. (1973) using ^{125}I labeled PTH and by DiBella et al. (1974). 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 polypeptide & amine hormones leads to the activation of this system and subsequent rise in the intracellular cAMP production, the receptors for these hormones are highly specific and probably located on distinct cell types (Heath & Aurbach,1974).PTH stimulates adenyl-cyclase predominantly in the renal cortex (Chase et al. 1970), and recently it has been shown to be preferentially distributed in 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). With the exception of the pars recta, these are also the known sites of PTH induced alterations in the transport of electrolytes.

Vasopressin, another hormone whose effects are mediated by cAMP, stimulates adenyl-cyclase of renal medulla only, (Chase et al. 1970), in keeping therefore with its effect on the permeability to water in the collecting system. Calcitonin, which like PTH alters renal transport of electrolytes, although its exact effects and the

mechanism of action are still rather poorly defined, is also believed to act via the adenyl-cyclase cAMP system. Its effect however has been shown to be specific and distinct from that of PTH, since when the hormones were administered together, an additive effect was observed (Heersche et al. 1974). Other hormones which regulate functions by activating adenyl-cyclase of their target tissues (e.g. corticotropin and glucagon acting on the adrenal and liver respectively) were not found to bind to the PTH-specific receptors of the renal tissue (Sutcliffe et al. 1973), or to influence urinary levels of cAMP (Chase & Aurbach 1967).

iv. Protein Kinase

Several studies support the hypothesis that in cells responding to hormones through the activation of adenyl-cyclase and mediation of cAMP, cyclic nucleotides cause activation of protein kinases (Marx et al. 1973). 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 were performed, in an attempt to reproduce the known effects of PTH.

a. 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. DBcAMP was shown to be much more potent than cAMP in the intact liver cells but not in cell extracts (Posternak et al. 1962). Furthermore, Heershe et al. (1971) showed DBcAMP to be more phosphodiesterase resistant, while the results of Aurbach et al. (1972) suggest it acts as an inhibitor of the phosphodiesterase, causing thereby an accumulation of endogenous cAMP.

The concentration of exogenously administered nucelotide also appears to be of vital importance. Dose response

studies with DBcAMP in the intact cells of different tissues (adipose, liver, bone) gave a bell shaped curve over a relatively narrow concentration range (Vaes et al. 1969). Similarly, 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.

Thus, 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.

b. In vivo Studies

Effects of cAMP on the transport of phosphate

The physiological effects of cyclic nucleotides were also investigated. The phosphaturic action of PTH on the kidney has been consistently reproduced with both cAMP and DBcAMP (Rasmussen et al. 1968, Russell et al. 1969, Agus et al. 1971, Kuntziger et al. 1974). 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 Na;thus supporting the view that phosphaturia resulted from the inhibition of proximal Na 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 (Kuntsiger et al. 1974). The effects of cAMP were localized to the terminal nephron. These are the same sites as those thought to be involved in the effect of PTH on the transport of phosphate, and localization of PTH sensitive adenylcyclase (Chabardes et al. 1975).

Effects of cAMP on the Transport of Calcium

There is little evidence that the effect of PTH on Ca clearance by the kidney also involves mediation by cAMP. Rasmussen et al. (1969) reported that changes in urinary calcium and phosphate during cAMP infusion were

identical to those during administration of PTH. These were long term experiments however, and a rise, rather than a drop in urinary calcium was observed with both cAMP and PTH, while PTH and cAMP had an opposite effect on magnesium excretion. An inhibitory effect of DBcAMP on proximal calcium reabsorption similar to that of PTH was reported (Agus et al. 1972), but unlike PTH treated animals, where hypocalcuria was observed, no change in urinary calcium excretion was present in group infused with DBcAMP. Kuntziger in a study where he clearly demonstrated an effect of cAMP on the transport of phosphate along the nephron, failed to show any effect of the nucleotide on the transport of either calcium or magnesium. Plasma levels of these electrolytes were also unaffected. The hypercalcemic response to both nucleotides was investigated (Wells and Lloyd 1969), acutely and chronically in TPTX rats. Whereas DBcAMP caused a rise in plasma calcium and a drop in phosphate similar to that shown with PTH, neither effect could be shown with cAMP. However, theophylline, a known cyclic nucleotide phosphodiesterase inhibitor, which causes a rise in the intracellular cAMP concentration produced a significant increase in serum calcium, and when added with DBcAMP the effect was additive. On the contrary,

imidasole, a potent activator of phosphodiesterase, antagonised the hypercalcuric action of DBcAMP as well as PTH. A dose-response study showed the hypercalcemic effect of DBcAMP to be dose dependent, an observation similar to that of the in vitro studies of Raisz discussed previously, thus further emphasising the importance of the dose, when investigating effects of the nucleotides.

In summary, these observations, although in strong support of the involvement of cAMP in the phosphaturic effect of PTH, are inconclusive with respect to their role in mediating the action of PTH on the renal transport of calcium and magnesium.

Chapter III - GOLDEN SYRIAN HAMSTER (MESOCRICETUS AURATUS)- A MODEL FOR THE INVESTIGATION OF THE MECHANISM OF PTH ACTION ON THE KIDNEY

As discussed previously, the evidence that PTH regulates calcium clearance by the kidney is overwhelming. However, the exact mechanism of this effect is not known. In designing experiments to further complement a rather fragmentary knowledge regarding the sequence of the cellular events involved in this action of the parathyroid hormone, the golden hamster appears to be an ideal model,

as its renal calcium transport has been shown to be unusually sensitive to PTH (Biddulph et al. 1970, 1973). In the course of their investigation the hamster kidney was observed to play a dominant role in the marked and rapid changes that occur in serum calcium concentration after TPTX or exogenous PTH. A drop in plasma calcium with a concomitant rise in urinary calcium after acute TPTX could be prevented by either infusion of PTH or nephrectomy, whereas nephrectomy alone had no effect on plasma calcium levels (Biddulph et al. 1970).

Dose-response characteristics of urinary calcium levels in response to exogenous PTE were also studied to determine renal sensitivity to PTH. A linear drop 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 dosage required to restore calcium excretion levels to those observed in the intact animals. The urinary levels of calcium in the hamster were shown to be highly influenced by levels of dietary calcium as plasma calcium was maintained 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. However, unlike in other species, where PTH causes a

rapid fall in plasma phosphate, in the hamster, PTH failed to lower levels of serum phosphate. This may indicate a difference in the mechanism of the phosphaturic effect of PTH in the two species (Biddulph et al. 1969). Further investigations are required however, before any conclusion can be drawn.

Chapter IV - METHODS

1. Animal Preparation

Standard clearance experiments were performed on 54 male, golden hamsters (Mesocricetus auratus), 90 - 130g body weight, allowed free access to food and water. Animals were anesthetized intra-peritoneally (ip) with Inactin (Promonta, Hamburg, Germany) (18, mg/100 g.b.wt.), tracheotomized, and volume expanded to 5% body weight with o.5% saline (ip). Acute thyroparathyroidectomy (TPTX) was performed by cauterisation in all but the intact controls. Polyethylene catheters (PE50) were inserted into a jugular vein for the infusion of inulin, and into the carotid artery for blood sampling. Urine was collected from a catheter (PE50) inserted into the bladder. Animals were kept at constant body temperature (36 - 37^{o}C).

2. Experimental protocol

Animals were divided into five groups. The protocol employed was identical in all TPTX animals (group I-IV), except for a different drug infused in the experimental phase of each group. Group V were the intact controls.

Clearance periods were started at two hours post TPTX and consisted of two 60 minute phases (control and experimental) separated by 60 minute interval. The control and experimental phases each comprised two 30 minute urine collection periods. Blood samples were taken at the end of each phase.

Throughout the experiments all animals were infused with 3.5% inulin in 0.9% saline at 0.02 ml/ml. The equilibration of inulin concentration in the extracellular fluid compartment was assured by infusing it for one hour before commencement of the first phase. Group I (the TPTX control group) received only this infusion throughout both phases. In group II-Iv, administration of PTH, cAMP, or DBcAMP respectively was commenced at the completion of the first phase and the experimental clearances were begun one hour later.

Group II (n= 14) received purified bovine PTH (Wilson Laboratories, Chicago Ill.) at 5U/hour.

Group III (n = 8) received cAMP (Sigma Chemical Company, St.Louis, Mo.) ($5x10^{-8}$M prime followed by 10^{-9}M/min)

Group IV (n=9) received DBcAMP (5×10^{-8}M prime followed by 10^{-9}M/min).

To determine effects of acute TPTX, a group of eight animals with intact parathyroid glands was included in the study.

Experiments with DBcAMP (group IV), in which a drop in plasma calcium was observed, were repeated on an additional group of four animals that were infused with 1.6 mEq/l $CaCl_2$ together with DBcAMP in the experimental phase. This low background infusion of calcium prevented a drop in plasma calcium and filtered load of calcium.

