EFFECTS OF DIPHOSPHONATES IN DISORDERS OF BONE TURNOVER

Submitted for the degree of Doctor of Medicine

in the University of Sheffield

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SUMMARY OF THESIS

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This thesis describes several interrelated studies concerning the effects of diphosphonates in the treatment of disorders of bone turnover.

Diphosphonates have previously been shown to suppress bone turnover in Paget's disease when given orally for at least three months. However, five daily intravenous infusions of the diphosphonates, clodronate, etidronate and a newer agent, aminohexane diphosphonate (AHDP), induced marked and sustained biochemical and clinical changes similar to those achieved with long-term oral treatment. These new regimens may be useful in the management of Paget's disease.

The assessment of biochemical response in Paget's disease, particularly in re-treated patients, is more complex than hitherto described. The pre-treatment serum alkaline phosphatase and, in re-treated patients, the extent of biochemical relapse are important determinants of response.

Little was previously known of the effects of high doses of diphosphonates on mineral metabolism.

Inhibition of mineralisation occurred following all 3 intravenous diphosphonates. However, this effect was partial and short-lived following clodronate or AHDP, but was complete and much more sustained following etidronate. All three diphosphonates induced hyperphosphataemia by increasing renal tubular reabsorption of phosphate. This latter effect was related, both temporally and quantitatively, to the effects of these agents on skeletal mineralisation suggesting a possible causal relationship.

Hypocalcaemic responses were consistently observed in pagetic patients following intravenous clodronate or AHDP, but not following etidronate. Similarly, infusions of either clodronate or AHDP, but not etidronate, induced normalisation of mean serum calcium in patients with hypercalcaemia of malignancy. Furthermore, oral clodronate, but neither high-dose nor low-dose oral etidronate, induced significant hypocalcaemic responses in patients with primary hyperparathyroidism. The degree of inhibition of bone resorption was similar for each of the diphosphonates in all of the disorders studied, suggesting that the attenuated hypocalcaemic effects of etidronate resulted from the greater impairment of mineralisation that occurred following treatment with this agent.

ACKNOWLEDGEMENTS AND STATEMENT OF ORIGINALITY

This thesis is based on various studies I conducted in the Department of Human Metabolism and Clinical Biochemistry at Sheffield University Medical School. For the initial 10 months I had tenure of a grant from the United Sheffield Hospitals Special Trustees and thereafter, for 32 months, I became a Training Fellow of the Medical Research Council. I am indebted to the generosity of these bodies for the opportunities they provided to undertake this work.

My main thanks goes to Dr. John Kanis, Reader in the department, who has guided my studies and encouraged the development of my interest in clinical in clinical research. My thanks also go to Professor Graham Russell who as head of department has provided a fertile soil for research activity.

In the work embodied in this thesis, I designed and coordinated the various studies. The concepts of using short intravenous courses of diphosphonates in the treatment of Paget's disease of bone and of the use of a triple tetracycline bone labelling technique for study of the acute effects of these treatments on mineralisation were original and my own. With the exception of a minority of the patients treated with oral clodronate 1.6g daily for 6 months I have personally treated each of the patients used in the clinical studies described in

this thesis. In addition, the collection and interpretation of data have been entirely my own.

The studies described involved a large number of patients and would not have been possible without the help of several clinical colleagues. These included Mr. Richard Percival, Dr. Richard Gray, Mr. Graeme Urwin, Dr. Neveen Hamdy and Mr. Roger Atkins. Although these colleagues maintained separate clinical interests, each has shared in the extensive clinical workload of the department and contributed to the clinical care of many of the patients described in this work.

Some specialised laboratory techniques, including quantitative histomorphometry of bone and hormone assays were used to investigate these patients. During the tenure of my training fellowship I became familiar with bone histomorphometric techniques required for the measurement of mineral apposition rate and also with laboratory techniques for measurement of immunoreactive parathyroid hormone (iPTH). However, in order to obtain sufficiently reproducible results, particularly from bone histomorphometry, prolonged experience is required. Thus I am grateful to Ms. Monique Beneton for the histomorphometric analyses and also to Dr. John Galloway who performed the measurements of iPTH. Mrs. Ragini Vaishnav performed the measurement of $1,25(OH)_2D_3$ in serum samples from two patients with immobilisation hypercalcaemia. The Department of Clinical Chemistry has also been very helpful both in providing routine biochemical measurements on serum as

well as performing measurements of urinary hydroxyproline.

Finally, I owe my gratitude to Mrs. Ina Underwood, research nurse, for her valuable assistance in collecting blood samples and to the medical and nursing staff and patients of the Royal Hallamshire Hospital and Western Park Hospital for their patience, help and cooperation.

List of abbreviations

AHDP	aminohexane diphosphonate
APD	3-amino-1-hydroxypropylidene diphosphonate
Cl_2MDP	clodronate; dichloromethylene diphosphonate
EHDP	etidronate; ethane-1-hydroxy 1,1 diphosphonate
PPi	inorganic pyrophosphate
IL-1	interleukin-1
OAF(s)	osteoclast activating factor(s)
Ca/Cr	fasting urinary calcium/creatinine ratio
OHP/Cr	fasting urinary hydroxyproline/creatinine ratio
CaE	calcium excretion per unit volume of glomerular filtrate
TmP/GFR	maximum amount of phosphate reabsorbed per unit volume of glomerular filtrate
PTH	parathyroid hormone
iPTH	immunoreactive parathyroid hormone
GFR	glomerular filtration rate
ECF	extracellular fluid
$25(OH)D_3$	25 hydroxy vitamin D_3
$1,25(OH)_2D_3$	1,25 dihydroxy vitamin D_3

SECTION ONE

INTRODUCTION AND METHODS

CHAPTER ONE

INTRODUCTION

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THE PHYSIOLOGY OF BONE AND CALCIUM

The skeleton provides a strong rigid framework for the body. It is capable of growth and self repair and acts as a reservoir of mineral for the regulation of calcium concentration in the extracellular fluid. It achieves these various tasks by virtue of complex and interrelated control mechanisms which act to modulate the activities of both bone forming cells, the osteoblasts, and bone resorbing cells, the osteoclasts. The processes of bone formation and bone resorption are continuous and require to be well regulated in order both to preserve calcium homeostasis and to maintain the structural integrity of the skeleton.

Known physiological influences on these processes include hormones, especially parathyroid hormone (PTH), 1,25 dihydroxy vitamin D ($1,25(OH)_2D_3$) and calcitonin. Many more factors, such as prostaglandins and various cytokines can be demonstrated to have potent effects on bone in vitro and have been implicated in the pathogenesis of skeletal disease although their physiological importance is not known. Mechanical stress is a further physiological bone regulatory signal and is likely to be of primary importance in maintaining an efficient bone structure (Jaworski, 1984). When stresses are reduced, as during bed rest or weightlessness in space, bone resorption greatly exceeds formation and

rapid bone loss ensues (Lutwak et al, 1969). Conversely, skeletal mass is increased in marathon runners compared with normal controls and even in osteoporotic subjects modest exercise can be shown to delay or reverse bone loss (Aloia et al, 1978a+b).

In addition to these influences it is clear from the organised activity of osteoblasts and osteoclasts that these cells are capable of interacting with each other in order to coordinate their activities in the remodelling process. To date the precise nature of these coordinating intercellular signals has not been determined but there has been considerable progress in the understanding of the processes involved in bone remodelling. This knowledge has contributed greatly to our understanding of disease processes in skeletal disorders.

Remodelling of bone

In adults, in whom longitudinal growth has ceased, much of the skeletal turnover (>95%) is accounted for by remodelling of bone. The remodelling process is comprised of a series of discrete events which are well characterised morphologically, but physiologically ill understood (for review see Parfitt, 1983). The process is important for the self-repair of microdamage in skeletal tissue (Frost, 1981). The remodelling sequence,

which was first described by Frost (1970) in cortical bone, was based on observations that a resorption phase preceded a formation phase and both were definable as discrete cellular and metabolic events in both space and time. It has been estimated that at any one time there are about 1.5 million active remodelling sites in an average adult skeleton with normal turnover (Parfitt, 1983). A phase of osteoclast activation and osteoclastic bone resorption results in the formation of a resorption cavity which in trabecular bone has a final depth of about 60um (Parfitt, 1984). Mononuclear cells are found deep within resorption bays, and this is presumed to represent a later event in the resorption sequence (Baron et al, 1983). These cells move off the resorption cavity and may be responsible for the signals that ultimately attract osteoblasts to the site of previous resorption (Baron et al, 1983). The surface is smoothed and a cement substance is deposited, but the responsible cells are not known. The secondary attraction of osteoblasts, the mechanism of which is poorly understood, is known as coupling and is important both for the maintenance of skeletal mass and of structural integrity (Parfitt, 1982).

Once on the resorption surface, osteoblasts synthesise osteoid matrix, largely composed of type I collagen. In addition to collagen, osteoblasts synthesise other bone matrix components. These include

the gamma-carboxyglutamic acid containing protein, osteocalcin, which is dependent on vitamin K for carboxylation of three glutamate residues which enables it to bind calcium (Price et al, 1980). Production of osteocalcin is dependent on $1,25(OH)_2D_3$ (Price et al, 1981). Osteonectin is a bone derived phosphoprotein which binds both calcium and collagen (Termine, 1981). These proteins appear to inhibit hydroxyapatite formation but their physiological role, and in particular whether they might be involved in the coupling process, is unknown (Menanteau et al, 1982). Many other bone proteins have been identified which might regulate skeletal metabolism. Of these, human skeletal growth factor, isolated from human bone (Farley & Baylink, 1981) appears to stimulate bone cell proliferation. Although such chemical signals may be important for osteoblast recruitment the site specificity for cell attachment is likely to be due to other factors such as a chemotactic constituent within the cement substance (Parfitt, 1982, 1984). The rate of matrix apposition is most rapid at the beginning of bone formation (2-3um/day) when the osteoblasts are columnar or "plump". Later this process slows and the cells become flattened (Parfitt, 1984). Newly formed matrix does not mineralise immediately suggesting that it may have to undergo changes before mineralisation can occur. There is evidence that in growing bone osteoblasts produce matrix vesicles which

may be responsible for initiating mineralisation (Anderson, 1978). On completion of mineralisation the bone site enters a quiescent phase. Quiescent surfaces account for 80% or more of total trabecular surface in normal adults (Parfitt, 1984).

In the healthy young adult, in whom bone mass is constant, the rate of bone resorption must be equal to the rate of new matrix formation and mineralisation. There is no direct method for measurement of the rates of bone formation and resorption. Radio-calcium kinetic studies, which measure total calcium movement into bone, produce estimates for calcium accretion of around 12mmol/day (Aubert & Milhaud, 1960). However, considerable exchange of bone mineral, possibly mediated by the presence of brushite in areas undergoing mineralisation, occurs independently of cellular bone turnover (Lauffenburger et al, 1977; Neuman et al, 1982). Therefore, such techniques probably overestimate the rate of bone formation (Krane & Schiller, 1979) and the true rate for a normal adult is probably less (around 5mmol calcium per day). A closely similar amount of bone must be resorbed in order to maintain a relatively constant bone mass.

Remodelling activity occurs on all bone surfaces and it is therefore not surprising that bone turnover is much higher in trabecular bone, which has a very high surface to volume ratio, than in cortical bone where this

ratio is much lower. At any one time approximately 15-20% of the bone surface is undergoing bone remodelling, the remainder being relatively inert.

A consideration of the remodelling process is important for understanding how skeletal turnover may become disturbed in disease. In a number of metabolic bone disorders despite increases in bone turnover the normal close quantitative relationship between the rates of bone formation and bone resorption is maintained. A good example is provided by Paget's disease of bone where although bone turnover may be 10-20 times greater than normal, overall skeletal balance is usually close to zero, as the anatomical coupling process ensures close matching of formation and resorption. This close matching of the rates of formation and resorption is demonstrated by the preserved relationship between serum alkaline phosphatase and urinary hydroxproline excretion (markers of bone formation and resorption repectively) in patients with Paget's disease (Figure 1.1). A similar "coupled" increase in bone turnover occurs in the majority of patients with hyperparathyroidism (Parsons, 1979).

In other skeletal disorders, such as osteoporosis or skeletal involvement in malignancy, bone formation and resorption rates are imperfectly matched and there is a resultant skeletal imbalance for calcium. Bone loss may occur either because osteoblasts fail to

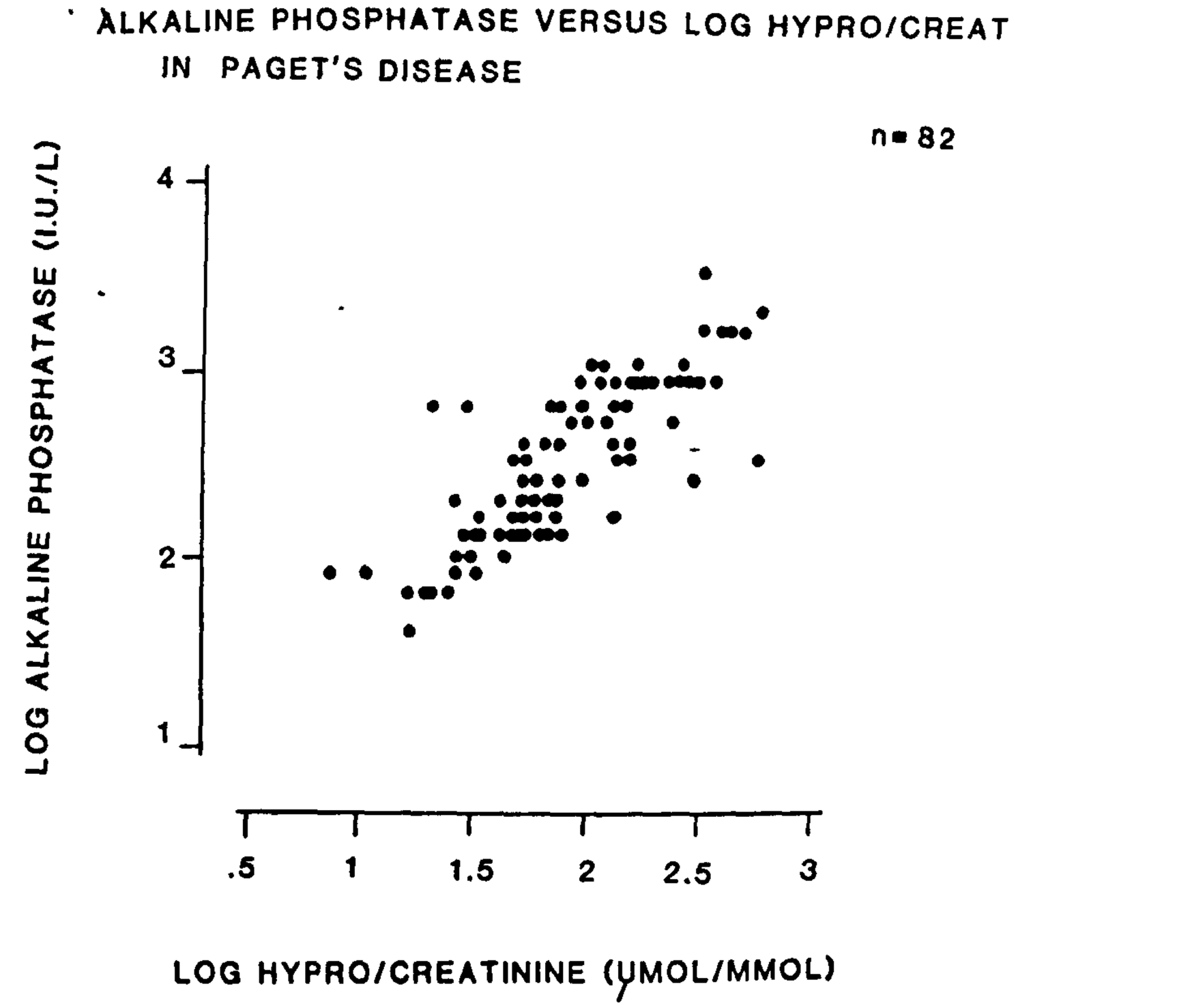


Figure 1.1 Relationship between serum alkaline phosphatase and urinary hydroxyproline in patients with Paget's disease of bone. (From Douglas, 1983).

become attracted into the resorption cavities (true anatomical uncoupling) or because the amount of bone formed in each remodelling unit is quantitatively less than the amount of bone resorbed to leave a net bone deficit. It is likely that both of these mechanisms contribute to the development of postmenopausal osteoporosis (Baron, 1981). A relative failure of the coupling process may lead to a reduced osteoblast population on the resorption surface. In such circumstances the amount of new bone would be reduced even if each osteoblast produced the same quantity of bone matrix as normal (Parfitt, 1982). In multiple myeloma osteoclastic bone resorption is stimulated by the local production of osteoclast activating factors (OAFs) whereas the expected secondary increase in bone formation is suppressed (Mundy et al, 1974a, 1974b; Stepan et al, 1984). This disease represents a true disorder of coupling in the sense that resorption cavities are produced that will never subsequently attract osteoblasts (Stewart et al, 1982).

Calcium homeostasis

Calcium is widely distributed throughout the tissues of the body, although by far the greatest proportion is found in bone (approximately 99% of total). Almost 1% is found in the ECF and a variable amount is

present inside cells. Actual free cytosolic concentrations of calcium are extremely low (between 10^{-8} and 10^{-7}M) but have been found, particularly in conjunction with the phosphoinositol pathway, protein kinase C and calmodulin, to be of fundamental importance in the regulation of a multitude of cell functions, including cell division, protein synthesis and exocytosis, in various tissues. Some aspects of this rapidly expanding area have been the subject of recent reviews (Brown et al, 1985; Rubin et al, 1982).

Regulation of the ECF concentration of calcium is critical to maintain normal neuro-muscular activity. A fall in plasma calcium concentration results in tetany and convulsions whereas hypercalcaemia results in many adverse effects which are reviewed subsequently.

Plasma calcium

For over fifty years it has been appreciated that serum (and plasma) calcium is composed of three major fractions (McLean and Hastings, 1935). These are, free or ionised calcium (around 50 per cent), calcium bound to protein (mainly albumin) which is non-diffusible (approximately 40 per cent) and a remaining 10 per cent or so which is complexed to anions such as bicarbonate and citrate. It is the ionised fraction which has greatest physiological and therefore clinical relevance.

Unfortunately, until recently, direct measurement of this fraction was rarely performed because of technical difficulties requiring the anaerobic collection of plasma samples and the use of ion selective electrodes which was tedious and time consuming. Although in recent years many of these problems have been partially overcome, most clinicians still have to rely on the measurement of total rather than ionised calcium.

Many algorithms have been proposed to give a value which is more closely proportional to the concentration of ionised calcium than is the total calcium concentration. Such adjustments, which "correct" the total plasma calcium to a chosen "normal" value of serum protein or albumin, may be useful, for example, in gross hypoalbuminaemia such as occurs in hepatic failure, where the consequent reduction in protein bound calcium results in a low total serum calcium value despite a normal ionised calcium. Conversely, haemoconcentration due to dehydration or prolonged venous stasis during venepuncture is associated with apparent hypercalcaemia due to an increase in the protein bound fraction. There is a close correlation between serum calcium and serum albumin and in different studies correlation coefficients of between 0.71 and 0.87 have been reported when patients with a large range of serum albumin concentrations are studied (Payne et al, 1973; Orrell, 1971). The slope of such regressions approximate 0.02mmol calcium per 1g/l

change in albumin. This forms the basis for the commonly used adjustments such as adding or subtracting 0.02mmol/l from the measured total calcium for every 1g/l of albumin below or above 40 respectively (Anonymous, 1977; Payne 1973). However, in order for a regression to have useful predictive value a correlation coefficient of greater than 0.9 is required (Morgan, 1983; Armitage, 1971)

All such algorithms have several additional limitations in that they do not account for changes in pH (acidosis reduces protein binding of calcium) or the wide interindividual differences in the degree of binding to albumin and other proteins (Pain et al, 1975). This is of particular relevance to the measurement of calcium in multiple myeloma where a total of six case reports have documented grossly raised concentrations of serum calcium due to calcium binding to paraproteins (Annesley et al, 1982; Spira et al, 1980; Soria et al, 1976; Jaffe & Mosher, 1979; Mazzaferri et al, 1978; Lingarde & Zetterval, 1973). "Correction" of the serum calcium in these cases, because of the associated hypoalbuminaemia, may be misleading rather than useful. The importance of such calcium-immunoglobulin binding in measurement of serum calcium in patients with myeloma or other dysproteinaemias is unknown.

In a large study comparing total serum calcium with values adjusted according to several algorithms none of the adjustments significantly enhanced the correlation

with ionised calcium (Ladenson et al, 1978). Thus, caution should be exercised in the interpretation of adjusted serum calcium results, particularly when these appear to be out of keeping with the clinical condition of the patient.

Recently there has been renewed interest in the measurement of dialysable (ionised and complexed) fraction of serum calcium as this measurement may be achieved by simple adaptation of the currently used continuous automated colorimetric techniques for measuring total calcium (Prince & Langton, 1985). The technique simply involves substituting a buffer for the acid used to dissociate calcium from protein in the standard automated assay. By excluding the protein bound fraction the major source of variability is eliminated. It is likely that this technique will become widely adopted in future.

Regulation of plasma calcium

The concentration of ionised calcium in the ECF is regulated by homeostatic control mechanisms. These include changes in the three major calcium regulatory hormones, PTH, calcitonin and $1,25(OH)_2D_3$, the major active metabolite of vitamin D. All three hormones alter fluxes of calcium between the ECF and other organs. The major movements of calcium to and from the extracellular fluid are accounted for by calcium fluxes to and from the gut, bone and kidney (Figure 1.2).

Intestinal absorption

Calcium entry into the body by intestinal absorption is dependent on both the dietary intake of calcium and the efficiency of gut absorption which occurs by both active and passive transport mechanisms. Active transport is a vitamin D dependent, high affinity, low capacity mechanism for transport of calcium against an electro-chemical gradient. This system is most vigorous in the duodenum and proximal jejunum. However, there is some evidence that active transport of calcium also occurs in the ileum, albeit at only about one third of the upper jejunal rate (Krejs et al, 1983). Passive diffusion of calcium is a relatively slow but non-saturable process. Thus the active transport

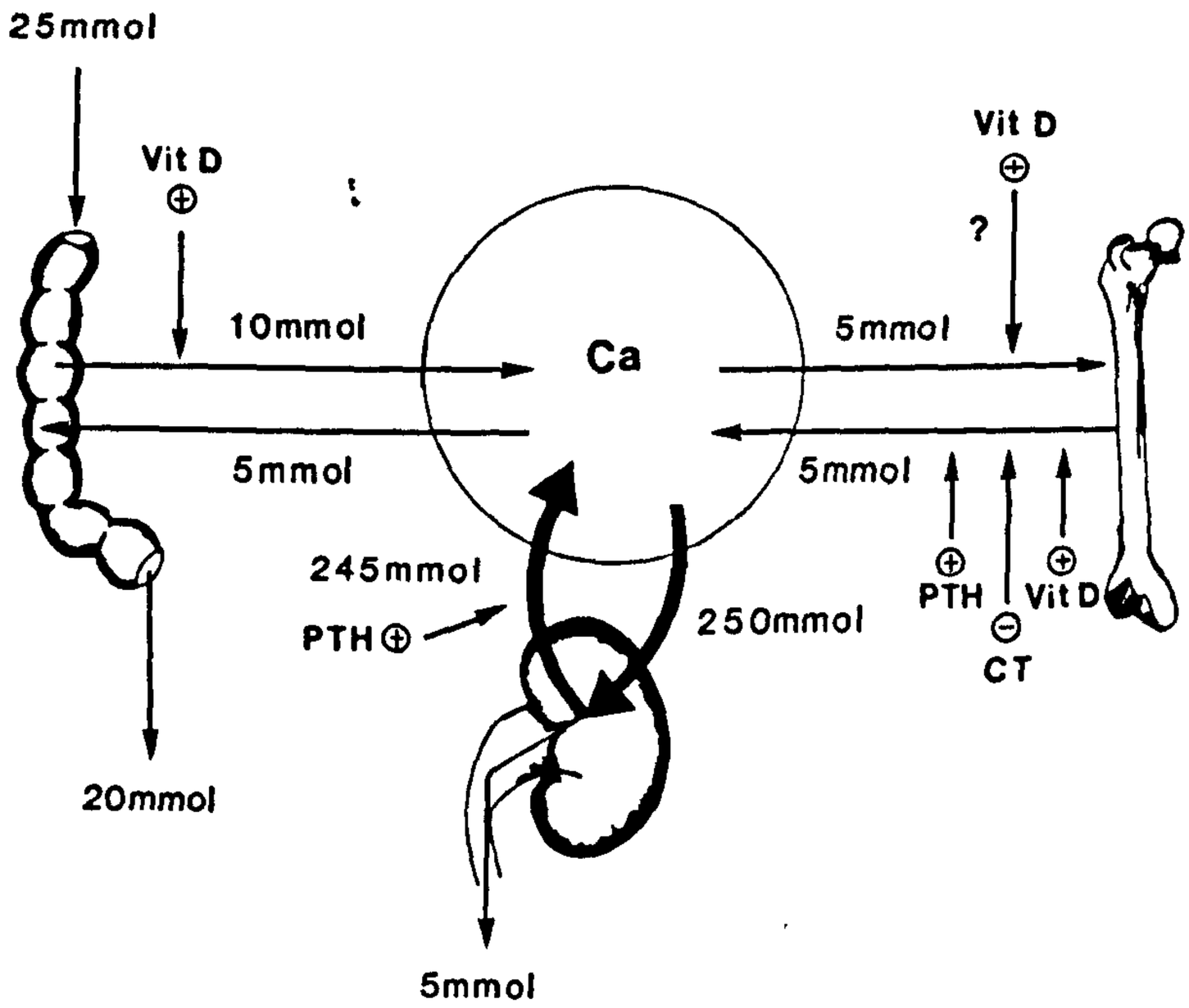


Figure 1.2 Flux diagram to show the exchange of calcium between the ECF and the major organs involved in calcium homeostasis and the sites of action of the major calcium regulatory hormones. (From Kanis, 1982).

mechanism is resposible for most of the calcium absorption at low intraluminal concentrations of calcium whereas the diffusional mechanism becomes relatively more important with high dietary intakes of calcium (Nordin, 1976). Although transport of calcium is most efficient in duodenum and jejunum the intraluminal calcium concentrations and transit times at these sites are considerably less than those found in the ileum (Cramer, 1965). For these reasons, on a normal diet the greatest proportion of intestinal calcium absorption is probably accomplished in the ileum, whereas on a low calcium diet fractionally more may be absorbed in duodenum and jejunum (Cramer, 1965). With a dietary intake of around 25mmol (1g) of calcium per day approximately 40% (10mmol) is absorbed. However, intestinal loss of calcium in intestinal secretions reduces the net absorption to approximately 5mmol per day (Kenny, 1981).

$1,25(OH)_2D_3$ has an important role in the regulation of calcium absorption. During calcium deprivation more 25 hydroxy vitamin D ($25(OH)D_3$) is converted to the active metabolite $1,25(OH)_2D_3$ because of an increase in activity of $25(OH)\ D_3$ 1-alpha hydroxylase. The activity of this enzyme, which is localised within the kidney, is stimulated both by hypocalcaemia and by the secondary hyperparathyroidism that ensues (Norman et al, 1982). $1,25(OH)_2D_3$ directly stimulates active transport of calcium in the intestine thus limiting the

effects of dietary deprivation (Krejs et al, 1983).

In chronic renal failure, damage to the renal parenchyma and hyperphosphataemia reduce the 1-alpha hydroxlase activity despite increased PTH and hypocalcaemia (Cheung et al, 1983). Thus, concentrations of $1,25(OH)_2D_3$ and, consequently, intestinal absorption of calcium are low in patients with renal insufficiency (Chesney et al, 1983). Conversely, patients with sarcoidosis or other granulomatous disorders may have raised concentrations of $1,25(OH)_2D_3$ due to the presence of 1-alpha hydroxlase activity within alveolar macrophages (Barbour et al, 1981; Kozeny et al, 1984). The resultant hyperabsorption of calcium leads to hypercalcaemia, which has been estimated to occur in between 2 and 10 per cent of patients with sarcoidosis, and hypercalciuria, and thus an increased risk of renal stone formation, in up to 40 per cent of these patients (Goldstein et al, 1971; Sandler et al, 1984).

Calcium fluxes across bone

As discussed above, bidirectional fluxes in the order of 5mmol per day occur in the normal adult skeleton due to bone formation and resorption (Figure 1.2). During hypocalcaemia parathyroid secretion of PTH is stimulated. One of the actions of this hormone is to stimulate osteoclastic bone resorption. <u>In vitro</u> the

presence of osteoblasts is required before PTH effects to stimulate osteoclast activity are observed (Chambers et al, 1985). Indeed, receptors for PTH and increased adenylate cyclase activity in response to this hormone are demonstrable in osteoblasts but not in osteoclasts (Wong et al, 1977). These findings suggest that the in vivo action of PTH to stimulate bone resorption may be indirect, probably mediated by osteoblasts (Rodan & Martin, 1981). In vitro PTH inhibits collagen synthesis but, in contrast, bone formation is stimulated by PTH in vivo (Kream, 1980; Parsons, 1979). Thus, despite an increase in bone turnover, in patients with primary hyperparathyroidism net bone calcium balance commonly remains close to zero (Parsons, 1979). It is also interesting to note that intermittent treatment with PTH has been found, paradoxically, to have predominantly anabolic effects on trabecular bone (Reeve et al, 1980).

As indicated above, radiocalcium kinetic studies suggest that calcium fluxes occur due to calcium exchange between ECF and accessible calcium deposits within bone without involving matrix turnover. These may be of only minor importance in terms of overall body calcium economy as there is probably no long-term net exchange of calcium by this route (Lauffenburger et al, 1977). However, there is some evidence, reviewed by Parfitt (1976), that rapid changes in the calcium flux between this pool and the ECF may occur in response to acute changes in PTH

concentrations. Thus the hypercalcaemic effect of PTH is more rapid (10-20mins) than can be accounted for by PTH effects on osteclastic bone resorption. It is thought that this effect may be mediated by osteocytes as these cell show very rapid structural and metabolic responses to PTH and, because of their canalicular processes, these cell have a very large surface area of bone contact.

In response to a calcium load, either dietary or by intravenous infusion, there is an increase in calcitonin secretion by the C-cells which are mainly localised within the thyroid. Calcitonin is a 32 amino acid peptide hormone which has a direct action to rapidly suppress osteoclast activity and, particularly if bone turnover is increased, this results in net entry of calcium into bone and thus an effect to lower ECF calcium (MacIntyre, 1983). However the inhibitory effect of calcitonin is not sustained despite continued high concentrations of calcitonin probably due to the down-regulation of calcitonin receptors (Tashjian et al, 1978). Thus the most likely physiological role for calcitonin is to attenuate the acute hypercalcaemic effects of dietary calcium loads rather than as a long-term regulator of the overall rate of bone resorption (Raisz, 1983). This hypothesis is supported by findings in rats that thyroidectomy results in an impairment of postprandial calcium uptake into bone and that this defect can be reversed by calcitonin

administration (VanderWiel & Talmage, 1981).

Renal regulation of calcium homeostasis

From an examination of the magnitude of the calcium fluxes shown in Figure 1.2 it can be seen that the kidney is likely to have important role in calcium homeostasis. Thus the kidney filters approximately 250mmol of calcium daily of which about 98% is reabsorbed. Small changes in the efficiency of the reabsorptive process may, therefore, have a very much amplified effect on urinary calcium excretion. The two determinents of calcium excretion are first, the filtered load of calcium and second, the tubular reabsorption of calcium (% of filtered load).

Unlike the reabsorption of solutes such as glucose or other ions such as phosphate it is not possible to define a renal tubular transport maximum for calcium as calcium excretion increases in a curvilinear fashion with increasing filtered load (Peacock & Nordin, 1968; Peacock et al, 1969; Figure 1.3). It should be noted from this relationship that, even in the absence of any other regulatory influences, quite modest degrees of hypercalcaemia can be expected to increase urinary calcium excretion several fold whereas hypocalcaemia might cause the opposite effect.

In vivo the sensitivity of this mechanism is

further enhanced by the effects of the calcium regulatory hormones, particularly PTH. In hyperparathyroidism (primary, secondary or tertiary) the efficiency of tubular calcium reabsorption is increased and thus for any given filtered load of calcium less calcium is excreted. The converse is true when PTH concentrations are reduced.

From a consideration of calcium fluxes (Figure 1.2) it is important to realise that, under steady-state conditions, urinary calcium excretion is equal to the net entry of calcium from gut and bone into the ECF and is therefore not determined by changes in renal tubular reabsorption of calcium. Thus, the effect of changes in PTH status is to change the set point of plasma calcium for any given net flux of calcium entering the ECF. Figure 1.3 shows the relationship between plasma calcium and fasting urinary calcium excretion for hypoparathyroid, euparathyroid and hyperparathyroid subjects. Throughput of calcium (calcium excretion) is similar for all three groups but the plasma calcium concentrations at which this level of excretion is achieved differ. Incidentally, it should be noted that the normal or near normal fasting calcium excretion in hyperparathyroid subjects indicates that net bone resorption is not greatly increased and, as a corollary, the renal effects of PTH are likely to be more important than the skeletal effects for sustaining hypercalcaemia

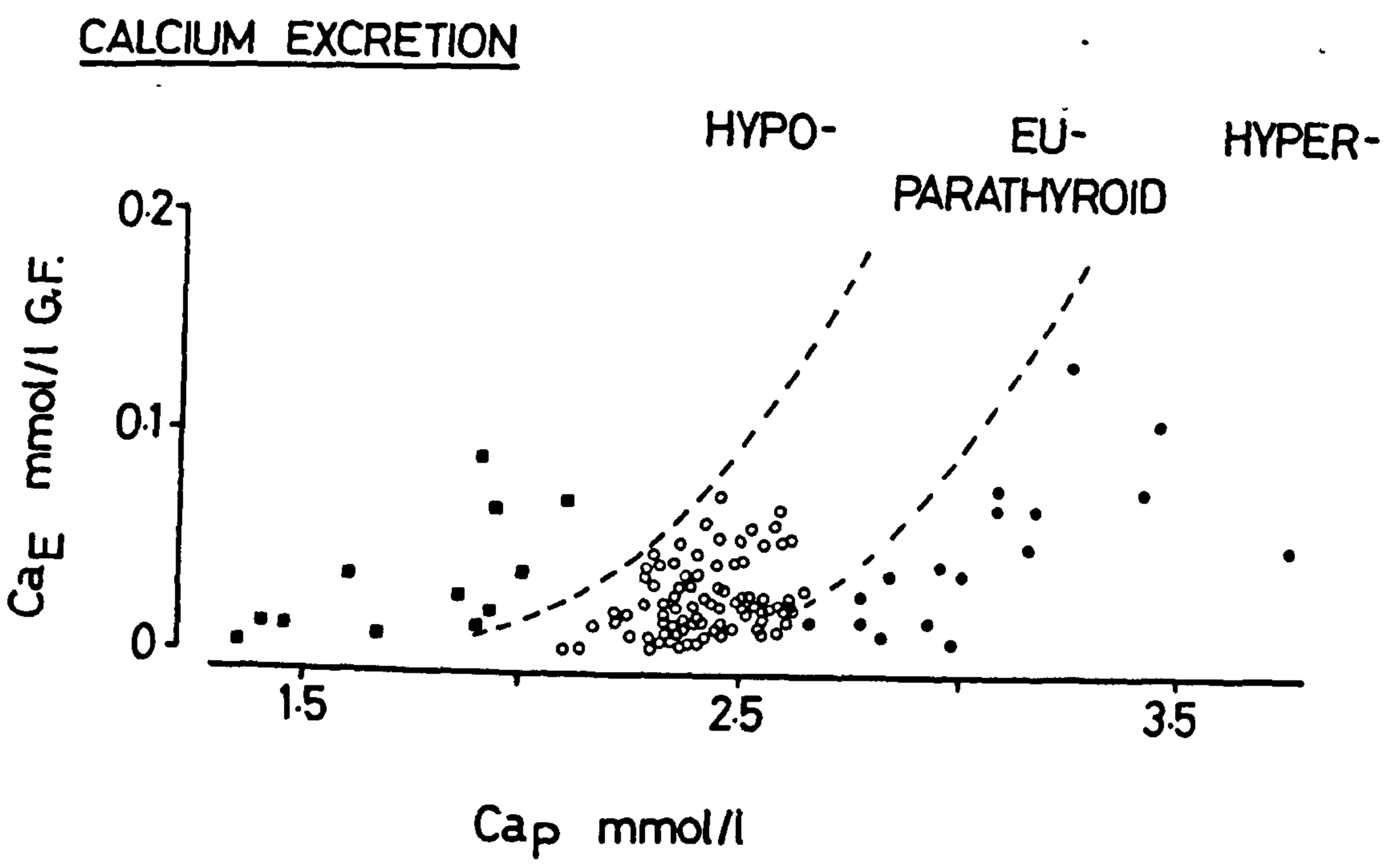


Figure 1.3 Relationship between plasma calcium (which is proportional to the filtered load of calcium) and fasting urinary calcium excretion (CaE) in hypoparathyroid, euparathyroid and hyperparathyroid subjects. (From Kanis et al, 1982).

in hyperparathyroidism (discussed later).

In normal subjects the secretion of PTH increases with a fall in the concentration of ionised calcium in the ECF and decreases during hypercalcaemia, thus conserving or enhancing excretion of calcium respectively. This remains true in primary hyperparathyroidism although the response curve is shifted so that higher calcium concentrations are required to reduce PTH secretion to a given rate (Rudberg et al, 1982; LeBoff et al, 1985). The rate of secretion of PTH is related to the cytosolic concentration of calcium in parathyroid cells which in turn is dependent on the ECF calcium concentration (LeBoff et al, 1985).

In summary, three organs, gut, bone and kidney, are involved in the homeostatic regulation of plasma calcium. The calcium fluxes between these organs and the ECF are modulated by PTH, $1,25(OH)_2D_3$ and calcitonin. Because of the very large fluxes of calcium handled by the kidney this organ appears to have a central role in calcium homeostasis.

DISORDERS OF BONE TURNOVER

As discussed above, skeletal turnover in the adult results from the resorption of bone by osteoclasts and its subsequent replacement with new bone formed by osteoblasts. Rarely, there may be abnormally low rates of turnover. Much more commonly, however, the rate of bone turnover is increased in skeletal disorders. This high turnover group of bone diseases includes Paget's disease, bone disease in neoplasia and hyperparathyroid bone disease. In each of these disorders increased bone resorption is the most important underlying mechanism for the development of skeletal disease. Consequently, therapy aimed at inhibiting this excessive bone resorption, such as treatment with the diphosphonates, might be expected to have beneficial effects in these conditions. The following section will provide some background information on the three disorders (Paget's disease, hypercalcaemia of malignancy and primary hyperparathyroidism) which form the main areas of study of this thesis.

Paget's disease of bone

Epidemiology

Paget's disease affects in the region of 5 per

cent of the population aged 55 or over in England and Wales with an area of peak prevalence in Lancashire of around 7 per cent (Barker, 1981). The prevalence is higher in men (6.2%) than in women (3.9%). The disease is also common in Western Europe, Australia, New Zealand and the USA but rare in Africa,the Middle East and Far East and, somewhat surprisingly, in Scandanavian countries. Migrants between areas of low and high prevalence have an intermediate prevalence of the disease suggesting the importance of both hereditary and environmental factors. The aetiology of the disease remains uncertain, but the findings of Rebel (1981) of viral inclusion bodies present within pagetic osteoclasts suggest that a slow viral cause is likely.

Morphologically the viral inclusion bodies appear to be of the paramyxoviridae family which includes measles virus, respiratory syncytial virus and canine distemper virus (Singer & Mills, 1983, Harvey et al, 1982). Further distinction of the exact virus involved has not been possible using purely morphological criteria. Immunocytological studies have not produced consistent results although positive immunocytochemical staining for both measles and respiratory syncytial viruses have been reported (Mills et al, 1984).

Epidemiologically, however, there seems little to suggest measles as the causative agent. Thus the incidence of measles in Scandinavia and in the U.K. is

similar whereas Paget´s disease is much more common in the U.K. (Harvey et al, 1982). Of interest is a recent study of the association of pet ownership and Paget´s disease which found that previous ownership of dogs, but not of other pets or current dog ownership, was significantly higher in pagetic patients than in a control population of maturity onset diabetics (O´Driscoll & Anderson, 1985). If confirmed by further studies this would suggest that canine distemper virus could be the aetiological agent and this explanation might fit better with the known epidemiology of the disease.

The viral inclusions are thought to be specific for Paget´s disease and are not found, for example, in the osteoclasts of patients with hyperparathyroidism. Recently, however, similar inclusions have been seen in osteoclasts from patients with pycnodysostosis, a rare inherited generalised osteosclerotic condition, in whom there was no evidence of coexisting Paget´s disease (Beneton et al, in press). If, as this finding suggests, such inclusion bodies are not specific for Paget´s disease then it may be that their presence is the result of abnormal osteoclast function rather than the cause.

Clinical features

Paget's disease is a patchy disorder which can affect virtually any bone but occurs most frequently in the pelvis (56% of affected individuals), spine (50%), femora (46%), tibiae (38%) and skull (28%; Schmorl, 1932). The small bones of the hands and feet are rarely involved. The disease may be monostotic or, at the other extreme, may involve almost the entire skeleton. Changes are frequently asymmetrical with, for example, localisation within one hemipelvis.

The majority of patients with the disorder are asymptomatic and the diagnosis is frequently made during the radiological or biochemical investigation of unrelated symptoms. The radiographic features of Paget's disease are well known and, except for very early changes, are diagnostic. The earliest phase is the appearance of a slowly progressive wave of bone osteolysis which may be well seen in skull radiographs as an area of osteoporosis circumscripta. In long bones the disease tends to spread from one end to the other with a flame shaped lytic front which advances at a fairly constant rate of around 1cm per annum (Doyle et al, 1974). This lytic front is followed by a phase of predominant bone formation with the production of expanded, osteosclerotic coarsely trabeculated bone often

associated with bony deformity. In weight bearing long bones bowing occurs and transverse fissure fractures may be seen particularly on the convex aspect of the bowed bones. The deformity is not due to bone softening (pagetic bone is in fact extremely hard) but may be related to the loss of normal bony architecture and the very rapid rate of bone turnover. Osteoarthritic changes are commonly seen in adjacent joints and may result in part from deformity of joint contours and redistribution of mechanical loads. In later stages of skull involvement the vault becomes grossly thickened and the base may invaginate leading to platybasia which may result in brain stem compression or hydrocephalus.

The major clinical complications of Paget's disease are pain, fracture, neurological complications, joint disease and sarcoma. Pagetic pain occurs in only a minority of patients with the disorder and even then may be present in only a proportion of involved sites in an individual patient. The pain may arise from pagetic bone itself, from microfractures, from associated joint disease or from neurological complications. The bone pain of Paget's disease is often described as burning or deep seated, is characteristically not relieved by rest and may be troublesome at night. In contrast, osteoarthritic pain tends to be exacerbated by movement and weight-bearing and to be relieved by rest. However, the distinction may be very difficult in some patients.

The mechanism of bone pain is unknown but possible causes include increased intramedullary pressure, stretching of the periosteum or the production of local factors such as prostaglandins (Russell et al, 1982).

Pathological fracture through pagetic bone is reasonably common particularly with more advanced Paget's disease as bony deformity gives rise to abnormal stresses. Usually fracture healing is normal but there is an increased incidence of delayed or non-union of up to 40% (Dove, 1980).

Neurological complications arise mainly from skull or spinal involvement. Deafness, which usually has both conductive and neuronal components, is the commonest complication and is found in up to 50 per cent of patients with skull involvement (Hamdy, 1981). However, pagetic patients are usually elderly and deafness due to causes other than Paget's disease is also common in this age group. Vertebral Paget's may cause nerve root or spinal cord syndromes, either by direct compression of expanded bone or by a vascular steal effect of the highly vascular pagetic bone which shares the same blood supply as the spinal cord. That this latter mechanism may be important is suggested by the excellent and rapid results of the medical treatment of spinal cord syndrome (Douglas et al, 1981) which has been shown to rapidly reduce bone blood flow (Wootton et al, 1981; Walton et al, 1983). As a result of the greatly increased bone vascularity

cardiac output is increased and, rarely with very active disease, this can precipitate a high output cardiac failure.

The incidence of osteogenic sarcoma is much higher in pagetic patients than in the elderly population as a whole but this usually rapidly fatal complication occurs in fewer than 1 per cent of patients (Poretta et al, 1957).

Monitoring of pagetic activity

The extent and activity of pagetic involvement may vary considerably between patients and in individual patients changes may occur either with progression of the disease (Woodard, 1959) or in response to specific treatment. Although changes in clinical and radiographic features are of importance they are not sufficiently sensitive to be of use in monitoring changes in the degree of pagetic activity. Fortunately, the indirect biochemical markers of turnover, serum alkaline phosphatase and urinary hydroxyproline excretion, provide objective and reliable reflections of skeletal turnover and thus of pagetic activity.

Alkaline phosphatase is an enyme system of multiple molecular forms in which heterogeneity is partly due to posttranslational modifications (Fishman, 1974). It is produced by osteoblasts where it is predominantly

associated with cell membranes (Posen et al, 1977). It is released into the circulation in direct proportion to osteoblast numbers and therefore acts as a marker of bone formation (Russell et al, 1981). The half-life of bone-derived alkaline phosphatase within the circulation is of the order of 1 to 2 days (Walton et al, 1975b). Hydroxyproline, an amino acid found almost exclusively within collagen, is derived mainly from the breakdown of collagen and excreted in small peptides in the urine (Krane, 1980). In disorders of increased turnover such as Paget's disease, by far the greatest proportion of collagen degradation occurs within the skeleton and, despite hepatic metabolism of over 50 per cent of hydroxyproline by the liver, urinary excretion of hydroxyproline provides a reasonable marker of the activity of bone resorption (Krane, 1980).

Although at focal sites in pagetic bone either resorption or formation may predominate, overall these processes remain well matched and skeletal balance as a whole remains close to zero. This balance is well demonstrated by the close correlation between serum alkaline phosphatase (formation) and urinary hydroxyproline (resorption) shown in Figure 1.1. This close correlation of these two independent biochemical measurements suggests that both alkaline phosphatase and urinary hydroxyproline excretion are valid markers of bone turnover.

Neither of these markers is entirely specific. Alkaline phosphatase is also derived from other sources, principally the liver, and serum values may be grossly increased in cases of biliary obstruction whereas hydroxyproline is released from non-skeletal as well as skeletal collagen degradation. Non-skeletal sources of these markers are important when the rate of bone turnover is low or normal but, as discussed in Chapter 3, these become proportionately less important with increasing rates of turnover. In addition, a small proportion of skeletal hydroxyproline (in the order of 10 per cent) is derived from the breakdown of newly synthesised collagen or procollagen to be excreted in the urine as non-dialysable peptides and thus this fraction may have a role as a marker of formation rather than of resorption (Bienkowski et al, 1978; Krane, 1980). Despite these reservations, these markers are extremely useful for monitoring pagetic activity. Excretion of hydroxyproline may be expressed as a ratio to urinary creatinine (OHP/Cr). Measurement of OHP/Cr in fasting urine samples has been found to correlate well with OHP/Cr in 24 hour urinary specimens (Figure 1.4) and provides a more convenient index of bone resorption (Russell et al, 1981). The treatment of Paget's disease will be reviewed subsequently.

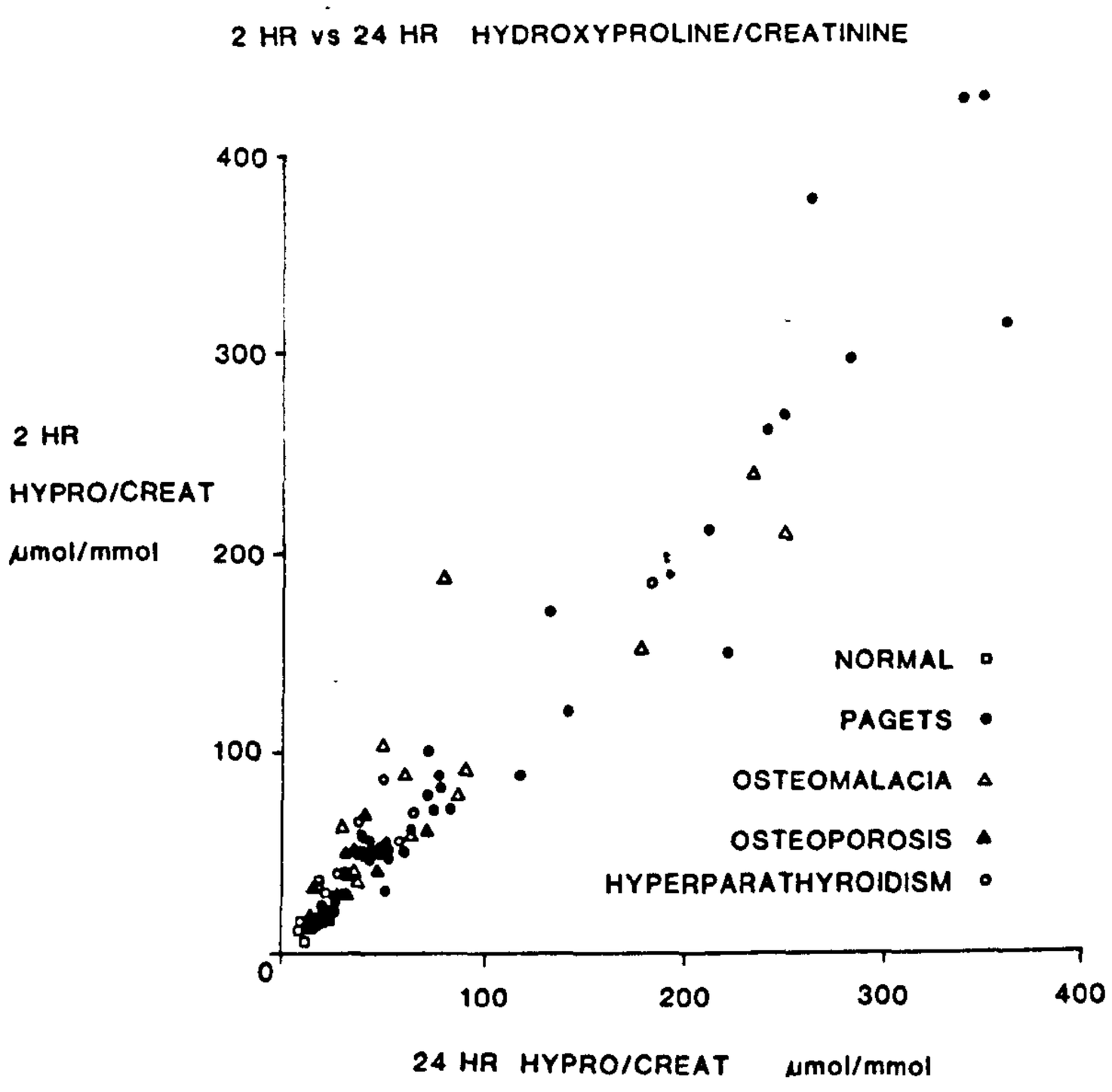


Figure 1.4 Relationship between hydroxyproline/creatinine ratio (OHP/Cr) in a fasting urine specimen and OHP/Cr in a 24 hour collection in normals and patients with various disorders of bone turnover. (From Douglas, 1983).

Skeletal complications of malignancy

Malignant disorders are extremely heterogeneous in virtually all aspects of their behaviour including their effects on the skeleton. For example, gastric cancer rarely metastasises to bone or causes hypercalcaemia. At the other extreme, carcinomas arising from breast or prostate almost invariably spread to bone at some stage of the disease. Carcinoma of breast most commonly induces predominantly lytic bone lesions and is frequently the cause of hypercalcaemia whereas prostatic carcinoma is associated with osteosclerosis and hypercalcaemia is rare. Yet other solid tumours, notably squamous carcinoma of the lung, hypernephromas and squamous carcinoma of the head and neck, may induce generalised bone loss and hypercalcaemia by humoral mechanisms not involving skeletal metastasis (Myers, 1960). Haematological malignancies, in particular multiple myeloma, are frequent causes of skeletal complications and hypercalcaemia (Kyle, 1975; MRC, 1973). This is perhaps not surprising in view of the juxtaposition of trabecular bone and the bone marrow and the functional interrelationships that exist between the two tissues. Thus there is considerable heterogeneity and there can be no unifying hypothesis for the skeletal effects of malignant disorders. It is likely that not

only do the mechanisms responsible for skeletal effects differ for the various forms of malignancy but that even within one diagnostic group more than one factor will be operating. The clinical effects of skeletal involvement of malignancy will be discussed following a review of the known mechanisms of bone loss in these disorders.

Mechanisms of bone loss and hypercalcaemia in malignancy

When considering mechanisms of hypercalcaemia and bone loss in malignancy it is useful to divide patients into three major subgroups. These are solid tumours without bony metastases (around 10%), solid tumours with metastases (70%) and haematological malignancies (20%; Myers, 1960). Most information is available with regard to the mechanisms in haematological malignancies, and these will be reviewed first.

Myeloma is by far the most frequent haematological cause of hypercalcaemia. Hypercalcaemia is present in between 15 and 30 per cent of myeloma patients at the time of presentation and probably occurs in over 50 per cent of patients at some point during the course of the disease (Kyle, 1975). Radiographic evidence of increased bone lysis is present in over 80 per cent of cases (MRC, 1973; Kyle, 1975). Over the last 13 years there has been much work to implicate the

release of an osteoclast activating factor (OAF) from myeloma plasma cells in the production of bone lysis.

OAF was discovered as a bone resorbing lymphokine in 1972 by Horton and colleagues (1972) who were investigating the cause of periodontal bone lysis. Originally the term was used to describe bone resorbing activity derived from cultures of peripheral blood leukocytes activated by the mitogen, phytohaemagglutanin A, or by other lectins (Horton et al, 1972). Subsequently Mundy and co-workers (1974a,b) were able to demonstrate the release of a similar factor from marrow aspirate cells taken from patients with myeloma and also from a human myeloma cell line in culture. Other lymphoid cell lines are known to be capable of OAF production and OAF activity has been demonstrated in various lymphomas, including Burkitt's lymphoma, as well as in association with lymphosarcoma (Mundy et al, 1974b, 1978). In myeloma there is a close relationship between estimates of the total production of OAF and the extent of skeletal involvement, and specific treatment of the myeloma is associated with a significant decrease in OAF production (Durie et al, 1981).

The production of OAF by lymphocytes is dependent on the synthesis of prostaglandins by monocytes which may therefore have a regulatory role in OAF synthesis (Yoneda & Mundy, 1979a,b). Although in vitro OAF synthesis can be inhibited by cyclo-oxygenase

inhibitors such as indomethacin *in vivo* these drugs are ineffective in reducing hypercalcaemia in most patients. In contrast, corticosteroids at physiological concentrations have no effect on OAF production *in vitro* although both *in vitro* and *in vivo* they inhibit the effects of OAF on bone resorption (Strumpf et al, 1978) and are of use in treating hypercalcaemia in myeloma.

It is now apparent that there are different OAFs produced by T cells and B cells and it is probably more appropriate either to consider an OAF "family" of lymphokines or to use the term OAF as a descriptive label for several otherwise unrelated factors that stimulate osteoclasts (Mundy, 1984a). Recent studies suggest that T cell OAF may be related to lymphotoxin, the structure of which has been elucidated. Colony stimulating factor is a further lymphocyte product which appears to enhance bone resorption by causing increased recruitment or proliferation of osteoclast progenitors rather than by direct activation of pre-existing osteoclasts (Mundy et al, 1985). In addition, cells of the monocyte/macrophage lineage may also produce OAF-like substances which are probably related to interleukin-1 (IL-1). IL-1 can stimulate bone resorption by at least two mechanisms. It increases synthesis of prostaglandins which induce osteoclastic bone resorption, but also stimulates osteoclasts independently of prostaglandin synthesis (Gowen et al, 1983).

It is of interest that in human T-cell leukaemia associated with the virus HTLV-1, hypercalcaemia and destructive bone lesions can develop suggesting that production of bone resorbing factors may be enhanced by the presence of tumour virus (Grossman et al, 1981). Interferon gamma has the capacity to inhibit cytokine stimulated bone resorption (but not resorption stimulated by either PTH or $1,25(OH)_2D_3$) and thus may be a further regulator of resorption (Gowen et al, 1986). In some patients with non-Hodgkin's lymphoma hypercalcaemia appears to be mediated by increased concentrations of $1,25(OH)_2D_3$ which is presumably a result of synthesis by the lymphoma cells (Breslau et al, 1984).

There is even less certainty with regard to the mechanisms that produce excess bone resorption in patients with solid tumours. Although the osteoclast is probably the cell primarily responsible for bone resorption other cells may be involved, and both tumour cells and monocytes may be capable of bone resorption (Eilon & Mundy, 1978; Mundy et al, 1977). Tumours can produce local factors such as prostaglandins and transforming growth factors (TGFs) which stimulate osteoclasts. Monocytes and activated lymphocytes associated with the tumour may produce IL-1 and there is scope for many complex cellular interactions (Mundy, 1984b).

Much interest has centred around the

non-metastatic effects of tumours to produce hypercalcaemia and increased bone resorption. This syndrome of humoral hypercalcaemia was initially thought to be due to ectopic production of PTH by tumour tissue. However, tumour PTH production has not been convincingly demonstrated (Skrabenek et al, 1980) and techniques for demonstrating PTH messenger RNA have failed to detect substantial tumour synthesis of PTH (Simpson et al, 1983). Prostaglandins have also been proposed as possible mediators of the humoral effect as certain prostaglandins, particularly PGE2 and prostacyclin (PGI2), have been found to be potent stimulators of bone resorption (Martin & Partridge, 1980). However, breakdown of prostaglandins to inactive metabolites, particularly within the pulmonary circulation, occurs very rapidly with almost complete inactivation in a single transit (Piper et al, 1970). In a small minority of patients treatment with potent inhibitors of prostaglandin synthesis, such as indomethacin, may have a significant hypocalcaemic effect (Seyberth et al, 1975). However, these agents do not reduce serum calcium in the majority of patients with malignant hypercalcaemia. Furthermore it has proved difficult to increase serum calcium by infusing prostaglandins into experimental animals (Robinson & Parsons, 1974). Thus it is likely that prostaglandins do not have a major role in the mechanism of humoral hypercalcaemia of malignancy

although they may act as important local mediators of bone resorption.

More recently, two other groups of factors have been suggested to account for the humoral effects on bone. These are the "PTH-like" factors and the TGFs. A PTH-like factor has been isolated from a human renal adenocarcinoma which appeared to bind to PTH receptors although not cross-reacting with antibodies to PTH (Strewler et al, 1983). Like PTH this factor increased renal tubular cyclic AMP production. Such a mechanism may account for the observation that the majority of patients with hypercalcaemia of malignancy have increased excretion of nephrogenous cAMP despite low plasma concentrations of iPTH (Stewart et al,1980; Rude et al, 1981). It might also help to explain the occurrence of phosphaturia and possibly the increased renal tubular reabsorption of calcium that occurs in some patients (Ralston et al, 1984). That this latter effect may be an important mechanism of hypercalcaemia in a proportion of patients with solid tumours is suggested by our studies of patients with breast cancer in whom a tendency to increased renal tubular reabsorption of calcium was observed despite continued saline treatment (Percival et al, 1985). However, the adequacy of rehydration, which has important effects on renal tubular reabsorption of calcium, is very difficult to assess in these patients, particularly while calcium remains increased. Such

uncertainties may be lessened by reducing serum calcium using specific inhibitors of bone resorption. For this reason I have studied renal tubular reabsorption of calcium in patients with solid tumours or haematological malignancies both before and after treatment of hypercalcaemia with diphosphonates (Chapter 5).

TGFs of the alpha and beta type are produced by a variety of experimental tumours in vitro (Ibbotson et al, 1985). Alpha TGF is closely related to epidermal growth factor and competes for the same receptor. Beta TGF is produced by both normal and tumour tissues and reacts with its own distinct receptor. These factors may act synergistically to stimulate bone resorption by either endocrine or paracrine effects (Ralston et al, 1984).

Thus although the precise nature of bone-resorbing factors in malignant disorders has not been fully elucidated there is rapid progress in this field. Improved understanding of the intercellular mechanisms might lead to more specific therapy for the treatment or prevention of skeletal complications of malignancy including malignant hypercalcaemia.

Clinical features of skeletal involvement in malignancy

The important clinical consequences of skeletal involvement in malignancy are bone pain, fracture and hypercalcaemia. Bone pain is often severe and intractable and adequate analgesia is often only achieved with the use of opiates. Several mechanisms have been proposed to account for the pain including increased intraosseous pressure, periosteal involvement, production of local factors such as prostaglandins, nerve entrapment and overt or occult fracture.

In patients with multiple myeloma, in whom bone pain is common (Bergsagel, 1977), the skull is rarely a site for bone pain despite the frequent occurrence of osteolytic foci at that site (Whitelaw, 1963; Snapper et al, 1953) suggesting that tumour pressure is an unlikely mechanism. Radiographic abnormalities are present in only about 50 per cent of sites of bone pain in myeloma patients (Whitelaw, 1963), but it is of interest that, in a small study, increased uptake of bone seeking isotopes has been noted at painful sites (Charkes et al, 1972). This finding was interpreted as evidence of bone infraction. Overt pathological fracture is very common in myeloma, being noted in 60 per cent of the 824 patients reviewed by Kyle (1975). The spine is the site most frequently involved, with consequent pain, loss of

height and the development of kyphosis. Other common fracture sites are the ribs and the proximal ends of humerus and femur.

Studies of fracture incidence in association with solid tumours have shown this complication to be surprisingly uncommon. Fracture occurred in 6 per cent of autopsied patients with skeletal metastases reviewed by Johnston (1970), but Galasko (1972) reported pathological fracture in less than 1 per cent of patients with advanced carcinoma. Carcinoma of the breast accounts for approximately 60 per cent of fractures associated with solid tumours (Galasko, 1974; Fitts et al, 1953). Once fracture of a long bone has occurred it may be very difficult to treat as the surrounding bone may be grossly abnormal, making fixation difficult, and fracture healing may be impaired particularly in femoral transcervical or subcapital fractures (Galasko, 1974). Pathological fracture is often considered as a near terminal event in patients with malignant disease. However, almost half the patients survive for at least one year from the time of first fracture (Galasko, 1974) and appropriate orthopaedic surgery may be very valuable in reducing morbidity.

The clinical effects of hypercalcaemia in general will be considered following an account of primary hyperparathyroidism, which is the most common cause of hypercalcaemia amongst the general population

(Heath et al, 1980; Mundy et al, 1980).

Primary hyperparathyroidism

Primary hyperparathyroidism is a common condition which is characterised by chronic hypercalcaemia. It is primarily a disease of postmenopausal women although both sexes and all age groups may be affected (Heath et al, 1980; Mundy et al, 1980). Surveys of the incidence of primary hyperparathyroidism show an apparent increase over the last 10 to 15 years. This phenomenon has generally been attributed to the routine use of multi-channel serum autoanalysers for patient screening. Thus, in a study of the resident population of Rochester, Minnesota between 1965 and 1976, Heath and colleagues (1980) reported the apparent incidence during the initial 9.5 years to be only around 8 per 100,000 population per year. Immediately following the introduction of routine serum calcium measurement in 1974 the apparent incidence rose to 51 per 100,000 per year. This was presumed to be due to a catch-up effect whereby patients who had the disease for in excess of one year, but who may not have had specific symptoms, were diagnosed on routine biochemical screening. Subsequently, this effect appears to have abated as the average annual incidence fell to 27.7 per

100,000 for the last 18 months of this study (Heath et al, 1980). This incidence is similar to that reported by Mundy and colleagues (1980) for the population of Birmingham, England of 22.2 per 100,000 per year. From such estimates of incidence it can be inferred that approximately 10,000 new cases of primary hyperparathyroidism will be diagnosed in England each year (Anonymous, 1980). Other studies have produced even higher incidence figures for this disease, ranging fom 50 per 100,000 (Haff et al, 1970) to 100 per 100,000 per year (Boonstra & Jackson, 1970).

With the apparent change in incidence there has been a marked change in the mode of presentation of this disease. Whereas, before 1975 renal stones or nephrocalcinosis were the most common presenting features and bone disease was also relatively common (McGeown, 1969; Watson 1974) in more recent studies the majority of patients have had no symptoms which can be related to primary hyperparathyroidism and bone disease has become distictly uncommon (Mundy et al, 1980; Heath et al, 1980). This could reflect a true change in the nature of the disorder. However, it is more likely that the improved ability to diagnose mild, more benign forms of primary hyperparathyroidism together with earlier diagnosis and treatment of the subpopulation of patients that would have developed specific symptoms is responsible for the observed change in the patterns of

clinical presentation.

Many of the symptoms of primary hyperparathyroidism result from the effects of hypercalcaemia and are thus similar to the symptoms in other forms of hypercalcaemia including hypercalcaemia of malignancy. The clinical effects of hypercalcaemia will be discussed below.

The frequency with which asymptomatic patients with primary hyperparathyroidism are being diagnosed raises several important questions regarding the indications for treatment of this condition. This question will be addressed later in this chapter. The possible role of active medical treatment of primary hyperparathyroidism will also be considered. It is pertinent, however, to first review the mechanisms of hypercalcaemia in primary hyperparathyroidism as an understanding of these is likely to lead to a more rational approach towards medical treatment.

Mechanisms of hypercalcaemia in primary hyperparathyroidism

It was noted in the section on regulation of plasma calcium that PTH has actions on all three major organs involved in calcium homeostasis (i.e. gut, bone and kidney). The importance of the contributions of each of these mechanisms to the development of hypercalcaemia

in primary hyperparathyroidism will now be discussed.

Studies which have measured serum concentrations of 1,25(OH)2D3 and intestinal absorption of calcium in patients with primary hyperparathyroidism have found these to be increased (Peacock 1975, Broadus et al, 1980). However, there is considerable interindividual variation and in a proportion of patients these indices are normal. Thus, calcium absorption, as judged by the increase in urinary calcium excretion following an oral calcium load, was found to be normal in 20 of 50 unselected patients with primary hyperparathyroidism studied by Broadus and colleagues (1980). This index of calcium absorption correlated strongly and positively with concentrations of $1,25(OH)_2D_3$ in these patients. Thus, they divided their patients into a "non-absorptive" group (normal calcium absorption) in which the mean value for $1,25(OH)_2D_3$ was within the normal range, and an "absorptive" group in which $1,25(OH)_2D_3$ concentrations were markedly increased. 24 hour urinary excretion of calcium on a 1g calcium diet was normal or near normal in the "non-absorptive" group and was consistently high in the "absorptive" group. In spite of these differences there were no significant differences in serum calcium, phosphate or iPTH between the two groups. These findings indicate that, although intestinal calcium absorption is increased in a proportion of patients, hypercalcaemia can exist in patients with primary hyperparathyroidism in the

absence of any increase in calcium absorption. Even in patients in whom intestinal calcium absorption is increased, this mechanism alone would be unlikely to be sufficient to cause hypercalcaemia if other homeostatic mechanisms for regulating plasma calcium remained intact.

Both PTH and $1,25(OH)_2D_3$ remain sensitive to variations in dietary intake of calcium which, partly as a consequence, have little effect on the serum calcium in patients with primary hyperparathyroidism (Insogna et al, 1985).

The skeletal effects of PTH in patients with primary hyperparathyroidism, like those on the gut, are variable. As indicated above PTH stimulates both bone formation and bone resorption in vivo (Parsons, 1979). Hyperparathyroid patients in general have increased bone turnover, as judged by increased serum alkaline phosphatase although in most patients this index remains within the normal reference range (Dent, 1962; Marcus et al, 1984). Most patients with primary hyperparathyroidism are postmenopausal women in which group, even in the absence of parathyroid disease, osteoporosis is common (Melton & Riggs, 1983; Lewis, 1981). Primary hyperparathyroidism appears to be a further risk factor for the development of osteoporosis as bone loss, predominantly from the axial skeleton, is accelerated (Seeman et al, 1982). However, although important for skeletal integrety, reduction in bone mass

occurs over a prolonged period and the short-term net losses of calcium, as judged by fasting urinary calcium excretion, remain within or just above the normal range in the majority of patients (Broadus et al, 1980; Peacock et al, 1969; also see Figure 1.3). Thus, although net bone loss may contribute to the development of hypercalcaemia in some patients, it seems unlikely that, overall, this is an important mechanism.

At least theoretically the renal effects of PTH, in contrast to its effects on gut and bone, appear to be necessary for the maintenance of hypercalcaemia in primary hyperparathyroidism (Peacock et al, 1969). The importance of this effect may be illustrated by a hypothetical example. Consider a patient in whom both the net fluxes of calcium entering the ECF (or calcium throughput) and the plasma calcium concentration were normal (around 5mmol/24h and 2.4mmol/l respectively). If this patient developed only the renal tubular effects of primary hyperparathyroidism initially the urinary calcium losses would fall to much less than 5mmol/24h (see Figure 1.3). Consequently, the plasma calcium concentration, and thus the filtered calcium load, would rise. This process would stop once the filtered load had risen sufficiently to ensure that once again the net calcium entry into the ECF from gut and bone were matched by the urinary calcium losses and thus a new steady state was reached. Thus the renal tubular effect of PTH alone is

sufficient to produce hypercalcaemia. If, in addition, net entry of calcium into the ECF from gut and/or bone (calcium throughput) also occurred this would compound the hypercalcaemia by increasing the filtered load of calcium (and thus plasma calcium) required to reach a new steady state. However, because of the shape of the calcium excretion curve with increasing filtered load (Figure 1.3), the effects of increased calcium throughput are likely to be relatively minor in comparison with the effects of increased renal tubular reabsorption of calcium, at least for the majority of patients with primary hyperparathyroidism (Peacock et al, 1969).

This concept of the primary importance of the renal mechanism for maintaining hypercalcaemia is supported by the relative failure of treatments aimed at reducing either intestinal calcium absorption or bone resorption to normalise serum calcium (see below).

Clinical effects of hypercalcaemia

Although primary hyperparathyroidism is the commonest cause of hypercalcaemia among the population as a whole (Heath et al, 1980), within hospital populations malignancy is a more frequent cause (Fisken et al, 1981). As both primary hyperparathyroidism and maligant disorders are common it is not surprising that these conditions occasionally coexist (Francis et al, 1982; Stone et al, 1982). Patients with hypercalcaemia of malignancy usually have clinical or laboratory evidence of their neoplasia. However, the differential diagnosis between these two conditions, and other less common forms of hypercalcaemia, can be difficult. (For review see Habener & Potts, 1979).

Hypercalcaemia, regardless of aetiology, is associated with a complex range of largely nonspecific symptoms which are mainly the result of effects on the kidney, nervous system and gastrointestinal tract. Although in health the kidney has an important role in the regulation of the concentration of calcium in the ECF (see above), hypercalcaemia itself may offset this sparing mechanism by its renal effects (Lins, 1979; Benabe & Martinez-Maldonado, 1978). Thus, hypercalcaemia impairs the ability to form concentrated urine, and experimentally a renal resistance to the action of

vasopressin has been found (Levi et al, 1983). Clinically this results in excess thirst, polyuria, polydipsia and clinical signs of dehydration. The fall in ECF volume is associated with a reduced glomerular filtration rate (GFR) as is hypercalcaemia itself, which may induce calcium precipitation particularly in the renal medulla. The ability of the kidney to eliminate large loads of calcium falls in proportion to the fall in GFR.

A further consequence of volume depletion is an effect to increase the renal tubular reabsorption of calcium. There is a common co-transport mechanism for sodium and calcium in the proximal convoluted tubule, and when sodium delivery is decreased a greater fraction of both sodium and calcium is reabsorbed. In dehydrated patients this effect to increase renal tubular reabsorption of calcium will tend to offset the renal sparing effect of a homeostatic suppression of PTH secretion.

Differences in the underlying mechanisms of hypercalcaemia are important determinants of the natural history of this abnormality. Thus, as discussed above, in primary hyperparathyroidism hypercalcaemia is sustained largely due to the PTH-induced increase in tubular calcium reabsorption. The ability of the kidney to eliminate more calcium in response to an increase in the filtered load (a function of plasma calcium) is

preserved (Peacock et al, 1969). Thus, these patients generally remain in a steady state and serum calcium may remain elevated, but largely unchanged, for many years. In contrast, where fluxes of calcium into the ECF are very large, as in many cases of malignant hypercalcaemia, the impaired renal handling of calcium due to the mechanisms discussed above will produce a non-steady state situation with a progressive increase in the concentration of ECF calcium (Parfitt, 1979). Thus patients with primary hyperparathyroidism often report mild hypercalcaemic symptoms lasting for many months or years, but in contrast those with malignant hypercalcaemia usually have an acute onset of symptoms which are rapidly progressive and become severe.

Neurological complications range from inability to concentrate or mild confusion to coma. Occasionally patients present with altered behaviour or psychosis and rarely focal neurological signs may be produced. The severity of these complications does not correlate well with the concentration of plasma calcium and other factors, such as the rate of rise of the calcium, may be important. Reversal of these neurological sequelae may take several days after normalisation of plasma calcium.

Gastrointestinal effects of hypercalcaemia include anorexia, nausea, vomiting and constipation. It is probable that these are partly mediated by impairment of autonomic function. Pancreatitis is an occasional,

but serious, complication. Its aetiology is multifactorial, but probably includes dehydration, altered coagulation, activation of trypsinogen and focal deposition of calcium within pancreatic tissue.

Other clinical changes include electrocardiographic changes with shortening of the Q-T interval and deposition of calcium in the limbus of the eye, particularly when the hypercalcaemia has been long-standing.

DIPHOSPHONATES IN THE TREATMENT OF DISORDERS OF BONE TURNOVER

Diphosphonates, calcitonin and mithramycin are each inhibitors of bone resorption and each of these drugs has been used both in the treatment of Paget's disease and in hypercalcaemia of malignancy. Other forms of therapy may also be of value in the treatment of skeletal disorders. However, in view of the potential importance of the diphosphonates as therapeutic agents and their central role in the studies reported in this thesis it is appropriate to consider this group of drugs in some detail here. In the following section the discussion of treatment of skeletal disorders will mainly centre around the use of diphosphonates, although mention will be made of the role of other treatments.

DIPHOSPHONATES

Historical perspective

Extensive studies on inorganic pyrophosphate have formed the basis of the development of the diphosphonates over the past 20 years (Russell & Fleisch, 1976; Fleisch, 1983; Fleisch & Felix, 1979). The theory

put forward originally was that constituents of the inorganic matrix of bone played a crucial role in the process of calcification by inducing calcium phosphate precipitation (Neuman & Neuman, 1958). Thus, it became important to explain why the extracellular matrix of soft tissues does not normally mineralise. From this it was postulated that inhibitors of calcification might exist which would be broken down locally at sites of mineralisation.

In the search for factors which might counteract the initiation of the crystal phase in bone and cartilage matrix, a serum factor was found to inhibit calcium phosphate precipitation from solution (Fleisch & Neuman, 1961). The activity of this factor could be destroyed, in part, by alkaline phosphatase which was subsequently shown to possess pyrophosphatase activity. Urine was found to contain even higher amounts of crystal inhibitory activity than serum, and using this source the factor was identified as inorganic pyrophosphate (PPi), a compound not previously isolated from biological fluids (Fleisch & Bisaz, 1962a). Subsequently, PPi was isolated from serum (Fleisch & Bisaz, 1962b), synovial fluid (Russell et al, 1970) and saliva (Hausmann et al, 1970). Further studies on the physico-chemical effects of PPi revealed that inhibition of calcium phosphate precipitation occurred both in solution and in the solid phase of hydroxyapatite crystals (Hansen et al, 1976).

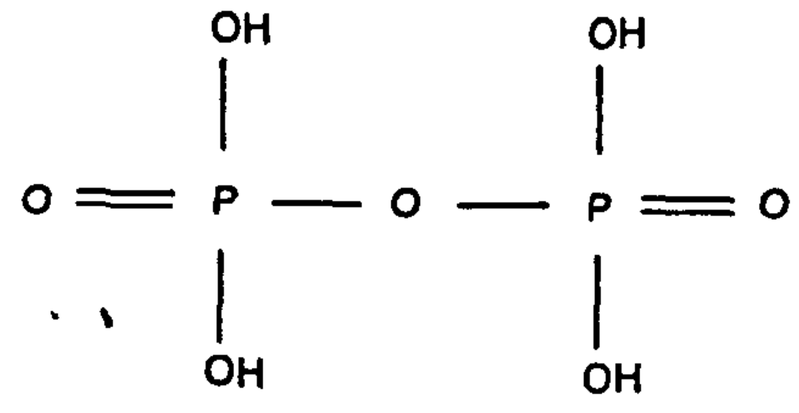
Also the conversion of amorphous calcium phosphate into crystalline hydroxyapatite was slowed by PPi (Fleisch et al, 1968), although it had no effect on the amorphous phase. PPi was further found to inhibit dissolution of hydroxyapatite crystals (Fleisch et al, 1966a). These effects appear to be related to the high affinity of PPi for hydroxyapatite (Jung et al, 1973).

The pronounced effects of PPi on calcium phosphates in vitro at concentrations found in biological fluids suggested that it could be of significance in both normal and disease states. PPi might protect soft tissues from mineralisation, and in bone it could influence the calcification process as well as the distribution of the mineral phase (Russell & Fleisch, 1976). Parenteral, but not oral, administration of PPi was found to inhibit the calcification of tissues in vivo (Fleisch et al, 1966b; Schibler et al, 1968; Schibler & Fleisch, 1966), but no effect was observed on the calcification or resorption of bone. Further studies of PPi effects were hampered by the finding that PPi was not absorbed intact from the gut and was rapidly destroyed enzymatically when given parenterally. This led to a search for PPi analogues which would be stable to both chemical and enzymatic degradation and effective when given by mouth. Among the analogues which turned out to be effective were the diphosphonates, some of which had previously been synthesised and studied as potential

additives to detergents. A large number of diphosphonates have now been synthesised and a few of these have been used clinically. These include dichloromethylene diphosphonate (clodronate; Cl2MDP), ethane-1-hydroxy-1,1 diphosphonate (etidronate; EHDP; Didronel), aminopropylidine diphosphonate (APD), aminohexylidine diphosphonate (AHDP) and aminobutylidine diphosphonate (ABuDP). The structures of the diphosphonates studied in this thesis and of PPi are shown in Figure 1.5.

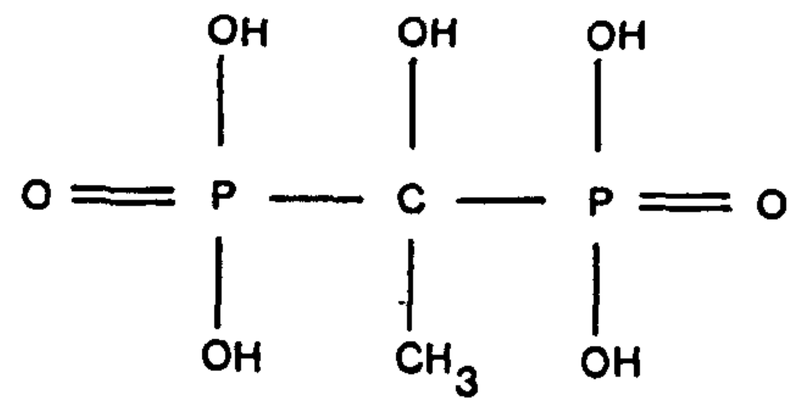
Effects of diphosphonates in experimental systems

Like PPi, the diphosphonates inhibit precipitation of calcium and phosphate from solution (Fleisch et al, 1970), and antagonise the transformation of the amorphous form of calcium phosphate into hydroxyapatite (Francis, 1969). They retard aggregation of hydroxyapatite crystals (Hansen et al, 1976) and disaggregate crystal clusters (Bisaz et al, 1976). They also delay the dissolution of hydroxyapatite crystals <u>in vitro</u> (Jung et al, 1973). These properties of diphosphonates probably relate to their adsorption onto the surfaces of hydroxyapatite crystals for which they have a high affinity (Jung et al, 1973). <u>In vivo</u> diphosphonates inhibit a variety of forms of experimentally induced soft tissue calcification



PPi

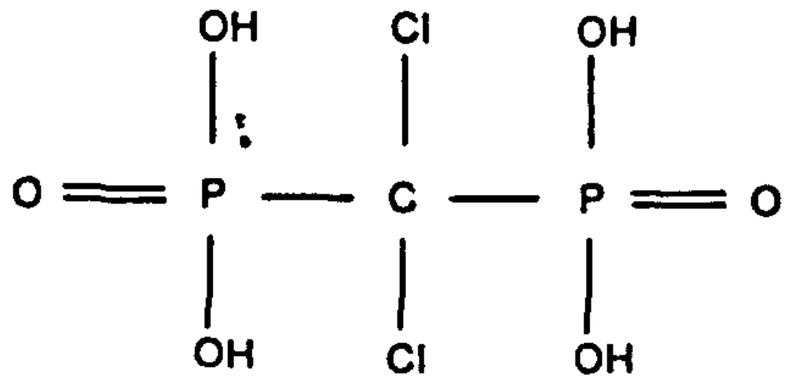
INORGANIC PYROPHOSPHATE



EHDP

ETHANE-1-HYDROXY-1,

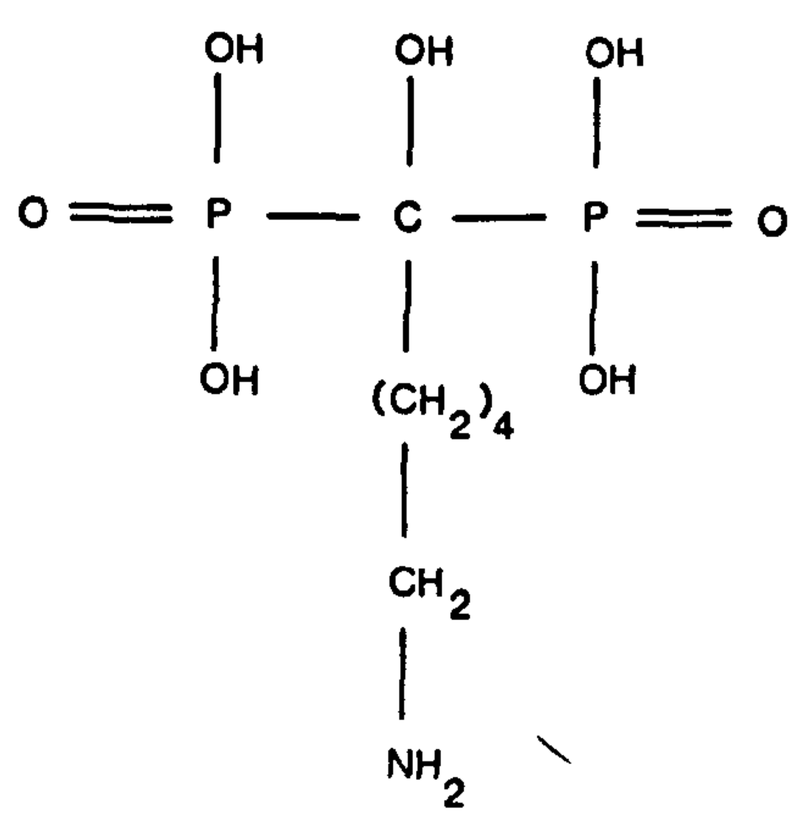
1-DIPHOSPHONATE



Cl_2MDP

DICHLOROMETHYLENE

DIPHOSPHONATE



AHDP

AMINOHEXANE

DIPHOSPHONATE

Figure 1.5 Molecular structures of inorganic pyrophosphate and of the three diphosphonates studied in this thesis.

(Fleisch, 1983) and there is a close relationship between these *in vivo* effects and the ability of the various diphosphonates *in vitro* to inhibit crystal formation and growth (Fleisch et al, 1970). This suggests that the *in vivo* actions might be primarily explained by physico-chemical mechanisms. Diphosphonates possessing a 1-alpha hydroxy group, such as etidronate, appear to be the most effective inhibitors of mineralisation.

However, unlike PPi, several diphosphonates, including etidronate, prevent the normal calcification of bone and cartilage (Rosenblum, 1974; Russell et al, 1973). The impaired mineralisation does not appear to be related to abnormalities in vitamin D metabolism as production of $1,25(OH)_2D_3$ in experimental animals is normal or increased unless very large doses of etidronate are given (Baxter et al, 1974). Such doses are associated with hypercalcaemia in growing animals (Gasser et al, 1972) which acting both directly and through reduced secretion of PTH would inhibit 1-alpha hydroxylation of $25(OH)D_3$. Of the diphosphonates in clinical use, etidronate is by far the most potent inhibitor of mineralisation *in vivo*. For example, following subcutaneous administration to rats, APD is about 50 times less potent than etidronate in this respect (Reitsma et al, 1980). Such differences in relative potency of various diphosphonates to inhibit mineralisation may have important implications for their

clinical use which are examined in this thesis (Chapters 4, 5 and 6).

Experiments in vitro, for example in cultured calvaria, indicate that various diphosphonates inhibit basal bone resorption as well as resorption stimulated by a variety of agents, including PTH, prostaglandins and $1,25(OH)_2D_3$ (Fleisch et al, 1969; Fleisch, 1983; Gebauer & Fleisch, 1980). When diphosphonates are administered to growing animals, remodelling of long bones is blocked in the metaphysis leading to a club shaped appearance (Schenk et al, 1973). The inhibition of bone resorption gives rise to appearances which resemble osteopetrosis (Reynolds et al, 1973). This effect, seen with clodronate, APD and other alkyl-amino compounds, is less marked with etidronate (Schenk et al, 1973; Trechsel et al 1977; Reitsma, 1982). Although the architectural consequences are similar in animals given etidronate, the amount of mineralised bone does not increase since, at these doses, inhibition of mineralisation as well as inhibition of bone resorption are prominent features (Schenk et al, 1973). Clodronate, APD and AHDP, unlike etidronate, have very little effect on skeletal mineralisation (Flora et al, 1980; Reitsma et al, 1980; Felix et al, 1982).

There is little correlation between the activity of diphosphonates to inhibit bone resorption in vivo and their ability to inhibit the dissolution of

hydroxyapatite in vitro (Shinoda et al, 1979). This suggests that the action of diphosphonates to inhibit bone resorption may involve mechanisms other than their physico-chemical effects on hydroxyapatite. Potencies vary considerably and, for instance, on a molar basis APD is approximately 10 times more potent than etidronate in vivo in inhibiting bone resorption when given subcutaneously.