3. Chemical Methods

In all experiments, the glomerular filtration rate was measured by inulin clearance. 100 µl of plasma was deproteinized with 10 µl of 25% trichloroacetic acid prior to analysis for inulin and phosphate. Plasma inulin was determined by the fluormetric dimedone method of Vurek & Pegram (1966) and urine inulin by the anthrone method of Fuhr, Kaczmarczyk and Kruttgen (1955). Plasma and urine Ca and Mg were determined by atomic absorption spectrophotometry (Perkin Elmer 303), and phosphate by Chen method (1956). Fractional excretions of calcium and magnesium were based on mean ultrafilterable values of 60% and 70% respectively. These figures were derived from ultrafiltration of pooled TPTX hamsters, blood through artificial

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

4. Methods of analysis

Significance of the difference between the means in the two phases was obtained by unpaired Student T Tests. Unpaired T tests were used for comparison of different groups of animals. Fractional excretion rates were calculated according to the following standard formula :

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

Chapter V - RESULTS

1. Comparison of intact and TPTX hamsters

The effects of acute TPTX in the hamsters are shown in Table I. No significant differences in GFR and urine flow rate were observed between the two groups of animals. In TPTX hamsters total plasma calcium was lower than intact animals, whereas fractional calcium excretion of 2.8 ± 1.0% in the intact animals was markedly different from that of 11.9 ± 1.6% in TPTX hamsters ($p < 0.001$).

Similar differences in magnesium handling were observed between the groups. Total plasma Mg was lower in TPTX than intact hamsters (1.6 ± 0.1 mg% vs 2.4 ± 0.1 mg% $p < 0.02$), while FEMg in the intact was higher (5.4 ± 1.8 vs 18.7 ± 2.5% $p < 0.01$).

The fractional phosphate excretion of 41.6 ± 6.9% in the intact group was significantly different from that of 2.9 ± 0.9% in TPTX hamsters ($p < 0.001$). However, there was no significant difference in the plasma phosphate concentration although it tended to be higher in the TPTX group (3.2 ± 0.11 mg% in the intact vs. 4.5 ± 0.7 mg% in TPTX).

2. Effects of PTH and cyclic nucleotides in TPTX hamsters

i. Hemodynamic changes (Table II)

Neither PTH, cAMP nor DBcAMP caused significant changes in GFR or urine flow rate, but a significant fall in hematocrit and plasma protein was observed in all groups, including the control. This was possibly attributable partly to removal of blood for analysis and partly to volume expansion, as the infusion rate was higher than the urinary output.

ii. Changes in calcium handling (Table III Fig. I)

While there was no change in either plasma Ca, filtered load of Ca or fractional Ca excretion in the control group (I), following PTH infusion fractional calcium excretion decreased from 13.0± 2.2% to 1.5 ±0.3% ($p < 0.001$) (Fig.I). Decreases in FE Ca were also observed following cAMP infusion (18.9 ±2.5% to 9.9 ±1.9% ($p < 0.01$), and DBcAMP (17.5± 2.9% to 6.9± 0.9% ($p < 0.01$). The calculated filtered calcium load was unchanged in each group. A modest rise in total plasma calcium with PTH (3.6 ±0.2 to 4.1 ±0.2 mEq/l $p < 0.01$) could not be mimicked with either cAMP or DBcAMP. In fact, a small, but significant fall in total plasma calcium was observed with DBcAMP (3.5± 0.1 to 3.3± 0.1 mEq/l $p < 0.05$). In all groups plasma protein fell, so that the protein bound

component of plasma Ca presumably also fell. The fall in total Ca with DBcAMP may be attributable to this effect rather than indicating a fall in ionised plasma Ca.

iii. Changes in magnesium handling (Table IV Fig.2)

The effect of all the three agents on renal Mg handling was similar to that on Ca. PTH significantly increased total plasma magnesium (1.6 ± 0.1 to 2.3 ± 0.1 mg%, $p < 0.001$) restoring it to the levels observed in intact animals. A small rise in total plasma Mg was observed with cAMP (1.9 ± 0.2 to 2.0 ± 0.2 mg%) and DBcAMP (1.5 ± 0.1 to 1.6 ± 0.1 mg%) but these changes were not significant. The calculated filtered load of magnesium was not significantly altered by PTH or DBcAMP but was singificantly increased by cAMP infusion. Nevertheless, like PTH, (fig. 2) which decreased FEMg from 15.6 ± 4.0 to 2.3 ± 1.0% ($p < 0.01$) both cAMP and DBcAMP significantly decreased FEMg (20.4 ± 1.9 to 13.2 ± 2.5% p 0.01 and 21.5 ± 3.6 to 8.6 ± 1.2% $p < 0.01$ respectively).

iv. Changes in phosphate handling (Table V Fig. 3)

A phosphaturic effect was observed with all three agents (Fig.3). PTH caused an increase in FE_{PO4} from 4.3 ± 1.2 to 15.4 ± 2.1%, $p < 0.001$), cAMP from 0.1 ± 0.1 to

3.2 ± 0.9% $p < 0.02$) and DBcAMP from 2.0 ±0.1 to 12.7 ± 2.6% ($p < 0.01$), while no change in FE_{PO4} of the control group (2.2 ± 1.0 to 2.4 ± 1.4%) was observed. Plasma phosphate rose throughout the experiment in all groups of animals, including the control. However, there was no significant difference between the plasma phosphate in the experimental phase of groups II, III and IV, and the experimental phase of the control group I. Calculated filtered load of phosphate was constant in all but the DBcAMP treated group, where a small, but significant rise was observed (from 11.1 ± 2.3 µg/min to 14.6 ± 2.5 µg/min.).

v. Effects of DBcAMP and Ca Cl_2 Infusion (Table VI)

In group IV (DBcAMP), a small but significant drop in plasma Ca was observed. Therefore, these experiments were repeated on four animals infused with 1.6 mEq/l Ca Cl_2 solution along with DBcAMP in the experimental phase. This infusion of low concentration calcium prevented a drop in plasma calcium, but did not influence the PTH-like effect of DBcAMP on Ca excretion. While plasma Ca increased from 2.6 to 2.9 mEq/l, FE Ca dropped from 24.5% to 11.2%, with no change in the filtered load of calcium. Changes in magnesium handling were similar, as the FEMg dropped from 33.9% to 12.6% and plasma Mg rose from 1.4 to 1.7 mg%.

Chapter VI

DISCUSSION

Parathyroid hormone is known to produce phosphaturia and to reduce calcium clearance in several species (Pullman et al. 1960, Amiel et al. 1970, Agus et al. 1973, Biddulph et al. 1970, Kleeman et al. 1961). The effects of the hormone on renal magnesium handling are similar to those on calcium (MacIntyre et al. 1963, Massry et al. 1970). The phosphaturic effect of PTH is thought to be mediated via stimulation of renal cortical adenyl cyclase (Chase & Aurbach 1968). PTH sensitive adenyl cyclase has been localised in several segments of the rabbit cortical nephron (Chabardes et al. 1975) and during PTH infusion an increase in the renal excretion of cyclic AMP precedes the onset of phosphaturia (Chase & Aurbach 1968). Infusion of both cyclic AMP and its butyrated derivative have been shown to increase fractional phosphate excretion (Rasmussen et al. 1968, Agus et al. 1972, Kuntziger et al. 1974).

However, there is little evidence to suggest that the enhancement by PTH of calcium and magnesium reabsorption is mediated by cAMP. Neither infusion of cyclic AMP

in the rat (Kuntziger et al. 1974) nor of DBcAMP in the dog (Agus et al. 1972) significantly reduced calcium clearance. Rasmussen et al. (1968) claimed that cAMP mimicked the effects of PTH on calcium but not magnesium handling in the conscious rat. However, as already discussed, in this comparatively long term experiment , PTH was observed to increase rather than decrease calcium clearance.

PTH administration to TPTX animals generally increases plasma calcium concentration . Whereas such an effect was shown after cAMP administration in the rat by Wells & Lloyd (1969), no increase was observed by Buttlen and Jard (1972) and Kuntziger et al. (1974). It is probable that the dose of cAMP used is of critical importance in this respect, as the hypercalcemic response to cAMP has been shown to be dose dependent over a relatively narrow range (Wells & Lloyd 1969).

The present studies were designed to further investigate the role of cAMP as a mediator of PTH action. The experiments were performed in the hamster, as the kidney of this species has been shown to be highly sensitive to the Ca retaining effect of PTH and to play an important role in the maintenance of plasma calcium levels (Biddulph et al. 1970). Although Dousa et al. (1976) were unable to

show a phosphaturic effect of PTH in this species, we observed that PTH infusion caused phosphaturia and a decrease in the fractional excretion of both Ca and Mg. All of these effects were mimicked by both cAMP and DBcAMP, although the phosphaturic effect of cAMP was less striking than that of PTH and DBcAMP. Both nucleotides significantly enhanced tubular reabsorption of Ca and Mg, again the effects of cAMP were less pronounced than these of PTH and DBcAMP. The butyrated derivative decreased the fractional calcium and magnesium excretion by 66% and 60% respectively, whereas decreases after cAMP were 48% and 36%.