Diphosphonates adsorb strongly onto hydroxyapatite and are localised in vivo to sites of mineral deposition such as the calcification front (Kahn et al, 1979). It is likely that this activity is related to their basic P-C-P configuration (compare with the P-O-P configuration of PPi shown in Figure 1.5) since there are no differences in gross distribution of these agents (Larsson, 1982). Pharmacokinetics of the diphosphonates are difficult to study, in part owing to the unavailability of sufficiently sensitive and specific assays for these agents. Also it is likely that concentrations in the ECF may not reflect concentrations at target tissues which may be much higher. The disposition of diphosphonates within bone is uneven (the basis of bone scanning which utilises 99Tc-tagged diphosphonates), so that the concentration of diphosphonate presented to particular cells is largely a matter of conjecture (Larsson & Rohlin, 1980). Diphosphonates are internalised within some cells (Fast

et al, 1978), and an example of this may be the renal secretion of diphosphonates, which has been documented in the case of etidronate and clodronate (Trohler et al, 1975). However, the degree to which different diphosphonates are internalised by different skeletal cell types *in vivo* is largely unknown, so that it becomes difficult to relate any differences in effect observed *in vitro* to different cellular activity.

A variety of biochemical actions of the diphosphonates have been described (Felix et al, 1982a). Of possible relevance to their ability to inhibit bone resorption is that they inhibit *in vitro* the activity of various lysosomal enzymes, including acid phosphatase and pyrophosphatase (Felix et al, 1976). They also inhibit the release of these enzymes stimulated by PTH *in vitro* (Morgan et al, 1973) and *in vivo* (Doty et al, 1972). Several of the diphosphonates, including etidronate, appear to inhibit the production of prostaglandins by bone cells *in vitro* (Felix et al, 1981). However, APD has been shown to increase prostaglandin synthesis, and thus the relevance of this effect to bone resorption is unclear (McGuire et al, 1982). Both clodronate and etidronate appear to decrease glycolytic activity in bone cells *in vitro* as judged by glucose consumption and lactate production (Fast et al, 1978; Shinoda et al, 1979).

It should be noted, however that many of the *in*

vitro studies were performed on osteoblast-like cells. Moreover, cell separation techniques suggest that osteoclast-like, rather than osteoblast-like, cells are sensitive to clodronate (Guenther et al, 1982) and thus the relevance of these effects in vivo is not clear. In this respect it is interesting that cells with phagocytic properties may engulf diphosphonates with avidity, particularly if they are bound to hydroxyapatite. Since osteoclasts are derived from potentially phagocytic cells (of the monocyte series), it is possible that diphosphonates influence not only osteoclast metabolism, but also their differentiation.

Macrophages and monocytes are able to phagocytose and digest bone particles in vitro (Kahn et al, 1978) and several diphosphonates inhibit the proliferation of macrophages (Cecchini et al, 1984). The attachment of macrophages to bone particles coated with APD or clodronate is not affected, but resorption is inhibited (Reitsma et al, 1982). However, resorption of bone particles is not inhibited when clodronate is added after macrophage attachment indicating that this diphosphonate acts before cell attachment. In contrast, APD activity is not solely dependent on prior adsorption onto bone suggesting an additional mechanism of action. Such differences in effects, and thus in mechanisms of action, of the various diphosphonates may prove important in the clinical use of these agents.

Effects of diphosphonates in man

Etidronate is the only commercially available diphosphonate in this country, although clodronate has recently been licenced for use in Finland and Italy and may soon be available in the rest of Europe. The therapeutic uses of these two agents have been widely investigated whereas clinical studies on the other diphosphonates (APD, AHDP and ABuDP) have been less extensive and confined to fewer centres. Etidronate has been most used in the medical management of Paget's disease and in the prevention of heterotopic calcification (Finerman & Stover, 1981). Experience with this drug is more limited in other disorders, including skeletal complications of malignancy, osteoporosis, hyperparathyroidism and other disorders associated with increased bone resorption. In contrast, the other diphosphonates, including clodronate, have been widely used in these latter disorders, as well as in Paget's disease, with encouraging results.

General pharmacology

Diphosphonates in general are poorly absorbed from the gastrointestinal tract. In the case of etidronate, absorption is between 1 and 10 per cent, but

this is reduced to zero in the presence of calcium-containing foods which chelate the diphosphonate (Fogelman et al, 1984). Oral clodronate bioavailability has been estimated to be around 1 to 2 per cent (Yakatan et al, 1982). It appears that geographical factors affect the degree of absorption as etidronate absorption was found to be much higher in Glasgow than in Madison for normal individuals (Fogelman et al, 1984). This may be one of the reasons for disparate observations of effects of etidronate between different centres (Kanis, 1984).

Diphosphonates are avidly retained by the skeleton, and the fraction appearing in the urine is related to skeletal turnover. This forms the basis for the use of 99Tc-tagged diphosphonate retention to monitor bone metabolism (Fogelman et al, 1978). The 24 hour whole body retention may be considerably increased in disorders such as Paget's disease and metastatic bone disease and correlates well with biochemical indices of bone turnover (Fogelman et al, 1978; Smith et al, 1984). It is thought that diphosphonates are excreted unchanged in the urine, although the possibility that some is metabolised has not been satisfactorily resolved. As previously mentioned, there is evidence for renal tubular secretion of diphosphonates since renal clearance may exceed the glomerular filtration rate (Trohler et al, 1975).

Even in health, skeletal uptake of diphosphonates is not uniform, and in bone disorders such as Paget's disease or metastatic bone disease the patchy distribution is even more marked. This may have important therapeutic implications since a proportionately greater dose of diphosphonate is delivered to the site of the disorder than elsewhere in the skeleton. This targeting effect may have obvious advantages in sparing non-affected sites, but also causes problems in assessing the adequacy of effect of a particular dose on skeletal metabolism. Thus, in very extensive Paget's disease a given dose of diphosphonate will deliver less diphosphonate to any one site of disease activity than the same dose delivered to a patient with limited skeletal involvement. In support of this suggestion a study of the use of etidronate in Paget's disease by Fromm and co-workers (1979) has suggested that moderate to high doses may be associated with a greater incidence of side effects in patients with "oligostotic" Paget's than in those with more widespread disease.

Once incorporated into the skeleton the half life of diphosphonates may be very long. Prolonged treatment may lead to cumulative uptake which could account for the sustained biochemical suppression of pagetic activity that is observed with these agents even following withdrawal of treatment. Thus in the process

of bone resorption osteoclasts may cause local release of diphosphonate previously accreted in bone mineral. Such a mechanism might account for the highly specific effects of diphosphonates on bone resorbing cells. However, in other disorders associated with increased turnover, such as hypercalcaemia of malignancy, relapse may be rapid once treatment has been stopped (Chapter 5).

Treatment of Paget's disease

As recently as 15 years ago there were no generally available specific treatments for Paget's disease which therefore remained as something of a medical curiosity. However, since the discovery of calcitonin and its earliest use in Paget's disease (Bijvoet et al, 1967) and the development of etidronate, first used in Oxford in 1969 (Smith et al, 1971) there has been a very rapid increase in the availability of specific treatments for this disorder. In addition to etidronate the newer diphosphonates, particularly clodronate and APD have been reasonably extensively evaluated in the treatment of Paget's disease.

Indications for treatment

It is generally agreed that the use of specific anti-pagetic treatment is indicated in patients with bone pain. However, as mentioned previously, it may be difficult to distinguish between pain of pagetic origin and pain from an osteoarthritic joint. Intra-articular lignocaine may be useful in deciding the source of the pain but in many patients this remains uncertain and a trial of treatment is often justified. Furthermore, if patients with osteoarthrosis associated with Paget's disease require orthopaedic surgery, prior anti-pagetic treatment may be indicated to reduce bone vascularity and thus improve the ease and safety of operation (Bowie & Kanis, 1976; Meyers & Singer, 1978). A further indication for medical treatment is spinal cord dysfunction in Paget's disease where results are as good as, or better than, those obtained by surgical decompression (Douglas et al, 1981).

However, in the majority of individuals with Paget's disease (approximately 90 per cent) the disorder is not associated with significant symptoms and the risk of development of clinically important complications is small. Therefore the finding of Paget's disease per se is not an indication for treatment. Nonetheless, where pagetic involvement is extensive, particularly in a

relatively young patient, or in cases of Paget's involving the skull or thoracic or cervical spine, the risk of development of complications may justify treatment even in the absence of symptoms.

Use of calcitonin

This 32 amino acid polypeptide hormone displays considerable interspecies differences in amino acid sequence and potency. Pharmacologically, its major action is to specifically inhibit osteoclastic bone resorption (MacIntyre, 1983). To be effective as a therapeutic agent calcitonin has to be given parenterally, usually as daily or thrice weekly subcutaneous injections. More recently the possible use of salmon calcitonin given by nasal spray has been investigated, albeit in very small numbers of patients (Nagant de Deuxchaisnes et al, 1985b; Reginster et al, 1985). These preliminary studies have suggested that pagetic activity can be significantly suppressed with this mode of administration and, moreover, that this route may be associated with fewer side effects than is subcutaneous administration. Certainly nasal insufflation would seem likely to be more acceptable than subcutaneous injections for many patients.

Porcine, salmon and human calcitonin, given by subcutaneous injection, have all been shown to be

effective agents in the short term treatment of Paget's disease (DeRose et al, 1974; Evans et al, 1977). Effects include relief of bone pain, suppression of the indirect biochemical markers of bone resorption and a reduction in blood flow through pagetic bone (Wootton et al, 1981). However it is usual for serum alkaline phosphatase and urinary hydroxyproline to fall only to about half of the pretreatment values. Thus, whereas normal values may be achieved in patients with mild pagetic activity this is seldom the case in patients with more active disease. In careful radiological studies treatment with calcitonin has been shown to prevent the advance of osteolytic fronts and produce an increase in radiodensity at lytic sites (Nagant de Deuxchaisnes et al, 1980).

A proportion of patients develop resistance to treatment with biochemical and/or symptomatic relapse despite continued administration of the drug. Various explanations for this resistance have been proposed, including secondary hyperparathyroidism (Burckhardt et al, 1973), development of neutralising antibodies (Haddad & Caldwell, 1972) and down-regulation of calcitonin receptors (Tashjian et al, 1978). The first of these mechanisms is unlikely as although PTH concentrations increase following acute administration of calcitonin they do not change significantly following long-term treatment (DeRose et al, 1974). The development of antibodies which block binding of calcitonin to its

receptor may be responsible in some patients, particularly those treated with salmon calcitonin. However, many patients possess antibodies but remain responsive, whereas others become calcitonin resistant in the absence antibodies (Singer & Mills, 1983; Tashjian et al, 1978). That down-regulation of calcitonin receptors may occur is evidenced in patients with medullary carcinoma of thyroid who, despite often massively raised concentrations of circulating calcitonin, continue to have a normal or even increased rate of bone turnover, possibly due to associated hyperparathyroidism (Saad et al, 1984). It is likely that this mechanism is responsible for aquired resistance in some patients.

A further problem with the use of calcitonin is the relatively rapid rate of biochemical relapse (within months) that follows cessation of treatment (Avramides, 1977; Evans et al, 1980). Symptomatic response may, however, outlast the biochemical response and in many patients intermittent treatment for a few months at a time may be satisfactory.

Side effects of calcitonin, which occur in about 30 per cent of patients, include nausea, facial flushing, tingling in the extremities, febrile reactions and a metallic taste in the mouth. These may subside after several weeks of therapy but in some patients demand withdrawal of treatment. A further consideration is that the calcitonins are relatively expensive and this is

especially true if a district nurse is required to give the injections.

Diphosphonates in Paget's disease

Despite poor intestinal absorption the diphosphonates that have been investigated clinically have all been demonstrated to be effective when given by mouth. In general they are capable of inducing a more marked suppression of the biochemical markers of bone turnover than is seen following calcitonin. Furthermore, the suppression is sustained not only during a period of treatment but often for in excess of one year thereafter. As indicated previously, however, important differences exist in the spectra of effects of the various diphosphonates, and some may be more suitable than others for the treatment of Paget's disease. The greatest volume of experience has been gained with the use of etidronate.

Etidronate has been used at doses of between 1 and 20mg/kg body weight/day. A dose dependent reduction in the biochemical markers of pagetic activity is seen throughout this dose range with the highest dose achieving a 70 to 80 per cent fall in serum alkaline phosphatase and urinary hydroxyproline excretion (Canfield et al, 1977). The recommended dose (5mg/kg/day) induces approximately a 50 per cent

reduction in disease activity although the response of individual patients is very variable (Johnston et al, 1980; Russell et al, 1974; Altman et al, 1973). The biochemical suppression of disease activity is associated with pain relief which may occur in a dose dependent manner (Altman et al, 1973).

As with calcitonin, the treatment of Paget's disease with etidronate is associated with a reduction in skeletal blood flow and in cardiac output (Walton et al, 1983). However, whereas significant reductions in blood flow occur after only 2 weeks from the start of calcitonin treatment, no such effect is observed with etidronate treatment at this time, although by 3 to 4 months from the start of treatment the effects on blood flow of the two treatments are similar (Walton et al, 1985).

Suppression of disease activity with low dose etidronate (5mg/kg/day) is also associated with the formation of lamellar rather than woven bone, a reduction of the calcification rate from high to normal or subnormal and a reduction in the number of osteoblasts and osteoclasts (Alexandre et al, 1981). When doses higher than 5mg/kg/day are used, mineralisation of osteoid is impaired and this gives rise to the histological appearance of osteomalacia in both pagetic and normal bone (Meunier et al, 1975) . Even at the recommended 5mg/kg/day for 6 months a recent study from

Glasgow demonstrated a high frequency of focal osteomalacia in pagetic bone (Boyce et al, 1984). It is notable, however, that many of these patients became hyperphosphataemic, an effect usually only seen at higher doses, suggesting that increased bioavailability of the drug may have been a major factor. Indeed, as previously mentioned, studies from the same centre have shown that the absorption of etidronate is on average two-fold higher in normal subjects from Glasgow than in normal subjects in Madison (Fogelman et al, 1984). Such differences in bioavailability probably account for much of the observed interindividual variability in response as well as differences in the general experience of different centres. In our own experience treatment with low dose (5mg/kg) etidronate has produced inconsistent results; some patients having responded well whereas in others treatment was ineffective (Preston et al, 1986; see Chapter 3).

It is important to know whether the impairment of bone mineralisation is of any clinical relevance. There are reports of increased bone pain with the use of etidronate, particularly when given in high doses (Canfield et al, 1977), and there are concerns that the risk from fracture may be increased (Krane, 1982; Kantrowitz et al, 1975). It has been suggested that the recommended dose of etidronate is not associated with increased fracture prevalence (Johnston et al, 1983).

Eleven per cent of the 737 etidronate treated pagetic patients reviewed by these authors sustained at least 1 fracture. Although this prevalence was comparable to retrospective data of untreated patients, no untreated controls were included in this study. Such comparisons may not be valid as there are likely to have been important differences in patient selection in the intervening years. Higher doses of etidronate were associated with a higher fracture incidence (Johnston et al, 1983). Recently Nagant de Deuxchaisnes and colleagues (1985) have reported a very low fracture incidence in untreated pagetic patients and suggested that the figures obtained in Johnston's study represent a real increase in fracture frequency due to etidronate. It is generally considered that rapidly advancing osteolytic Paget's in the appendicular skeleton is a relative contra-indication to the use of etidronate.

Thus, for a given patient there is either a narrow therapeutic range or perhaps there is no dose of etidronate which will maximally suppress pagetic activity while having no significant effect on mineralisation of bone. Because of the inter-individual variability in drug absorption and in extent of pagetic involvement it is evident that no single dose will be appropriate for the entire population of Paget's patients. This suggests that, if oral etidronate is to be used, some form of monitoring may be required to prevent overdosing or

underdosing of individual patients.

One possibility is to use the hyperphosphataemic effect of etidronate as a guide to the systemic availability of etidronate (Kanis, 1984). Etidronate induces hyperphosphataemia via a dose dependent effect to increase renal tubular reabsorption of phosphate which becomes maximal at about 2 weeks from the start of treatment (Walton et al, 1975a). The mechanism by which this interesting effect occurs is unknown but appears to be unrelated to changes either in PTH or in renal sensitivity to PTH (Walton et al, 1975; Recker et al, 1973). Treatment with other diphosphonates is generally associated with a fall rather than a rise in serum phosphate. However, in a study by Nagant de Deuxchaisnes and colleagues (1982) using high oral doses of APD (600mg/day) significant, albeit transient, increases in serum phosphate and TmP/GFR were observed. Moreover, radiological evidence in this study suggested a degree of impairment of skeletal mineralisation. Thus, there may be a relationship between the effects of diphosphonates in general on renal phosphate transport and their effects on bone mineralisation. Whether this relationship is causal, or whether these effects are unrelated epiphenomena is unknown. This question is addressed in Chapter 4 in which the effects of very high intravenous doses of etidronate and other diphosphonates on mineral metabolism are examined. Whatever the association,

monitoring of serum phosphate may enable titration of the dose of etidronate to more appropriate levels for individual patients. Etidronate induced hyperphosphataemia does not appear to be associated with any important clinical consequences and in particular, calcium phosphate precipitation within soft tissues does not occur.

Some authors have advocated the use of low doses of etidronate combined with human or salmon calcitonin (Hosking et al, 1981; Bijvoet et al, 1978). Studies with this combination have suggested a more marked and/or a more consistent suppression of pagetic activity than may be achieved with either calcitonin or low-dose etidronate used alone.

Both clodronate at doses of between 400mg and 3,200mg per day and APD at doses of 50 to 600mg per day induce a dose-dependent rapid suppression of urinary hydroxyproline excretion with a later fall in serum alkaline phosphatase similar to that observed with etidronate treatment (Douglas et al, 1980; Frijlink et al, 1979; Heynen et al, 1982). A transient positive skeletal calcium balance is thus produced and these treatments are associated with hypocalcaemia and a secondary increase in serum iPTH (Harris et al, 1982). In contrast, treatment with oral etidronate is not associated with any significant change in serum calcium (Russell et al, 1974; Canfield et al, 1977). Clodronate

and APD, like etidronate are associated with a reduction in numbers of osteoclasts and osteoblasts seen on bone histology towards normal (Meunier et al, 1979; Bijvoet et al, 1980). However, in contrast to the observations with etidronate, patients do not develop histological evidence of osteomalacia and, at least with clodronate, the rate of bone mineralisation, as judged by double tetracycline labelling techniques, remains normal (Meunier et al, 1979).

The lack of significant impairment of mineralisation may give these newer agents a major advantage over etidronate. However, it should be noted that very high doses of both etidronate and clodronate given to dogs are associated with fracture, and in the case of clodronate this is likely to be solely due to impaired bone remodelling and thus impaired healing of bone microdamage (Flora et al, 1980). This phenomenon has not been observed with the use of therapeutic doses in man.

It is difficult to compare the the relative efficacies of these diphosphonates as, with one exception, direct comparative studies have not been made. Dewis and colleagues (1985) in a small study, found that the clinical and biochemical effects of etidronate 20mg/kg/day and APD 4.5mg/kg/day for three months were indistinguishable.

Apart from osteomalacia induced by etidronate,

side effects of diphosphonates are generally minor. All three diphosphonates are associated with some gastrointestinal side effects (Nagant de Deuxchaisnes et al, 1982; Mautalen et al, 1984, Douglas et al, 1980). In the case of etidronate or clodronate these consist of nausea, abdominal discomfort and diarrhoea which may be troublesome, particularly when high doses are used. In clinical trials with clodronate 3 cases of leukaemia emerged from a total of around 900 patients which caused some concern over the safety of this agent. However, one of these patients had been exposed to benzene 20 years earlier, another patient had myeloma and the third came from an area in which the incidence of leukaemia is several times the average. Thus all 3 had predisposing factors for the development of leukaemia. No leukaemogenic or carcinogenic effects of clodronate were observed in animal studies with this agent and with continued patient follow-up no new cases of leukaemia have emerged. Thus, although continued vigilence is required, the association was probably fortuitous. The use of APD has been associated with severe gastrointestinal upset and oral ulceration although this may be a result of inadequate formulation of the drug (Mautalen, personal communication).

Treatment with APD may also be associated with a pyrexia persisting for several days, transient leukopenia and the release of acute phase proteins (Bijvoet et al,

1980; Nagant de Deuxchaisnes et al, 1982). This appears to be a dose dependent effect, since when doses of 250mg per day or less have been used fever has not been reported (Heynen et al, 1982). The mechanism of the pyrogenic reaction is not clear but would be consistent with the release of IL-1 (endogenous pyrogen). Despite the fall in total white cell count, monocytes increase transiently (Nagant de Deuxchaisnes et al, 1982). These immunomodulatory effects of APD could confer either therapeutic advantages or disadvantages. Comparable effects with either etidronate or clodronate have not been observed.

Other treatments

Mithramycin is a cytotoxic antibiotic which also inhibits osteoclastic bone resorption, although this action is not specific (Ryan, 1977). It is effective in producing both a symptomatic and biochemical response. However its usefulness is severely limited by the nature of the side effects which include hepatic and renal dysfunction and bone marrow suppression which may result in thrombocytopenia. Although these effects are uncommon at the low doses used to treat Paget's disease, in view of the availability of safer drugs mithramycin is probably only rarely, if ever, indicated for the treatment of this disorder. Actinomycin D is another RNA

inhibitor which has been used to treat Paget's disease but which suffers from similar disadvantages (Fennelly & Groarke, 1971). An alternative approach has been to attempt to stimulate endogenous calcitonin secretion using glucagon or calcium and thiazides. The latter form of therapy was reported to be effective in reducing bone pain and causing a sustained suppression of alkaline phosphatase in a small group of pagetic patients (Evans, 1977).

Apart from specific treatment, general measures such as the use of analgesic and non-steroidal anti-inflammatory drugs, orthopaedic appliances and supports can often be helpful.

Treatment of hypercalcaemia of malignancy

The most important aspect of the acute management of hypercalcaemia is adequate restoration of extracellular volume. This is normally achieved with the use of physiological saline. An adequate saline load decreases renal tubular reabsorption of calcium and increases GFR both factors combining to allow more efficient elimination of calcium and thus a fall in serum calcium (Hosking et al, 1981). Some patients, particularly those with myeloma, may have marked renal impairment and care should be taken not to overload the

cardiovascular system with saline. It may be necessary to give supplements of potassium or magnesium as these cations may become depleted during prolonged saline administration (Sleeboom et al, 1983). Loop diuretics are sometimes used since they increase the excretion of both sodium and calcium (Reimold, 1972). However, in patients who are not adequately volume expanded the effects of diuretics to increase dehydration will offset any beneficial effect (Parfitt, 1979) and thus these agents should be used cautiously if at all. Thiazides should be avoided as they increase renal tubular reabsorption of calcium, and occasionally may be the sole cause of hypercalcaemia. Only a minority of patients become normocalcaemic following treatment with saline alone (Hosking et al, 1981; Sleeboom et al, 1983). Even in these patients, if the cause for the increased load of calcium is not treated the hypercalcaemia is likely to recur (Parfitt, 1979).

Corticosteroids are frequently used in conjunction with saline rehydration. In myeloma they have effects both on the disease process and on the activity of bone resorbing factors (Strumpf et al, 1978). However, we have recently shown that their use with saline in hypercalcaemia associated with solid tumours does not confer any additional benefit over saline alone (Percival, 1984). Moreover, because treatment with high doses of steroids inhibit bone formation (Avioli et al,

1984) it is possible that in some cases their use may limit rather than enhance the hypocalcaemic effect of other agents. The hypocalcaemic effects of specific forms of chemotherapy tend to be of slow onset although such treatment may help to prevent recurrence of hypercalcaemia. Thus, specific additional treatment for hypercalcaemia is often required.

A reduction in serum calcium may be achieved with the use of inorganic phosphate, which may be given either orally or intravenously. The mechanism of action of oral phosphate may in part be related to decreased bone resorption (Mundy & Raisz, 1974). Increasing concentrations of phosphate inhibit bone resorption in vitro and the administration of phosphate by mouth to immobilised normal subjects decreases calcium losses from the skeleton (Hulley et al, 1971). In contrast, the major mechanism of action of intravenously administered phosphate is the formation of calcium phosphates in the ECF and their subsequent clearance by the reticulo-endothelial system. This treatment is associated with a risk of renal failure particularly in those with pre-existing renal impairment. With the development of specific inhibitors of bone resorption the use of this agent is probably no longer indicated.

The calcitonins, mithramycin and the diphosphonates have all proved useful in the management of malignant hypercalcaemia. Apart from inhibiting

osteoclastic bone resorption calcitonin also inhibits renal tubular reabsorption of calcium; both of these mechanisms contributing to the overall hypocalcaemic effect (Hosking & Gilson, 1984). Flushing and nausea occur in a proportion of patients treated with calcitonin which is, however, free from major side effects and produces a prompt hypocalcaemic response. Unfortunately, this effect is often incomplete and is short-lived with maximal hypocalcaemia being seen at 4 to 6 hours after a single injection (Behn & West, 1977). Thus injections need to be repeated frequently. When calcitonin is given repeatedly its effects diminish progressively. This may be due to down-regulation of receptors but, if so it occurs very much more quickly than in patients with Paget's disease. The combination of corticosteroids with calcitonin may decrease the rapidity with which this resistance occurs (Binstock & Mundy, 1980; Ralston et al, 1985).

Infusion of mithramycin in lower doses than those used for tumour chemotherapy (around 25ug per kg of body weight) lowers plasma calcium concentrations (Stamp et al, 1975; Smith et al, 1975; Ralston et al, 1985). However, the effect is relatively slow and responses in serum calcium may not be seen for one to two days (Ralston et al, 1985; Mundy & Martin, 1982). Moreover, at these doses mithramycin may induce hepatic damage and thrombocytopenia.

There has been much recent interest in the use of diphosphonates in the treatment of malignant hypercalcaemia. However, perhaps surprisingly, there has only been one small study of the use of oral etidronate, the only commercially available form, in the management this complication (Mundy et al, 1983). These authors found that only 1 of 4 hypercalcaemic patients responded to treatment with oral etidronate. Two studies on the use of intravenous etidronate have demonstrated hypocalcaemic responses in the majority of patients (Jung, 1982; Ryzen et al, 1985) although the mean serum calcium for the entire etidronate treated group remained above normal in the second of these studies. Other diphosphonates, particularly clodronate and APD have been successfully employed in the treatment of malignant hypercalcaemia (van Breukelen et al, 1979; Paterson et al, 1983; Jacobs et al, 1981) but, with one exception (Jung et al, 1982) direct comparative studies between diphosphonates have not been performed. Jung and colleagues (1982), treated patients with either intravenous clodronate or etidronate. They noted a delay in the fall of serum calcium in the etidronate group although there was no difference in the hypocalcaemic response after 10 days. However, known differences in the mechanisms of action of the various diphosphonates may be expected to affect their suitability as hypocalcaemic agents and thus such comparisons are of

considerable importance. In my own studies, reported in this thesis, I have compared the effects of three diphosphonates (etidronate, clodronate and AHDP) given intravenously in the treatment of malignant hypercalcaemia (Chapter 5). In addition, I have compared the hypocalcaemic effects of etidronate and clodronate given by mouth to patients with primary hyperparathyroidism (Chapter 6).

High-dose bolus intravenous injection of diphosphonates may impair renal function in dogs and this effect has also been reported in man (Bounameaux et al, 1983). However, as we have previously reported, these renal effects may be avoided by the use of slow intravenous infusion rather than bolus injection (Kanis et al, 1983b).

Long-term management of skeletal involvement in malignancy

In many patients with skeletal metastases the value of chemotherapy is questionable and even in hormone responsive tumours the effects of hormonal manipulation are usually only short-lived. Bone pain, fractures and hypercalcaemic episodes are a major cause of morbidity and account for some of the mortality of malignant diseases. Although, as previously reviewed, different forms of malignant disease affect the skeleton by

different mechanisms, they all induce an increase in osteoclastic bone resorption. Thus if specific inhibitors of osteoclast activity, such as the diphosphonates, are capable of producing effects regardless of the mechanism of osteoclast activation then they may have a role in the prevention of skeletal complications.

There are anecdotal reports of the relief of bone pain following etidronate treatment (Schnur, 1984; Zalcberg personal communication) but this agent is probably not suitable for long-term control of osteolysis because of the associated mineralisation defect. Several short-term studies with the newer diphosphonates (APD and clodronate) have shown encouraging results with reduced calcium release from the skeleton and suppression of hypercalcaemia (Siris et al, 1980; Chapuy et al, 1980; Douglas et al, 1980; van Breukelen et al, 1979; Delmas et al, 1982; Siris et al, 1983). Recently Elomaa and her colleagues (1983) have performed a placebo controlled trial of longer-term oral clodronate in the treatment of women with carcinoma of the breast and multiple osteolytic metastases. Bone pain, new bone metastases and development of hypercalcaemia as well as bone resorption, as judged by urinary hydroxyproline excretion, were suppressed in the treatment group. Further follow-up of these patients has shown a significantly increased survival of clodronate treated

patients compared to placebo controls (Elomaa et al, 1984). It seems very likely that such treatments will become a useful addition to currently employed methods for control of malignant disease.

Treatment of primary hyperparathyroidism

In the presence of specific symptoms or complications referable to primary hyperparathyroidism, such as renal stones, bone disease, thirst and polyuria, there is general agreement that parathyroidectomy is required. Worsening renal function or an increase in serum calcium to above 3.0mmol/l are also usually accepted as sufficient justification for surgery. However, as discussed above, the widespread availability of routine serum calcium measurement, and the consequent change in the pattern of clinical presentation has produced a situation in which over 50% of patients with proven primary hyperparathyroidism are asymptomatic or at least have no specific symptoms (Mundy et al, 1980; Heath et al, 1980). The most appropriate management of these patients (i.e. those that fill none of the above criteria) has been a topic of much recent debate (Coe & Favus, 1980; Anonymous, 1984).

The two major alternative approaches are, on the one hand, early parathyroidectomy and, on the other, simple observation with subsequent referral for

parathyroidectomy if the patient becomes symptomatic or develops other evidence of significant disease. A third alternative, that of active medical treatment (rather than just observation), is discussed below. The decision for or against surgery for asymptomatic patients should be based on three principal considerations. Firstly, what is the natural history of the disease in mildly hypercalcaemic asymptomatic individuals? Secondly, what are the risks and benefits of parathyroidectomy? Finally, what is the relative acceptability of either surgery or long-term medical follow-up to the patient?

The question of the natural history of asymptomatic patients has most extensively investigated in a prospective 5 year study by Purnell and co-workers (1974) which included 147 such patients. Six patients died of causes unrelated to primary hyperparathyroidism and 27 were lost to follow-up. Of the remainder, 29 required parathyroidectomy by the end of 5 years. Reasons for parathyroidectomy included deteriorating renal function in 5 patients, psychological problems in 4 patients, increasing hypercalcaemia in 2 patients, osteitis fibrosa in 1 patient and preparation for hip surgery in 1 patient. Thus, over one third of the original study group (56 of 147 patients) were either lost to follow-up or eventually required surgery anyway and might be considered as failures of the conservative medical approach.

In a smaller study, Van't Hoff and colleagues (1983) followed 32 untreated asymptomatic patients for a mean period of 4.2 years. One patient was referred for surgery because of increasing serum calcium and hypertension and 2 patients were operated on because they were relatively young although they had no complications. Operation was advised, but refused, in another patient who subsequently developed pancreatitis followed by diabetes mellitus. Three patients died through unrelated causes. These authors also studied a control group of 60 patients who underwent successful parathyroidectomy. Neither serum creatinine nor blood pressure changed significantly in either group of patients. However, 5 patients (of 21 non-hypertensive patients) in the medical management group became hypertensive during the study period compared with only 3 (of 42 non-hypertensive patients) in the surgically treated group. The numbers are too small to be able to exclude a significant risk of developing hypertension due to primary hyperparathyroidism. Thus larger numbers of patients are required to determine the relative risks for the development of hypertension in conservatively managed versus parathroidectomised patients.

In experienced hands the outcome of parathyroidectomy in asymptomatic patients is generally excellent, with very high cure rates (over 98%) and minimal morbidity (Taylor, 1980; Attie & Khafit, 1976;

Gaz & Wang, 1984). Persistant postoperative hypoparathyroidism and recurrent laryngeal nerve damage are currently rare, at least in interested centres (Taylor, 1980; Gaz & Wang, 1984). However, the importance of choosing a surgeon with considerable experience in neck explorations to achieve such good results has been stressed (Cope, 1966). Recently surgery has been facilitated by improved imaging techniques for parathyroid tissue including thallium/technetium subtraction scanning, ultrasound and computerised tomography. As confidence in these techniques has improved some surgeons have adopted the practice of initially only exploring the side of the neck in which a single adenoma has been localised. If the surgical findings conform to those predicted by the imaging techniques (i.e. one normal and one adenomatous gland) then the contralateral side may not need to be explored (Gaz & Wang, 1984).

Although the main reason for surgery in asymtomatic patients is prophylactic, some patients notice an improvement in their general well-being after successful parathyroidectomy even though no specific symptoms were noted beforehand. However, despite the safety and high rate of success of parathyroidectomy many patients are understandably reluctant to submit themselves to surgery, and this is particularly so when patients are asymptomatic. Most patients will, however,

consent to operation if they are strongly advised to do so by their doctor. Thus, the clinician has a difficult role in trying to balance the best interests of the patient in these circumstances. In some cases surgery will be clearly inappropriate, either because of a short life expectancy or because the patient is unfit for anaesthesia.

The reluctance of patients to have surgery must be balanced against the problems of long-term follow-up, particularly if they are seen in a routine clinic where mechanisms for ensuring continued surveillance may be inadequate. Even within the prospective study of Purnell and colleagues (1974), despite "heroic efforts", 27 of the original 147 patients (18%) were lost to follow-up. A further consideration noted by these authors, and emphasised by Heath and colleagues (1980), is the relative cost of the two approaches to management. The latter authors estimated that, in the USA, the costs of surgery would be outweighed by the costs of follow-up for 5.5 years.

On balance the risks of conservative medical management of asymptomatic patients with primary hyperparathyroidism, though probably small, appear to be greater than the risks of surgery for the majority of asymptomatic patients with primary hyperparathyroidism. However, these relative risks need to be further evaluated with a prospective controlled study as

advocated by Coe & Favus (1980). At present there can be no rigid rules and the decision to advise parathyroidectomy must be made on an individual basis.

The role of medical treatment of primary hyperparathyroidism obviously depends on the efficacy, acceptability and side effects of the treatment. Several different forms of treatment have been proposed including oral phosphate, cimetidine, beta-adrenergic blockade, oestrogens, progestagens, and the diphosphonates. Oral phosphate, which may act partly by limiting intestinal calcium absorption and partly by inhibiting bone resorption (Hulley et al, 1971), was the first form of medical treatment to be used (Albright et al, 1932). Although this form of therapy might have a minor role in treatment of a subgroup of patients with hyperabsorption of calcium its effects on serum calcium are generally small and side effects, including diarrhoea and possibly renal impairment, preclude its general use (Broadus et al, 1983; Purnell et al, 1974).

Bearing in mind the multiple mechanisms by which PTH acts to produce hypercalcaemia in primary hyperparathyroidism, the most rational medical approach would be at the level of the parathyroid gland itself to inhibit PTH secretion. An initial report on 12 patients by Sherwood and colleagues (1980) suggested that treatment with cimetidine 1.2g/day may result in consistent falls in iPTH. In addition, these authors

stated that serum calcium was reduced in all patients, although data was not shown to confirm this. Subsequent studies, however, have failed to substantiate any significant response to cimetidine treatment either in terms of iPTH concentration or serum calcium (Wiske et al, 1983; Fisken et al, 1982). Similarly, although the use of beta-adrenergic blocking agents has theoretical potential to reduce PTH secretion these drugs have not be found to have any significant effects on serum calcium in patients with primary hyperparathyroidism (Ljunghall et al, 1982).

Recent studies with the use of an organic thiophosphate compound, WR-2721, are of interest. WR-2721 is a radioprotective and chemoprotective agent of potential use in patients with malignant disease undergoing treatment with ionising radiation or alkylating agents. In a phase 1 clinical trial this drug was discovered to induce reversible hypocalcaemia and at the same time reduce serum iPTH concentrations in previously normocalcaemic patients with malignant disorders (Glover et al, 1983). The hypocalcaemia appeared to result purely from an acute inhibition of PTH secretion. Furthermore, in <u>in vitro</u> studies by these authors, WR-2721 produced consistent inhibition of release of iPTH from dispersed bovine parathyroid cells . The mechanism of action of this agent on the parathyroid glands is unknown. Side effects of this treatment, which

was administered by slow intravenous infusion, were reported to be minor, and thus a possible role of this agent in the management of parathyroid disorders was suggested (Glover et al, 1983).

Treatment with ethinyloestradiol 50ug/day significantly reduced the serum calcium in 10 women with primary hyperparathyroidism, although in none of these patients did serum calcium return to within the normal range (Gallagher & Nordin, 1972). In a more recent study of 6 patients treated with ethinyloestradiol, Selby & Peacock (1986) reported a fall in the mean serum calcium to the upper end of the normal range. Similar results have been achieved with the use of the conjugated oestrogen, Premarin, at an average dose of 1.25mg/day although 4 of the 14 patients studied failed to respond to this treatment (Marcus et al, 1984). In these various studies treatment with oestrogens produced falls in urinary hydroxyproline excretion and both fasting and 24h urinary calcium excretion. Neither intestinal calcium absorption nor concentrations of free $1,25(OH)_2D_3$ were affected by oestrogen treatment (Gallagher & Wilkinson, 1973; Selby & Peacock, 1986). Thus these studies suggest that the primary effect of oestrogens is to inhibit bone resorption. Such an effect is probably mediated indirectly as no oestrogen receptors have yet been localised within bone cells (Yoshioka et al, 1980).

Treatment with norethisterone, appears to have effects

similar to those of oestragens, producing significant, but incomplete, reductions in serum calcium (Selby & Peacock, 1986; Horowitz et al, 1986).

In a small study of the use of high-dose oral etidronate (20mg/kg/day) there was no significant change in serum calcium (Kaplan et al, 1977). However, a report of a single case suggested that treatment with low-dose etidronate (400mg/day) may be more effective (Licata & O'Hanlon, 1983). This suggestion has been examined in the studies reported in Chapter 6. There have been two studies of the use of oral clodronate (Douglas et al, 1983; Shane et al, 1981). In both studies serum calcium fell significantly but mean values remained above the normal range.

The hypocalcaemic effects of oestrogens, norethisterone, and the diphosphonates in primary hyperparathyroidism each appear to mediated, at least principally, by their effects to inhibit bone resorption. From a consideration of the mechanisms of hypercalcaemia, outlined previously, it would seem likely that inhibition of bone resorption, alone, would only be partially effective in reducing plasma calcium as both the gut and the renal mechanisms of hypercalcaemia would be unchecked. The studies of the use of gonadal steroids and diphosphonates quoted above appear to conform to this expectation.

Perhaps more surprising than the lack of

complete response is the fact that hypocalcaemic responses can be so consistently observed following treatment with these agents. However, bone turnover is generally increased in primary hyperparathyroidism and part of the observed hypocalcaemic response may be a transient effect due to dissociation of the rates of bone formation and bone resorption. This phenomenon is well demonstrated in patients with Paget´s disease treated with clodronate (see Figure 1.6). Acute inhibition of bone resorption in this disorder is only later followed by a reduction in the rate of bone formation, as indicated by the delayed fall in serum alkaline phosphatase. During this period of dissociation, bone formation exceeds bone resorption and the net entry of calcium into bone results in hypocalcaemic and hypocalciuric effects. However, after several months the rates of bone resorption and formation again become matched and the hypocalcaemic and hypocalciuric effects gradually become attenuated. Such a sequence might be predicted in patients with primary hyperparathyroidism treated with inhibitors of bone resorption. Indeed, attenuation of the hypocalcaemic response has been observed in a single patient treated with clodronate for several months (Douglas et al, 1983) although resposiveness to oestrogens has been reported to be sustained for up to 2 years (Marcus et al, 1984). Thus, in order to examine the importance of the dissociation

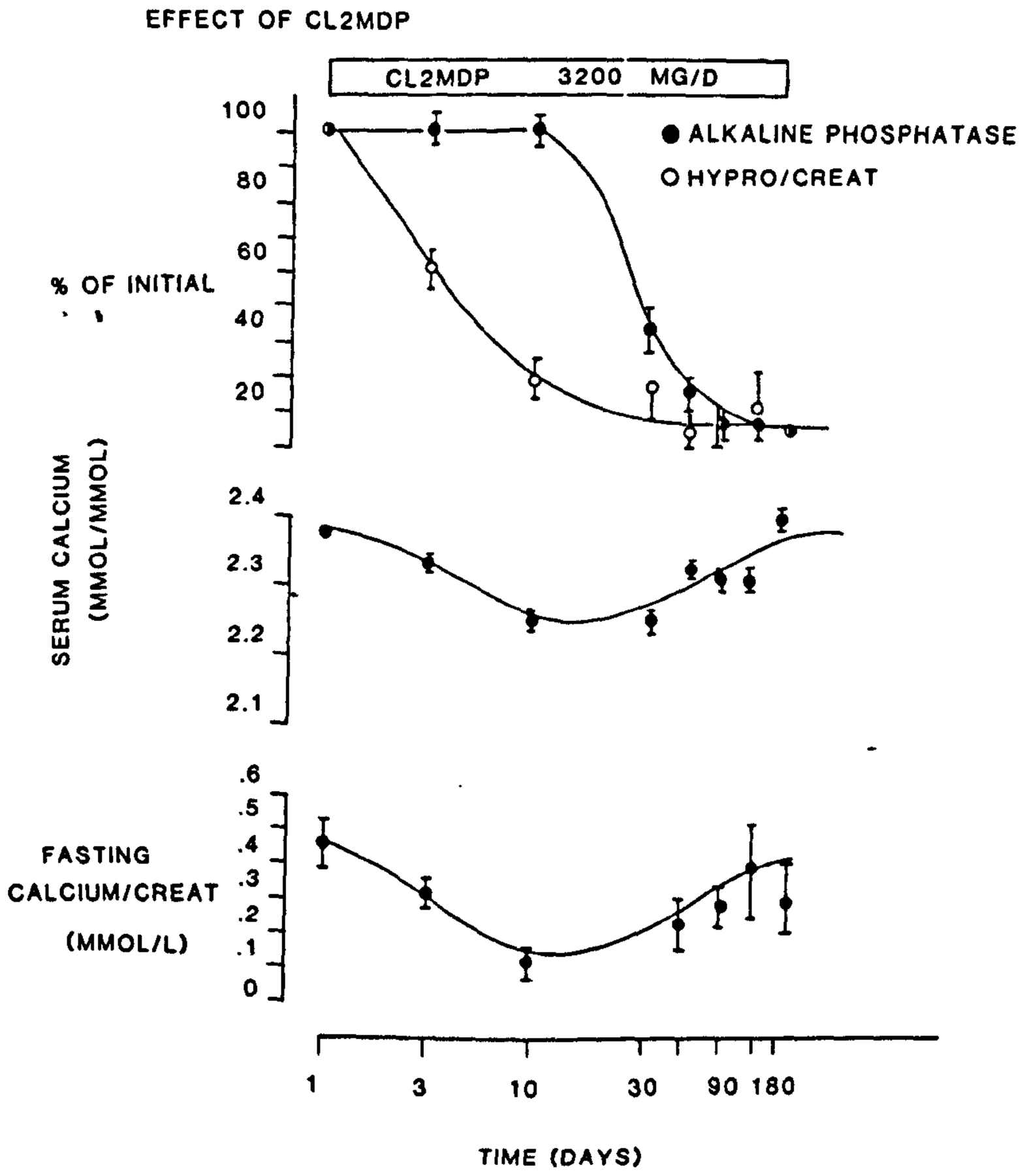


Figure 1.6 Effect of diphosphonate treatment on skeletal calcium balance. Rapid suppression of bone resorption is associated with a transient phase of net bone formation and calcium accretion. Due to the coupled remodelling process the rate of bone formation subsequently decreases to match the rate of resorption and skeletal balance is restored. The transient dissociation of resorption from formation causes significant hypocalcaemic and hypocalciuric effects. (Kanis et al, unpublished observations).

mechanism and the duration of hypocalcaemic responses I have studied the long-term effects of clodronate in patients with primary hyperparathyroidism (Chapter 6).

CHAPTER TWO

OBJECTIVES AND METHODS

OBJECTIVES

The clinical uses of diphosphonates have not yet been widely investigated. However, the published studies that are available clearly suggest that they may be of considerable benefit in the treatment of Paget's disease of bone and in the treatment of hypercalcaemia associated with malignancy. The role of diphosphonates in other disorders in which bone resorption is excessive, such as hyperparathyroidism, remains to be established.

Several questions regarding the use of diphosphonates remain. As discussed above, structural differences between the various diphosphonates result in marked differences in their effects both *in vitro* and *in vivo*. Even the mechanisms by which these drugs inhibit osteoclast function appear to differ depending on which analogue is used. In addition, at least some diphosphonates have actions at sites other than bone. These include effects on renal tubular reabsorption of inorganic phosphate and immunomodulatory effects. Such differences in action are likely to have important consequences for their use in disease and it is for this reason that direct comparisons of the clinical effects of different diphosphonates are required. Furthermore, the pharmacokinetics of diphosphonates have proved difficult to study. Because of these uncertainties and the limited number of clinical studies so far performed with these

agents, in no disorder has the optimum diphosphonate treatment regimen been established. Studies of alternative modes of administration might not only provide more effective ways in which to employ these agents but also shed further light on differences between the effects of different diphosphonates and their mechanisms of action.

Interest lies, not only in the use of diphosphonates in treatment, but also in their role as tools for investigating the physiological regulation of bone turnover and the mechanisms of bone disease. This thesis attempts to examine many of these questions in the light of original clinical studies of the use of diphosphonates in disorders of bone turnover. Thus, there were several objectives at the inception of these studies :-

1) To study the differences between the effects of different diphosphonates. The therapeutic effects of different diphosphonates were compared directly. In addition, other actions of the diphosphonates, in particular their effects on the mineralisation of newly formed bone and effects on calcium and phosphate metabolism were examined. The mineralisation effects of diphosphonates, if prolonged, might cause a significant reduction in skeletal integrity, and their effects on calcium homeostasis are relevant to their use as

hypocalcaemic agents.

2) To compare effects of diphosphonates in different disorders. Thus, although Paget's disease, hypercalcaemia of malignancy and primary hyperparathyroidism are each characterised by increased bone resorption there are important differences in terms of skeletal balance and effects on calcium metabolism. Potentially, a study of responses to diphosphoanates in one disorder, such as Paget's disease, might provide insights into probable responses in other disorders, such as hypercalcaemia of malignancy, which are inherently less susceptible to controlled study. The validity of the use of such disease models was assessed.

3) To investigate the use of novel treatment regimens. Previous diphosphonate regimens for Paget's disease have consisted of continuous oral treatment with these drugs for a duration of three months or longer. However, the bioavailability of orally administered diphosphonates is very low and variable. By using the intravenous route of administration systemic loading with diphosphonate similar to that achieved following several months of oral diphosphonate therapy might be achieved within days. Thus, one aim was to investigate the relative effects of acute pulses of high-dose diphosphonates compared with the much lower, but more sustained, systemic dosing

achieved with continuous oral diphosphonate treatment.

4) To study the assessment of biochemical response in Paget´s disease. Existing methods for assessment of response are relatively crude, either utilising percentage change in serum alkaline phosphatase with treatment or numbers of patients in whom values enter a "normal" range. Little attention has been paid to the potential importance of factors such as the the initial degree of pagetic activity or of previous treatment with diphosphonates which have been studied in this work.

5) Some of the variability in response to diphosphonates may be due to differences in bioavailability or drug compliance between patients. These differences would be eliminated by the use of intravenous administration leading to more accurate dosing. This might enable a clearer assessment of dose-response relationships which were studied in the case of treatment of Paget´s disease with intravenous clodronate.

6) To examine mechanisms of disease. The pathogeneses of hypercalcaemia, both in malignancy and in primary hyperparathyroidism, are complex and involve renal as well as skeletal mechanisms. Because of the selective effect of diphosphonates to inhibit bone resorption, responses to these drugs may be useful as a tool for

dissecting out the mechanisms of hypercalcaemia in individual patients.

7) To investigate new clinical uses for diphosphonates.
The use of the diphosphonates in the treatment of immobilisation hypercalcaemia had not previously been described and was studied in two patients reported in this thesis.

METHODS

Several different patient groups with disorders of bone or calcium metabolism were studied. Details of patients are provided either in the appropriate chapters or, in the case of patients with Paget's disease of bone, within the Appendix. The majority of patients were studied on the Metabolic Unit of the Royal Hallamshire Hospital, Sheffield either as inpatients or on a regular outpatient basis. However, a proportion of patients, particularly those with hypercalcaemia of malignancy, were studied in other wards either in the Royal Hallamshire Hospital or at Western Park Hospital by courtesy of the consultants in charge of their primary care. All patients gave informed consent and all studies had been approved by the local Ethics Committee.

Diagnostic criteria

Paget's disease was diagnosed on the basis of typical radiographic features. This was supported in all but a few cases, in whom the extent of Pagetic involvement was very limited, by increased serum alkaline phosphatase (>105U/l) in the absence of evidence of liver dysfunction and increased urinary hydroxyproline excretion (>30mmol/mol creatinine). Skeletal scintigraphy was performed in the majority of patients.

A typical distribution of areas of increased uptake of the ^{99}Tc-tagged diphosphonate provided further support for the diagnosis.

Hypercalcaemia of malignancy was characterised as hypercalcaemia (calcium >2.63mmol/l) in the presence of proven malignant disease (haematological or solid tumour). Concomitant primary hyperparathyroidism was excluded either on the basis of recent normocalcaemia with a return of serum calcium into the normal range following therapy or on the presence of low or undetectable values of iPTH during the period of hypercalcaemia.

Primary hyperparathyroidism was diagnosed when persistent elevation of serum calcium occurred in patients with high or inappropriately normal values of iPTH and no evidence of malignancy or other causes of hypercalcaemia.

Serum and urine sample collection

All samples were collected after an overnight fast, patients having had water only since the previous evening. Approximately 40ml of blood was obtained using a "Vacutainer" system (Becton Dickenson). 10ml was sent to the department of Clinical Chemistry for routine

biochemical measurements on serum including calcium, phosphate, albumin, creatinine, alkaline phosphatase and hepatic transaminases. The remaining blood was allowed to clot, centrifuged and the serum separated and stored at -20^{o}C for later analyses. Patients were asked to void urine at around 0700h. A fasting urine sample was then collected approximately 2 hours later and stored in a 200ml bottle containing 1ml of 0.1 normal hydrochloric acid to prevent precipitation of calcium phosphate crystals. Urine analyses, which were performed by the Department of Clinical Chemistry, were for calcium, phosphate, creatinine and hydroxyproline. During periods of inpatient admission to the Metabolic Ward urine collection continued for the remaining 22 hours of each day. 22 hour urine samples were analysed separately for calcium, phosphate and creatinine and the results were expressed as excretion per 24 hours (i.e. multiplied by 24/22).

Biochemical measurements

Serum Measurements

Serum calcium, inorganic phosphate, creatinine, albumin, alkaline phosphatase and liver transaminases were measured using a Technicon SMAC multichannel analyser by standard methods. Serum alkaline phosphatase

was used as an index of the rate of bone formation. The coefficient of variation for this measurement in patients with Paget's disease both in untreated patients and in the plateau phase following diphosphonate treatment was approximately 10%. As discussed in Chapter 3, alkaline phosphatase is a useful marker of bone formation, particularly when this is greatly increased. Nearer the normal range, however, the variable contribution of liver-derived alkaline phosphatase is proportionately greater and thus the usefulness of this enzyme as a marker of bone formation becomes more limited.

Immunoreactive parathyroid hormone (iPTH) in stored samples was measured using an antiserum cross-reacting predominantly with the mid-molecule region of human parathyroid hormone (amino acid numbers 44-68) using a kit supplied by Immuno Nuclear Corporation Ltd. The characteristics of this sensitive assay have been described by Mallette and colleagues (1982). In common with other immunoassays for PTH, values are increased in patients with recent renal impairment (3-4 times normal in patients with non-hyperparathyroid hypercalcaemia and serum creatinine above 440umol/l) due to altered peripheral metabolism of PTH. Chronic renal failure is associated with very high concentrations of iPTH (up to 100 fold higher than normal) due both to altered degradation and to true hyperparathyroidism (Mallette et

al, 1982)

1,25-dihydroxy-vitamin D_3 ($1,25(OH)_2D_3$) was measured by the method previously described by Clayton and colleagues (1982). Using this measurement, together with measurements of other metabolites of vitamin D, we have previously been able to describe disturbances of vitamin D metabolism in a variety of disorders including myeloma and Paget's disease (Lawson-Matthew et al, 1985; Yates et al, 1985) as well as to investigate seasonal trends in normal individuals (Clayton et al, 1982). Thus, in hypercalcaemic myeloma patients serum concentrations of $1,25(OH)_2D_3$ were low whereas normocalcaemic myeloma patients had values which were in the lower end of the normal range (Lawson-Matthew et al, 1985; Yates et al, 1985).

Urine measurements

Urinary calcium, creatinine and phosphate were measured using a Technicon II AutoAnalyser.

Total urinary hydroxyproline was measured by the method of Stegeman and Stalder (1967). The principle of this method depends on the neutralisation of the hydrolysate, prepared by hydrolysing urine samples at

100˚C with 50% hydrochloric acid, prior to oxidation of hydroxyproline to pyrrole and pyrrole-2-carboxylic acid by chloramine T. Both products form red complexes on addition of P-amino-dimethylbenzaldehyde (Erlich's reagent). The dialysable fraction of hydroxyproline (about 90% of total) is related to bone resorption whereas the non-dialysable component is related to bone formation (Krane, 1980). However, total rather than dialsable hydroxyproline was measured as the former measurement is more convenient and provides an adequate index of bone resorption (Russell et al,1981).

Derived measurements

Total Serum calcium concentrations were adjusted for changes in serum albumin with the addition of 0.02mmol/l for each 1g/l of albumin below 42g/l and a similar subtraction for albumin values above 42g/l (Payne, 1973). Unless otherwise stated the values referred to in this thesis are of adjusted rather than total calcium.

Fasting urinary excretion of calcium was expressed as a ratio to urinary creatinine (Ca/Cr). Under steady-state conditions the net amount of calcium entering the ECF pool from bone and gut is equal to the amount leaving this pool by urinary excretion. After an

overnight fast the net contribution of intestinal absorption of calcium is negligible and thus at least the greater part of a net calcium flux into the ECF is likely to be bone derived. Thus fasting Ca/Cr provides an index of net calcium loss from bone and hence, where skeletal mineralisation is unimpaired, an index of net bone resorption. The Ca/Cr has been previously used as an index of bone resorption in several disorders including primary hyperparathyroidism and osteoporosis (Gallagher & Wilkinson 1973; Selby & Peacock, 1986).

In order to study renal tubular handling of calcium, excretion was also expressed per unit of glomerular filtrate (CaE of Peacock & Nordin, 1968). This may be calculated simply by multiplying the Ca/Cr by the serum creatinine concentration. Thus:-

Ca/Cr	x	serum creatinine	=	calcium excretion (CaE)
(mmol/mmol)		(mmol/l)		(mmol/l GF)

<u>Urinary hydroxyproline</u> excretion in fasting urine was expressed as a ratio to urinary creatinine (OHP/Cr). Previous studies have found very close correlations between OHP/Cr from fasting urine samples and OHP/Cr from 24 hour collections as shown in figure 1.4 (Russell et al, 1981). Inhibition of bone resorption with diphosphonates results in a rapid suppression of OHP/Cr which becomes significant as early as 1 day from

the start of treatment (Chapter 3). Bone formation does not become suppressed within the first week or so from the start of treatment and thus the fall in OHP/Cr is due to changes in bone resorption rather than bone formation. As discussed in Chapter 1, close correlations between this measurement and the level of bone formation, as judged by serum alkaline phosphatase, in patients with Paget's disease suggest that it is a reliable marker of bone turnover.

Renal tubular reabsorption of phosphate was expressed as the maximal tubular reabsorption of phosphate per litre of glomerular filtrate (TmP/GFR) and was derived from the equation of Bijvoet (1977) for measurement of TmP/GFR without phosphate infusion. This measurement was used in preference to the fractional tubular phosphate reabsorption (TRP) as the latter index is highly dependent on the filtered load of phosphate per nephron and thus on the plasma phosphate concentration.

Creatinine clearance, expressed as ml/min, was calculated using the equation:-

$$Cl_{Cr} = U_{Cr} \times V/S_{Cr}$$

where U_{Cr} is the urinary concentration of creatinine V is the urinary volume (average per minute from a 22 hour

sample) and S_{Cr} is the serum creatinine concentration.

Histomorphometric techniques

Bone biopsies were taken from patients with Paget's disease treated with intravenous diphosphonates. These patients had each previously received 3 courses of tetracycline for labelling bone surfaces undergoing mineralisation. To aid distinction of the labels ledermycin 600mg/day was for given for 4 days for the first label and oxytetracycline 1g/day for 2 days was used for the two subsequent labels. In all but 3 patients successive labels were each separated by a 10 day interval and intravenous diphosphonate infusions started on the first day following completion of the second tetracycline label. In 2 clodronate treated patients, however, the final label was given during the last 2 days of the treatment period and in one patient the final label was given 30 days from the start of etidronate treatment (Chapter 4). At between 2 and 5 days after completion of all labels patients underwent a bicortical trans-iliac biopsy using an 8mm Meunier trephine. All patients gave informed consent for the procedure which was carried out after valium premedication and local infiltration with lignocaine 2% of the standardised biopsy site 2-3cm below the iliac crest. Samples were fixed in 80% ethanol before being

embedded undecalcified in methyl methacrylate. Bone histomorphometry was performed on 7u sections. Measurements were made with polarised light microscopy using a Kettle MOP AMO 3 digitising table with a Leitz orthoplan microscope at a final magnification of x 300. Trabecular bone was examined utilising at least 2 sections and 5 contiguous fields per section. The mineral apposition rate (MiAR) was derived from the distance between midpoints of two tetracycline labels, corrected for obliquity, divided by the time interval between the labels (Merz & Schenk, 1970). The administration of three tetracycline labels permitted two separate assessments of MiAR for each biopsy sample. Thus the distance between the first label (identified by the colour difference between labels as well as the relationship to the trabecular surface) and the second label was used to measure the MiAR before diphosphonate treatment whereas the distance between second and third labels was used to measure the MiAR following the start of treatment. Using this triple label technique acute changes in the rate of mineralisation could be determined from a single biopsy.

Other techniques

Bone scintigraphy and skeletal radiography were used extensively for the detection of skeletal metastases

in patients with hypercalcaemia of malignancy. Other hospital investigative facilities were used where appropriate.

Statistical methods

Some of the variables studied, in particular serum alkaline phosphatase and urinary OHP/Cr, conformed to a log-normal rather than a normal distribution as determined by probit analysis. Thus for the purpose of statistical analysis of change in these measurements the data were log-transformed. Other measurements, such as serum calcium and serum phosphate, were distributed in a Gaussian manner and therefore the significance of changes were analysed directly (Armitage, 1971).

Where an approximately Gaussian distribution could be achieved comparisons were made using a 2-tailed Student's t-test for either paired or non-paired data as appropriate. Data which did not conform to a Gaussian distribution was analysed using nonparametric tests including Wilcoxon and Fisher's exact tests. Correlation coefficients were determined by the method of least squares. Other statistical methods are detailed where used.

Reference ranges	Range
Serum measurements	
Calcium	2.12-2.63 mmol/l
Phosphate	0.6-1.5mmol/l
Creatinine	60-120umol/l
Albumin	35-53g/l
Alkaline phosphatase	35-105U/l
iPTH (midmolecule assay)	<130pg/ml
$1,25(OH)_2D_3$	20-60pg/ml
Derived measurements	
Urinary Ca/Cr	0.1-0.4mol/mol
Urinary OHP/Cr	9-30mmol/mol
TmP/GFR	0.80-1.35mmol/lGF
Mineral apposition rate (MiAR)	0.5-0.8um/day

Normal ranges (+/- 2SD) for iPTH, $1,25(OH)_2D_3$, urinary Ca/Cr and OHP/Cr are derived from a combined group of 30 healthy hospital staff member and 32 elderly healthy individuals (mostly spouses accompanying clinic patients). The normal range given for MiAR is derived from published data for normal subjects (Merz & Schenk, 1970). Other ranges are those quoted by the routine hospital laboratory.

CHAPTER THREE

THE USE OF NOVEL DIPHOSPHONATE REGIMENS IN THE TREATMENT OF PAGET'S DISEASE OF BONE

SECTION TWO

EFFECTS OF DIPHOSPHONATES IN PAGET'S DISEASE OF BONE

THE USE OF NOVEL DIPHOSPHONATE REGIMENS IN THE TREATMENT OF PAGET'S DISEASE OF BONE

Introduction

Etidronate, in tablet form, is the only commercially available diphosphonate, but when given in maximally effective doses this drug is associated with impaired mineralisation of bone (Meunier et al, 1975). In the recommended regimens treatment is continued for 3 to 6 months. High-dose treatment (20mg/kg/day) for this length of time produces reliable suppression in pagetic activity, but at a cost of clinically significant osteomalacia in a proportion of patients (Krane, 1982), whereas low doses are ineffective in some and may produce osteomalacia in others due to variable bioavailability (Boyce et al, 1984; Fogelman et al, 1984). However, as the mineralisation defect is reversible on stopping etidronate treatment, shorter courses, even of very high doses, of etidronate might not be expected to induce clinically significant osteomalacia as the amount of unmineralised osteoid is proportional to the duration of treatment. In addition, by far the greatest fall in bone resorption, as judged by urinary OHP/Cr, occurs within the first month of etidronate treatment and the later fall in alkaline phosphatase may be a consequence of the coupling process rather than of continued treatment.

These considerations suggested that short courses of high-dose treatment with etidronate might prove both effective and relatively safe. In a previous study we have shown that, over a period of 6 months, 1 month of high-dose oral etidronate (20mg/kg/day) produces a degree of biochemical suppression comparable to that seen with the same dose for 6 months (Preston et al, 1986). Moreover, the response to the 1 month regimen was more marked and more consistent than that achieved with low-dose treatment (5mg/kg/day) for 6 months despite a higher total dose in the latter regimen (see Figure 3.1).

Similarly, Delmas and colleagues (1982) found that a 1 month course of high-dose oral clodronate treatment produced suppression of bone resorption comparable to that seen with 6 months treatment at the same dose (1.6g/day). Thus continued biochemical suppression was not dependent on patients receiving long-term treatment and might possibly be related to early treatment effects on the remodelling process. The question thus arose as to whether even shorter courses of treatment with higher doses may prove effective in the treatment of Paget´s disease.

Major problems with the oral route are the inconvenience of having to take tablets away from food and poor and variable intestinal absorption of diphosphonates. Bioavailability has been estimated at 1-10 per cent for etidronate and 1-2 per cent for

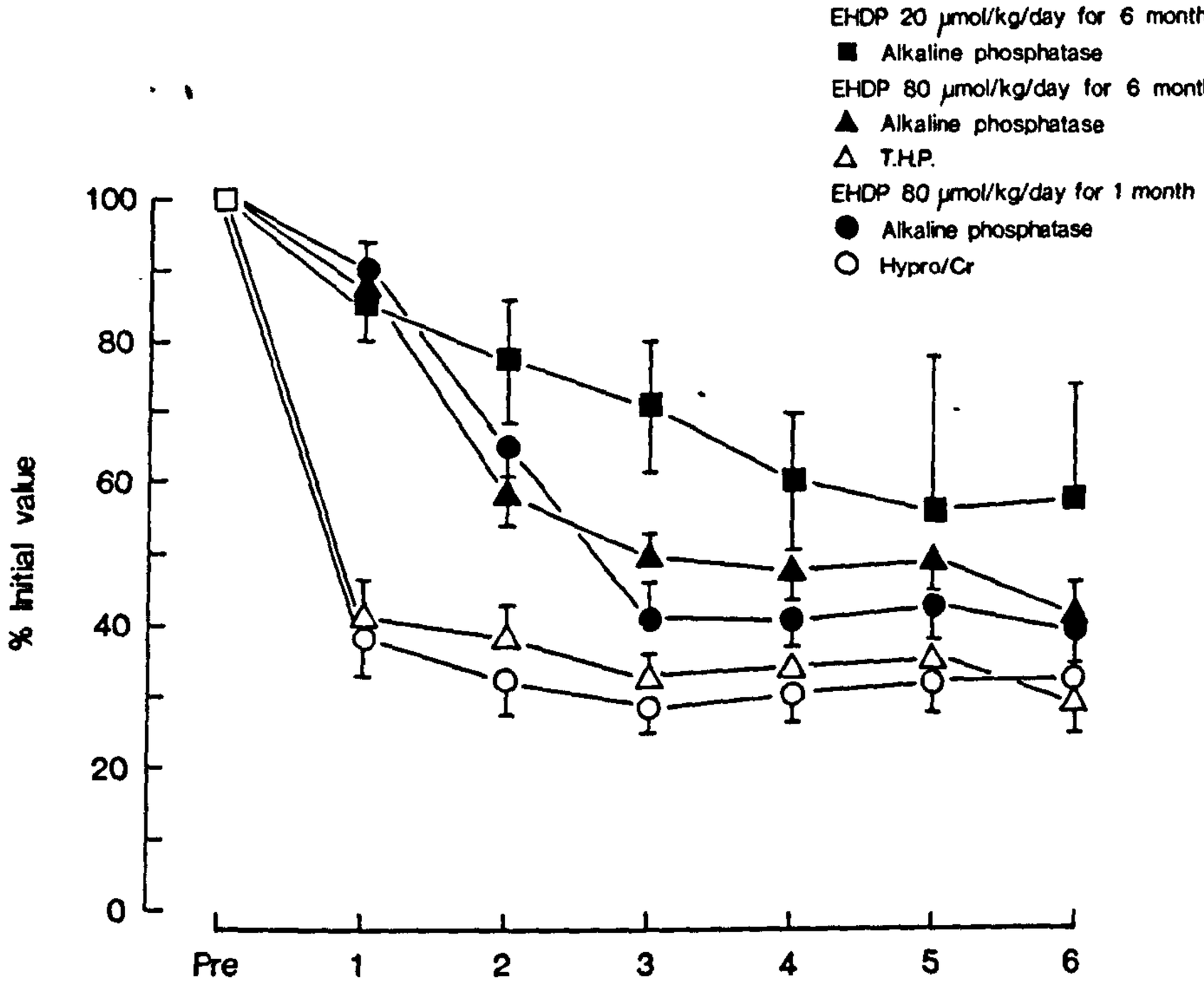


Figure 3.1 Comparison of the effects of 3 different etidronate treatment regimens in patients with Paget's disease of bone. Mean suppression of serum alkaline phosphatase was least marked in the low-dose group in which responses were very variable. Responses to 1 month and those to 6 months of high-dose etidronate were similar. THP = total 24h hydroxyproline excretion. 80umol/kg/day is equivalent to 20mg/kg/day. (Data from Preston et al, 1986).

clodronate (Fogelman et al, 1984; Yakatan et al, 1982). In addition gastrointestinal side effects are frequently encountered with high oral doses. Much higher and more consistent systemic loading can be achieved with parenteral administration of diphosphonates. Thus, assuming 2 per cent bioavailability, a 1 month course of oral clodronate 1.6g/day would result in a total systemic delivery of only around 1g of the drug. Using the intravenous route the same systemic loading may be achieved within a few days. However, with reduced duration of treatment uptake of the diphosphonate might be expected to be concentrated at more focal sites within the skeleton which may potentially affect its skeletal actions.

Thus, in the present study the use of short intravenous treatments with diphosphonates (clodronate and etidronate) was investigated. The results were compared with those obtained using high-dose long-term oral clodronate treatment. The aims of the study were not only to examine the effects of treatment on skeletal turnover in Paget's disease (this chapter) but also to investigate the effects of these agents on mineral metabolism (Chapter 4).

Aminohexane diphosphonate (AHDP) is a new diphosphonate which in animal studies has been shown to be a more potent inhibitor of bone resorption than either clodronate or etidronate and, like clodronate and APD,

appears to have little effect to impair mineralisation of osteoid (Felix et al, 1982b). This agent therefore shows promise as a treatment for Paget's disease and thus the effects of AHDP in this disorder were examined in the present study.

Patients and methods

45 patients were treated with clodronate 1.6g daily by mouth for 6 months. None of this group had received previous treatment with diphosphonates. Data from some of these patients have been previously reported (Douglas et al, 1980).

31 patients received clodronate 300mg/day by intravenous infusion. 15 of these patients had been previously treated with diphosphonates.

7 patients (5 previously treated) were given clodronate 100mg/day i.v.

16 patients (13 previously treated) received clodronate 600mg/day i.v.

21 patients received intravenous etidronate either at a dose of 300mg/day (n=7) or 7.5mg/kg/day (n=14; dose range 410 to 700mg/day).

17 patients (8 previously treated with diphosphonates) were treated with intravenous AHDP at a dose of 25mg/day. A further 16 patients received AHDP 400mg daily by mouth for 1 month.

Details of all patients who received intravenous diphosphonates are given in the appendix. All intravenous treatments were given as 3 hour infusions of the drug in 500ml of 0.9% saline repeated daily for 5 days. All patients gave informed consent for study which was approved by the local Ethics Committee.

Methods for study were as outlined in Chapter 2. Patients given oral clodronate were reviewed monthly as outpatients. Intravenously treated patients were admitted 3 days before the start of treatment for collection of baseline samples of blood and urine. These were continued daily during the 5 day infusion period and patients were seen regularly as outpatients thereafter.

Results

Comparison of oral clodronate and clodronate 300mg i.v.

The effects of these two treatments on biochemical markers of Pagetic activity are shown in Figure 3.2. There was a marked progressive fall in urinary OHP/Cr during the 5 days of intravenous treatment but this effect rapidly became attenuated on stopping treatment. Although at 1 month the degree of biochemical suppression induced by the two treatments was broadly comparable, by 6 months the response in the oral treatment group was clearly the more marked.

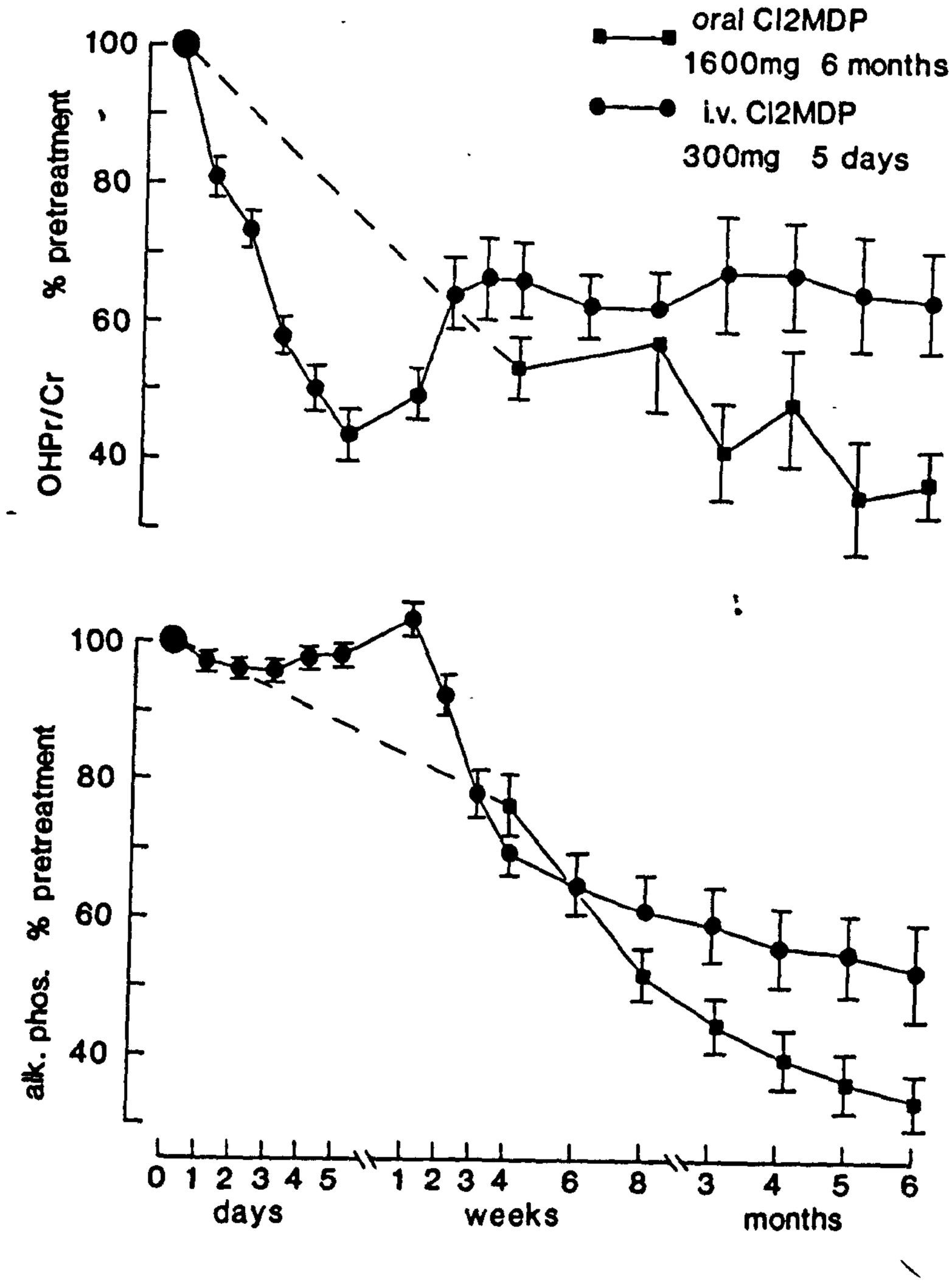


Figure 3.2 Comparison of changes in indirect markers of bone turnover (as a percentage of immediate pretreatment values for individuals) in pagetic patients treated with clodronate either by mouth 1.6g/day for 6 months or intravenously 300mg/day for 5 days.

However, there was a striking difference in the degree of biochemical suppression between the 16 previously untreated patients and the 15 patients who had already received at least 1 course of diphosphonate treatment. Thus there was no significant difference in terms of suppression of alkaline phosphatase between previously untreated patients and those given oral clodronate (also previously untreated), although the fall in urinary OHP/Cr was not as complete in the intravenously treated group (Figure 3.3). In contrast, patients who had previously received diphosphonates had a much attenuated biochemical response after intravenous treatment (Figure 3.3). This suggested that previous treatment with diphosphonates might render patients relatively resistant to subsequent treatments. However, although these patients had relapsed as judged by biochemical criteria, the majority had not attained the degree of Pagetic activity observed before their first course of treatment (mean 50% and 61% of initial alkaline phosphatase and OHP/Cr respectively). It should be noted, however, that the mean original pretreatment alkaline phosphatase was higher in the retreatment group than in either the previously untreated intravenous group or the oral treatment group (Table 3.1).

The effect of incomplete relapse on response to further treatment is illustrated by one patient who received oral clodronate for 6 months and, following

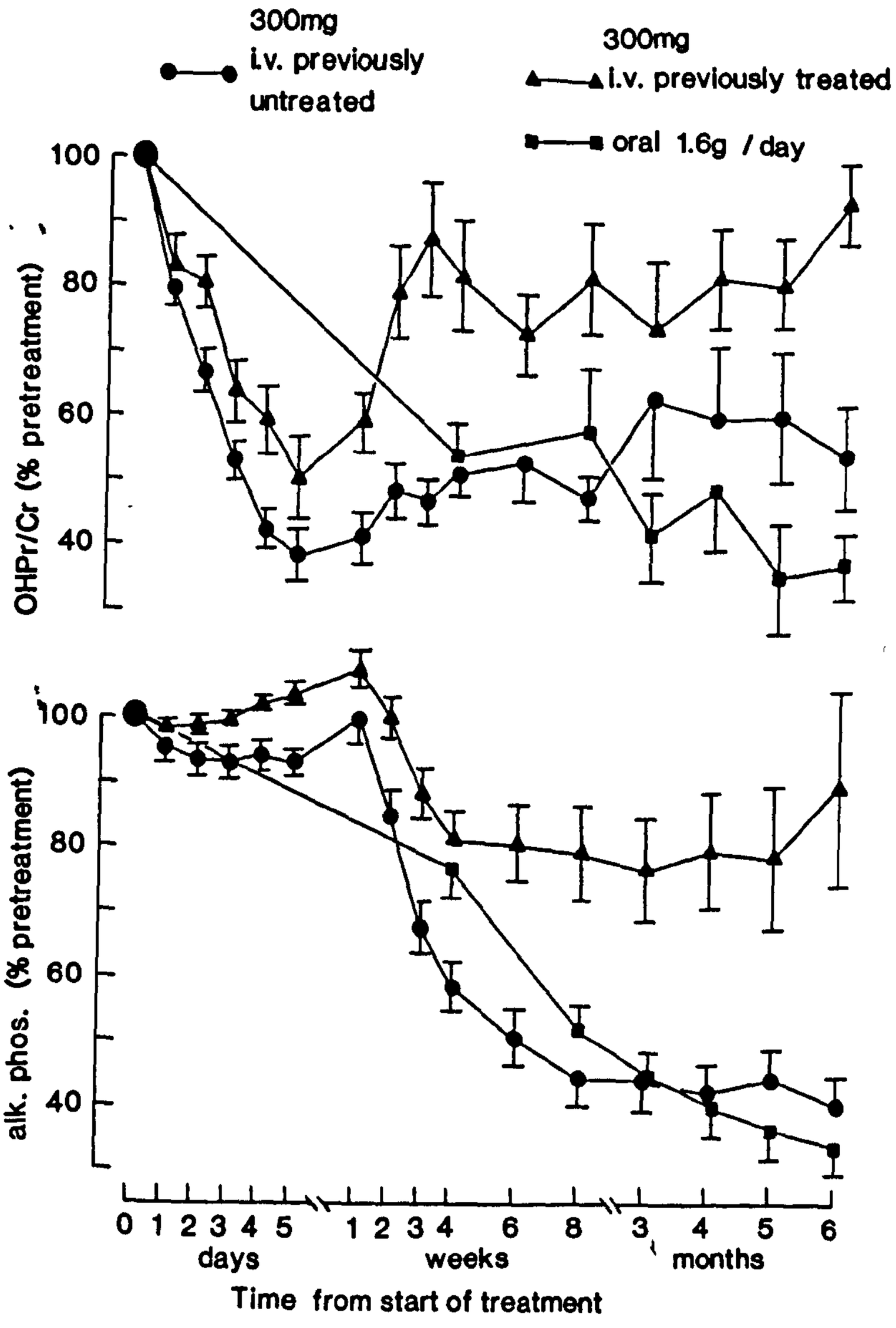


Figure 3.3 Effects of clodronate on biochemical markers of bone turnover. Patients who received intravenous clodronate 300mg/day were separated into previously treated (n=15) and previously untreated (n=16) groups. Compare with figure 4.2.

Table 3.1

Comparison of biochemical values before and 3 months after the start of oral or intravenous clodronate treatment

Treatment	No. of patients	Serum alkaline phosphatase (U/l) Before treatment	Serum alkaline phosphatase (U/l) After 3 months	Urinary OHP/Cr (mmol/mol) Before treatment	Urinary OHP/Cr (mmol/mol) After 3 months
clodronate 1.6g oral 6m	45	420 (313-563)	170 (124-233)	100 (74-135)	32 (25-41)
clodronate 300mg i.v. 5d					
Previously untreated	16	341 (220-459)	144 (105-197)	71 (55-93)	38 (30-49)
Previously treated	15	304 (201-459)	216 (138-338)	68 (42-110)	49 (30-79)
Original values		705 (408-1219)		124 (73-210)	

Mean biochemical values with 95% confidence estimates (in parentheses) derived from log-transformed values.

partial relapse, was retreated with intravenous clodronate (Figure 3.4). The percentage fall in biochemical markers upon retreatment appeared to depend to a great extent on the degree of biochemical relapse. This source of artefact was circumvented by replotting values as a proportion of biochemical values observed before the original diphosphonate treatment for each patient. The differences in final degree of biochemical suppression between previously untreated and previously treated patients disappeared (Figure 3.5).

Clinical observations

Bone pain improved in 20 of the 31 patients given intravenous clodronate (77% of those with bone pain) and improvement was substantial in 17. Patients reported no side effects related to intravenous treatment although transient proteinuria (<24 h) was observed in 4 patients following each infusion. There were no significant changes in serum creatinine with either regimen.

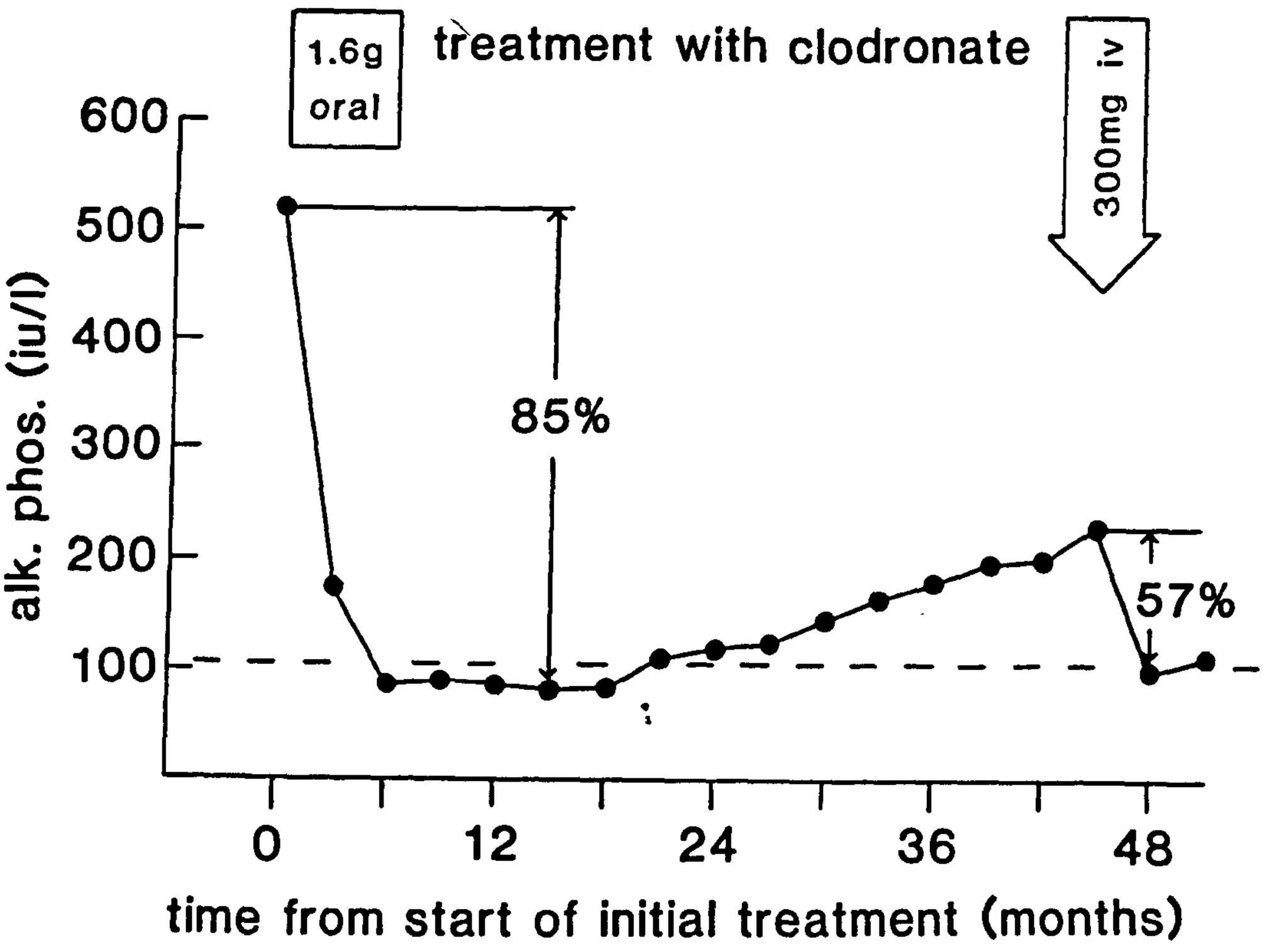


Figure 3.4 Responses to 2 successive diphosphonate treatments in one patient (JC). A reduced percentage fall on retreatment is largely due to incomplete biochemical relapse.