The doses of the cyclic nucleotides used were similar to those shown to produce phosphaturia in rats (Kuntziger et al. 1974). The butyrated derivative of cAMP was administered as well as cAMP as it has been shown to be more permeable in certain tissues (Posternak et al. 1962), more resistant to phosphodiesterase (Heersche et al. 1974), and possibly is itself an inhibitor of phosphodiesterases (Aurbach 1972), which are responsible for the breakdown of cAMP to the physiologically inert 5'AMP. Although cAMP has been shown previously to produce phosphaturia, this study is the first to indicate that cAMP may also mediate the effects of PTH upon Ca and Mg transport. The reasons for the disparity between these results and those of Kuntziger et al.

(1974) in the rat and Agus et al. (1972) in the dog are not apparent, though it may relate to the remarkable sensitivity of the hamster kidney to the calcium retaining effect of PTH. The increase in total plasma calcium and magnesium observed during PTH infusion was not observed with either nucleotide. In fact, a small, but significant decrease in total plasma calcium occurred during DBcAMP although the ultrafilterable calcium may actually have risen due to the decrease in plasma protein concentration. However, the decreased fractional excretion of Ca after DBcAMP is unlikely to be due to a drop in the filtered load, as the calculated filtered load was unchanged. This is further supported by the data from four TPTX hamsters which were infused with calcium together with DBcAMP in the second phase. The mean plasma Ca rose from 2.6 to 2.9 mEq/l, GFR from 0.29 to 0.35 ml/min. In spite of an increase in calculated filtered load of Ca from 0.46 to 0.59 μEq/min., the fractional excretion of calcium fell from 24.5 to 11.2%. There are several possible reasons for the failure to show a PTH-like effect of cAMP on plasma Ca and Mg. First, changes in ionized or UFCa (not measured for individual experiments) may not be reflected by changes in total PCa; second, the tubular effects on Ca and Mg are less than with PTH; third, the arbitrary dose used may

have been either too high or too low to completely mimic the effect of PTH (Wells & Lloyd 1968) and fourth PTH may cause an increase in plasma Ca and Mg by some renal or extrarenal mechanism not mediated by cAMP. There remains a fifth possibility that PTH may increase plasma calcium and magnesium concentration by activation of the 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 actions of many other hormones including ADH which acts primarily in the renal medulla (Chase & Aurbach 1972), and calcitonin which generally acts in an opposite direction to PTH on calcium metabolism (Heath & Aurbach 1974).

The tubular location of these mediatory effects of cAMP is at present unknown. Micropuncture experiments in the dog suggest that the Ca retaining effect of PTH is beyond the proximal tubule (Sutton et al. 1976) while preliminary micropuncture studies in the hamster (Harris et al. 1976) suggest that PTH acts at several sites in the nephron- the proximal tubule, ascending limb of Henle's loop and in the terminal part of the nephron. Further experiments are required to determine whether cAMP is active in the mediation at all these sites. Certainly these data fit well with those of Chabardes et al. (1975) who demonstrated the

presence of PTH sensitive adenyl cyclase activity in the proximal convoluted tubule and pars recta, the thick ascending limb of Henle,s loop, the distal convoluted tubule (to a lesser extent) and the first cortical segment of the collecting duct.

The reason for the increase in plasma phosphate concentration throughout each experiment including the TPTX controls is not known. Perhaps this is a function of diurnal variation, as it has been shown that in the rat, plasma phosphate rises through the day, despite a concomitant rise in endogenous PTH levels (Cohn 1970, Mudge et al. 1973). However, as suggested by Biddulph et al. (1969), who observed a similar rise in plasma phosphate after PTH administration to the intact hamsters, but not in the rat, it may reflect the existence of a different phosphaturic mechanism in this species.

In present studies however, the observed rise in plasma phosphate could not account for the phosphaturia as there was no such increase in the fractional phosphate excretion in the control group.

The differences in the base line excretion of the electrolytes in different groups cannot be explained either, as all animals were treated identically. It is possible that, as the metabolism of this size of animal is relatively

fast, any variation in daily food intake could change the urinary output of electrolytes. Also there may have been differences in the completeness of the parathyroidectomy between these groups.

Summary - Contribution to original knowledge.

Although the accumulating evidence strongly suggests that the mechanism of action of PTH on the renal tubular transport of phosphate involves cAMP, this study presents the first clear evidence that cAMP may mediate the effects of PTH not only on renal phosphate handling but also that of calcium and magnesium. Both cAMP and DBcAMP were shown to enhance tubular reabsorption of calcium and magnesium and to reduce that of phosphate. The quantitative differences in the urinary data and lack of PTH-like effects of either nucleotide on plasma calcium and magnesium levels may be due to the arbitrary doses of cAMP and DBcAMP used. A dose-response study would resolve this problem. Further studies are also required to localise these renal actions of cAMP.

TABLE I

COMPARISON OF INTACT AND TPTX HAMSTERS

	Intact (n=8)	TPTX (n=8)	P
Hct%	54.0 ± 1.0	53.0 ± 1.0	NS
Prot. g%	4.5 ± 0.1	4.6 ± 0.1	NS
GFR (ml/min)	0.32 ± 0.08	0.29 ± 0.03	NS
V(µl/min)	3.9 ± 1.2	3.2 ± 0.5	NS
P Ca (mEq/L)	5,1 ± 0.3	2.9 ± 0.1	< 0.001
P Mg (mg%)	2.4 ± 0.1	1.6 ± 0.1	< 0.02
P PO_4 (mg%)	3.2 ± 0.7	4.5 ± 0.7	NS
FE Ca (%)	2.8 ± 1.0	11.9 ± 1.6	< 0.001
FE Mg (%)	5.4 ± 1.8	18.7 ± 2.5	< 0.01
FE PO_4 (%)	41.6 ± 6.9	2.9 ± 0.9	< 0.001

Results are mean ± S.E.M.

TABLE II

HEMODYNAMIC EFFECTS OF PTH, cAMP AND DBcAMP

	GROUP I		GROUP II		GROUP III		GROUP IV	
	TPTX	CONTROL	TPTX +	PTH	TPTX +	cAMP	TPTX +	DBcAMP
Hct.%	53.0 ±1.0***	51.0 ±1.0	52.0 ±1.0***	49.0 ±1.0	53.0 ±2.0***	48.0 ±2.0	53.0 ±1.0**	51.0 ±1.0
Prot. g%	4.6 ±0.1***	3.9 ±0.2	4.5 ±0.1***	3.9 ±0.1	4.3 ±0.1***	3.6 ±0.1	4.7 ±0.2***	3.9 ±0.1
GFR ml/min	0.29 ±0.03	0.29 ±0.05	0.34 ±0.06	0.32 ±0.05	0.31 ±0.06	0.38 ±0.07	0.25 ±0.03	0.27 ±0.02
Urine Flow Rate µl/min	3.2 ±0.5	2.8 ±0.5	2.9 ±0.3	2.9 ±0.2	1.9 ±0.2	2.1 ±0.2	3.1 ±0.6	2.7 ±0.3

*** p<0.001

** p<0.01

TABLE III

EFFECTS OF PTH, cAMP and DBcAMP ON RENAL CALCIUM HANDLING

	GROUP I		GROUP II		GROUP III		GROUP IV	
	TPTX	CONTROL	TPTX	PTH	TPTX	cAMP	TPTX	DBcAMP
PCa mEq/l	2.9 ±0.1	3.0 ±0.1	3.6 ±0.2**	4.1 ±0.2	3.0 ±0.1	2.8 ±0.1	3.5 ±0.1*	3.3 ±0.1
FL Ca µEq/min	0.49 ±0.02	0.52 ±0.04	0.70 ±0.13	0.77 ±0.09	0.57 ±0.11	0.62 ±0.10	0.52 ±0.08	0.53 ±0.07
FE Ca %	11.8 ±1.6	14.7 ±2.3	13.0 ±2.2***	1.5 ±0.3	18.9 ±2.5**	9.9 ±1.9	17.5 ±2.9**	6.9 ±0.9

*** $p<0.001$

** $p<0.01$

* $p<0.05$

TABLE IV

EFFECTS OF PTH, cAMP AND DBcAMP ON RENAL MAGNESIUM HANDLING

	GROUP I		GROUP II		GROUP III		GROUP IV	
	TPTX	CONTROL	TPTX	PTH	TPTX	cAMP	TPTX	DBcAMP
P Mg mg%	1.6 ±0.1	1.9 ±0.2	1.6 ±0.1***	2.3 ±0.1	1.9 ±0.2	2.0 ±0.2	1.5 ±0.1	1.6 ±0.1
FL Mg µg/min	3.3 ±0.1	3.8 ±0.1	4.3 ±0.8	5.3 ±0.7	3.6 ±0.6*	4.9 ±1.1	2.5 ±0.4	2.9 ±0.4
FE Mg %	18.7 ±0.1	15.8 ±0.3	15.6 ±4.0**	2.3 ±1.0	20.4 ±1.9**	13.2 ±2.5	21.2 ±3.6**	8.6 ±1.2