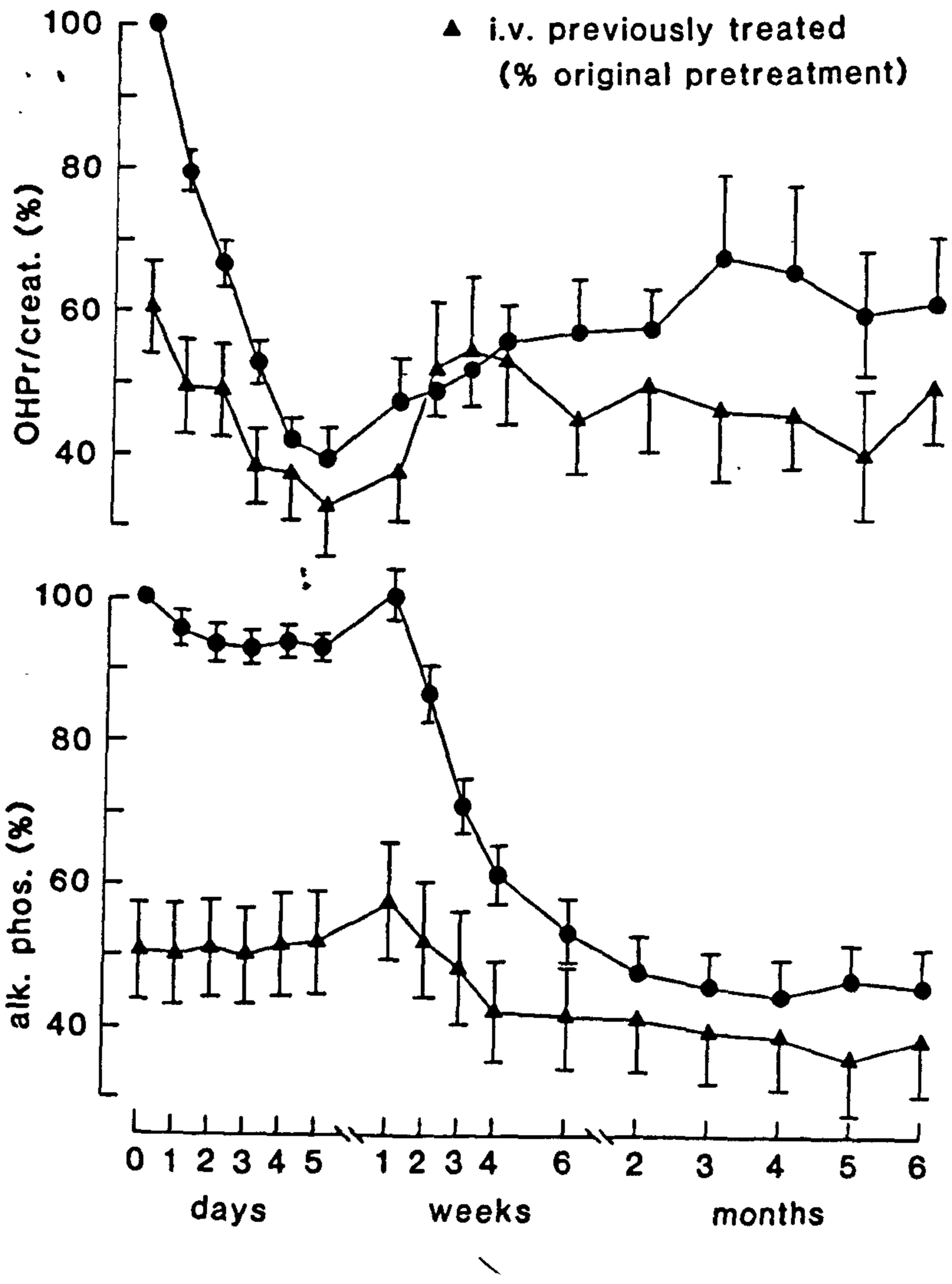


Figure 3.5 Biochemical markers of bone turnover expressed as a percentage of original pretreatment values in previously treated and previously untreated patients given clodronate 300mg/day for 5 days.

Duration of biochemical remission

Actuarial survival analysis (Kaplan & Meier, 1958) was used to examine the duration of biochemical remission following treatment with either oral or intravenous clodronate. The coefficient of variation for serial measurements of alkaline phosphatase taken either from the plateau response phase or before treatment was found to be approximately 10 per cent. On this basis response and relapse were defined as a fall or an increase respectively of at least 25% (2.5 x coefficient of variation) from previous mean values of alkaline phosphatase. The results (Figure 3.6) show that the duration of remission is significantly shorter following intravenous treatment than following 6 months of oral clodronate ($p<0.0001$ using Gehan's test; Gehan, 1965). The difference remained highly significant even when duration of remission is taken from the end rather than the beginning of treatment ($p<0.001$).

Dose response relationship with i.v. clodronate

With each of the three doses studied (100mg, 300mg and 600mg) the treatment groups contained both previously treated and previously untreated patients. Thus, in order to facilitate comparisons values were

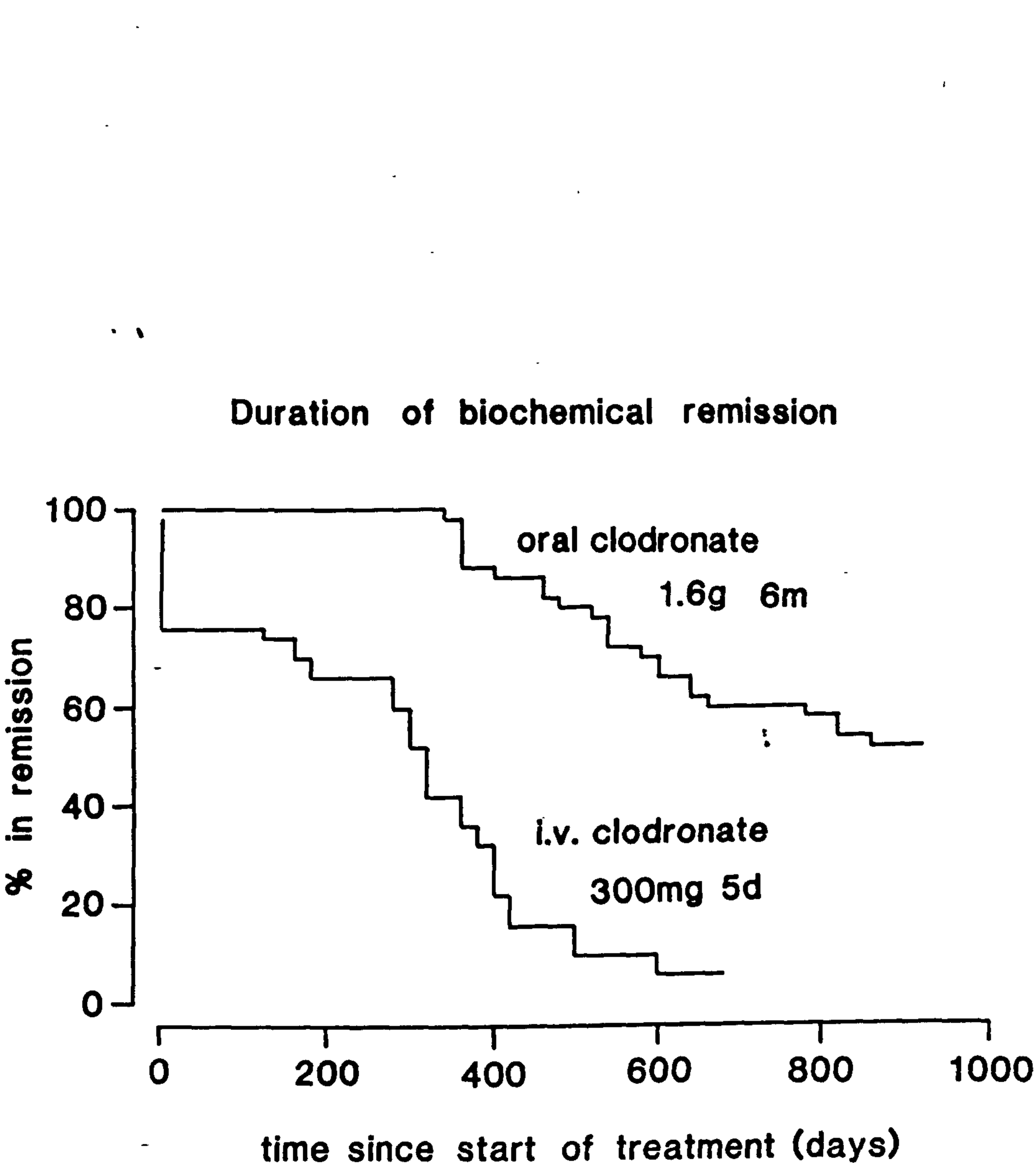


Figure 3.6 Duration of biochemical remission following treatment with clodronate either as 1.6g/day by mouth for 6 months or as 300mg/day intravenously for 5 days. Biochemical relapse (25% increase from minimum values) occurred in the majority of intravenously treated patients at around 1 year.

expressed as percentage of values seen before the initial course of diphosphonate treatment. The results of this comparison are shown in Figure 3.7. This appears to confirm a dose/response relationship with 100mg having no significant effect whereas 600mg induced a significantly more marked suppression in alkaline phosphatase at 3 months than did the 300mg dose ($p<0.01$ on non-paired t-testing of percentage values).

However, general experience with treatment of Paget´s disease has indicated that in patients with only minimally increased biochemical markers of Pagetic activity only a small percentage fall can be expected even after maximally effective treatment. Conversely, in some patients with very active Paget´s disease effective treatment can result in suppression of alkaline phosphatase to a very small percentage of the pretreatment values. This relationship is well demonstrated in the 6 month oral clodronate group by Figure 3.8. Thus, in order for a direct comparison between percentage falls in different groups to be valid it is important to match patients for the degree of Pagetic activity.

In fact, the three dose/response groups were poorly matched. Mean original pretreatment values for alkaline phosphatase (derived from log-transformed data) were 351U/l (266-704), 485U/l (356-699) and 760 (494-1170) U/l respectively for the 100mg, 300mg and

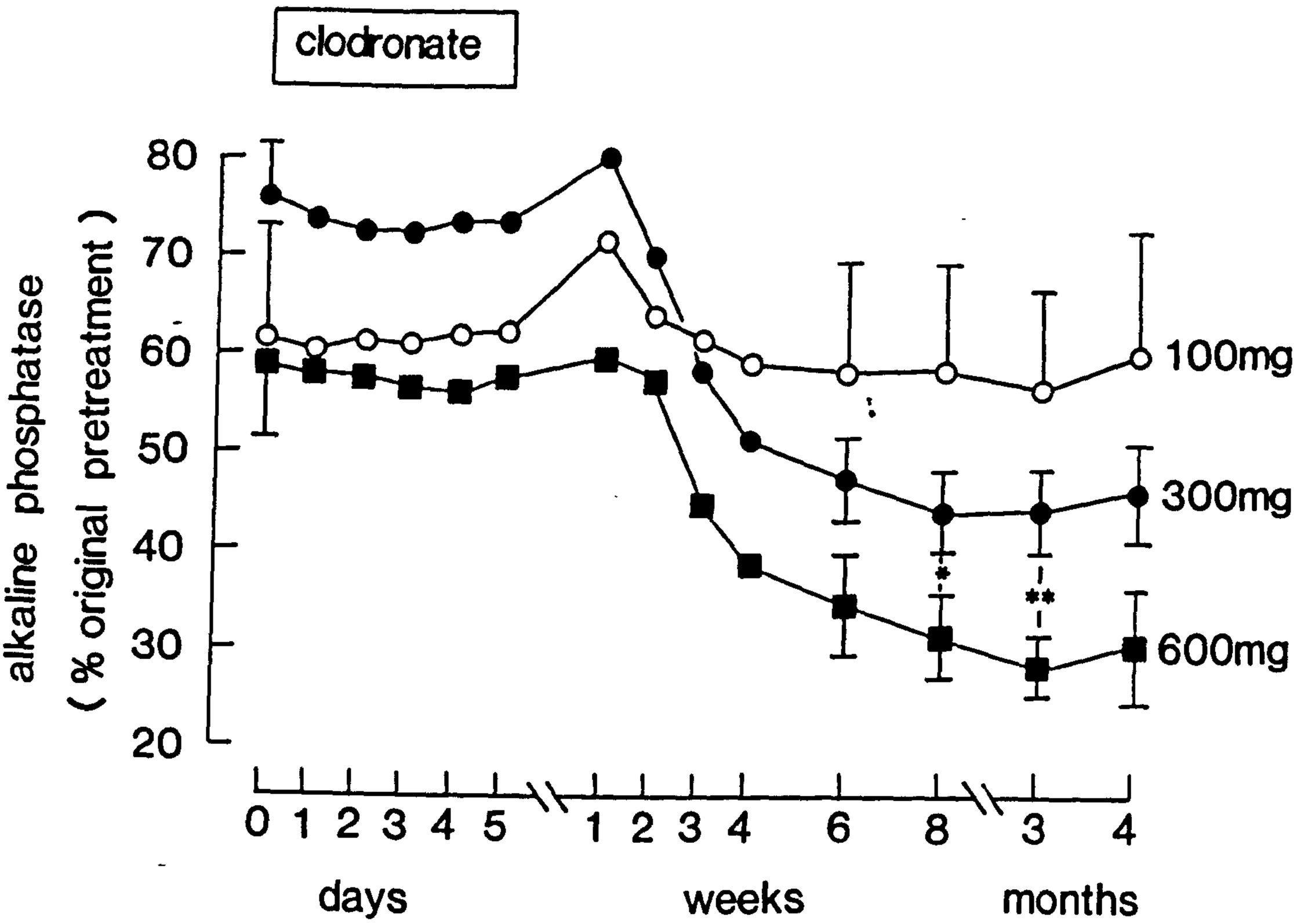


Figure 3.7 Dose/response relationship in pagetic patients treated with intravenous clodronate. Values for alkaline phosphatase are expressed as a percentage of values observed before the original course of diphosphonate treatment for each patient. * $p<0.05$ ** $p<0.01$ on non-paired t-testing.

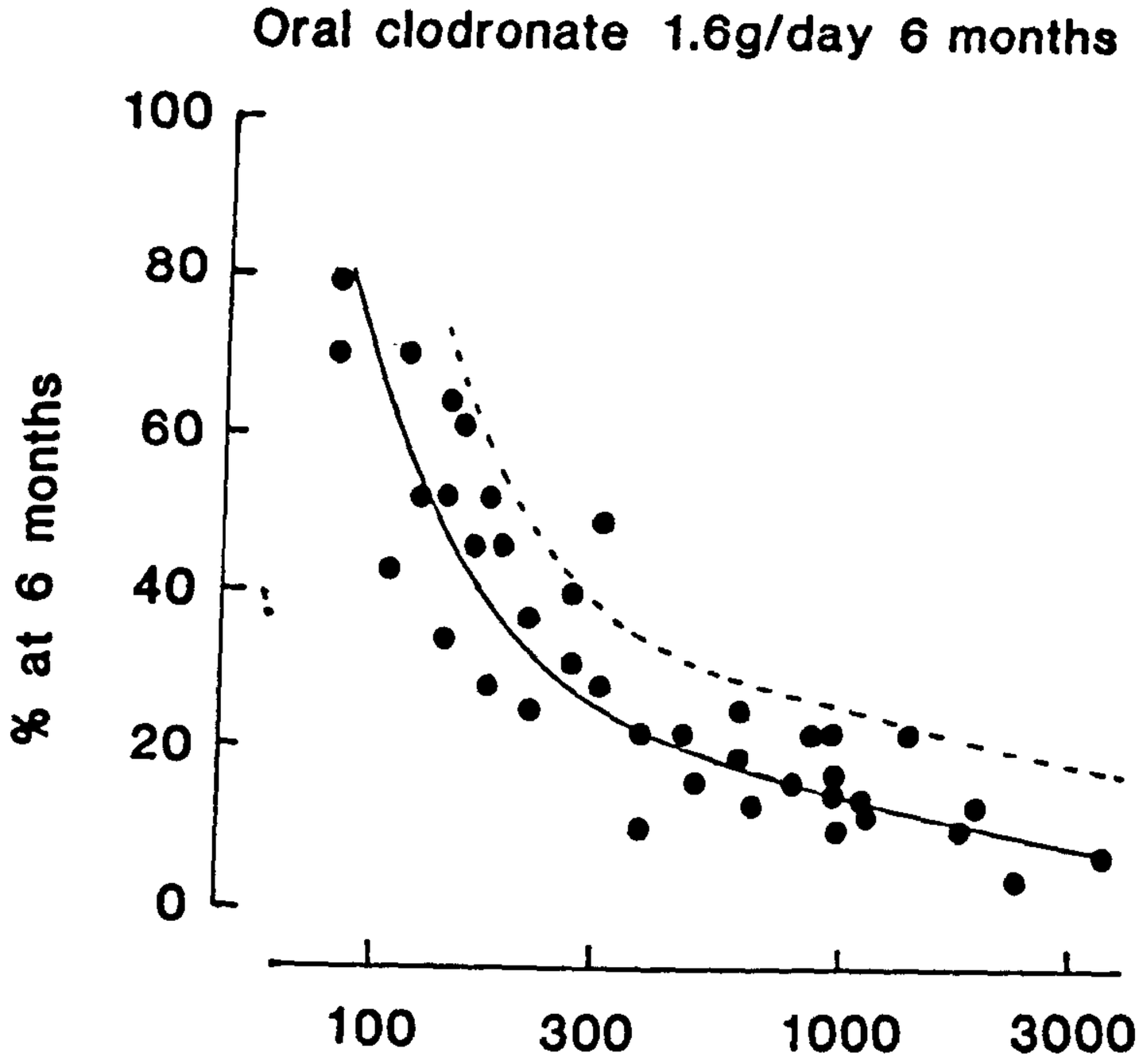


Figure 3.8 Relationship between original level of pagetic activity (pretreatment alkaline phosphatase) and the percentage of this value reached after 6 months of treatment with clodronate 1.6g/day by mouth. The lines, which were drawn by eye, approximate to mean values and the upper confidence estimate and are used for comparison of responses in other treatment groups (figure 3.9 and 3.13).

600mg groups (95% confidence estimates for the mean in parentheses). Thus much of the apparent dose/response relationship might be due to an artefact caused by differences in Pagetic activity between the groups. An alternative method is to compare response (in terms of percentage of original pretreatment values) against the curvilinear relationship seen in the oral treatment group (Figure 3.9). Values at 3 months for each group were used in this comparison as suppression was near maximal for all three dose groups at this time point and some patients relapsed rapidly thereafter. Exhibited in this way, all but 2 of the 600mg treated patients suppressed to values comparable to the oral treatment group, whereas half of the 300mg group and all of the 100mg group lie outside this range. This appears to confirm the existence of a dose/response relationship although other factors, in particular whether patients had been previously treated, may still be influential.

A further attempt to demonstrate a dose/response relationship is shown in Figure 3.10. In retreated patients, both immediate pretreatment and original pretreatment values of alkaline phosphatase are shown. From this analysis it can be seen that the 100mg dose did not cause significant further suppression in serum alkaline phosphatase in either previously treated or the 2 previously untreated patients. Treatment with 300mg induced a marked suppression in the majority of

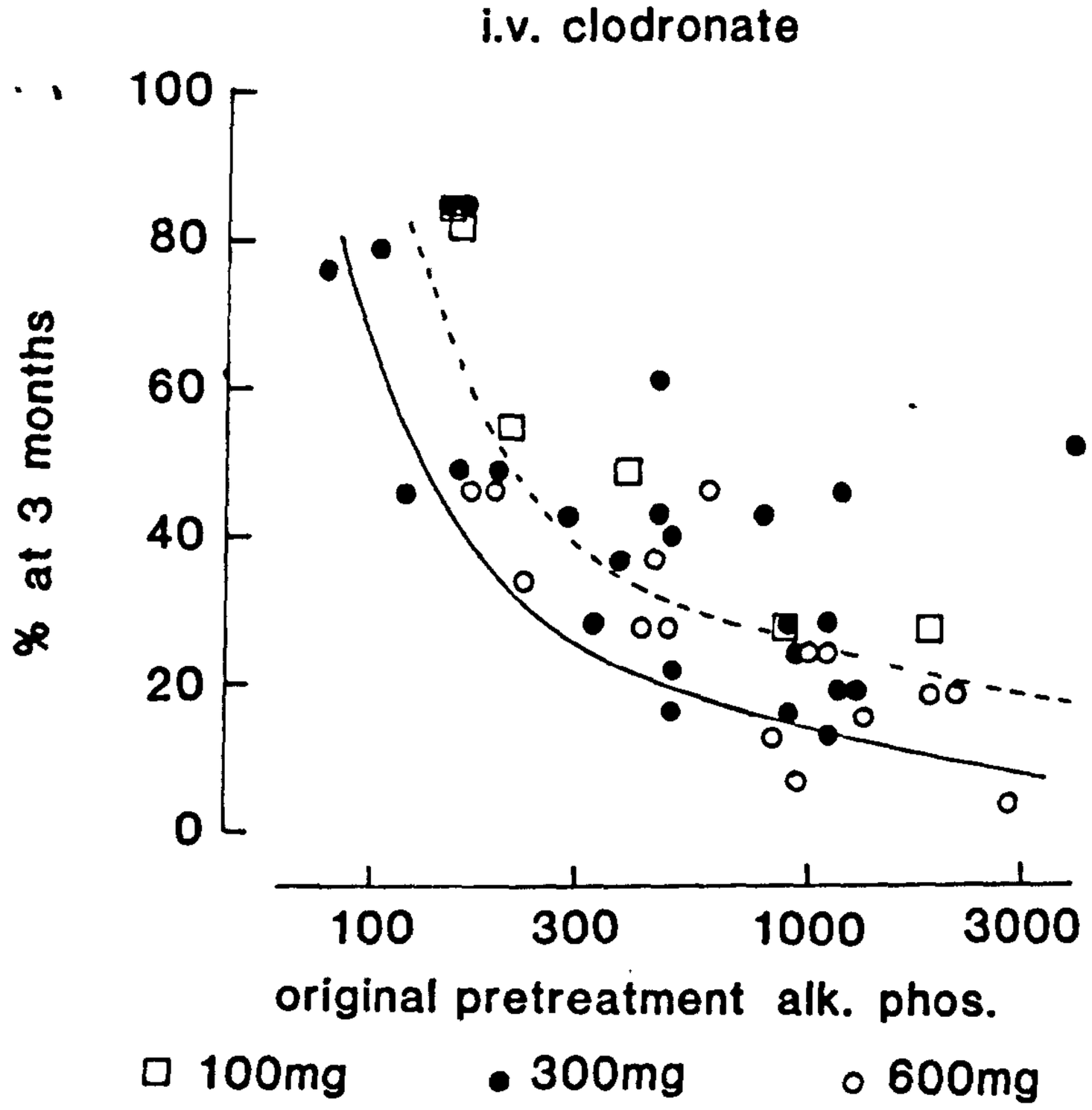


Figure 3.9 Serum alkaline phosphatase values at 3 months as a percentage of original pretreatment values in patients treated with intravenous clodronate at doses of 100mg, 300mg or 600mg/day. The continuous and dashed lines are derived from the relationship for the oral clodronate group shown in figure 3.8.

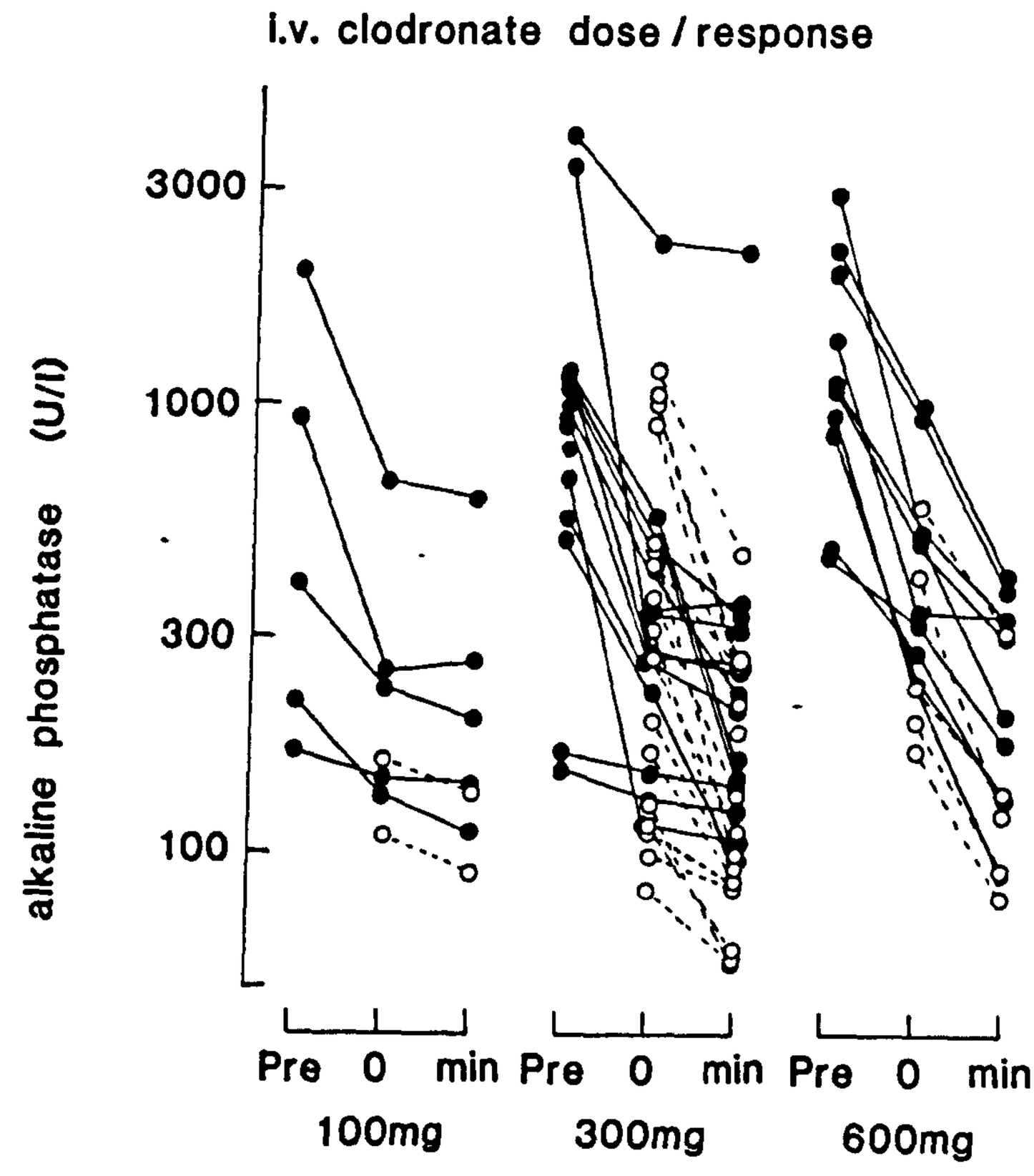


Figure 3.10 Changes in serum alkaline phosphatase (on a log scale) in individual patients treated with intravenous clodronate. Original pretreatment values are shown for retreated patients (closed circles). The final value shown for each patient is the minimum value of alkaline phosphatase observed between 3 and 6 months from the start of intravenous treatment.

previously untreated patients but this occurred in less than half of the retreated patients. The 600mg dose induced marked suppression in alkaline phosphatase in all but one (previously treated) patient. Thus, although the methods of analysis are, of necessity, somewhat arbitrary, there does appear to be a genuine dose/response relationship for intravenous clodronate at doses of between 100 and 600mg per day.

Effects of treatment with intravenous etidronate

The effects of intravenous etidronate on alkaline phosphatase and urinary OHP/Cr are shown in Figure 3.11. Only 7 of the 21 patients had been previously treated with diphosphonates and relapse was near completion in 5 of these patients. The fall in urinary OHP/Cr for the whole group was comparable to that observed following clodronate treatment but the suppression of alkaline phosphatase appeared to be less marked in the etidronate treated group (Figure 3.11). Thus there was a mean fall to 62.5% of original pretreatment at 3 months in the etidronate group compared with 44.1% at 3 months following clodronate 300mg ($p<0.02$ on non-paired t-testing of percentage values). 7 (previously untreated) patients had each received 300mg etidronate whereas the remaining 14 patients received a dose of 7.5mg/kg body weight to give a mean dose of 523mg/day. The group who received the higher dose had a greater degree of suppression in serum alkaline phosphatase than the lower dose group (Figure 3.12). Further evidence of a dose/response relationship to intravenous etidronate is shown in Figure 3.13 in which responses at 3 months were compared against the curvilinear relationship derived from the resposes in the oral treatment group. 6 of the 10 patients treated with the higher dose for whom data was available at 3 months

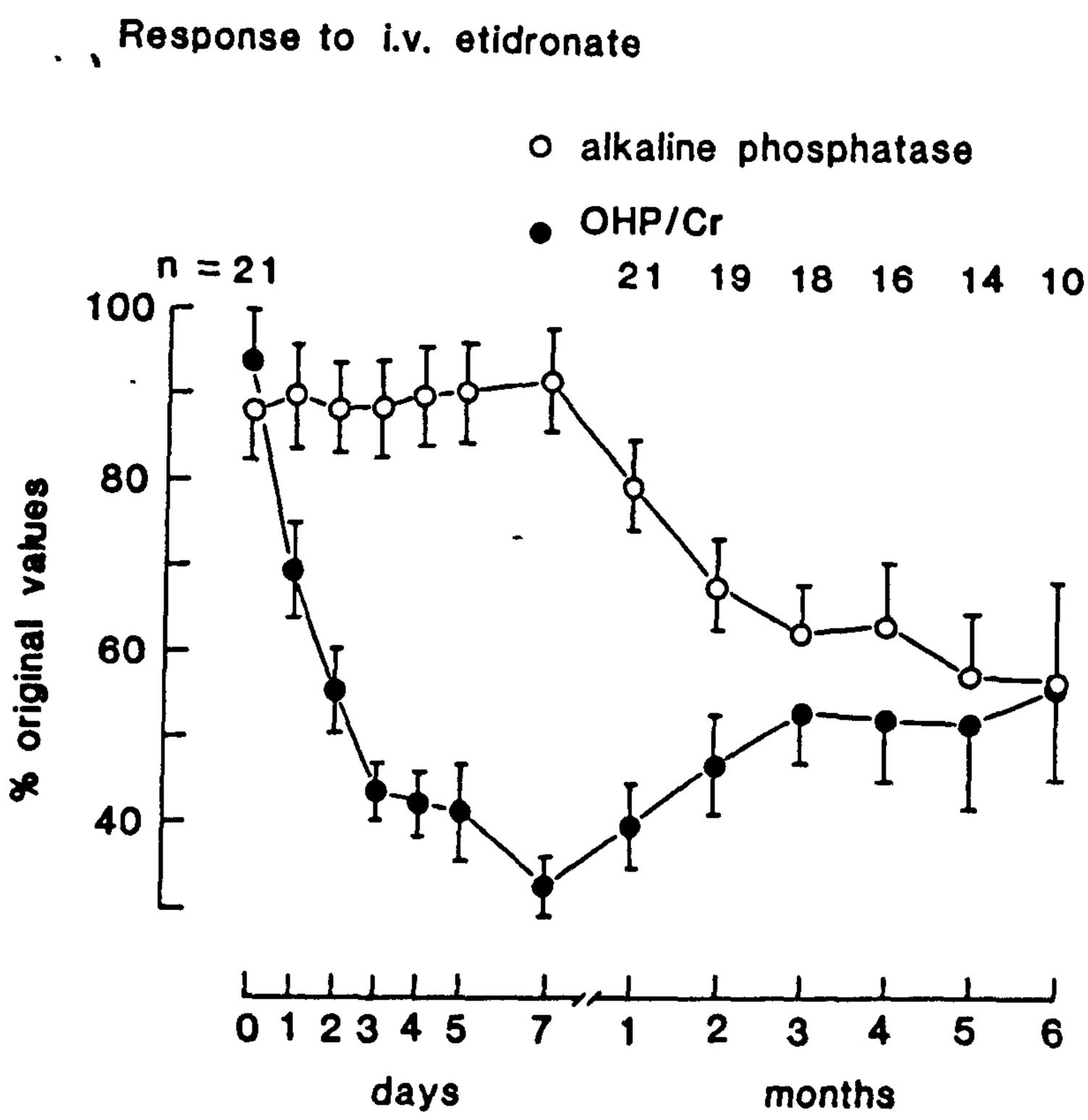


Figure 3.11 Effects of intravenous etidronate on biochemical markers of bone turnover.

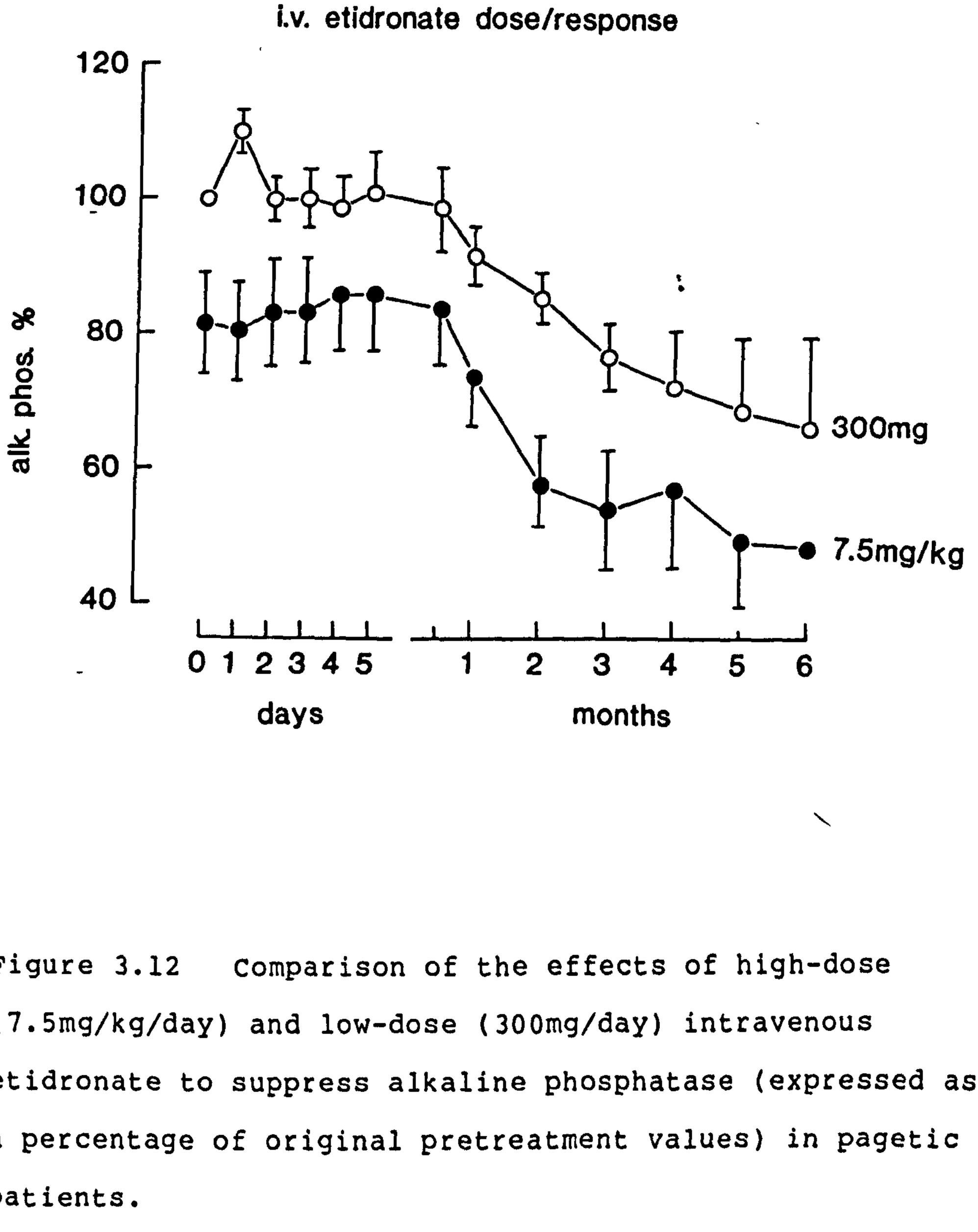


Figure 3.12 Comparison of the effects of high-dose (7.5mg/kg/day) and low-dose (300mg/day) intravenous etidronate to suppress alkaline phosphatase (expressed as a percentage of original pretreatment values) in pagetic patients.

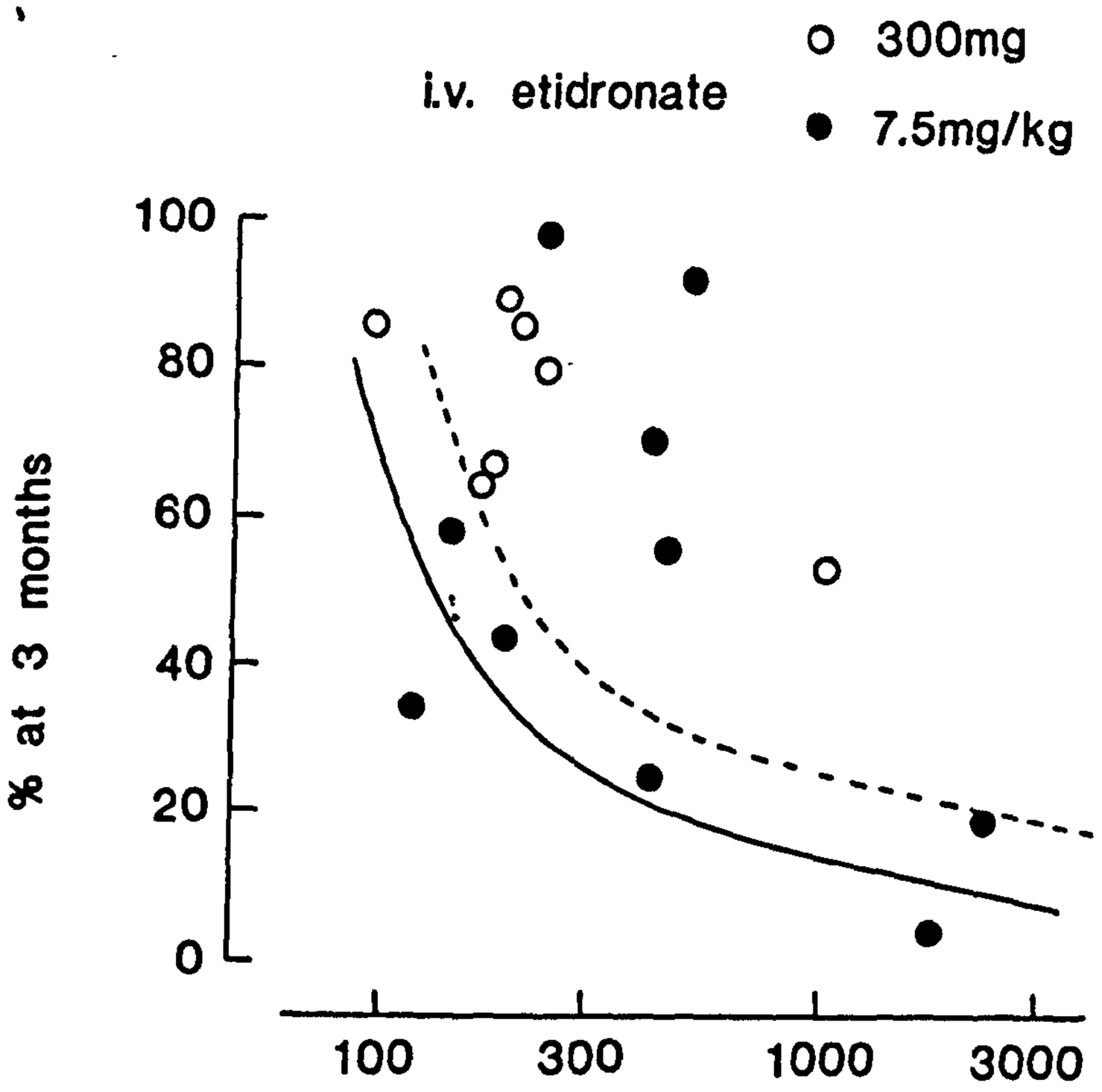


Figure 3.13 Percentage of original pretreatment alkaline phosphatase at 3 months from the start of etidronate treatment in patients given either 300mg/day or 7.5mg/kg/day. Solid and dashed lines are derived from the relationship in oral clodronate treated patients shown in figure 3.8.

had a degree of biochemical suppression comparable to the group treated with oral clodronate whereas responses were less marked in the lower dose group.

3 of the 21 patients (all of whom received the 7.5mg/kg dose) noted altered taste sensation during the treatment period. This feature was noticed shortly after the start of etidronate infusion and resolved within hours of the end of the infusion. Altered taste was not reported by any of the patients treated with the other diphosphonates studied. Transient proteinuria occurred in 1 patient treated with etidronate but this was not associated with any change in renal function as judged by serum creatinine.

Effects of treatment with AHDP

When given by mouth for 1 month, AHDP 400mg daily produced consistent suppression of both alkaline phosphatase and OHP/Cr to less than 50 per cent of pretreatment values (Figure 3.14). Intravenous AHDP 25 mg/day (Figure 3.15) or 50mg/day (not shown) also effectivly suppressed pagetic activity producing responses similar to that seen following the 300mg dose of intravenous clodronate. Thus, AHDP appears to be at least 6 times more potent than clodronate when given by the intravenous route.

There was no increase in body temperature in any

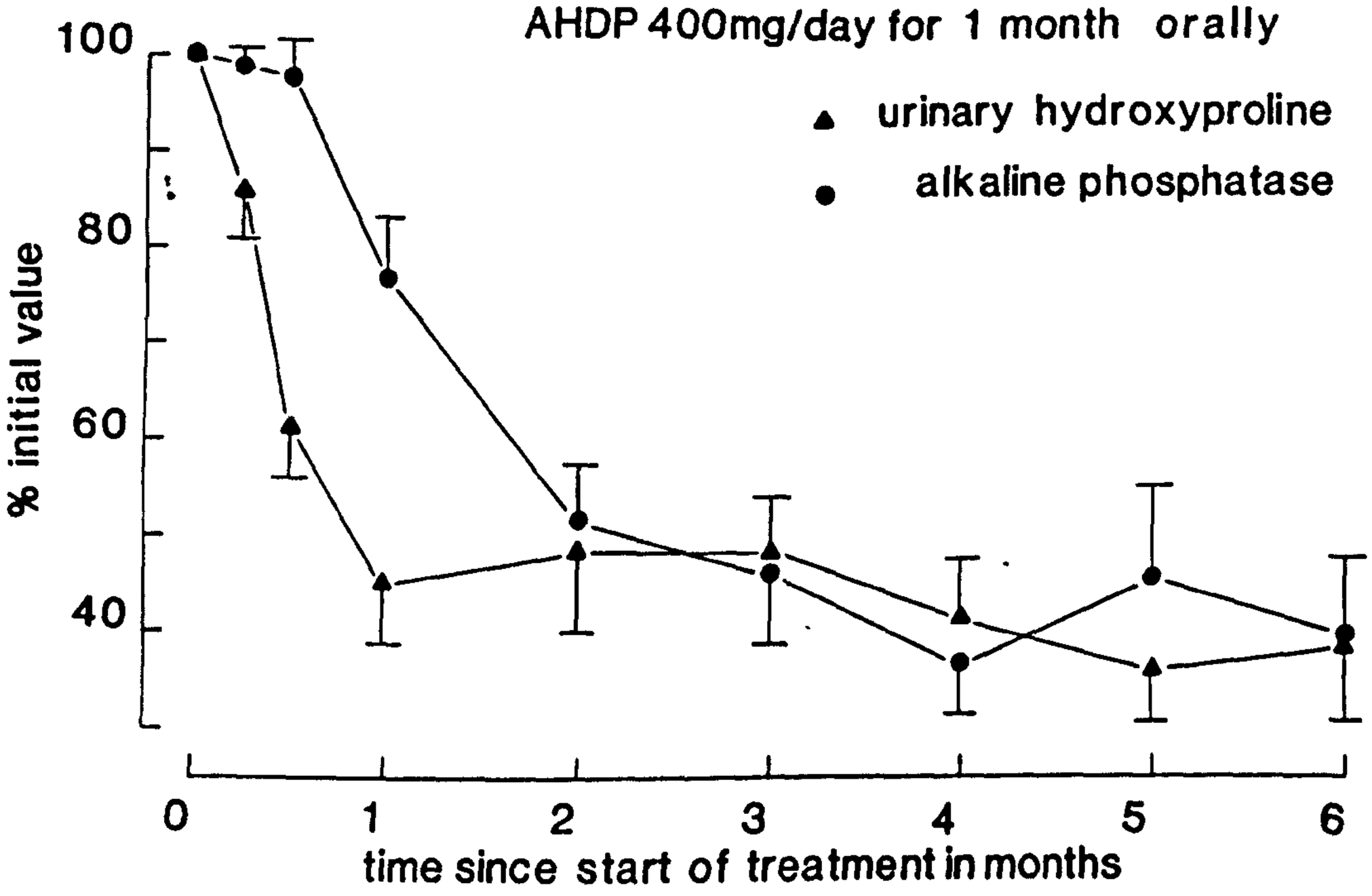


Figure 3.14 Effects of treatment with oral AHDP 400mg/day for 1 month on biochemical markers of pagetic activity.

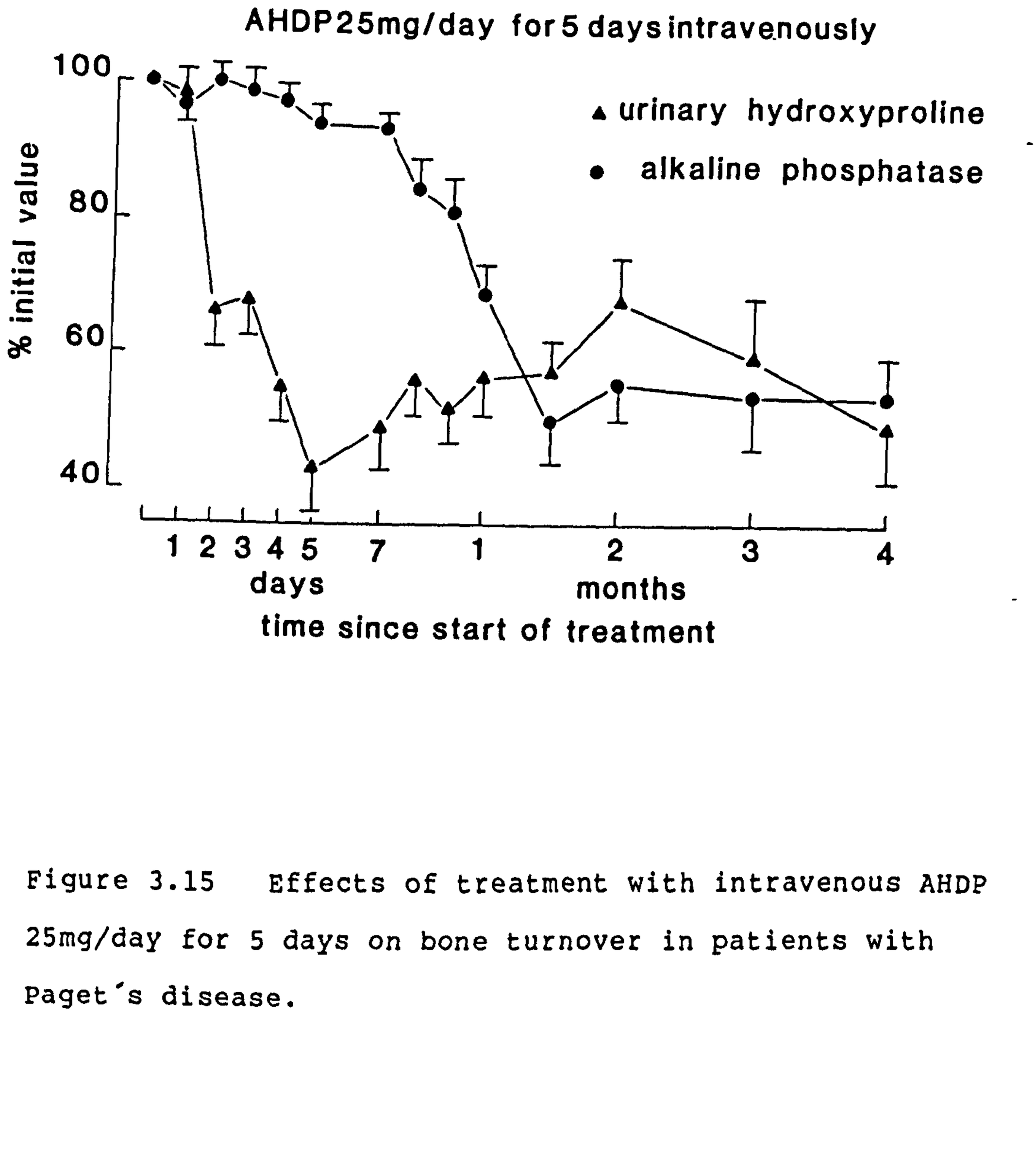


Figure 3.15 Effects of treatment with intravenous AHDP 25mg/day for 5 days on bone turnover in patients with Paget's disease.

of the intravenously treated patients and no effect on lymphocyte numbers was noted. As yet few data are available on the rate of biochemical relapse following AHDP treatment.

Discussion

Our previous experience with the successful use of 1 month high dose oral etidronate (Preston et al, 1986), and that of Delmas and colleagues (1982) with 1 month courses of oral clodronate, suggest that use of the currently recommended lengthy regimens of 3 to 6 months of treatment by mouth may be unnecessary. Skeletal uptake of diphosphonates, as shown by whole body retention of 99Tc-tagged diphosphonate, is proportional to the prevailing rate of skeletal turnover, being greatest during the early phase of treatment and diminishing thereafter as turnover decreases (Smith et al, 1984). This may explain, in part, why the effects of shorter courses of oral diphosphonate treatment appear to be similar to those of more prolonged treatments.

The present studies demonstrate that even very short courses of diphosphonate treatment may have profound and sustained effects to reduce bone turnover in Paget's disease. The inhibition of bone resorption is rapid (within days) but the fall in bone formation ,as judged by alkaline phosphatase, occurs over 3 months and

only commences after the end of intravenous treatment. A short delay in suppression of alkaline phosphatase might be expected as this enzyme has a half life in plasma of around 2 days (Walton et al, 1975b), but this could not account for the time course observed in these studies. The findings support the notion that the suppression of alkaline phosphatase is a consequence of the coupling mechanism, which is maintained in Paget's disease, rather than a direct result of the treatment per se.

The time course of the biochemical response to intravenous diphosphonates is of interest. Maximal suppression of OHP/Cr is achieved at the end of the treatment period, but it is impossible to predict whether a further fall would have occurred had intravenous treatment continued. Within weeks of stopping treatment there was a secondary increase in OHP/Cr suggesting either that reactivation of some osteoclasts occurred or that there was new recruitment of osteoclasts. At around 1 month from the end of treatment a new plateau for OHP/Cr was reached. The fall in alkaline phosphatase following treatment with intravenous clodronate 300mg may be slightly more rapid than that following oral therapy but by 3 months the degree of suppression was identical for the two groups.

In the intravenously treated groups in general no further suppression of mean values occurred after 3 months and after 6 months (data not shown) there is a

gradual increase due to biochemical relapse in a proportion of patients. In contrast, during 6 months oral treatment both OHP/Cr and alkaline phosphatase continue to fall. One possibility is that continued treatment prevents the secondary recruitment or activation of osteoclasts.

The actuarial survival analysis data on the duration of remission indicate that although intravenous clodronate 300mg has prolonged effects (median relapse free interval approximately 1 year) the length of remission is significantly shorter than that following long-term oral clodronate. Thus, although the ideal would be to be able to achieve reliable and complete suppression of disease activity which would last for many years following short intravenous courses of diphosphonate treatment this has not been fulfilled in practice. It is possible that some form of maintenance therapy, perhaps with intermittent intravenous doses of diphosphonate, could be used in order to prevent biochemical relapse. However the duration of remission following intravenous clodronate 300mg is comparable to that which we have observed following high dose oral etidronate for 3 to 6 months where only 58% remain in remission at 1 year (Gray et al, 1984). Thus, in real terms the duration of effect of intravenous clodronate treatment is comparable to that induced by the only generally available form of diphosphonate therapy.

The present study highlights several hitherto unresolved difficulties in the assessment of response to anti-pagetic treatment. Although previous treatment may be allowed for by always comparing values with those observed before the initial treatment (or highest observed), interpretation remains difficult in patients in whom the degrees of relapse, and subsequent response to re-treatment, are slight. Also the use of original pretreatment values assumes a lack of progression of the pagetic process, whereas this is probably not always so particularly if disease activity is incompletely suppressed (Woodard, 1959).

The possibility that a degree of resistance to retreatment with diphosphonates has not been entirely excluded by the present study as the mean value of original alkaline phosphatase was higher in the previously treated group than in untreated patients. Furthermore, many patients retreated with clodronate 300mg, unlike those given the 600mg dose, failed to show further biochemical suppression. This suggests that there may have been partial resistance which could be overcome with the use of the higher dose of clodronate. Apparent resistance might also be, in part, a consequence of patient selection. Responses to diphosphonates are heterogeneous and poor responders are likely to present for retreatment more often than good responders thus introducing a bias against retreated groups.

The extent to which the original pretreatment alkaline phosphatase is increased is clearly an important determinant of the percentage fall that may be expected. The observed relationship is to be expected as at least a proportion of total alkaline phosphatase (liver-derived) is not amenable to suppression with inhibitors of bone resorption. Thus the nearer the pretreatment value is to the value of the non-suppressible component of alkaline phosphatase the lower the percentage fall that can be expected. The position is complicated by the fact that patients with very high initial values of serum alkaline phosphatase often fail to suppress into the normal range even following prolonged effective treatment (Figure 3.16). This indicates that even the fraction of serum alkaline phosphatase which is derived from pagetic bone is not completely suppressed by treatment.

The concept of suppressible and non-suppressible alkaline phosphatase may be used as a simple bi-compartmental model. An equation may be formulated which approximates the observed percentage of pretreatment value achieved following treatment.

Thus :-

$$AP\% = \frac{APns + (k \times APs)}{APtotal} \times 100$$

Where AP% is the predicted percentage fall, APns and APs are the non-suppressible and suppressible components of

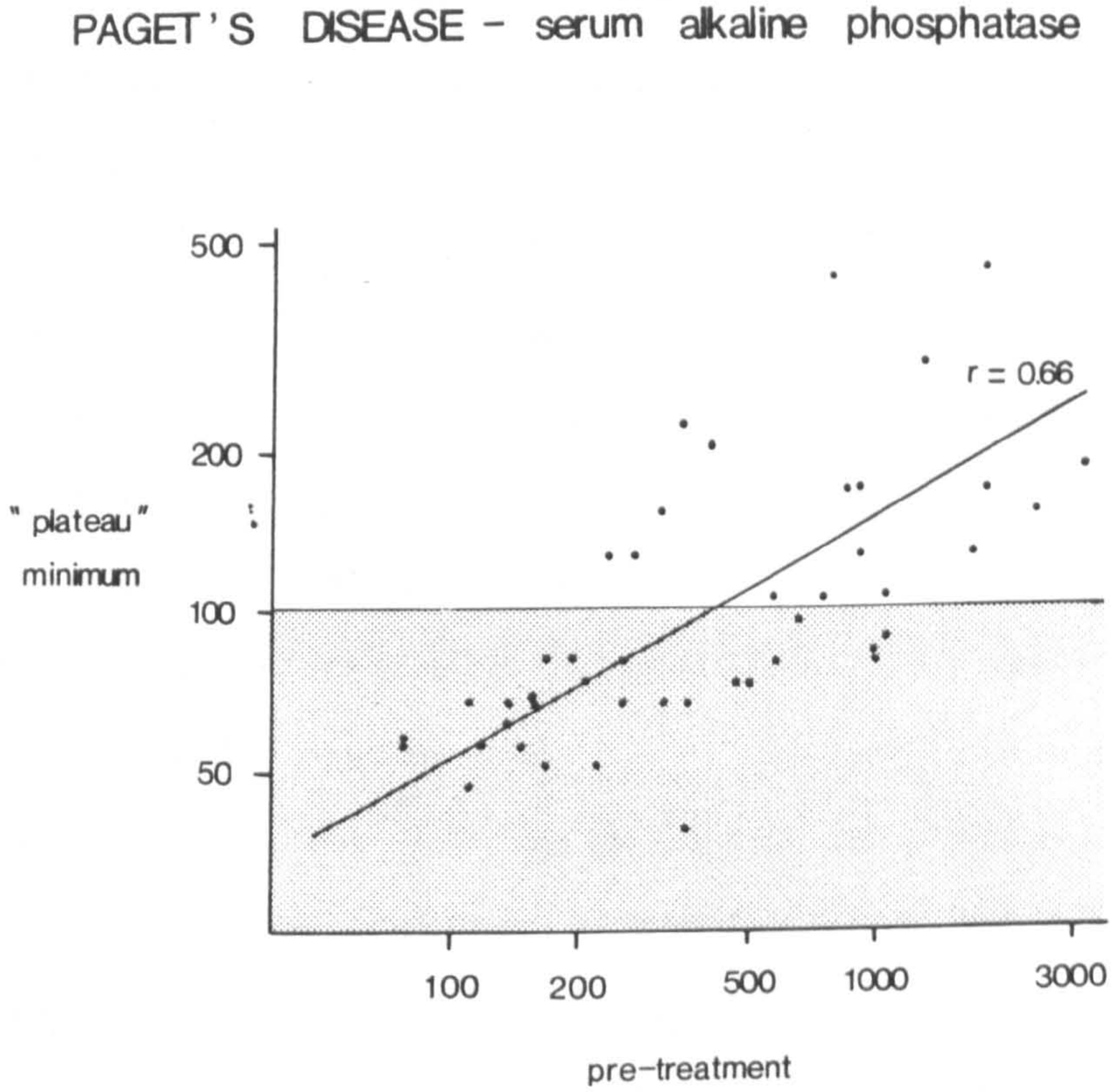


Figure 3.16 Relationship between pretreatment alkaline phosphatase and minimum values achieved in pagetic patients treated with clodronate 1.6g/day by mouth for 6 months ($P < 0.0001$). In general, patients with the most active disease failed to suppress into the normal range following treatment. However, there was considerable heterogeneity of response.

alkaline phosphatase respectively and k is the factor by which APs is reduced following treatment.

From an examination of Figure 3.8 it can be seen that for very high pretreatment values for alkaline phosphatase, where the contribution of the non-suppressible alkaline phosphatase is minimal, the values at 6 months are around 10% of the pretreatment values. Figure 3.17 shows this theoretical relationship and the effect of differences in the APns component on the percentage fall achieved. Note that at low values of pretreatment alkaline phosphatase quite small differences in the value of the APns component would result in large differences in percentage fall. In fact the line drawn by eye from Figure 3.8 approximates very closely to the theoretical line assuming a non-suppressible alkaline phosphatase of 60 U/l and a value for k of 0.1.

In reality there may be a spectrum of suppressibility of skeletal alkaline phosphatase with the fraction derived from non-involved bone being more resistant to suppression than that derived from pagetic bone where a very high concentrations of diphosphonate may be achieved. However, in terms of mathematical expression this is probably unimportant and a theoretical single non-suppressible component would remain valid in the same way that one can usefully consider a single "physiological dead space" in the lungs even though in reality there is a continuous spectrum between

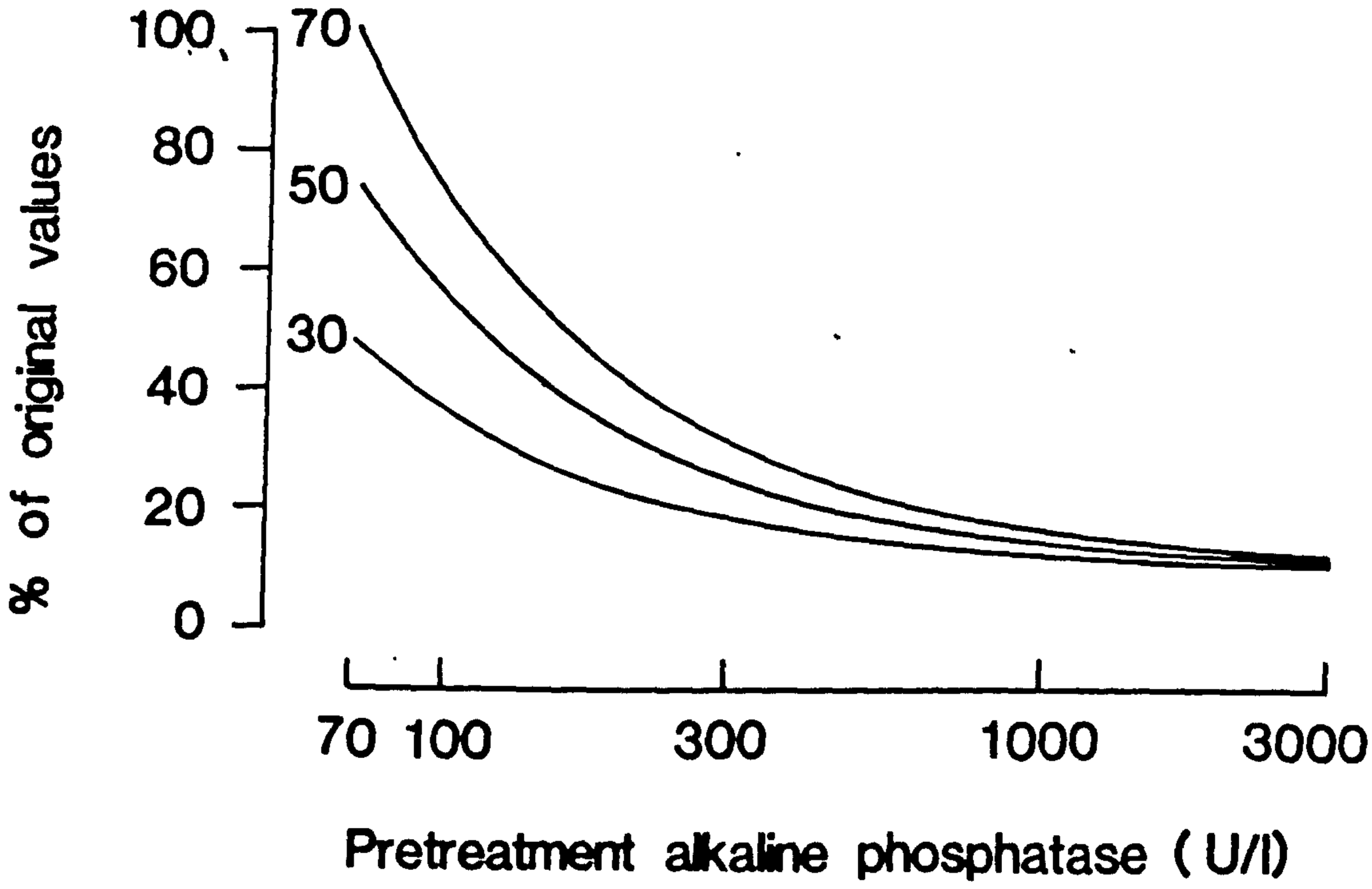


Figure 3.17 Theoretical relationship between pretreatment alkaline phosphatase and percentage achieved following treatment assuming a fall in the suppressible component of alkaline phosphatase to 10% of pretreatment (see text). The 3 lines represent predicted relationships for patients in whom the non-suppressible component of alkaline phosphatase is 70, 50 or 30U/l. The effect of these differences is greater at lower values of total pretreatment alkaline phosphatase.

functionless and fully functioning alveoli (West, 1977). Using this relationship it may be possible to construct an algorithm which could give an index of the adequacy of response for individual patients. However, this is unlikely to be useful in patients with pretreatment alkaline phosphatase values of less than 200 U/l as both the increased slope of the curve and the larger effect of interindividual variations in the non-suppressible component of alkaline phosphatase at lower values of total alkaline phosphatase will tend to reduce the predictive value (Figure 3.17).

Ideally, comparison of responses between treatment groups should be done on pairs of patients who are matched for both original pretreatment alkaline phosphatase and, if previously treated, patients should also be matched for alkaline phosphatase values immediately before retreatment. Although randomised controlled trials would also produce unbiased results, in order to ensure comparability of pagetic activity between groups larger numbers of patients would be required than in a study of matched pairs. In practice such matching is difficult. Furthermore, it is often desirable to be able to make retrospective comparisons in order to judge responses to new treatments against those of existing treatments and also to be able to compare treatment responses between two or more different studies. Therefore a more important use of the relationship

between pretreatment alkaline phosphatase and expected fall may be that it enables a more valid comparison of imperfectly matched treatment groups. This use has been demonstrated with regard to the dose/response relationship to intravenous clodronate.

Practical difficulties with oral diphosphonate treatment include the inconvenience of having to take tablets, often in considerable quantities, away from food (which impairs drug absorption) for prolonged periods. These problems are greater in patients who develop gastrointestinal intolerance and in those who have difficulty with drug compliance for other reasons. Thus the use of an intravenous regimen provides an attractive alternative to oral treatment. However, the relatively short duration of remission (at least in the clodronate 300mg group) suggests that a 5 day regimen may not be optimal.

Treatment of Paget´s disease with AHDP either by mouth or intravenously effectively suppresses the disease activity using doses considerably lower than those required with etidronate or clodronate treatment. It therefore shows promise as a treatment for this disorder. Apart from higher potency the effects of AHDP to suppress bone turnover appear to be very similar to those of clodronate. Biochemical relapse has occurred in some AHDP treated patients and although the follow-up interval on most patients is very short the duration of remission

appears to be similar to that following clodronate treatment. This treatment appears to be free from side effects and in particular, unlike APD, no immunomodulatory effects, either pyrexia or leucocyte suppression, were noted in the present study. Further studies are required to confirm this lack of effect of AHDP on the immune system. The effects of these agents on the mineralisation of newly formed osteoid is discussed in the next chapter.

The suppression of alkaline phosphatase following intravenous etidronate treatment appears to be less marked than that seen following treatment with intravenous clodronate or AHDP despite a similar degree of suppression of OHP/Cr. Thus, biochemically it appears that intravenous etidronate might produce a more sustained "uncoupling" effect than does clodronate. If this is a true reflection of events in terms of turnover of bone matrix then this might imply that etidronate is capable of producing a greater incremental increase in bone mass than clodronate and thus may be potentially useful in the treatment of bone loss. However, it is difficult to envisage a mechanism whereby differences in the degree of dissociation of bone formation and bone resorption lasting for several months may be produced by such short courses of treatment. It is possible that the apparent difference in the degree of "uncoupling" is artefactual. Further studies are required to examine

this point.

Future studies are planned, and have been initiated, to investigate the importance of the timing of intravenous diphosphonate administration and the possible role of a combined intravenous/oral approach. Thus, as a direct comparison to the 5 day intravenous regimens, some patients are receiving a once monthly intravenous infusion of clodronate 300mg or 600mg to be continued for 5 months. In other patients a 5 day intravenous course of clodronate will immediately be followed by a 1 month course of oral clodronate 1.6g daily. It will be interesting to see if either of these approaches results in more complete or more sustained responses than those obtained with 5 consecutive days of intravenous treatment alone.

The present studies have shed some light on the problem of optimising diphosphonate treatment and comparing treatment responses in Paget´s disease of bone. In addition, by confining treatment to such a short period, the biochemical effects of intravenous treatment provide information on bone turnover and the coupling mechanism in this disorder. Clearly there is much scope for further work in this area and it will be some time before optimal regimens will be known with confidence. Changes in bone turnover at a cellular or bone matrix level are inevitably associated with changes in calcium and phosphate homeostasis. The changes in mineral

metabolism observed following the short intravenous diphosphonate treatments described here are discussed in the next chapter.

Summary

1. We and others have previously shown that short courses (1 month) of high dose oral diphosphonate treatment induce biochemical suppression of pagetic activity comparable to that seen following 6 months treatment.

2. Thus the effects of very short (5 day), high dose, intravenous courses of diphosphonates to suppress pagetic activity were studied and compared with responses to long-term oral diphosphonate treatment.

3. Intravenous clodronate induced a marked and sustained suppression in the biochemical indices of pagetic activity. There appears to be a dose/response relationship; 600mg/day was almost universally effective, 100mg/day has very little effect whereas the response to 300mg/day was intermediate between the two.

4. Previous diphosphonate treatment caused an apparent resistance to intravenous clodronate which was, at least in part, an artefact due to incomplete biochemical

relapse before retreatment.

5. The percentage fall in biochemical markers following effective treatment for Paget's disease is highly dependent on the degree of pagetic involvement as judged by values of alkaline phosphatase observed before diphosphonate treatment. This relationship should be considered in order to facilitate comparisons between treatment groups.

6. The duration of biochemical remission following intravenous clodronate therapy was significantly shorter than that following 6 months oral clodronate treatment but similar to that observed following oral etidronate treatment.

7. Treatment of Paget's disease with the novel diphosphonate AHDP, given either orally or intravenously effectively suppressed pagetic activity when given by either route and when given intravenously has a potency of approximately 6 to 12 times greater than that of clodronate.

8. Intravenous etidronate was also effective in suppressing bone resorption although preliminary results suggest a less marked suppression of bone formation, as judged by alkaline phosphatase, than that seen with

either clodronate or AHDP.

9. Short intravenous courses of diphosphonates offer an attractive alternative form of treatment to the currently used long-term oral diphosphonate regimens.

CHAPTER FOUR

EFFECTS OF INTRAVENOUS DIPHOSPHONATES ON CALCIUM AND PHOSPHATE METABOLISM AND SKELETAL MINERALISATION IN PAGET'S DISEASE OF BONE

EFFECTS OF INTRAVENOUS DIPHOSPHONATES ON CALCIUM AND PHOSPHATE METABOLISM AND SKELETAL MINERALISATION IN PAGET'S DISEASE OF BONE

Introduction

The potent effects of diphosphonates on bone turnover in Paget's disease, discussed in Chapter 3, may be expected to result in changes in skeletal calcium balance and thus calcium homeostasis. While alterations in calcium homeostasis may be of little consequence in this disorder, Paget's disease forms a useful model for the study of the use of diphosphonates in treatment of hypercalcaemia of malignancy where conditions are less controlled. The known effects of etidronate at high dosage to impair mineralisation of bone may have important consequences for skeletal calcium homeostasis. Although diphosphonates other than etidronate given by mouth are not generally associated with impaired mineralisation, their effects when given intravenously, where very much higher systemic delivery may be achieved, have not previously been investigated. Thus the effects of intravenous diphosphonates on bone mineralisation as well as possible interrelationships between mineralisation effects and effects on calcium and phosphate homeostasis were investigated in these studies.

Several studies on the use of etidronate in the

treatment of Paget's disease of bone have noted a consistent hyperphosphataemic response in patients taking high oral doses (Altman et al, 1973; Russell et al, 1974). This effect, which becomes maximal at about 2 weeks from the start of treatment, has been shown to be mediated via an increase in renal tubular reabsorption of phosphate, expressed as TmP/GFR (Walton et al, 1975a). Clinical studies with other diphosphonates, including clodronate and APD, given by mouth have generally shown a decrease rather than an increase in serum phosphate and thus hyperphosphataemia is generally assumed to be an idiosyncratic effect of etidronate (van Breukelen et al, 1979; Douglas et al, 1980). However, using high doses of APD (600mg by mouth daily) Nagant de Deuxchaisnes and colleagues (1982) noted a small and transient increase in phosphate and TmP/GFR in pagetic patients. The question arose as to whether the hyperphosphataemic response might be a general property of all diphosphonates if given in sufficient dosage. Thus a further aim of the present study was to explore this possibility using the very high systemic doses of three diphosphonates which are achieved by intravenous infusion. Changes in TmP/GFR following diphosphonate treatment may be influenced by changes in calcium homeostasis and thus in PTH status. In order to investigate effects of diphosphonates on TmP/GFR independently from changes in PTH a small number of patients with hypoparathyroidism were also studied.

Patients and Methods

The studies on calcium and phosphate metabolism in Paget's disease were of the same patients and treatments with intravenous diphosphonates as reported in the last chapter except that an additional 9 patients who were treated with intravenous AHDP 50mg/day are included here. Quantitative histology of bone was studied in 29 patients with Paget's disease (8 given etidronate, 13 given clodronate and 8 given AHDP) using methods outlined in Chapter 2. In a further 3 patients the timing of the tetracycline labelling was altered to investigate mineralisation either during clodronate treatment (2 patients) or 1 month following the end of etidronate therapy (1 patient) as described in Chapter 2.

Two of the pagetic patients receiving their fifth day of etidronate were infused with 200U of human PTH (1-84) for 30 minutes during a period of half hourly blood and urine sampling for the detection of acute changes in renal tubular reabsorption of phosphate measured as TmP/GFR.

A further 4 patients (all female) with hypoparathyroidism were studied. Three received clodronate 300mg/day whereas the remaining patient was given AHDP 50mg/day. Each was treated for 5 days. All patients gave informed consent for study which had been

approved by the local Ethics Committee.

Results

Effects on calcium metabolism

The comparative effects of intravenous clodronate (300mg/day), etidronate (300-700mg/day) and AHDP (25mg/day) on serum calcium, fasting Ca/Cr and urinary OHP/Cr are shown in Figure 4.1. There was an initial slight but significant ($p<0.01$) increase in serum calcium in the etidronate treated group by day 1 which was sustained (apart from the drop at day 4) for at least 19 days. Urinary Ca/Cr also increased significantly on day 1 ($p<0.05$) but overall changed very little from pretreatment values. As in the etidronate treated group, significant, albeit modest, increases in calcium and Ca/Cr occurred at day 1 in the clodronate group ($p<0.01$ and $p<0.05$ respectively). However, the predominant effects in both clodronate and AHDP treated patients were of marked and consistent hypocalcaemia and hypocalciuria. These effects were maximal at 5 to 12 days from the start of treatment, both indices gradually returning to pretreatment values at around 3 months (data not shown). The similarity of changes in calcium and changes in Ca/Cr in all three groups suggests that calcaemic changes were not due to primary changes in renal tubular reabsorption

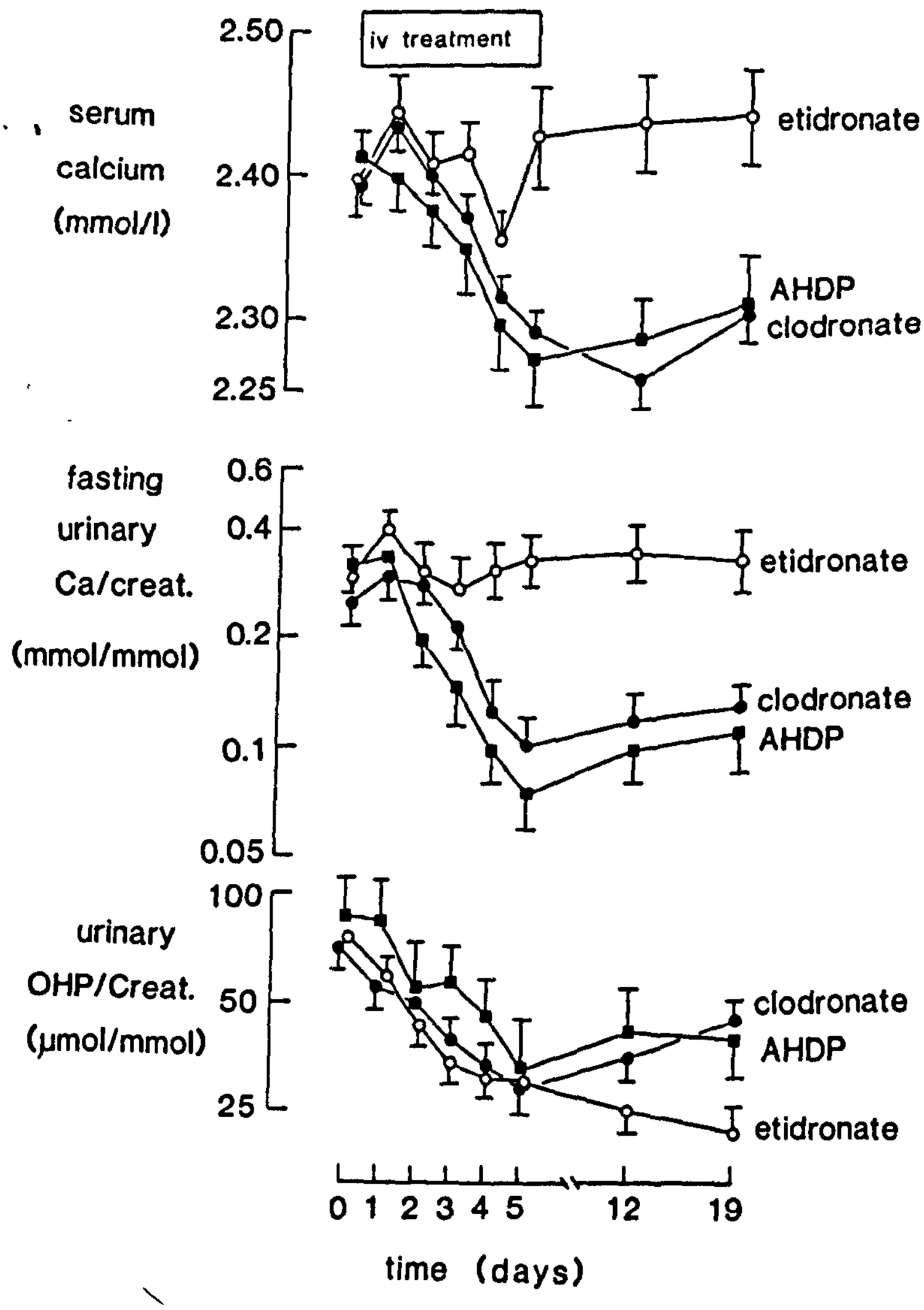


Figure 4.1 Effects of treatment with 3 diphosphonates given intravenously (etidronate 300-700mg/day, clodronate 300mg/day and AHDP 25mg/day) for 5 days on serum calcium, urinary Ca/Cr and urinary OHP/Cr in patients with Paget's disease.

of calcium. All three diphosphonates induced a similar degree of suppression of bone resorption as judged by urinary OHP/Cr.

The most likely explanation for the observed hypocalcaemic responses to clodronate and AHDP was that the rapid suppression of bone resorption with a delayed fall in bone formation, described in Chapter 3, would lead to a transient phase of net bone formation with calcium accretion and consequent hypocalcaemia. If this was the case the degree of the hypocalcaemic effect should be a function of the degree of suppression of bone resorption. Figure 4.2 shows that such a relationship did indeed exist among patients treated with clodronate 300mg/day but not among the 7 patients given etidronate 300mg/day. Moreover, in patients treated with clodronate 100mg/day, in whom suppression of bone resorption was minimal, no significant hypocalcaemic effect was observed, whereas the 600mg dose, which induced the greatest suppression of bone resorption, was also associated with the most marked hypocalcaemic effect (Figure 4.3).

Changes in iPTH in the different treatment groups were appropriate homeostatic responses to the observed changes in serum calcium for each group (Figure 4.4). Thus, there were marked increases in iPTH in both clodronate and AHDP treated patients due to a secondary hyperparathyroid effect whereas there was no significant

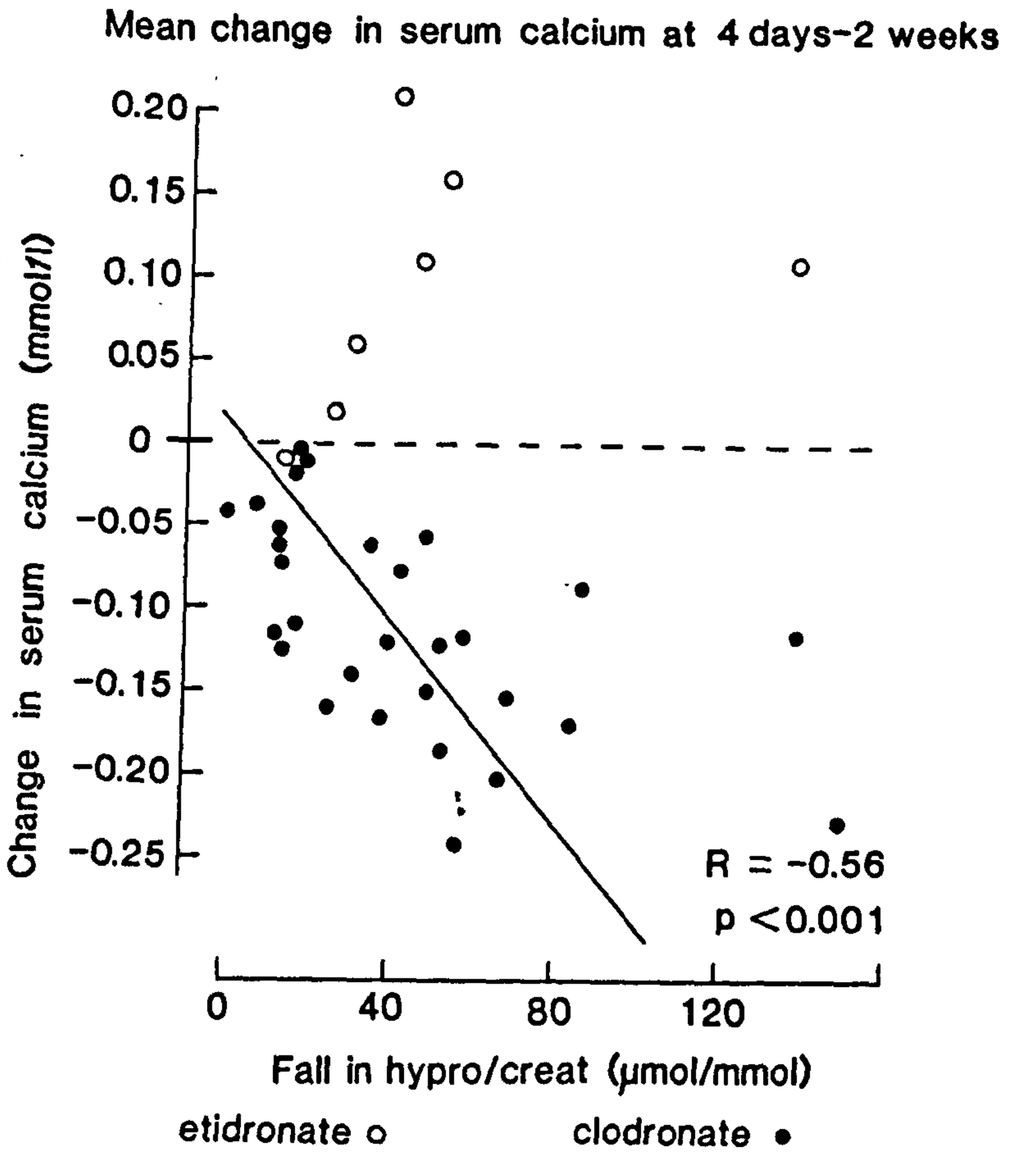


Figure 4.2 Relationship between the mean change in serum calcium and the mean fall in OHP/Cr by 4 days to 2 weeks from the start of treatment in patients treated with either intravenous clodronate 300mg/day or intravenous etidronate 300mg/day. Correlation coefficient and P value for clodronate treated patients only. No significant correlation in etidronate treated group.

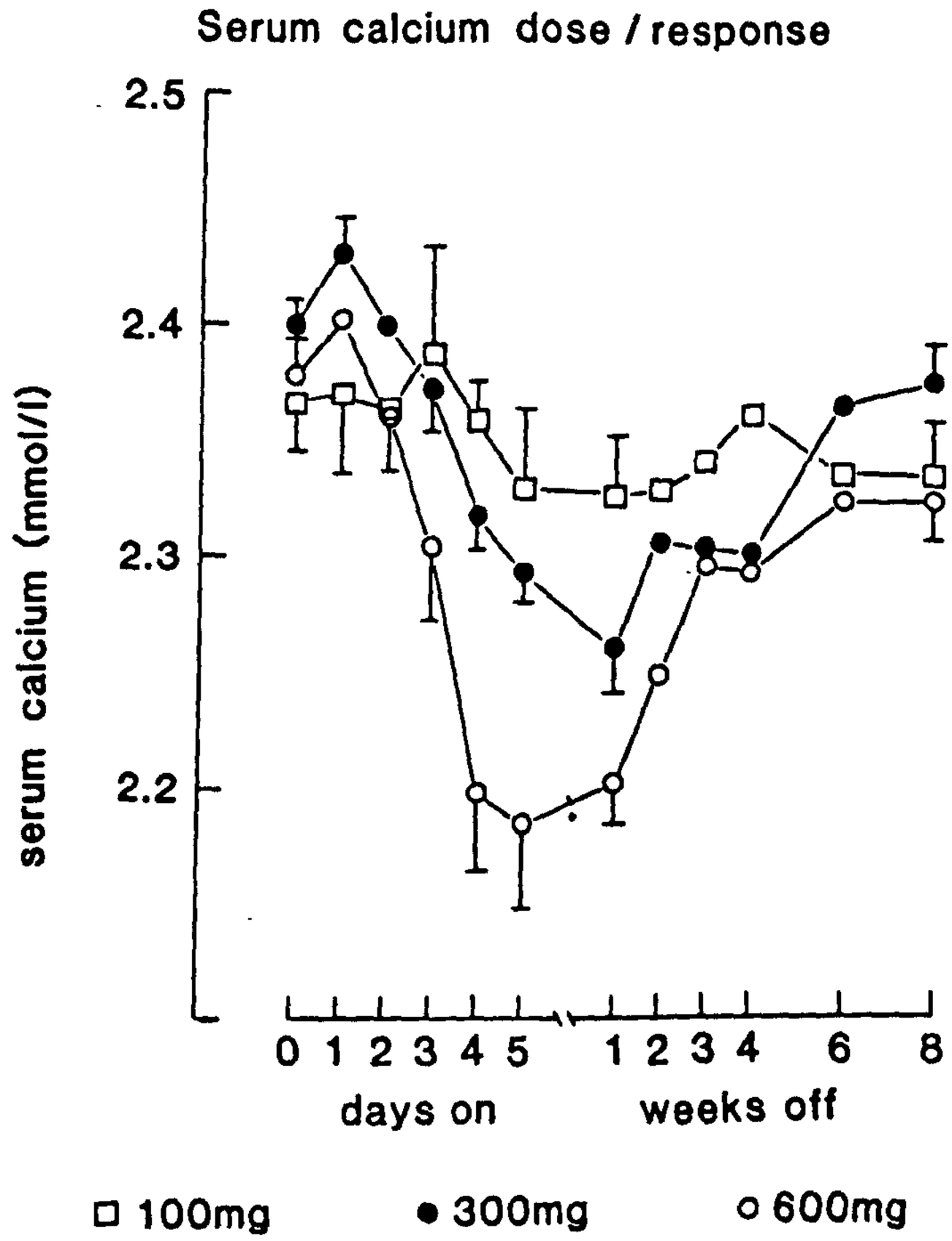


Figure 4.3 Comparison of hypocalcaemic responses to three doses of intravenous clodronate in patients with Paget's disease.