*** $p<0.001$

** $p\leq0.01$

* $p<0.05$

TABLE V

EFFECTS OF PTH, cAMP AND DBcAMP ON RENAL PHOSPHATE HANDLING

	GROUP I		GROUP II		GROUP III		GROUP IV	
	TPTX	CONTROL	TPTX	PTH	TPTX	cAMP	TPTX	DBcAMP
P_{PO4} mg%	4.7 ±0.7 *	5.7 ±0.6	5.2 ±0.7	5.9 ±0.5	4.8 ±0.6 *	7.1 ±0.7	4.2 ±0.5 *	5.2 ±0.6
FL_{PO4} µg/min	13.5 ±2.6	12.6 ±2.4	19.3 ±4.5	19.7 ±3.1	16.5 ±3.9	27.0 ±6.9	11.1 ±2.3 *	14.6 ±2.5
FE_{PO4} %	2.4 ±1.0	2.2 ±1.4	4.3 ±1.2 ***	15.4 ±2.1	0.1 ±0.1 *	3.2 ±0.9	2.0 ±0.1 **	12.7 ±2.6

*** $p<0.001$

** $p<0.01$

* $p<0.02$

TABLE VI

EFFECTS OF DBcAMP ON RENAL HANDLING OF CALCIUM AND MAGNESIUM WITH 1.6 mEq/L CALCIUM CHLORIDE INFUSION IN THE EXPERIMENTAL PHASE

	TPTX	DBcAMP + $CaCl_2$
Hct.%	5.7 ± 1.0	5.6 ± 1.0
Prot. g%	4.7 ± 0.1	4.2 ± 0.2
GFR ml/min	0.29 ± 0.01	0.35 ± 0.05
Vol. µl/min	7.1 ± 2.2	5.4 ± 0.9
PCa mEq/L	2.6 ± 0.2	2.9 ± 0.2
FL Ca µEq/min	0.45 ± 0.04	0.60 ± 0.09
FE Ca %	24.5 ± 5.6	11.2 ± 2.2
P Mg - mg%	1.4 ± 0.2	1.7 ± 0.2
FL Mg µg/min	5.3 ± 0.4	7.0. ± 1.0
FE Mg %	33.9 ± 8.3	12.6 ± 3.1

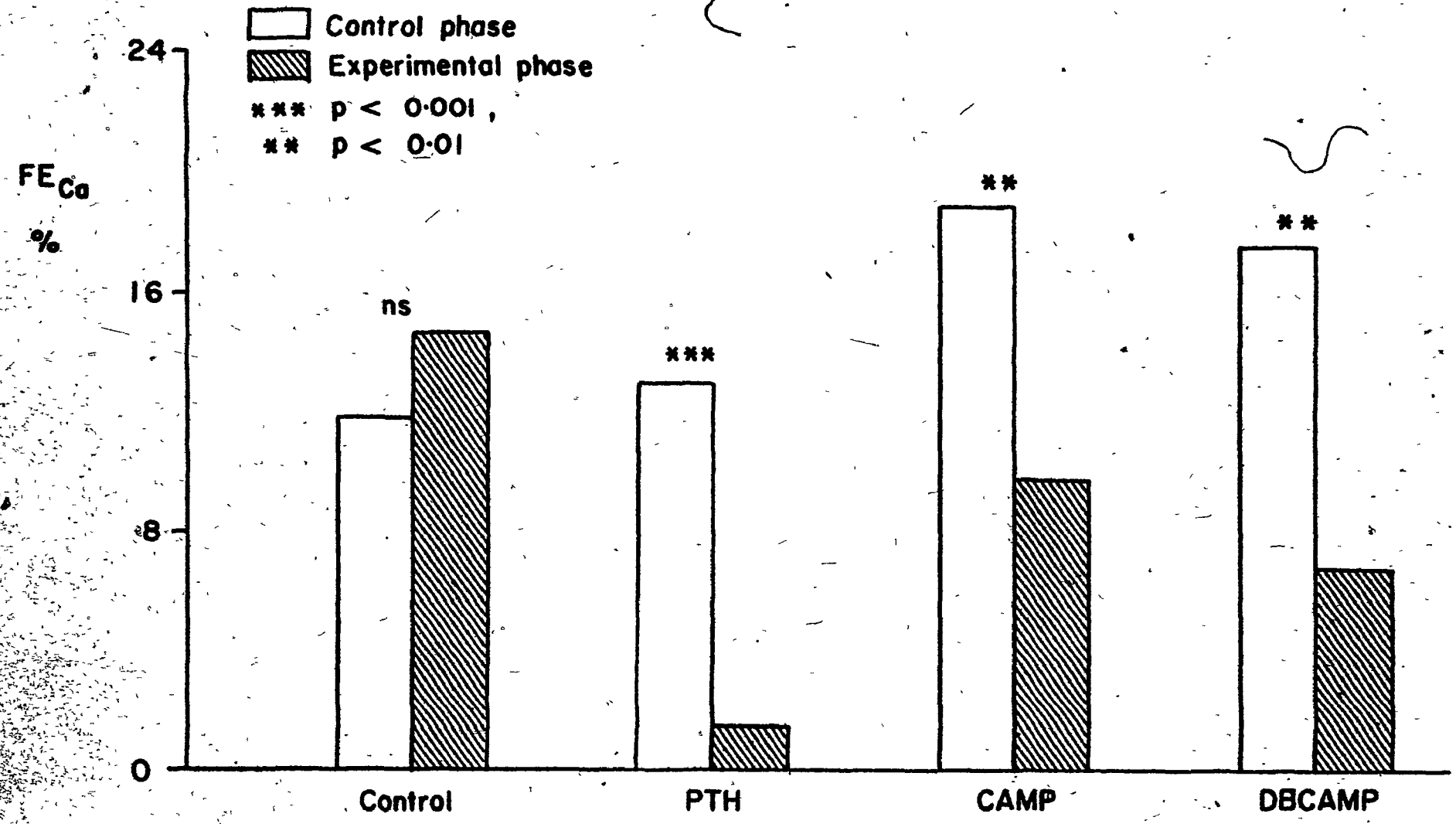

Figure I. Effects of PTH, cAMP and DBcAMP infusion on fractional calcium excretion.