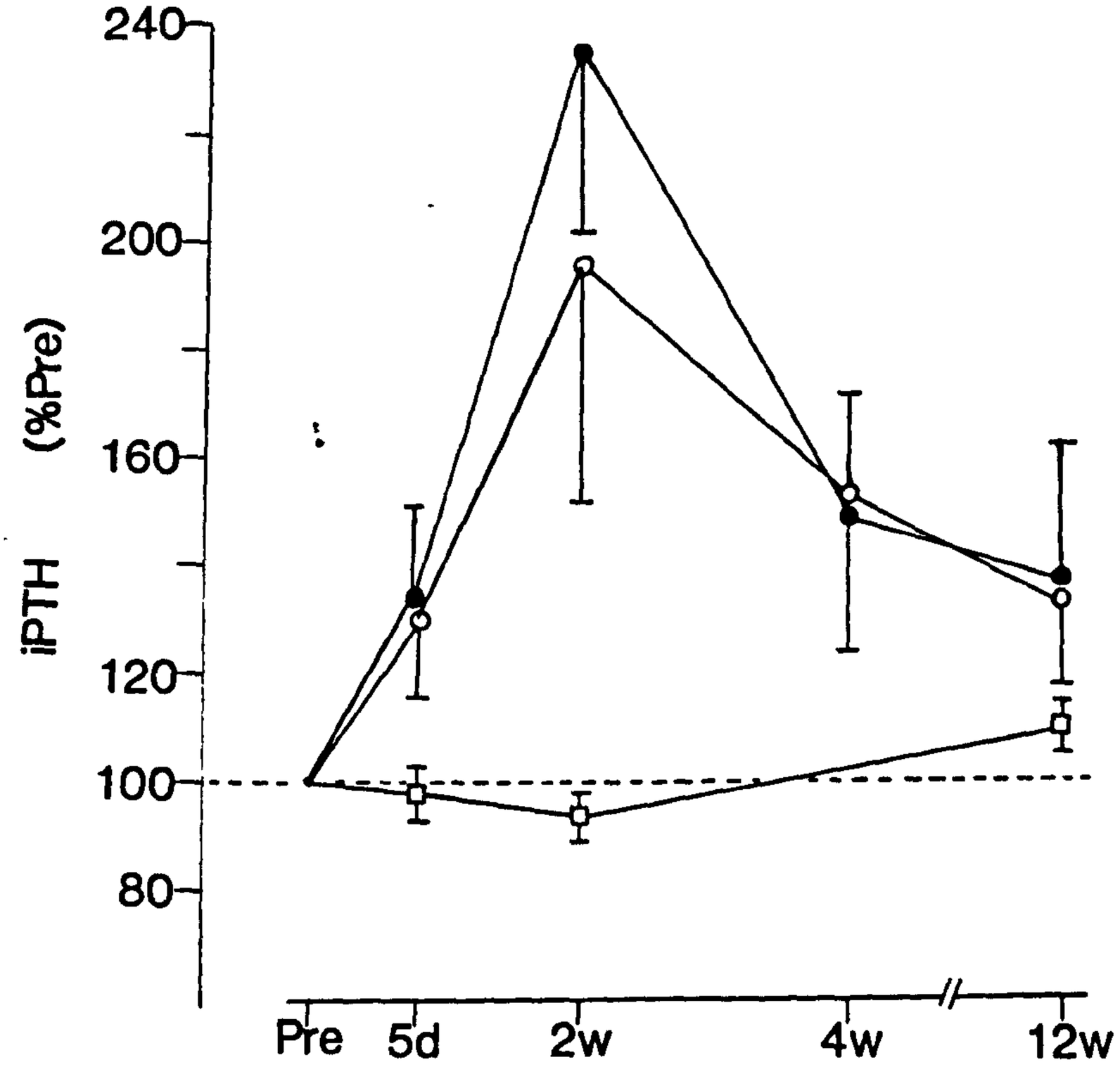


Figure 4.4 Changes in concentrations of immunoreactive PTH (as percentage of pretreatment values; mean +/- SEM) in response to intravenous treatment with etidronate (squares), clodronate (closed circles) or AHDP (open circles).

change in iPTH in the etidronate group.

Effects on mineralisation of bone

Mineral apposition rates (MiAR) before the start of diphosphonate treatment and for the 10 days after this point, determined from the triple tetracycline labelling technique, are shown in Figure 4.5. Rates before treatment were similar for etidronate, clodronate and AHDP treated patients. Intravenous treatment was associated with a consistent and highly significant fall in MiAR in all three groups. However, whereas mean values fell by 30% and 25% in clodronate and etidronate treated patients respectively, there was complete loss of uptake of the final tetracycline label (commenced 5 days after the end of intravenous treatment) in all 8 of the etidronate treated patients (Figure 4.5). This finding suggests that etidronate induced a complete inhibition of mineralisation and that this effect was sustained for at least 7 days from the end of the treatment period (i.e. to the end of the third label). In the one patient given the final tetracycline label 30 days from the start of etidronate treatment uptake of this label was present, but diminished, indicating that reversal of the mineralisation defect was occurring at around that time.

Changes in MiAR in clodronate treated patients given either 100mg (n=2) or 600mg (n=2) were also

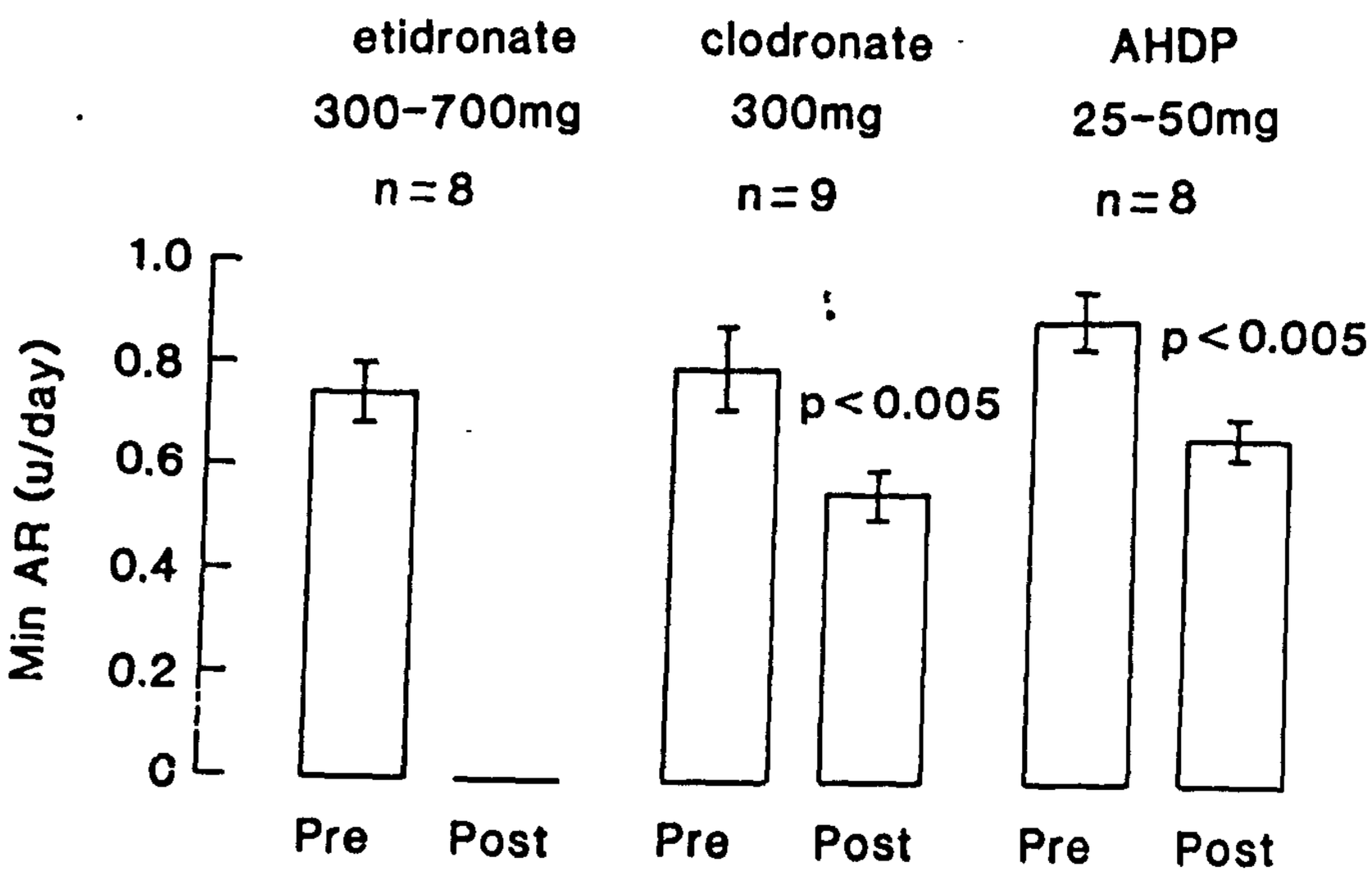


Figure 4.5 Effects of intravenous diphosphonate treatments of Paget's disease on mineral apposition rate (MiAR) determined by a triple tetracycline labelling technique. Both intravenous clodronate and intravenous etidronate produced a significant suppression of MiAR on paired t-testing. Uptake of the final label was completely absent in all etidronate treated patients.

examined and are compared with the individual responses of 300mg clodronate treated patients in Figure 4.6. These results suggest that a dose/response relationship exists so that 100mg has little or no effect on mineralisation 600mg produces a very marked suppression (mean fall of 66%). However, no such dose-dependence could be detected in AHDP treated patients. In fact the 5 patients given 25mg had a slightly greater mean reduction (29%) than did the 3 given 50mg/day (19%).

The reduced MiAR following clodronate or AHDP treatment might have resulted either from a transient but complete inhibition of mineralisation during the treatment period or from an incomplete but more sustained suppression of mineralisation. In order to examine these possibilities 2 patients were studied. Both patients, who received clodronate (300mg/day), were given their final tetracycline label during the last 2 days of the treatment period. In both cases uptake of this final was observed but separation between labels was markedly reduced suggesting that suppression but not complete inhibition of mineralisation occurred during the treatment period.

Effects on phosphate metabolism

Figures 4.7 and 4.8 show the changes in serum phosphate and TmP/GFR in response to intravenous

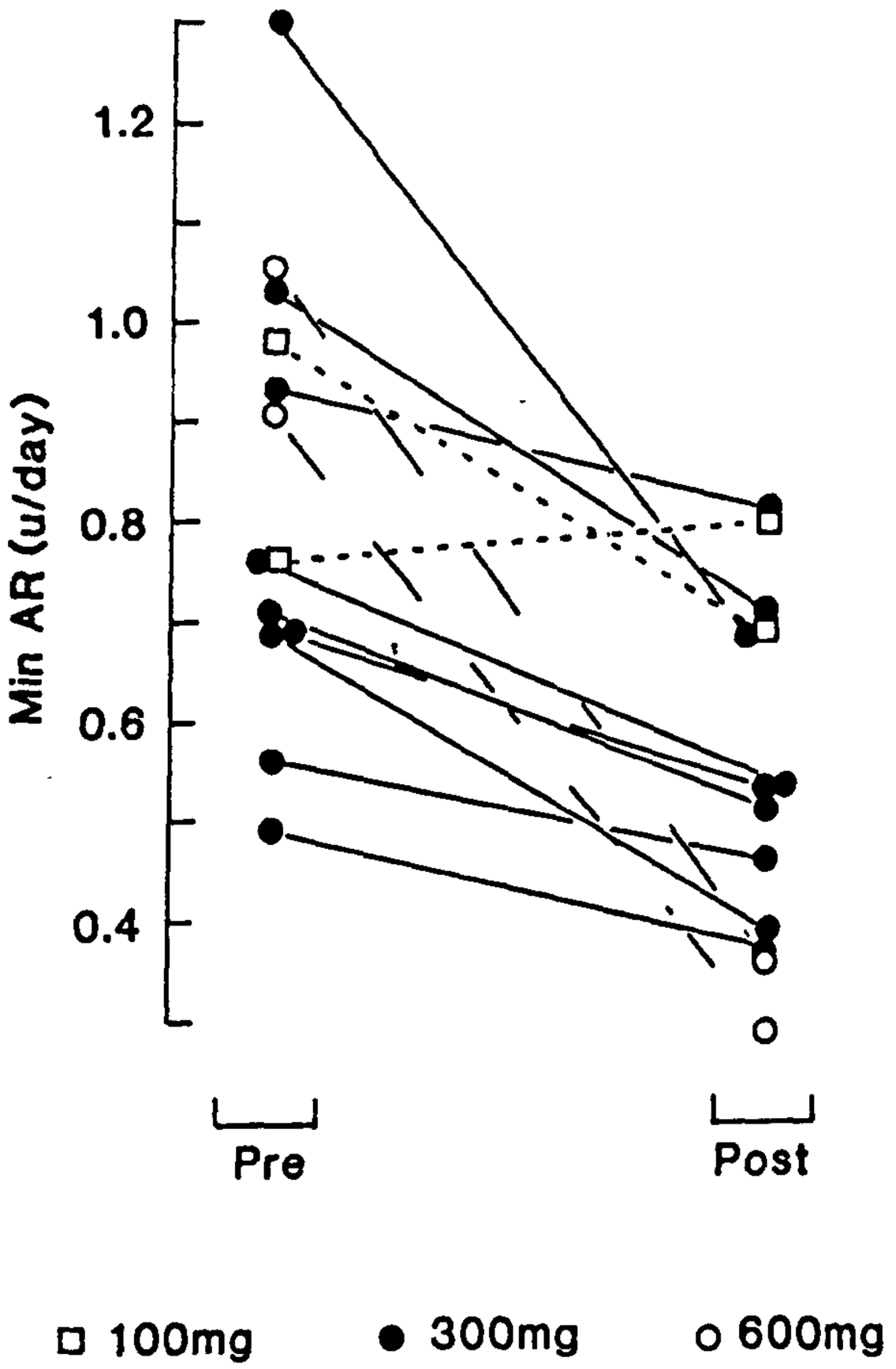


Figure 4.6 Changes in MiAR in individual clodronate treated patients. Marked decreases were observed in 2 patients who were treated with the 600mg dose whereas a fall in MiAR occurred in only 1 of the 2 patients treated with clodronate 100mg/day.

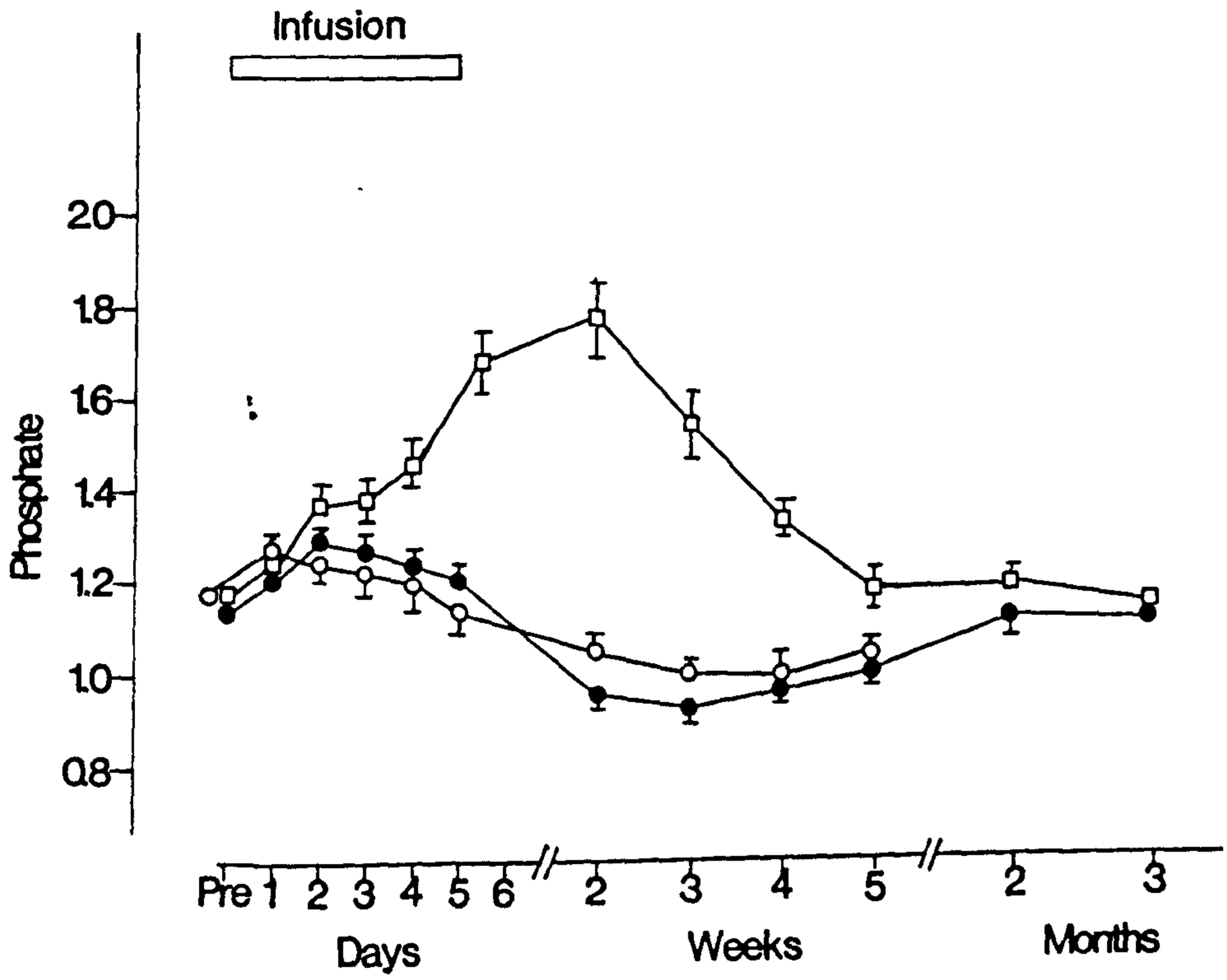


Figure 4.7 Effects of daily 3h intravenous infusions with etidronate (squares), clodronate (closed circles) or AHDP 50mg/day (open circles) for 5 days on serum phosphate in patients with Paget's disease. Early increases were significant in all three groups (at day 1 only in the AHDP group).

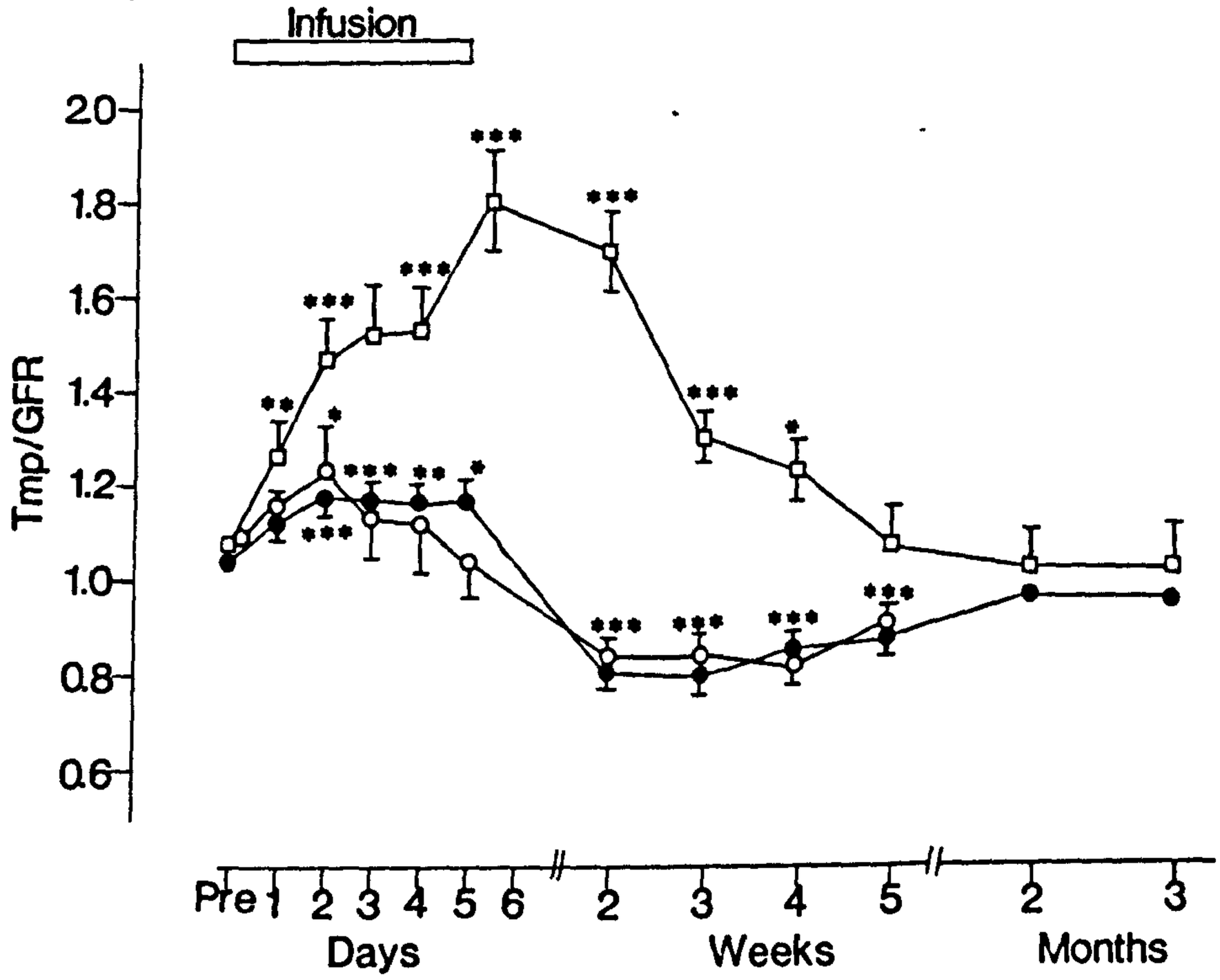


Figure 4.8 Effects of daily 3h intravenous infusions with etidronate (squares), clodronate (closed circles) or AHDP 50mg/day (open circles) on TmP/GFR. * $p<0.05$ ** $p<0.01$ *** $p<0.0001$ on paired t-testing.

treatment with the three diphosphonates (data shown for AHDP 50mg/day dose). Etidronate treatment induced a rapidly progressive increase in both of these indices to reach a maximum at between 6 and 14 days from the start of treatment with a gradual fall thereafter. Values for both phosphate and TmP/GFR remained significantly increased until 5 weeks from the start of treatment.

Significant, although less marked, increases in serum phosphate and TmP/GFR were seen in patients treated with the other two diphosphonates (although this failed to reach significance in the AHDP 25mg group). Increases in those given clodronate were sustained for the period of treatment whereas these effects were seen only for the first few days following treatment with AHDP 50 mg/day. In both the clodronate group and the AHDP group a highly significant hypophosphataemic effect was observed following the end of treatment which gradually diminished over 2 to 3 months. The time courses of changes in iPTH and of the secondary hypophosphataemic responses seen in these two groups were similar suggesting that the fall in phosphate was likely to be due to a PTH mediated fall in TmP/GFR.

The hyperphosphataemic response to clodronate did not appear to be dose dependent as the degree of the hyperphosphataemia was indistinguishable for the three doses (Figure 4.9). However, a dose/response relationship was apparent for the subsequent

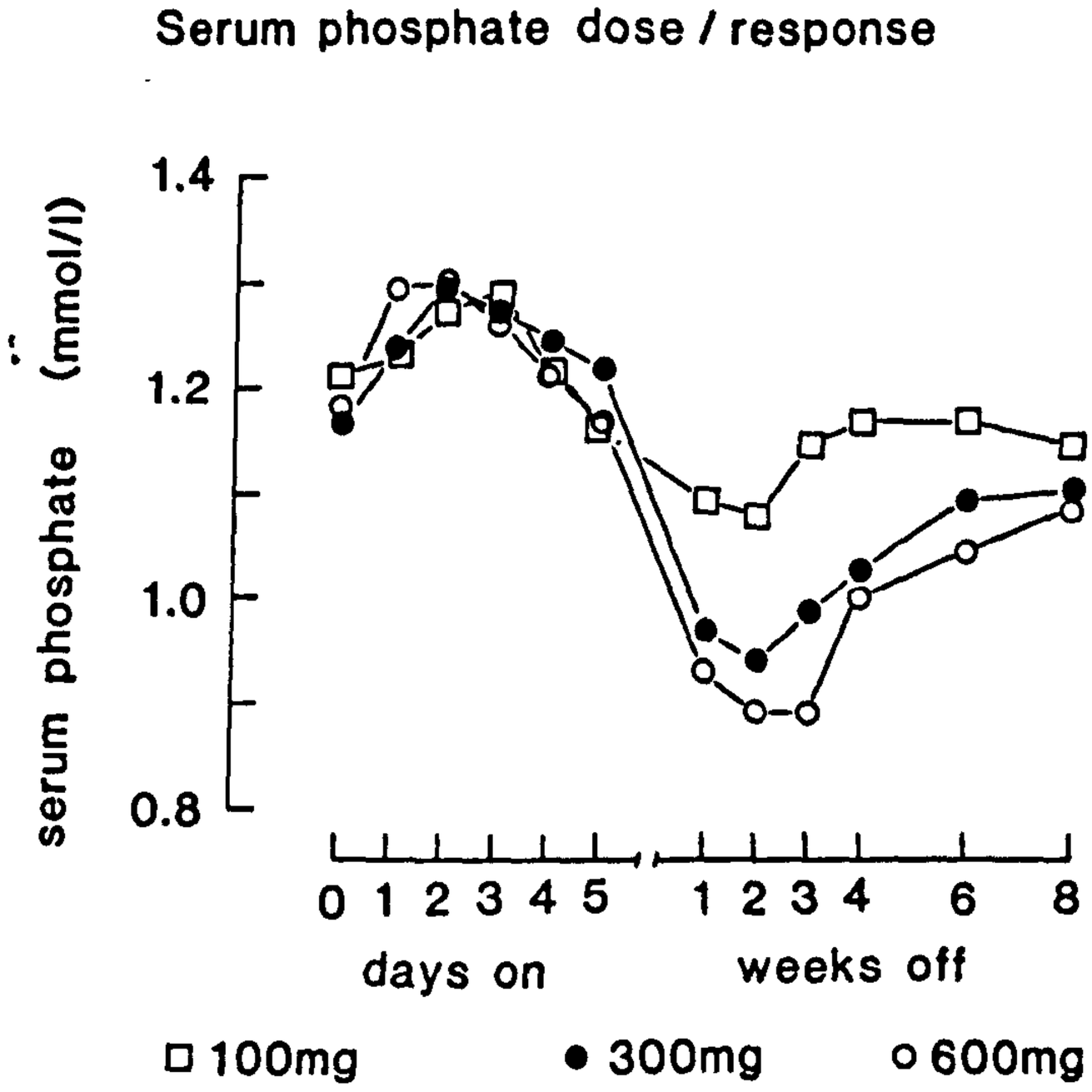


Figure 4.9 Mean values for serum phosphate in pagetic patients treated with either 100mg, 300mg or 600mg of clodronate per day for 5 days.

hypophosphataemia which is likely to reflect the different degrees of hypocalcaemia and consequent secondary hyperparathyroidism for the three dosage groups.

Effects of exogenous PTH on TmP/GFR

The fall in TmP/GFR induced by exogenous administration of human PTH in 2 etidronate treated patients is shown in Figure 4.10. The presence of this continued renal tubular responsiveness to PTH, even following very high intravenous doses of diphosphonates, indicates that at least part of the hyperphosphataemic response to clodronate and AHDP may be masked by the observed concomitant secondary hyperparathyroidism. In order to examine the effects of these agents given intravenously in the absence of changes in PTH status 4 patients with hypoparathyroidism were studied.

Effects in hypoparathyroid subjects

In all 3 hypoparathyroid patients treated with clodronate 300mg/day for 5 days serum phosphate and TmP/GFR became markedly increased during treatment (Figure 4.11). The extent of these increases was comparable to that seen following etidronate treatment and much greater than that observed in pagetic patients

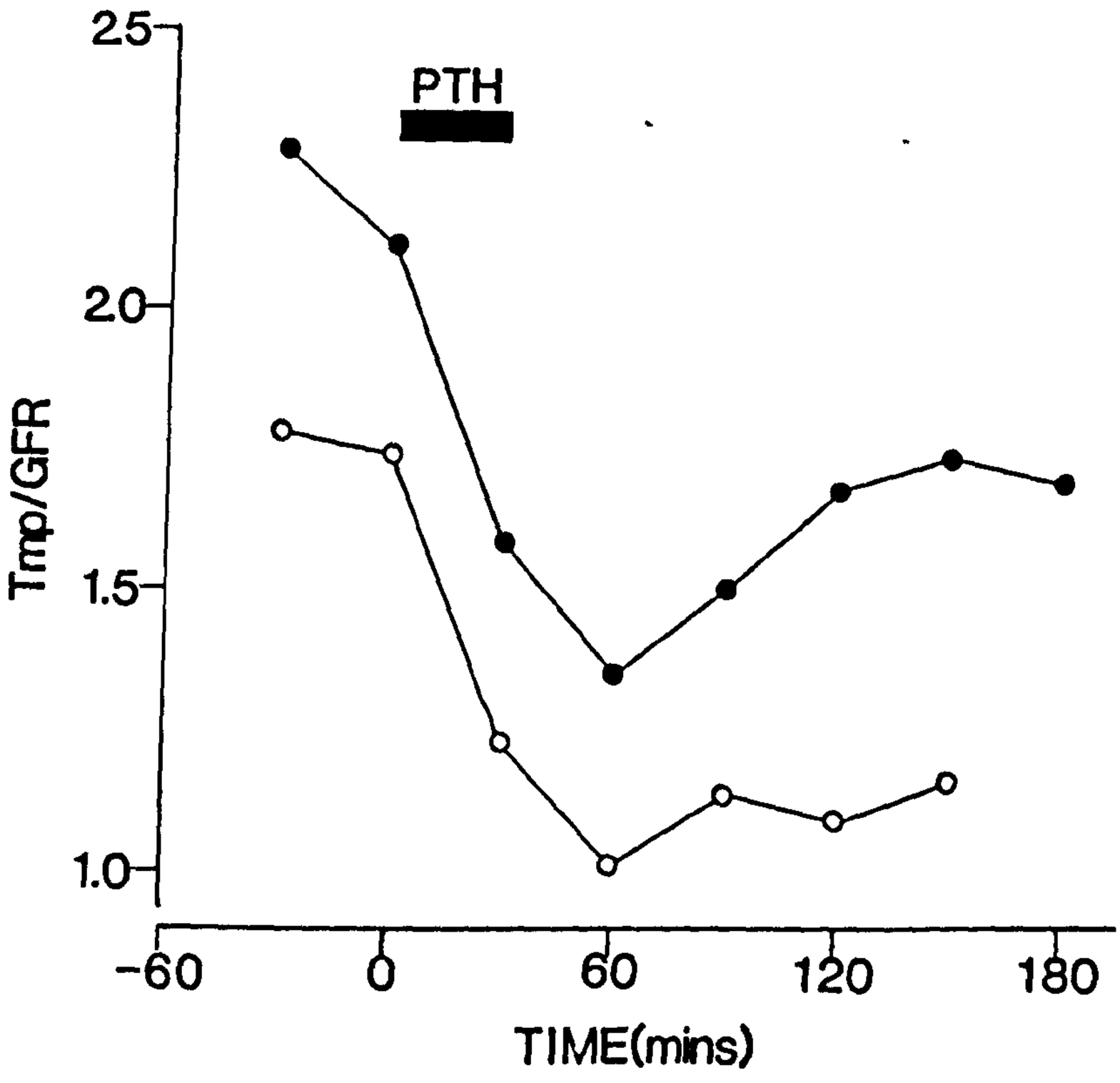


Figure 4.10 Acute changes in TmP/GFR in 2 patients with hyperphosphataemia due to treatment with intravenous etidronate 7.5mg/kg in response to exogenous PTH 200U.

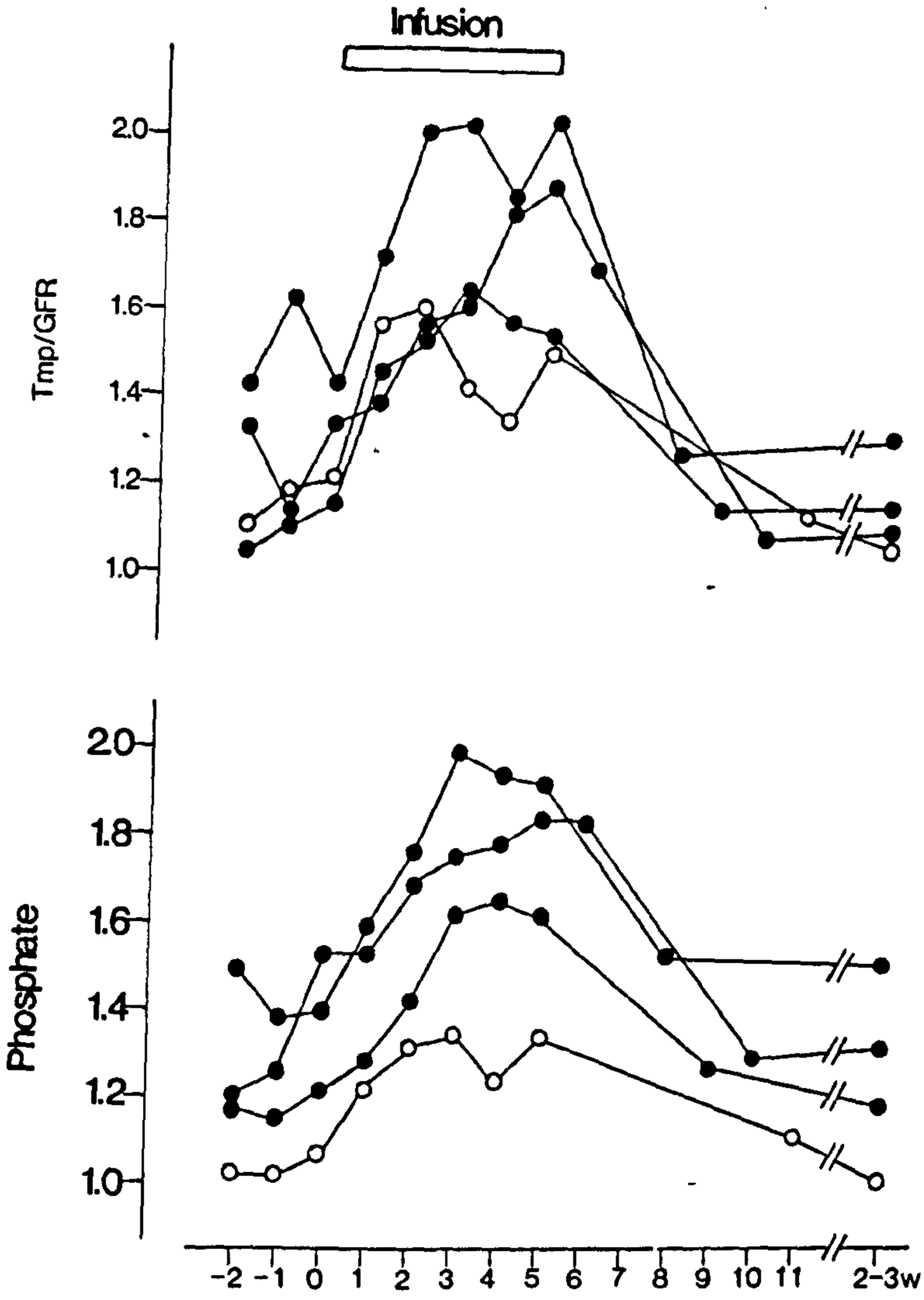


Figure 4.11 Changes in serum phosphate and TmP/GFR in 4 hypoparathyroid subjects treated with daily 3h intravenous infusions of either clodronate 300mg/day (closed circles) or AHDP 50mg/day (open circles) for 5 days.

given the same dose of intravenous clodronate. The single hypoparathyroid subject treated with intravenous AHDP also had increases in both phosphate and TmP/GFR although these changes were less marked than those seen in the other 3 patients. However, both from the increase in iPTH observed in this subject (Figure 4.12) and the initial serum phosphate, which was within the normal range, it seems likely that she possessed some residual parathyroid function which may have partially offset the effect of AHDP on TmP/GFR.

Discussion

This study demonstrates major differences in calcaemic and calciuric effects of intravenous etidronate treatment compared with those of the other diphosphonates studied. These findings are similar to those of previous studies using oral diphosphonates in pagetic patients in which no change in serum calcium has been noted following etidronate treatment (Khairi et al, 1974; Russell et al, 1974) but both clodronate and APD treatments have hypocalcaemic effects (Frijlink et al,1979; Douglas et al, 1980). Such marked differences between diphosphonates could have important implications when these drugs are being used for their hypocalcaemic effects, for example in the treatment of hypercalcaemia

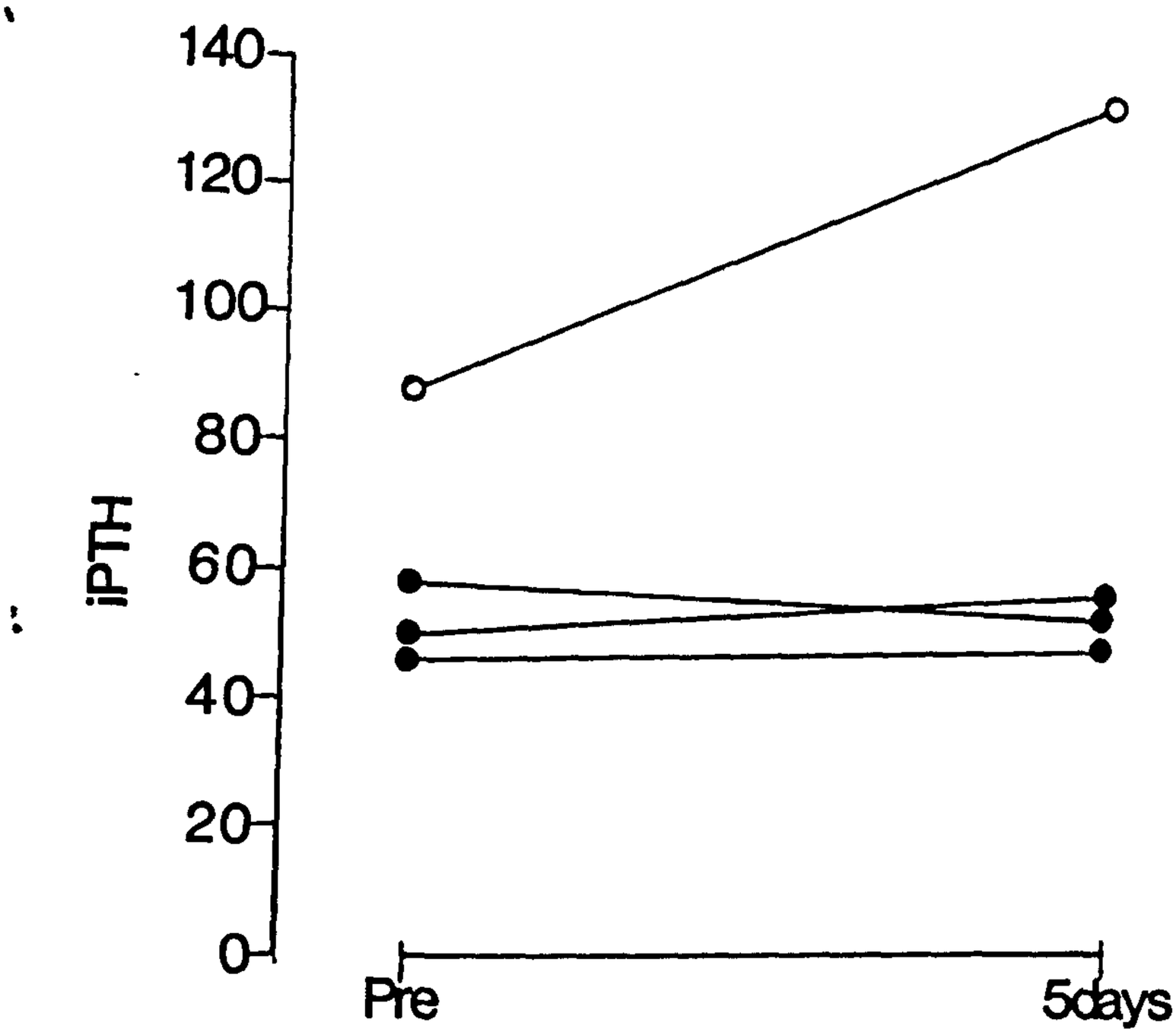


Figure 4.12 Serum concentrations of iPTH before and at the end of diphosphonate treatment in 4 hypoparathyroid subjects. The pretreatment value was in the mid- normal range in the 1 patient treated with AHDP (open circles) and increased following treatment suggesting that there was at least some parathyroid activity.

of malignancy which is examined in Chapter 5.

In the present study changes in urinary calcium excretion were in the same direction as changes in serum calcium in all groups. Thus, although renal tubular transport of calcium has not been studied directly, it is unlikely that differences in calcaemic response were caused by differences in diphosphonate effects on renal handling of calcium. All three diphosphonates studied produced a marked early suppression of bone resorption followed by a later fall in bone formation (Chapter 3). These changes would be expected to result in a period of positive net bone balance and thus net entry of calcium into bone and a tendency towards hypocalcaemia. However, the present study indicates that etidronate treatment results in a profound and sustained inhibition of bone mineralisation, and thus calcium entry into bone, and this probably accounts for the lack of hypocalcaemic response and the slight tendency towards hypercalcaemia in this group. Figure 4.13 represents diagramatically the changes in calcium fluxes between bone and the ECF that are likely to occur in response to these treatments.

The measurements of MiAR show that both clodronate and AHDP can also induce significant impairment of bone mineralisation, a finding which has not previously been reported in man. In the case of clodronate, this appears to be a dose-dependent effect. Such an effect may account for the significant increases

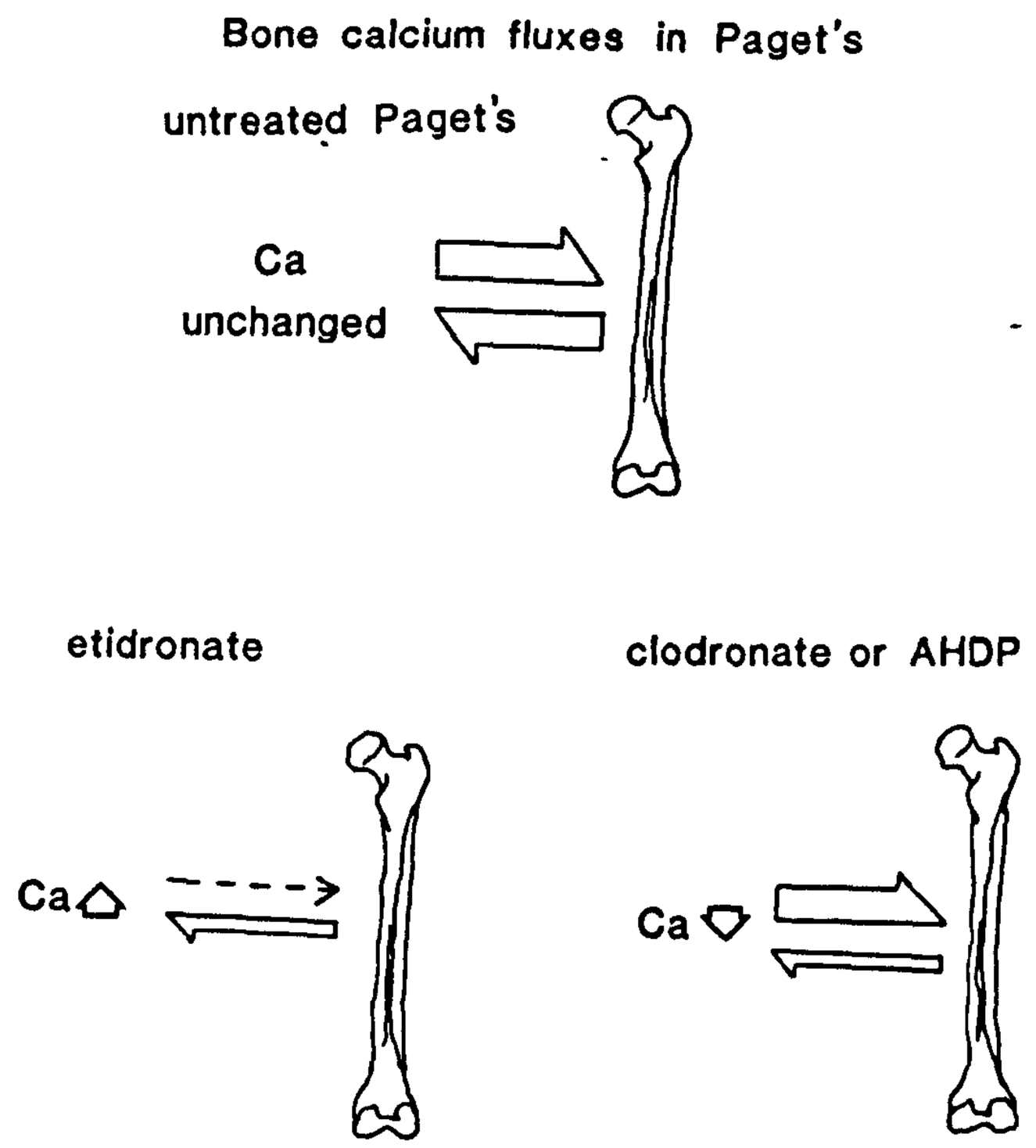


Figure 4.13 Schematic diagram of changes in calcium fluxes following diphosphonate treatment of Paget's disease. Inhibition of bone resorption results in net calcium entry into bone following clodronate or AHDP whereas this net flux is reversed following etidronate due to complete inhibition of mineralisation.

in serum calcium and urinary Ca/Cr at day 1 noted in the clodronate 300mg and 600mg groups. Thus it may be inadvisable to give very high intravenous doses of clodronate in the treatment of patients with hypercalcaemia of malignancy as impaired skeletal uptake of mineral at such doses may potentially offset the hypocalcaemic effect.