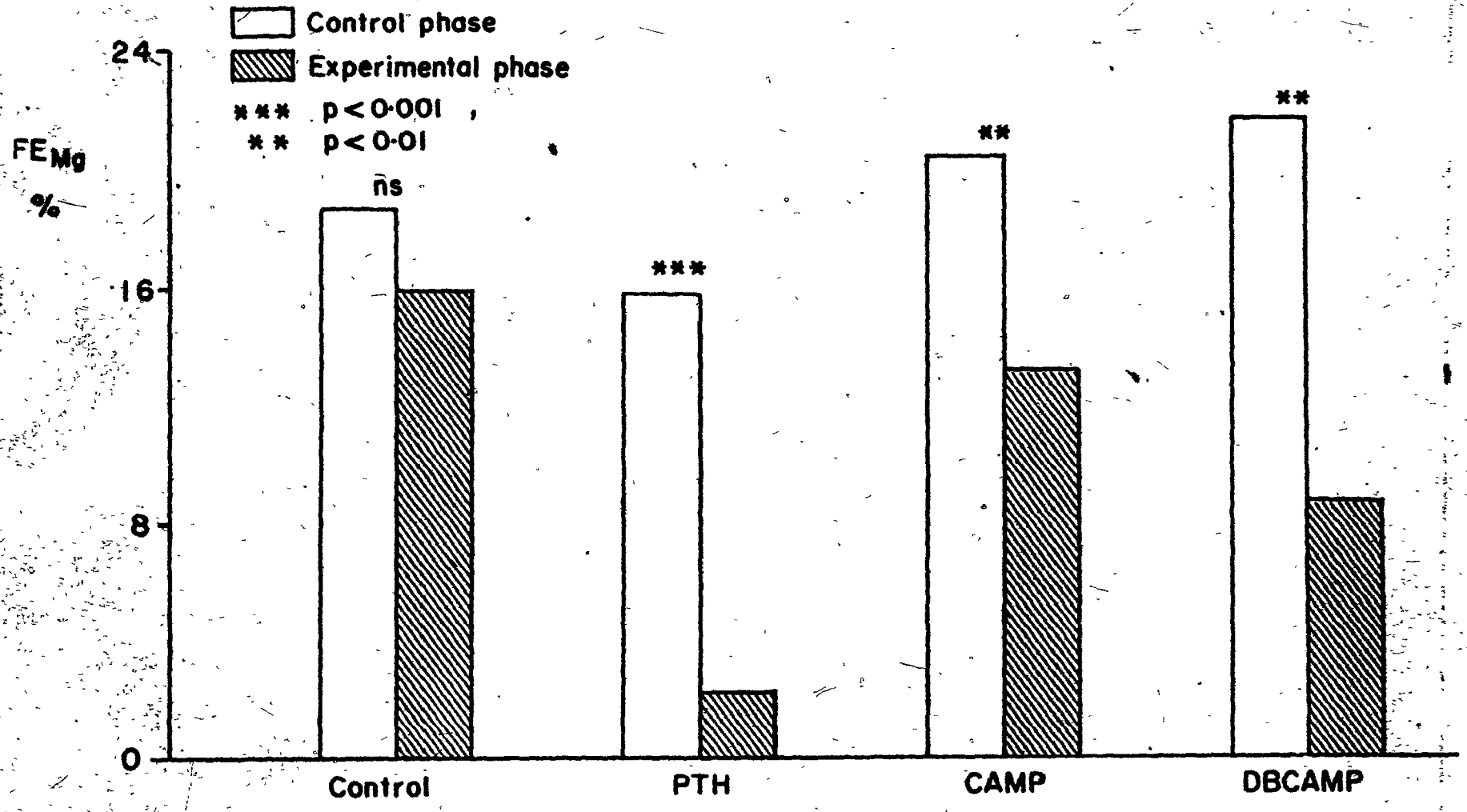

Figure 2. Effects of PTH, cAMP and DBcAMP on fractional magnesium excretion.

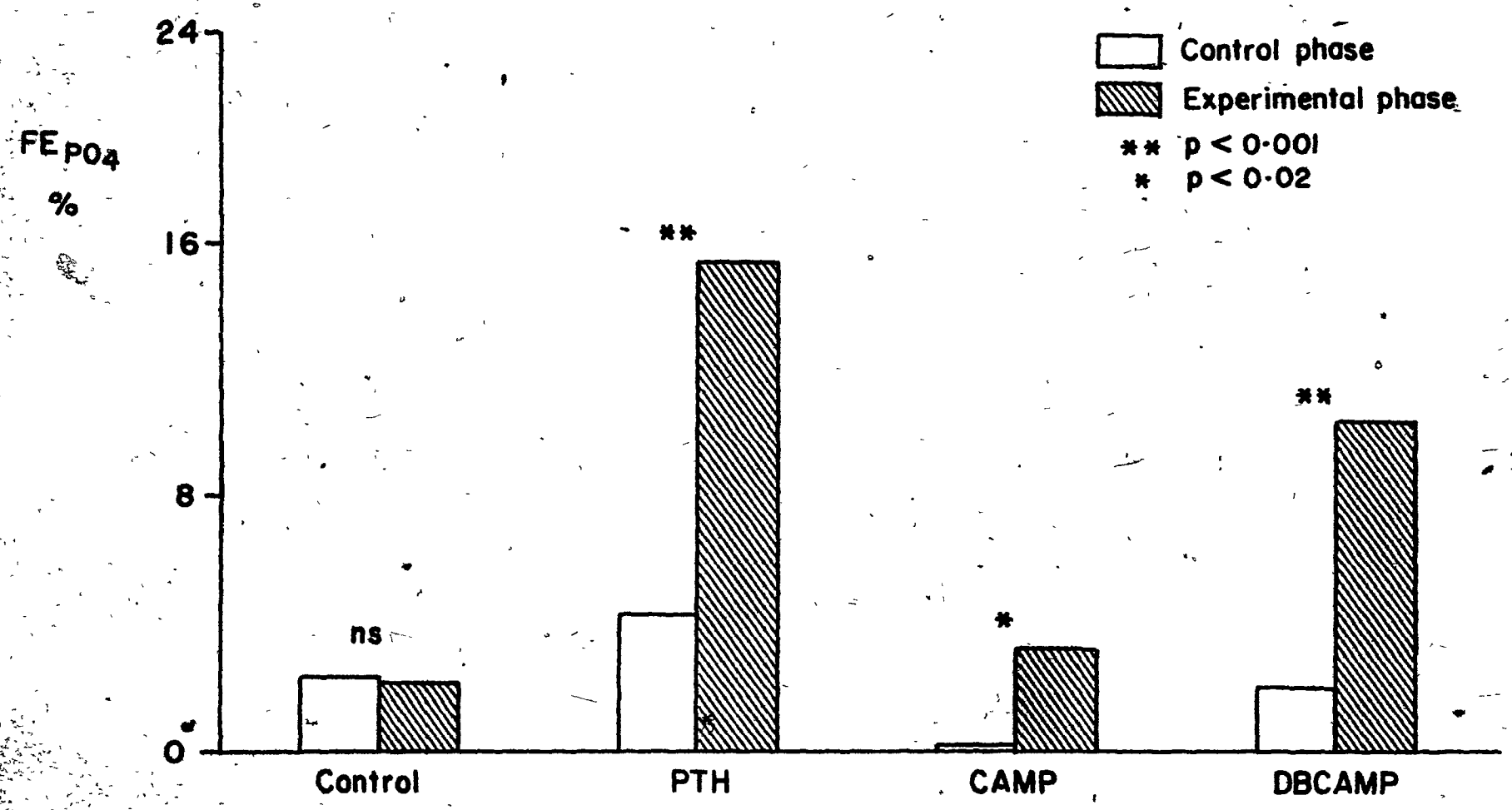

Figure 3. Effects of PTH, cAMP and DBcAMP on fractional phosphate excretion.

BIBLIOGRAPHY

1. Agus, J.B., D.B. Puschett, D. Senesky, M. Goldberg. Mode of action of PTH and cyclic adenosine 3'5 monophosphate on renal tubular phosphate reabsorption in the dog. J. Clin. Invest. 50: 617-626 (1971).

2. Agus, J.B., L.B. Gardner, L.H. Beck, M. Goldberg. Effects of parathyroid hormone on renal tubular reabsorption of calcium, sodium and phosphate. Am. J. Physiol. 1143-1148 (1973).

3. Albright, F., Reifenstein, E.C. Jr. "The Parathyroid Gland and Metabolic Bone Disease". Williams & Wilkins Company, Baltimore, p.13-23, 1948.

4. Amiel, C., H. Kuntziger, G. Richet. Micropuncture study of handling of phosphate by proximal and distal nephron in normal and parathyroidectomized rats. Evidence for distal reabsorption. Pflügers Arch. 317: 93-109 (1970).

5. Amiel, C., H. Kuntziger. Renal Tubular handling of phosphate. in les Colloques - (Inserm. 30: 37-50, 1974).

6. Aurbach, G.D. Isolation of parathyroid hormone after extraction with phenol. J. Biol. Chem. 234: 3179-3181 (1959).

7. Aurbach, G.D., Marcus, R., Heersche, J.N.M., Winickoff, R.N. Marx SH. Cyclic nucleotides in the action of native and synthetic parathyroid and calcitonin peptides. In

parathyroid hormone and the calcitonins, ed. Talmage, R.V. Munson, P.L., Amsterdam Excerpta Medica Foundation p.502-510, 1972.

8. Aurbach, G.K, Keutman, H.t,, Niall, M.D. Tregear, G.W., O'Riordan, J.L.N.,Marcus,R.,Marx, S.J., Potts, J.T. Jr. Structure, synthesis and mechanism of action of parathyroid hormone. Recent Progr. Horm. Res. 28: 353-398 (1972).

9. Aurbach, G.D., Heath, D.A. Parathyroid Hormone and Calcitonin regulation of renal function. Kidney Int. 6: 331-345, 1974.

10. Averill, C.M. and Heaton, F.W. The renal handling of magnesium. Clin. Sci. 31: 353-356 (1966).

11. Barcklay, J.A., Cooke, W.T., Kenney, R.A. The renal excretion of inorganic phosphate in man and dog. Acta Med. Scand. 134: 107-116 (1949).

12. Barnicot, N.A., The local action of parathyroid and other tissues on bone in the intracerebral grafts. Journal of Anat. 82: 233-248 (1948).

13. Bartter, F.C., , The effects of the parathyroid in phosphate excretion. In "The Parathyroids" ed. Greep, R.O. and Talmage, R.V. Springfield, Illinois: Thomas. p.388-405, 1961.

14. Beck, L.M., Goldberg, M. Effects of acetazolamide and parathyroidectomy on renal transport of sodium, calcium and phosphate. Am. J. Physiol. 224: 1136-1141 (1973).

15. Bethune, J.E., Turpin, R.A., Ingue, H. Effects of parathyroid hormone extract on divalent ion excretion in man. J. Clin. Endo. Metab.. 28: 673-678 (1968).

16. Biddulph, D.M., Hirsch, P.F., C.W. Cooper and P.L. Munson. Effect of thyroparathyroidectomy and parathyroid hormone on urinary excretion of calcium and phosphate in the golden hamster. Endocrinology 87: 1346-1350 (1970).

17. Biddulph, D.M., Gallimore, L.B. Jr. Sensitivity of the kidney to parathyroid hormone and its relationship to serum calcium in the hamster. Endo. 94: 1241-1246 (1973).

18. Biddulph, D.M., Hirsch, P.F., P.L. Munson. Thyrocalcitonin and parathyroid hormone in the hamster. In Calcitonin, Proceedings of the Second International Symposium. Ed. Taylor, S., G. Foster p.392-399 (1969).

19. Bijvoet, O.L.M. Relation of plasma phosphate concentration to renal tubular reabsorption of phosphate. Clin. Sci. 37: 23. (1969).

20. Brewer, H.B. Jr., Fairwell, T., Ronan, R., Sismore, G.W., Arnaud, C.D. Human parathyroid hormone: amino acid sequence of the amino terminal residues 1-34. Proc. Nat. Ac. Sci. USA 69: 3585-3588 (1972).

21. Brunette, M., Wen, S.F., Evanson, R.L., Dirks, J.H. Micropuncture study of magnesium reabsorption in the proximal tubule of the dog. Am. J. Physiol. 216: 1510-1516 (1969).

22. Brunette, M. Aras, D.M. A microinjection study of nephron permeability to calcium and magnesium. Am. J. Physiol. 221: 1442-1448 (1971).

23. Brunette, M.G., L. Taleb, Carriere, S. Effects of parathyroid hormone on phosphate reabsorption along the nephron of the rat. Am. J. Physiol. 225: 1076-1081 (1974).

24. Buckle, R.M., Core, A.D., Cooper, C.W., Gitelman, H.J. The influence of plasma magnesium concentration on parathyroid hormone secretion. J. Endo. 42: 529-534 (1968).

25. Bulger, H.A., Gausmann, F. Magnesium metabolism in hyperparathyroidism. J. Clin. Invest. 12: 1135-1142 (1933).

26. Butcher, R.W., Sutherland, E.W. Adenosine 3'5', phosphate in biological materials. I. Purifications and properties of cyclic 3'5' nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3'5' phosphate in human urine. J. Biol. Chem. 237: 1244-1249 (1962).

27. Buttlen, D., Jard, S. Renal handling 3'5' cAMP in the rat. The possible role of luminal 3'5' - cyclic AMP in the tubular reabsorption of phosphate. Pflügers Arch. 331: 172-190 (1972).

28. Carrasquer, G. and Brodsky, W.A. Transient secretion of phosphate in relation to underlying plasma level in the dog kidney. Am. J. Physiol. 199: 1239-1243 (1960).

29. Chang, H. Grafts of parathyroid and other tissues to bone. Anat Record 111: 23-48 (1951).

30. Chabardes, D., Imbert, M., Clique, A., Montegut, M., Morel, F. PTH sensitive adenyl cyclase activity in different segments of rabbit nephron. Pflugers Arch. 354: 229-234 (1975).

31. Chase, L.R. Aurbach, G.D. Parathyroid function and the renal excretion of 3'5' adenylic acid. Proc. Nat. Acad. Sci. 58: 518-525 (1967).

32. Chase, L.R. and Aurbach, G.D. Renal adenyl cyclase: Anatomically separate sites for parathyroid hormone and vasopressin. Science (Washington) 159: 545-547 (1968).

33. Chase, L.R., Melson, G.L., Aurbach, G.D. Pseudo-hyparathyroidism, defective excretion of 3'5' AMP in response to PTH. J. Clin. Invest. 48: 1832-1844 (1969).

34. Chen, P.S. Jr., Toribara, T.G., and H. Warner. Microdetermination of phosphorus. Anal. Chem. 28: 1756-1758 (1956).

35. Chu, L.L.H., MacGregor, R.M. Nast, C.S., Hamilton, J.W., Cohn, D.V. Studies on the biosynthesis of rat parathyroid hormone and proparathyroid hormone adaptation of the parathyroid glands to dietary restriction of calcium. Endo. 93: 915-924 (1973).

36. Coburn, J.V., Massry, S.G., Kleeman, C.R. The effect of calcium infusion on renal handling of magnesium with normal and reduced glomerular filtration rate. Nephron 7: 131-143 (1970).

37. Cohn, C.W. Diurnal rhythms in urinary electrolyte excretion by the rat. Life Sci. 9: 803-808 (1970).

38. Collip, J.B. The extraction of parathyroid hormone which will prevent or control parathyroid tetany and which regulates the level of blood calcium. J. Biol. Chem. Vol. 63: 395-438 (1925).

39. Cramer, C.F. Suiker, A.P., Copp, D.H. Parathyroid influence on calcium and phosphorus absorption by the gut. In the Parathyroids. Ed. R.O. Greep & R.V. Talmage. p.158-166 (1961).

40. Di Bella, F.P. Dousa, T.P., Miller, S.S., Arnaud, C.D. Parathyroid hormone receptors of renal cortex: Specific binding of biologically active 125 I-labeled hormone and relationship to adenyl-cyclase activation. Proc. Nat. Acad. Sci. 71: 723-726 (1974).

41. Dousa, T., Rychlik, I. The effect of parathyroid hormone on adenyl cyclase in rat kidney. Biochem. Bio. Phys. Ach. 158: 484-486 (1968).

42. Dousa, T.P., Preiss, J., Kim, J.K., Hui, Y.S.F., Knox, F.G. Activiation of cAMP system and protein kinase (PK) by parathyroid hormone (PTH) and calcitonin (CT) without phosphaturia in hamster. Clin. Res. 24 p. 399 A (Abstract) (1976).

43. Edwards, B.R., Sutton, R.A.L., Dirks, J.H. Effect of calcium infusion on renal tubular reabsorption in the dog. Am. J. Physiol. 227: 13-18 (1974).

44. Eisenberg, E. Renal effects of parathyroid hormone. In Parathyroid hormone and Thyrocalcitonin. Ed. Talmage, R.V. Belanger, L.F. Excerpta Medica Amsterdam. pp.465-475 (1965).

45. Ellsworth, R., Nicholson, W.M. Further observations upon the changes in the electrolytes of the urine following the injection of parathyroid extract. J. Clin. Invest. 14: 823-827 (1935).

46. Fang, V.S., Tashian, A.M. Jr. Studies on the role of the liver in the metabolism of parathyroid hormone. Effects of partial hepatectomy and incubation of the hormone with tissue homogenate. Endo. 90: 1177-1184 (1972).

47. Fischer, J., Oldham, S., Sisemore, C., Arnold, C. Calcium regulated parathyroid hormone peptidase. Proc. Nat. Acad. Sci. U.S.A. 69: 2341-2345 (1972).

48. Forte, L.R. Characterization of adenyl-cyclase of rat kidney plasma membrane. Bioch. Bioph. Acta 266: 524-542 (1972)

49. Frick, A. Proximal tubular reabsorption of inorganic phosphate during saline infusion in the rat. Am. J. Physiol. 223: 1034-1040 (1972).

50. Frick, A. et al. Microperfusion study of calcium transport in the proximal tubule of the rat kidney. Pflügers Arch. 286: 109-117 (1965).

51. Frick, A. Mechanism of inorganic phosphate diuresis secondary to saline infusions in the rat. Pflugers Arch. 313: 106-110 (1969).

52. Fuhr, J., J. Kaczmarczyk and C.D. Kruttgen. Eine einfache colorimetrische Methode zur Inulinbestimmung fur Nieren-clearance - untersuchungen bei Stoffwecheselgesunden Diabetikern. Klin. Wochschr. 33: 729-730 (1955).

53. Gaillard, P.J. Parathyroid and bone in tissue culture. In the Parathyroids. Ed. Greep, R.O. and Talmage, R.V. pp. 20-45, Springfield, Illinois (1961).

54. Goldberg, M., Beck, L.M., Puschett, J.P., Schubert, J.J. Site of action of Benzothiadiazi[redacted] Furosemide, Ethacrynic Acid. In Modern Diuretic Therapy. Excerpta Medica, Amsterdam p. 135 (1973).

55. Greenberg, D.M., Mackey, M.A. The effect of parathyroid extract on blood magnesium. J. Biol. Chem. 98: 765-768 (1932).

56. Greenwald, I., Gross, J. The effect of administration of a potent parathyroid extract upon the excretion of nitrogen, phosphorous calcium and magnesium with some remarks on the solubility of calcium phosphate in serum and on the pathogenesis of tetany. J. Biol. Chem. 66: 217-227 (1925).

57. Habener, J.F., Kempler, B., Potts, J.T. Jr., Rich, A. Proparathyroid hormone: biosynthesis by human parathyroid adenomas. Science 178: 630-633 (1972).

58. Hamburger, R.J., Lawson, N.L., Denis, J.W. Effects of cyclic adenosine nucleotides on fluid absorption by different segments of proximal tubule. Am. J. Physiol. 227: 396 (1974).

59. Hamilton, J.W., Spierto, F.W., MacGregor, R.M., Cohn, D.V. Studies on the biosynthesis in vitro of parathyroid hormone. II. The effect of calcium and magnesium on synthesis of parathyroid hormone isolated from bovine parathyroid tissue and incubation medium. J. Biol. Chem. 246: 3224-3233 (1971).

60. Handler, J.S. A study of renal phosphate excretion in the dog. Am. J. Physiol. 202: 787-790 (1962)

61. Harris, C.A., Baer, P.G., Chirto, E., Dirks, J.H. Composition of mammalian glomerular filtrate. Am. J. Physiol. 227: 972 (1974).

62. Harris, C.A., Burnatowska, M., Sutton, R.A.L., and Dirks, J.H. Evidence for parathyroid hormone (PTH) enhancement of calcium and magnesium reabsorption in the terminal nephron segment of the hamster. A Clin. Res. 24 401 A (Abstract) (1976).

63. Heaton, F.W. The parathyroid glands and magnesium metabolism in the rat. J. Clin. Sci. 28: 543-553 (1965).

64. Hellman, D.E., Baird, H.R., Bartter, F.C. Relationship of maximal tubular phosphate reabsorption to filtration rate of the dog. Am. J. Physiol. 207: 89-96 (1964).

65. Heersche, J.N.M., Fedak, S.A. and Aurbach, G.D. The mode of action of dibutryl adenosine 3'5' monophosphate on bone tissue in vitro. J. Biol. Chem. 246: 6770-6775 (1971).

66. Heersche, J.N.M., Marcus, R. and Aurbach, G.D. Calcitonin and the formation of 3'5' - AMP in bone and kidney. Endo. 94: 241-247 (1974).

67. Hellman, D.F., Au, W.U.W., Bartter, F.C. Evidence for a direct effect of parathyroid hormone on urinary acidification. Am. J. Physiol. 209: 643-650 (1965).

68. Henion, W.F., Sutherland, E.V., Posternak, T.H. Effects of derivatives of adenosine 3'5' - phosphate on liver slices and intact animals. Bioch. Bioph. Arch. 148: 106-110 (1967).

69. Ikkos, S.J, Woodward, C.J., Geinzella, C.A. The effects of human growth hormone in man . Acta. Endo. 32: 341 (1959).

70 Kalu, D.N., Hadji * Georgopoulos, A., Sarr, M.G., Solomon, B.A., Foster, G.V. Role of parathyroid hormone in the maintenance of plasma calcium levels in rats. Endo. 95: 1156-1165 (1974).

71. Kaminsky, N.L., Broadus, A.E., Hardman, J.C., Jones, D.V. Jr. Ball, J.H., Sutherland, E.W. and Liddle, G. Effects of parathyroid hormone on plasma urinary adenosine 3'5' monophosphate in man. J. Clin. Invest. 49: 2387-2395 (1970).

72. Kenny, A.D., Heiskell, C.A. Effects of crude thyrocalcitonin on calcium and phosphorous metabolism in rats. Proc. Sci. Exp. Biol. Med. 120: 269 (1965).

73. Keutmann, H.T., Dawson, B.E., Aurbach, G.D., Potts, J.I. Jr. A biologically active amino-terminal fragment of bovine parathyroid hormone prepared by dilute acid hydrolysis. Biochem. 11: 1973-1979 (1972).

74. Kleeman, C.R., Bernstein, O., Rockney, R., Dowling, J.T., Maxwell, M.H. Studies on the renal clearance of diffusable calcium and the role of the parathyroid glands in its regulation. Yale J. Biol. Med. 34: 1-30 (1961).

75. Kinne, R., Shaltz, L.J., Kinne-Saffran, E., Schwartz, I.L. Distribution of membrane bound cyclic AMP dependent protein kinase in plasma membranes of cells of the kidney cortex. J. Mab. Biol. 24: 145-159 (1975).

76. Knox, F.G., Lechene, C. Distal site of action of parathyroid hormone. Proc. Nat. Acad. Sci. U.S.A. 68: 63-67 (1971).

77. Lambert, P.P., Venderveiken, F., DeKosler, J.P., Kohn, R.J., DeMyttelnaere, M. Study of phosphate excretion by the stop flow technique. Nephron 1: 103-117 (1964).

78. Kuntziger, H., Amiel, C., Roinel, N., and Morel, F. Effects of parathyroidectomy and cyclic AMP on renal transport of phosphate, calcium and magnesium. Am. J. Physiol. 227: 905-911 (1974).

79. Lassiter, E., Gottschalk, C.W., Mylle, M. Micropuncture study of renal tubule reabsorption of calcium in normal rodents. Am. J. Physiol. 204: 771-775 (1963).

80. Le Grimellec, C., Roinel, N., Morel, F. Simultaneous magnesium calcium, phosphorus, potassium and chloride analysis in rat tubular fluid. IV During acute plasma phosphate loading. Pflügers Arch 346: 189-194 (1974).

81. Levinsky, N.G., Davidson, D.G. Renal action of parathyroid extract in the chicken. Am. J. Physiol. 191: 530-536 (1957).

82. MacCallum, W.B., Voegtlin, C. On the relation of tetany to the parathyroid glands and to calcium metabolism. J. Exp. Med. 11: 118-151 (1909).

83. MacIntyre, I., Boss, I., Troughton, V.A. Parathyroid hormone and magnesium homeostasis, Nature 198: 1058-1060 (1963).

84. Marcus, R., Aurbach, G.D. Adenyl cyclase from renal cortex. Bioch. Bioph. Acta 242: 410-421 (1970).

85. Marshal, E.K. Jr. and Graffin, A.L. Excretion of inorganic phosphate by the aglomerular kidney. Proc. Soc. Exp. Biol. Med. 31: [illegible] (1933).

86. Marx, S.J., Fedak, S.A., Aurbach, G.D. Preparation and characterization of a hormone responsive renal plasma membrane fraction. J.Bio . Chem. 247: 6913-6918 (1972).

87. Massry, S.G., Coburn, J.W., Kleeman, C.R. The influence of extracellular volume expansion on renal phosphate reabsorption in the dog. J. Clin. Invest. 48: 1237-1245 (1969).

88. Massry, S.G., Coburn, J.W. and Kleeman, C.R. Renal handling of magnesium in the dog. Am. J. Physiol. 216: 1460-1467 (1969).

89. Massry, S.G., Coburn, J.W., Chapman, L.W., Kleeman, C.R. Role of serum calcium, parathyroid hormone, and NaCl infusion on renal Ca and Na clearances. Am. J. Physiol. 214: 1403-1409 (1968).

90. Melick, R.A., Aurbach, G.D., Potts, J.T. Jr. Distribution and half life of I. 131 - Labelled parathyroid hormone in rat. Endo. 77: 198-202 (1965).

91. Mendel, L.B., Benedict, S.S. Evidence for relation between metabolism of magnesium and calcium in animals. Amer. J. Physiol. 25: 1323 (1909).

92. Melson, G.L., Chase, L.R., Aurbach, G.D. Parathyroid hormone sensitive adenyl cyclase in isolated renal tubules. Endo. 86: 511-518 (1970).

93. Morel, F., Roinel, N, LeGrimellec, C. Electron probe analysis of tubular fluid composition. Nephron 6: 350-364 (1969).

94. Mudge, G.H., Berndt, W.V., Valtin, M. Tubular transport of urea, glucose, phosphate, uric acid, sulphate and thiosulphate. in Orloff, J. and P.W. Berliner. Handbook of Physiology Renal

Physiology, Williams and Williamson, Baltimore p.587-752 (1973).

95. Muldowney, F.P., Carrol, D.V., Donohove, J.F. and Freoney, R. Correction of renal bicarbonate wastage by parathyroidectomy Q.J. Med. 40: 487-491 (1971)

96. Murayama, Y., Morel, F., LeGrimellec, C. Phosphate, calcium and magnesium transfers in proximal tubules and loops of Henle as measured by single nephron microperfusion. Pflugers Arch. 331: 1-9 (1972).

97. Murdough H.V.J. and Robinson, R.R. Magnesium excretion in the dog studied by stop-flow analysis. Am. J. Physiol. 198: 571-574 (1960).

98. Nordin, B.E.C. The effect of intravenous parathyroid extract on urinary pH, bicarbonate and electrolyte excretion. Clin. Sci. 19: 311-319 (1960).

99. Nordin, B.E.C., Peacock, M., Wilkinson, R. The relative importance of gut, bone and kidney in the regulation of serum calcium. In Parathyroid hormone and the calcitonins. Ed. Talmage, R.V., Munson, P.L. Proc. of the fourth Parathyroid conference p. 203 (1969).

100. Oldham, S.B., Fischer, J.A., Copen, C.C., Sizemore, G.W., Arnaud, C.D. Dynamics of parathyroid hormone secretion in vitro. Am. J. Med. 50: 650-657 (1971).

101. Pitts, R.F. The excretion of urine in the dog. Am. J. Physiol. 106: 1-8 (1933).

102. Pitts, R.F., and Alexander, R.S. The renal reabsorption of mechanism for inorganic phosphate in normal and acidotic dogs. Am. J. Physiol. 142: 648-652 (1944).

103. Posternak, T.M., Sutherland, E.W. Henion, W.F. Derivatives of cyclic 3'5' adenosine monophosphate. Biochm. Bioph. Acta. 65: 558-560 (1962).

104. Potts, J.T., Tregear, G.W., Keutmann, H.T., Niall, H.D., Sauer, R., Deftos, L.J., Dawson, B.F., Hogan, M.L., Aurbach, G.D. Synthesis of biologically active N-terminal tetratriacontapeptide of parathyroid hormone. Proc. Nat. Ac. Sci. U.S.A. 68: 63-67 (1971).

105. Pullman, T.R., Lavender, A.R. and Rasmussen, H. Direct renal action of purified PTH extract. Endo. 67: 570-582 (1960).

106 Puschett, T.B., Moranz, J., Kurnick, W.S. Evidence for direct action of cholecalciferol and 25-hydroxy cholcalciferol on the renal transport of phosphate, sodium and calcium. J. Clin. Invest. 51: 373 (1972).

107. Puschett, T.B., Agus, L.S., Senesky, D., Goldberg, M. Effects of saline loading and aortic obstruction on proximal phosphate transport. Am. J. Physiol. 223: 851-857 (1972).

108. Rall, T., Sutherland, E.W. Formation of cyclic adenine Ribonucleotide by tissue particles. J.B. Chem. 232: 1065-1076 (1958).

109. Raisz, L.V., Klein, D. Stimulation of bone resorption by dibutyryl cAMP in vitro. Fed. Proc. 28: 320 (19[redacted] (Abstract).

110. Rasmussen, H., Tenenhouse, A. Cyclic AMP, Ca and membranes Proc. Nat. Ac. Sc. 59: 1364-1370 (1968).

111. Rasmussen, H. The influence of parathyroid function upon the transport of calcium in isolated sacs of rat small intestine. Endo. 65: 517-519 (1959).

112. Rasmussen, H. Pechet, M., Fast, D. Effect of dibutyryl cyclic adenosine 3'5' monophosphate, theophylline and other nucleotides upon calcium and phosphate metabolism. J. Clin. Invest. 47: 1843-1850 (1968).

113. Rasmussen, H. Cell communication, calcium ion and cyclic adenosine monophosphate. Science 170: 404-412 (1970).

114. Rebhurn, L., Villar-Polasi, L. Stimulation of purified muscle protein kinase by cAMP and its butyrated derivatives. Biochem and Bioph. Acta 321: 165-170 (1973).

115. Robinson, G.A., Butcher, R.W., Sutherland, E.W. Cyclic AMP. Ann. Rev. Bioch. 37: 149-174 (1968).

116. Robinson, R.R., Portwood, R.M. Mechanism of Mg excretion by the chicken. Am. J. Physiol. 202: 309-312 (1962).

117. Rouffignac, D. de,, Morel, F., Moss, W., Roinel, N. Micropuncture study of water and electrolyte movements along the loop of Henle in psammomys with special reference to magnesium, calcium and phosphorus. Pflugers Arch. 344: 309 (1973).

118. Russel, R.G., Casey, P.A., Fleish, H. Stimulation of phosphate excretion by the renal arterial infusion of 3'5' AMP - a possible

mechanism of action of PTH. Calcil. Tissues Res. 2 (Suppl.) 54 (1968).

119. Samiy, A.H.E., Brown, J.L., Globas, D.L., Kessler, R.H. and Thompson, D.D. Interrelation between renal transport systems of magnesium and calcium. Am. J. Physiol. 198: 599-602 (1960).

120. Samiy, A.H., Hirsch, P.F., Ramsay, A.G. Localization of phosphaturic effect of parathyroid hormone in the nephron of the dog. Am. J. Physiol. 208: 73-77 (1965).

121. Sherwood, L.M., Mayer, G.P., Ramberg, G.F. Jr., Kronfeld, D.S., Aurbach, G.D., Potts, J.T. Jr. Regulation of parathyroid hormone secretion: proportional control by calcium, lack of effect of phosphate. Endo. 83: 1043-1051 (1968).

122. Shlatz, L.J., Schwartz, I.L, Kinne-Saffran, E., Kinne, R. Distribution of parathyroid hormone-stimulated adenylate cyclase in plasma membranes of cells of the kidney cortex. J. Memb. Biol. 24: 131-144 (1975).

123. Staum, B.B., Hamburger, R.T., Goldberg, M. Tracer microinjection study of renal tubular phosphate reabsorption in the rat. J. Clin. Invest. 51: 2271 (1972).

124. Steele, T.H. Increased urinary phosphate excretion following volume expansion in normal man. Metab. 19: 129-239 (1970).

125. Stewart, G.S., Bowen, H.F. The urinary phosphate excretion factor of parathyroid gland extracts: a hormone or an artefact: Endo: 51: 80-96 (1952).

126. Strickler, T.C., Thompson, D.D., Klose, R.M. Micropuncture study of inorganic phosphate excretion in the rat. J. Clin. Invest. 43: 1596-1607 (1964).

127. Sutcliffe, N.S., Martin, J.J., Eismon, D.A., Pilnyk, R. Binding of parathyroid hormone to bovine kidney-cortex plasma membranes. Biochem. J. 134: 913-921 (1973).

128. Sutherland, E.D., Rall, T.W. Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles. J. Biol. Chem. 232: 1077-1092 (1958).

129. Sutherland, E.D., Rall, T.W. The relation of adenosine 3'5' phosphate and phosphorylase to the actions of catecholamines and other hormones. Pharmac. Rev. p. 265-299 (1960).

130. Sutton, R.A.L., Wong, N.L.M. and Dirks, J.H. Effects of parathyroid hormone on sodium and calcium transport in the dog nephron. Clinical Science 51: 345-351 (1976).

131. Talmage, R.V., Kraintz, F.W. Progressive changes in renal phosphate and calcium excretion in rats following parathyroidectomy or parathyroid administration. Proc. Soc. Exp. Med. 87: 263-276 (1954).

132. Talmage, R.V., Kraintz, F.W., Buchanan, G.D. Effects of parathyroid extract and phosphate salts on renal calcium and phosphate excretion after parathyroidectomy. Proc. Soc. Exp. Med. 88: 600-604 (1955).

133. Talmage, R.V. Studies on the maintenance of serum calcium levels by parathyroid action on bone and kidney. Ann. N.Y. Acad. Sci. 64: 326-335 (1956).

134. Talmage, R.V. and Elliott, T.R. Influence of parathyroids on intestinal absorption of radiocalcium and radiostrontium. Fed. Proc. 17: 160(Abstract) (1958).

135. Targovnik, T.H., Rodman, T.S., Sherwood, L.M. Regulation of parathyroid hormone function in vitro: quantitative aspects of calcium and magnesium ion control. Endo. 88: 1477-1482 (1971).

136. Thomson, D.L., Collip, J.B. The parathyroid glands. Physiol. Rev. 12: 309-383 (1932).

137. Thomson, D.D., Hiatt, H.H., Renal reabsorption of phosphate in normal human subjects and in patients with parathyroid disease. J. Clin. Invest. 36: 550-555 (1975).

138. Vurek, G.L., Pegram, W.E. Fluoremetric method for the determination of nanogram quantities of inulin. Analytical Biochem. 16: 409-419 (1966).

139. Walker,A.M., Hudson, C.C. The role of the tubule in the excretion of inorganic phosphates by the amphibian kidney. Am. J. Physiol. 118: 167-174 (1937).

140. Webster, G.D., Mann, J.B., Hills, A.G. The effect of phosphate infusion upon renal phosphate clearance in man: evidence for tubular phosphate secretion. Metabolism. 16: 797-814 (1967).

141. Wells, M., Lloyd, W. Hypercalcemic and hypophosphatemic effects of dibutyryl cyclic AMP in rats after parathyroidectomy. Endo. 84: 861-867 (1969).

142. Wen, S.L., Evanson, R.L., Dirks, J.H. Micropuncture study of renal magnesium transport in proximal and distal tubule of the dog. Am. J. Physiol. 219: 570-576 (1970).

143. Wen, S.F. Micropuncture studies of phosphate transport in the dog. J. Clin. Invest. 53: 143 (1974).

144. Widrow, S.H., Levinsky, N.G. The effect of parathyroid extract on renal tubular calcium reabsorption in the dog. J. Clin. Invest. 14: 2151-2159 (1962).

145. Wolbach, R.A., Phlorizin and renal phosphate secretion in the spiny dog fish squalus acanthias. Am. J. Physiol. 219: 886-892 (1970).