The presence of a tetracycline label administered during treatment with clodronate indicated that mineral accretion into bone continued throughout the treatment period albeit at a reduced rate. This notion is supported by the hypocalcaemic response to clodronate treatment which suggests that there was a net flux of calcium entering bone. Although not studied directly, in view of the early hypocalcaemic response to AHDP continued mineralisation would also be expected in patients treated with this agent. These findings suggest that urinary Ca/Cr is insufficiently sensitive to be used as a screen to detect modest degrees of impairment of mineralisation following diphosphonates.

The present study demonstrates that hyperphosphataemia is not a unique feature of etidronate treatment but occurs when other diphosphonates are given in sufficient dosage. As previously mentioned, mild and transient increases in serum phosphate and TmP/GFR have been observed following the use of a high oral dose of APD (600mg/day) in the treatment of Paget's disease

(Nagant de Deuxchaisnes et al, 1982). In that study there was some radiographic evidence for treatment induced focal impairment of mineralisation.

Recker and colleagues (1973) have previously demonstrated unimpaired phosphaturic and urinary cAMP responses to exogenously administered PTH during oral etidronate-induced hyperphosphataemia in normal subjects. This observation has been extended in the present study to patients on intravenous etidronate treatment in whom the systemic dose may be as much as 20 times higher. These data suggest that a competitive mechanism, blocking the PTH mediated effect to reduce renal tubular reabsorption of phosphate, to cause the observed increase in TmP/GFR must be unlikely. In view of this continued responsiveness of renal tubular phosphate transport to PTH, much of the difference in degree of hyperphosphataemia between diphosphonates may be accounted for by the observed differences in calcaemic and PTH responses. When the effects of changes in PTH were removed, as in the hypoparathyroid subjects and in etidronate treated patients in whom iPTH remained unchanged, the full hyperphosphataemic effects of the diphosphonates became apparent.

However, despite similar increments in serum phosphate and TmP/GFR in the hypoparathyroid group compared with the etidronate treated Paget's patients there were marked differences in duration of responses.

Thus, serum phosphate and TmP/GFR had returned to normal within 1 week of stopping clodronate or AHDP, whereas these measurements remained maximally increased at 1 week following etidronate and only returned to pretreatment values after 1 month. It seems unlikely that the difference in time course is a result of fundamentally different mechanisms for the production of the hyperphosphataemic effect. The sustained effect following etidronate, which like other diphosphonates is rapidly taken up into bone or eliminated in the urine, in pagetic patients would appear to mitigate against a direct effect of the drug on the renal tubule to enhance phosphate transport. The time course of the hyperphosphataemic response to etidronate in hypoparathyroid patients has not been studied, but might be expected to be similar to that in pagetic patients in view of the lack of any significant change in PTH in the pagetic group.

One possible explanation for the different TmP/GFR effects of the various diphosphonates is that there may be a causal relationship between the impaired mineralisation and the renal tubular effects. Thus, the short-lived impairment of mineralisation with intravenous clodronate or AHDP treatment coincides with the transient hyperphosphataemia whereas both effects are sustained following cessation of etidronate treatment. As further circumstantial evidence for this hypothesis, whereas

neither increased phosphate nor defective mineralisation are commonly observed in patients treated with low dose etidronate (5mg/kg/day), higher doses usually produce both features (Recker et al, 1973; Russell et al,1974). Patients given the lower dose who have histological evidence for impaired mineralisation also develop hyperphosphataemia (Boyce et al, 1984). The relationship appears sufficiently close that the hyperphosphataemic response may in fact be a useful marker for measuring the dose requirements of etidronate for individual patients (Kanis, 1984b).

From a teleological viewpoint the production in bone of a factor in response to changes in mineralisation which could regulate renal phosphate handling might be beneficial for the control of the mineralisation process. However, on present evidence the existence of such a factor must remain entirely speculative.

Because of relatively wide fluctuations in serum phosphate in response to dietary phosphate loads it is often assumed that there is no specific homeostatic mechanism for the regulation of ECF phosphate concentrations. However, evidence from many studies using phosphate deprivation in both animals (Trohler et al, 1976) and man (Lotz et al, 1968; Dominguez et al,1976) indicates the existence of a rapid and highly sensitive mechanism for near complete conservation of inorganic phosphate by the kidney prior to any

significant change in serum inorganic phosphate. Conversely, TmP/GFR has been shown to fall in response to a dietary phosphate challenge in man (Bijvoet & Morgan, 1971). These homeostatic changes may result in part from a renal autoregulatory mechanism (Caverzasio et al, 1985). However, it is likely that humoral regulatory mechanism(s) also exist (Agus & Garrick, 1984). Whereas acute responses to phosphate depletion appear not to involve new protein synthesis, more prolonged increases in phosphate transport *in vitro* is inhibited by actinomycin D, an inhibitor of protein synthesis (Dousa et al, 1980) suggesting the presence of more than one regulatory mechanism.

Several hormones, including PTH, $1,25(OH)_2D_3$, sex steroids, corticosteroids and growth hormone, are known to induce changes in TmP/GFR (Steele et al, 1975). However, none of these appears to have a primary role in phosphate homeostasis, at least in the rat (Bonjour et al, 1978). Thus the existence of a specific, as yet unknown, phosphate regulatory hormone or hormones may usefully be invoked to account for the observed homeostatic changes in renal handling of phosphate.

A major difficulty in further study in this area arises because of wide interspecies variations in terms of phosphate responses to various probes. Thus whereas in man, and probably also in dogs and rabbits, etidronate induces an increase in inorganic phosphate the opposite

effect is observed in rats (Muhlbauer et al, 1981). There are also marked interspecies differences in calcaemic response to etidronate and in responses to phosphate deprivation. Studies to examine the effects of human bone cell culture media on phosphate transport in a pig renal epithelial cell line (LLC-PK1) are planned (Bevington, personal communication) but negative results in such a cross-species experiment obviously cannot exclude the presence of a bone derived phosphate regulatory factor in man. Whatever the mechanism responsible for diphosphonate-induced hyperphosphataemia, further investigation into this effect is likely to provide useful information about this poorly understood area of mineral metabolism.

Summary

1. The effects of daily intravenous infusions of three diphosphonates (etidronate, clodronate and AHDP for 5 days) on calcium and phosphate homeostasis were studied in patients with Paget's disease of bone.

2. Treatment with clodronate (100-600mg/day) and AHDP (25-50mg/day) induced hypocalcaemia and secondary hyperparathyroidism (increased iPTH). These changes were not observed with etidronate (300-700mg/day) despite similar degrees of suppression of bone resorption with

each of the three treatments.

3. The degree of the hypocalcaemic response in clodronate treated patients correlated significantly with the fall in urinary OHP/Cr suggesting that the fall in serum calcium was due to the inhibition of calcium efflux from bone. This relationship was not observed following etidronate treatment.

4. A dose/response relationship was found for intravenous clodronate in terms of the hypocalcaemic response reflecting the greater suppression of bone resorption induced with higher doses.

5. Intravenous etidronate induced marked increases in serum phosphate and TmP/GFR which remained significantly increased for 1 month after stopping intravenous treatment. Clodronate and AHDP induced smaller but significant increases in serum phosphate and TmP/GFR although these changes were ill-sustained and followed by decreases in both measurements.

6. Normal phosphaturic responses to exogenously administered PTH occurred in 2 patients with etidronate induced hyperphosphataemia.

7. In 4 hypoparathyroid subjects, intravenous clodronate

or AHDP produced increases in serum phosphate and TmP/GFR of comparable degree to, but shorter duration than, those seen following etidronate treatment.

8. Bone mineral apposition rates (MiAR), obtained from triple tetracycline labelled bone biopsies, fell from high to normal values following intravenous clodronate or AHDP but consistently decreased to zero both during treatment with etidronate and for at least 7 days thereafter.

9. In conclusion, clodronate and AHDP inhibit bone resorption with only minimal effects on mineralisation. These actions account for the hypocalcaemic response, secondary hyperparathyroidism and, in part, for changes in phosphate metabolism in pagetic patients.

10. There is an apparent relationship between diphosphonates effects to impair mineralisation and their effects to increase TmP/GFR suggesting the possibility of a bone/kidney link for regulation of phosphate homeostasis.

11. Despite similar effects on bone resorption, etidronate halts mineralisation, and this accounts for the attenuated effects on serum calcium.

12. Differences in the potencies of different diphosphonates to impair mineralisation relative to their effects on bone resorption may make etidronate less suitable than either clodronate or AHDP in the treatment of hypercalcaemic disorders.

SECTION THREE

THE USE OF DIPHOSPHONATES IN HYPERCALCAEMIC DISORDERS

CHAPTER FIVE

INTRAVENOUS DIPHOSPHONATES IN THE TREATMENT OF HYPERCALCAEMIA OF MALIGNANCY

INTRAVENOUS DIPHOSPHONATES IN THE TREATMENT OF HYPERCALCAEMIA OF MALIGNANCY

Introduction

The hypercalcaemia of malignancy is often marked, rapidly progressive and may be associated with severe symptoms. Treatment with intravenous saline, which increases renal elimination of calcium, is usually sufficient to halt the rise in serum calcium, but used alone saline treatment often fails to reduce serum calcium to normal (Hosking et al, 1981). Excessive bone resorption is the major mechanism responsible for hypercalcaemia in the majority of patients (see Chapter 1) and, therefore, inhibitors of bone resorption may be useful. Of the available agents calcitonin is often only partially effective and the usefulness of mithramycin is limited by liver and bone marrow toxicity caused by this agent. Diphosphonates, because of their potent and specific effects to inhibit bone resorption and their general lack of toxicity, may be very useful in the treatment of malignant hypercalcaemia. A small study of the effects of oral etidronate, the only generally available form of diphosphonate, in malignant hypercalcaemia found this agent to be effective in only 1 of the 4 patients treated (Mundy et al, 1983).

Similarly, oral etidronate was found to be ineffective in reducing calcium in patients with primary hyperparathyroidism (Kaplan et al, 1977; see chapter 6).

Treatment with clodronate or APD by mouth has been shown to be effective in normalising serum calcium (Chapuy et al, 1980; Douglas et al, 1980; Paterson et al, 1983; van Breukelen et al, 1979) or reducing net bone loss in patients with malignant disorders (Jung et al, 1983; Siris et al, 1980). However, the high doses required to ensure sufficient absorption are commonly associated with gastrointestinal side effects and thus may be poorly tolerated by some patients. Impaired absorption of diphosphonates from the intestine might also occur particularly in dehydrated or cachectic patients or in those on chemotherapy. Use of the intravenous route of administration avoids such problems and the much higher systemic loading achieved by this route might be expected to result in a more rapid or a more consistent suppression of excess bone resorption than occurs with oral administration.

Jacobs and colleagues (1981) using intravenous clodronate induced normocalcaemia in 11 of 12 treated hypercalcaemic patients. Intravenous APD has also been used with normalisation of calcium in 29 of 30 patients (Sleeboom et al,1983). However, hypercalcaemia persisted in "about half" of the patients treated with APD studied by Ralston and colleagues (1985). In a comparative

study, Jung and coworkers (1982) noted no difference in ultimate hypocalcaemic effect between patients given intravenous treatment with either etidronate or clodronate at doses ranging from 100 to 500mg per day. However, they did observe a greater delay by several days in the hypocalcaemic effect of etidronate compared with the response to clodronate. More recently Ryzen et al (1985) have reported normalisation of serum calcium in 19 of 26 patients treated with intravenous etidronate for 1 to 4 days. However, the mean serum calcium for their patients remained above the normal range because of the poor response of some patients. Thus, the available evidence is insufficient to determine whether there are real differences in therapeutic efficacy between these agents. An intravenous preparation of etidronate may soon become commercially available and thus it is important to know how the efficacy of intravenous etidronate compares with that of other diphosphonates.

Differences in the effects of the various diphosphonates in pagetic patients, examined in Chapters 3 and 4, suggest that some may be more suiTable than others for the treatment of malignant hypercalcaemia. These studies indicated that the hypocalcaemic effect of intravenous etidronate was attenuated due to the associated impairment of mineralisation whereas both clodronate and AHDP induced significant hypocalcaemia. Therefore, the aim of the present study was to compare

directly the hypocalcaemic responses to the three diphosphonates, etidronate, clodronate and AHDP, given intravenously to patients with hypercalcaemia of malignancy. The doses used were those which had been found to suppress bone resorption in pagetic patients.

In addition, control of serum calcium with the use of diphosphonates, which specifically inhibit bone resorption, facilitates an examination of the importance of renal tubular mechanisms in the genesis of hypercalcaemia in these patients. In particular, recent evidence has suggested that a humoral PTH-like mechanism to increase renal tubular reabsorption of calcium may be important in patients with solid tumours with or without metastases (Ralston et al, 1982, 1984; Percival et al, 1985b). For these reasons I have examined renal tubular reabsorption of calcium in patients with solid tumours and compared them with patients with haematological malignancies in order to investigate this mechanism.

Methods

48 hypercalcaemic episodes in 44 patients with malignant disease were studied. 17 patients had myeloma (20 episodes), 1 patient had a non-Hodgkins lymphoma (2 episodes), 13 had carcinoma of the breast, 4 had bronchial carcinoma whereas the remaining 9 patients had a variety of solid tumours (see Table 5.1). All patients

remained hypercalcaemic following at least 48 hours of continuous intravenous infusion of saline. A further 7 patients were excluded from the study on the basis of a fall in serum albumin concentration of more than 4g/l after the commencement of diphosphonate treatment suggesting that there was continued intravascular volume expansion. Informed consent was obtained either from the patient or, when their state of consciousness did not permit this, from a first degree relative. This study had prior approval of the local Ethics Committee. Fasting blood and urine samples were collected daily throughout the period of study and routine biochemical measurements were made as outlined in Chapter 2.

27 patients were treated with clodronate at doses of between 100 and 600mg daily for between 3 and 10 days. 13 patients received etidronate 100-500mg daily. AHDP was given to 8 patients at a dose of either 25mg (2 patients) or 50mg (6 patients) for 5 or 6 days (see Table 5.1). These drugs were added to 0.5l of saline and infused over a minimum period of 3 hours. Continuous infusion of physiological saline 3l daily was maintained either until the patients became normocalcaemic or at least for the duration diphosphonate treatment.

Results

Comparison of diphosphonate treatments

Table 5.1 I PATIENTS WITH HAEMATOLOGICAL MALIGNANCY

	Diagnosis	Age	Sex	Drug	Dose (mg)	Duration (days)	Serum calcium (mmol/l) Pre	5 days	7 days
1	Non-Hodgkins lymphoma	45	M	Cl2MDP	100	5	3.91	2.61	2.34
2	Myeloma	63	M	Cl2MDP	100	10	3.71	-	2.53
3	Myeloma	45	F	Cl2MDP	300	3	3.00	2.67	-
4	Myeloma	41	F	Cl2MDP	300	4	3.68	2.35	2.35
5	Myeloma	45	F	Cl2MDP	300	5	2.87	-	2.63
6	Myeloma	66	F	Cl2MDP	300	5	3.07	2.58	2.50
7	Myeloma	63	M	Cl2MDP	300	5	3.42	2.53	2.47
8	Myeloma	70	F	Cl2MDP	300	5	2.84	2.24	2.33
9	Myeloma	47	M	Cl2MDP	300	5	3.08	2.51	-
10	Myeloma	35	F	Cl2MDP	300	5	3.41	2.62	2.67
11	Myeloma	66	F	Cl2MDP	600	5	2.65	2.21	-
12	Myeloma	46	M	AHDP	25	5	3.07	2.54	2.31
13	Myeloma	47	M	AHDP	50	5	3.46	2.88	2.94
14	Myeloma	77	F	AHDP	50	5	2.84	2.09	1.95
15	Myeloma	59	M	AHDP	50	5	3.15	2.31	2.23
16	Myeloma	66	M	EHDP	500	5	2.88	2.54	2.51
17	Myeloma	55	M	EHDP	100	6	3.14	3.14	2.96
18	Myeloma	48	M	EHDP	500	5	3.08	2.79	2.85
19	Myeloma	59	F	EHDP	500	7	3.23	2.74	2.58
20	Myeloma	44	F	EHDP	200	3	3.11	3.05	-
21	Myeloma	55	F	EHDP	100	5	3.81	3.17	-
22	Non-Hodgkins lymphoma	46	M	EHDP	500	3	4.22	3.13	-

(continued over)

Table 5.1 (cont.) II PATIENTS WITH SOLID TUMOURS

	Diagnosis	Age	Sex	Drug	Dose (mg)	Duration (days)	Serum calcium (mmol/l) Pre	5 days	7 days
23	Breast Ca	62	F	Cl2MDP	100	5	3.42	2.50	-
24	Bronchial Ca	64	M	Cl2MDP	100	5	3.09	2.71	2.53
25	Breast Ca	53	F	Cl2MDP	100	8	3.50	2.57	2.15
26	Breast Ca	48	F	Cl2MDP	100	5	3.01	2.08	2.10
27	Parotid Ca*	64	M	Cl2MDP	100	5	3.19	3.13	-
28	Breast Ca*	41	F	Cl2MDP	300	5	4.00	2.72	2.31
29	Breast Ca	52	F	Cl2MDP	300	5	3.85	2.56	2.33
30	Primary unknown	69	M	Cl2MDP	300	5	3.34	2.50	2.19
31	Breast Ca	54	F	Cl2MDP	300	5	3.51	2.78	-
32	Breast Ca	49	F	Cl2MDP	300	6	3.12	2.27	2.23
33	Breast Ca	50	F	Cl2MDP	300	4	3.63	2.82	2.69
34	Post cricoid	62	F	Cl2MDP	300	5	3.44	2.38	2.19
35	Breast Ca	44	F	Cl2MDP	300	7	3.36	2.12	2.27
36	Ca cervix*	72	F	Cl2MDP	300	5	3.63	2.56	2.58
37	Breast Ca	46	F	Cl2MDP	300	5	3.34	2.37	-
38	Ovarian Ca	51	F	Cl2MDP	300	5	3.86	2.60	2.54
39	Bronchial Ca*	56	M	AHDP	25	5	3.00	2.52	2.41
40	Breast Ca	65	F	AHDP	50	5	2.64	2.08	-
41	Cholangio-Ca	66	M	AHDP	50	5	3.01	2.44	-
42	Hypernephroma	61	M	AHDP	50	5	3.44	2.70	2.66
43	Breast Ca	59	F	EHDP	200	5	3.60	2.69	3.33
44	Breast Ca	48	F	EHDP	500	3	3.09	-	2.82
45	Hepatoma	66	M	EHDP	500	3	3.32	-	4.13
46	Bronchial Ca	77	F	EHDP	450	4	2.90	2.78	2.72
47	Bronchial Ca	64	M	EHDP	200	5	3.66	3.21	3.22
48	Primary unknown	71	M	EHDP	200	6	3.39	2.87	2.85

* Denotes patients without evidence of skeletal metastases.

Changes in serum albumin concentration provided a guide to the adequacy of intravascular volume expansion. Mean values for albumin for all patients combined fell by 2.0g/l in the 48 hours preceding the start of diphosphonate infusion. No significant changes in serum albumin occurred following the start of diphosphonate treatment in any of the three treatment groups (Table 5.2) suggesting that intravascular volume expansion did not change markedly and was likely to be adequate in all groups.

Figure 5.1 shows the fall in serum calcium in the 3 treatment groups. The groups were well matched for the degree of hypercalcaemia at the start of diphosphonate treatment and mean concentrations of serum calcium had been sTable over the preceding 48 hours in each group. This stability of calcium concentrations despite continuous intravenous saline suggests that the majority of patients had reached a new steady state for calcium. A significant hypocalcaemic response was achieved within 2 days of the start of diphosphonate treatment in all three groups. Mean values for serum calcium fell to normal within 5 days in clodronate and AHDP treatment groups but remained above normal following etidronate treatment. Thus, whereas 24 of the 27 clodronate treatments (89%) and 6 of the 8 AHDP treatments (75%) resulted in normocalcaemia this was

Table 5.2 COMPARISON OF DIPHOSPHONATES IN HYPERCALCAEMIA OF MALIGNANCY

	Drug	Time since start of treatment (days)			
		-2	0	7	14
Serum	EHDP	3.31	3.34	3.00	2.92*
		(0.13)	(0.11)	(0.15)	(0.11)
calcium	Cl2MDP	3.45	3.37	2.42***	2.85*
		(0.10)	(0.07)	(0.04)	(0.13)
(mmol/l)	AHDP	3.19	3.08	2.39***	2.51*
		(0.10)	(0.10)	(0.12)	(0.13)
Serum	EHDP	0.96	0.99	1.17	1.14
		(0.08)	(0.07)	(0.13)	(0.15)
phosphate	Cl2MDP	1.09**	1.03	0.92	1.13
		(0.08)	(0.08)	(0.08)	(0.08)
(mmol/l)	AHDP	1.26	1.10	0.84	0.91
		(0.15)	(0.09)	(0.13)	(0.13)
Alkaline	EHDP	130	140	161	214
		(109-154)	(119-164)	(131-197)	(161-283)
phosphatase	Cl2MDP	121	116	131*	137
		(106-137)	(104-129)	(117-148)	(118-160)
(U/l)	AHDP	115	125	162	174
		(97-136)	(105-148)	(122-215)	(134-226)
Serum	EHDP	130	122	121	105
		(21)	(21)	(28)	(27)
creatinine	Cl2MDP	136	129	111*	92
		(17)	(15)	(19)	(10)
(umol/l)	AHDP	248	218	154	118
		(85)	(56)	(26)	(16)

*p<0.05 **p<0.01 ***p<0.0001 from pretreatment values on 2-tailed paired t-testing. Parentheses contain either SEM or range +/- SEM (for log-transformed data)

(continued over)

Table 5.2 (continued)

	Drug	Time since start of treatment (days) -2	0	7	14
Serum	EHDP	28.3	28.0	26.6	27.0
		(1.3)	(1.2)	(1.6)	(1.9)
albumin	Cl2MDP	33.6**	30.1	31.3	33.1
		(1.5)	(1.1)	(1.4)	(2.9)
(g/l)	AHDP	36.2*	32.7	34.7	34.2
		(1.8)	(1.6)	(1.7)	(2.3)
Urinary	EHDP	1.41	1.23	0.70	0.64
		(0.54)	(0.31)	(0.21)	(0.17)
Ca/Cr	Cl2MDP	1.56*	1.77	0.43**	0.63**
		(0.48)	(0.19)	(0.17)	(0.14)
(mol/mol)	AHDP	0.58	1.04	0.20	0.38
		(0.36)	(0.29)	(0.08)	(0.19)
Urinary	EHDP	69.5	69.7	57.9	62.1
		(56-86)	(59-82)	(43-78)	(46-84)
OHP/Cr	Cl2MDP	63.4**	81.3	40.9*	65.2
		(50-81)	(74-89)	(33-50)	(52-81)
(mmol/mol)	AHDP	60.8	64.6	32.9*	42.5
		(54-69)	(60-70)	(27-40)	(32-56)

*p<0.05 **p<0.01 ***p<0.0001 from pretreatment values on 2-tailed paired t-testing. Parentheses contain either SEM or range +/- SEM (for log-transformed data).

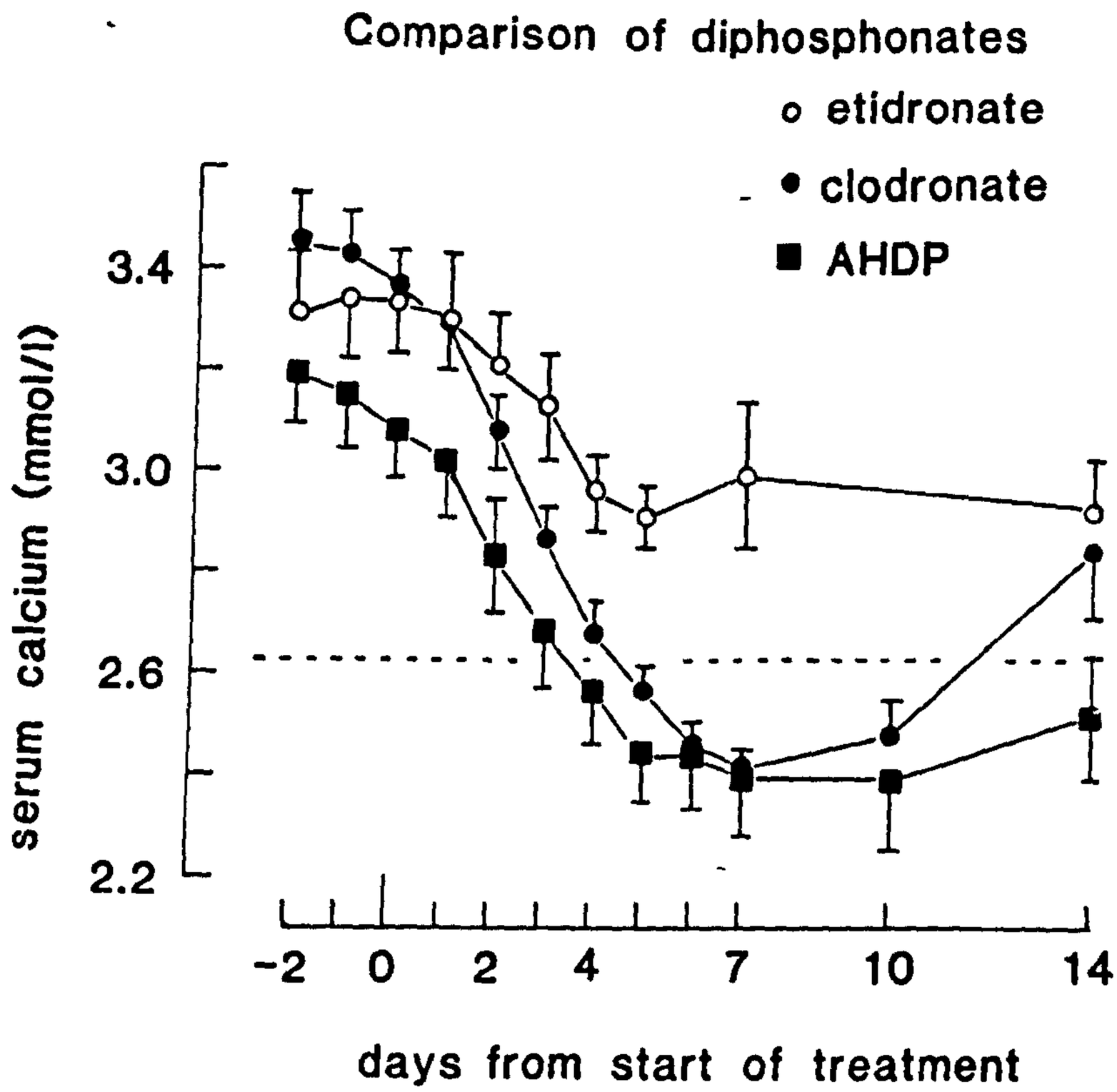


Figure 5.1 Effects of three diphosphonates given intravenously for 3 to 10 days on serum calcium in patients with hypercalcaemia of malignancy.

achieved in only 2 of the 13 episodes treated with etidronate (15%). This difference in response rate between etidronate and the other 2 treatments achieved statistical significance using Fisher's exact test ($p<0.001$).

Serum calcium in the etidronate group remained high even at 14 days (mean 2.92mmol/l). Mean serum calcium remained suppressed in the AHDP treated group but showed a secondary increase by 14 days in the clodronate group. It is not clear whether this late difference reflects true differences between clodronate and AHDP in duration of effect or is merely due to differences in the patient populations.

Changes in fasting urinary Ca/Cr were similar to those observed in serum calcium for each of the three groups (see Figure 5.2, Table 5.2). The lack of an early fall in Ca/Cr in the etidronate group suggests that net calcium release from bone remained high during etidronate treatment. In contrast, net calcium loss from bone, as judged by Ca/Cr, was decreased by both clodronate and AHDP treatment. However, the fall in Ca/Cr in the AHDP treated group did not reach statistical significance because of the low number of observations. Despite the difference in effects of the diphosphonates on calcium metabolism, bone resorption, as judged by urinary OHP/Cr, decreased significantly and by similar amounts in all three treatment groups (Figure 5.3).

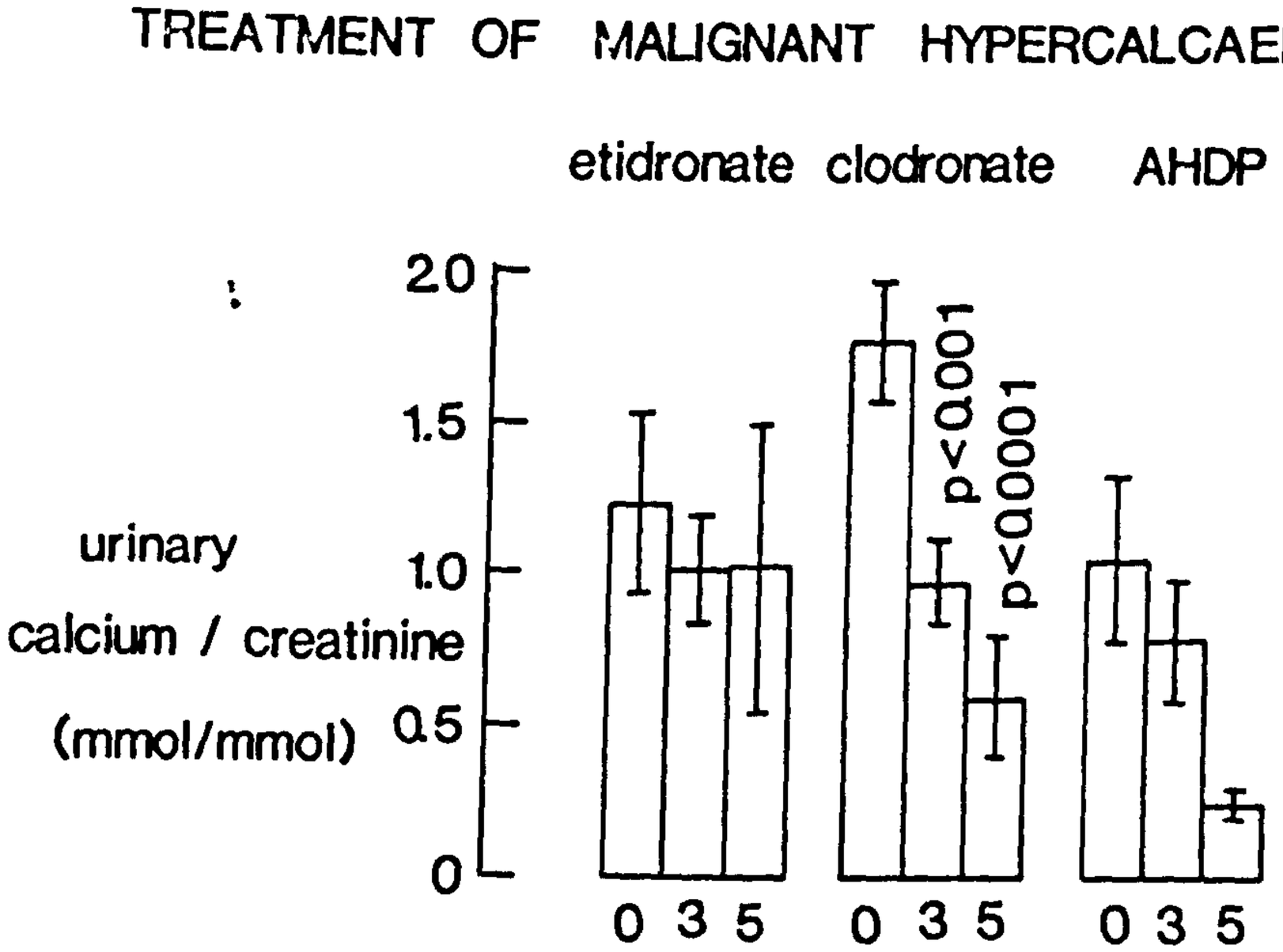


Figure 5.2 Effects of intravenous treatment with etidronate, clodronate or AHDP on fasting urinary Ca/Cr in patients with hypercalcaemia of malignancy.

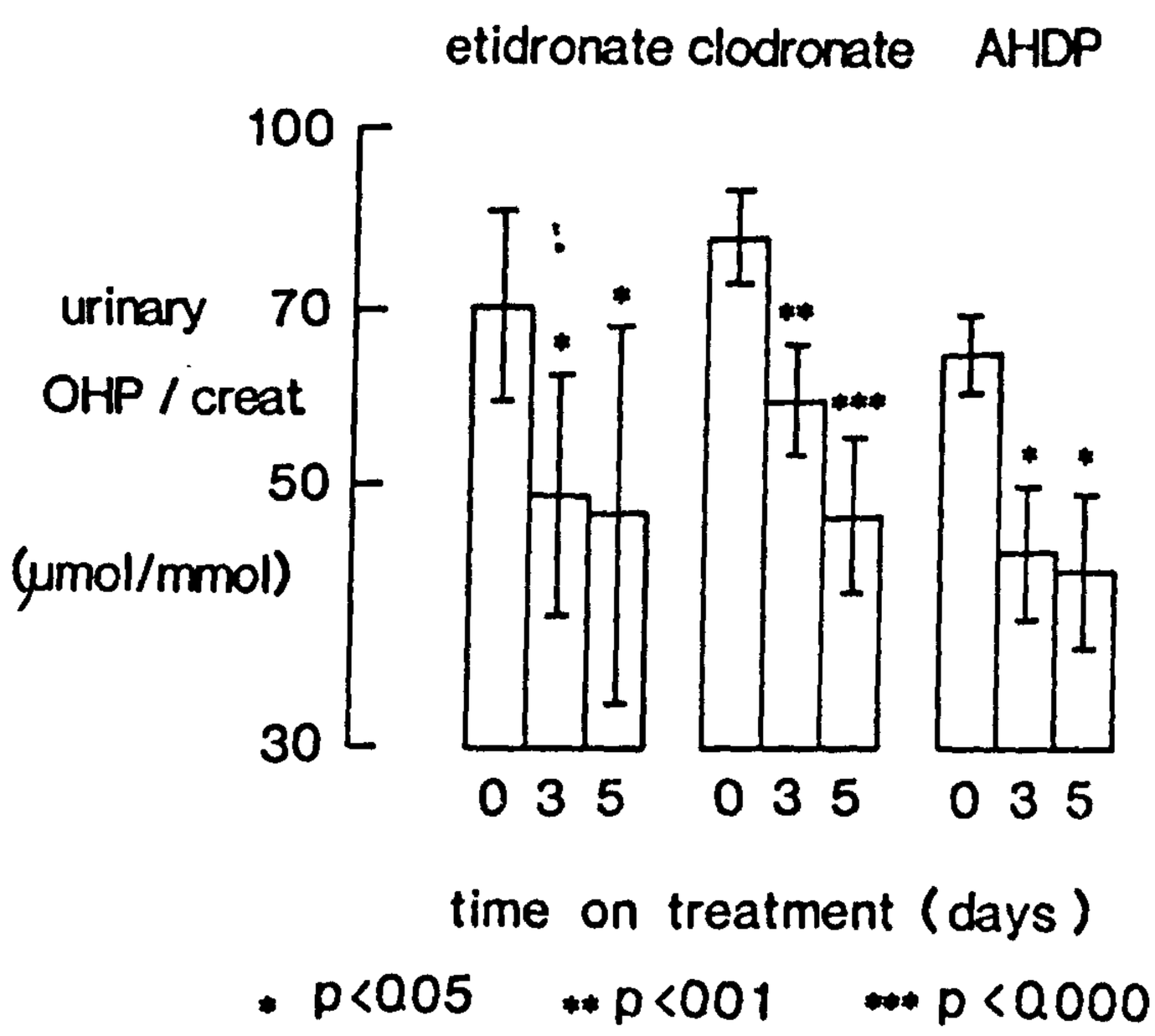


Figure 5.3 Effects of three diphosphonates on urinary OHP/Cr in patients with hypercalcaemia of malignancy.

Other biochemical changes are shown in Table 5.2. There were no significant changes in serum phosphate in any group, although mean values increased following etidronate and decreased following the other two diphosphonates. Serum creatinine fell in all 3 groups probably reflecting the fall in serum calcium with consequent increases in GFR in each group. The mean pretreatment serum creatinine concentration in the AHDP group was particularly high because of the inclusion of one patient with myeloma (No. 13) who had severe renal impairment (creatinine 688umol/l immediately before the start of diphosphonate treatment). 10 days after the start of treatment serum creatinine in this patient had fallen to 137umol/l. Although serum albumin did not change significantly following the start of treatment in any group, mean values were lowest in the etidronate group (Table 5.2).

Comparison of responses in haematological malignancies and solid tumours

Patients with haematological malignancy and those with solid tumours were well matched for the degree of hypercalcaemia before treatment. In response to treatment with either clodronate or AHDP highly comparable falls in serum calcium were seen in the two groups (Figure 5.4). In addition, both the mean

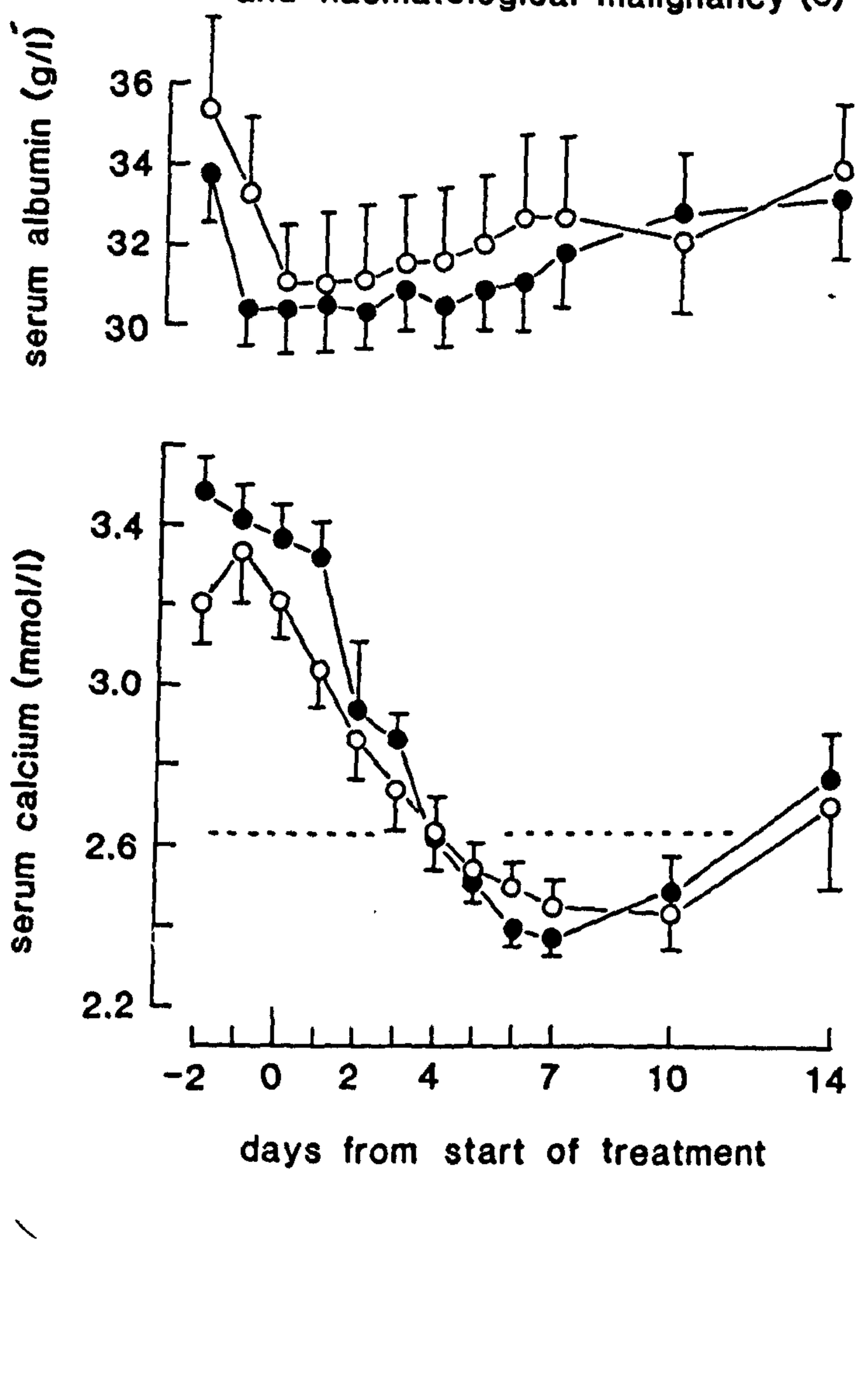


Figure 5.4 Comparison of changes in serum albumin and serum calcium between patients with haematological malignancy and those with solid tumours.

pretreatment values of urinary Ca/Cr and urinary OHP/Cr and the degree to which these measurements were suppressed following treatment was very similar in patients with haematological malignancy and in the solid tumour group (Table 5.3).

Whereas in 4 of the solid tumour patients given clodronate or AHDP serum calcium remained above the normal range this also occurred in 3 patients with myeloma. Of the 4 patients with solid tumours but no evidence of metastases (see Table 5.1) only 1 failed to become normocalcaemic. Thus separation of patients with different types of malignancy or on the basis of presence or absence of metastases was not useful in predicting degree of response to treatment.

Fasting urinary calcium excretion per unit of glomerular filtrate (CaE), both before treatment and after 5 to 10 days, were plotted against serum calcium for individual patients with haematological malignancy (Figure 5.5) or solid tumours (Figure 5.6). There was considerable heterogeneity, in terms of renal tubular reabsorption of calcium, present in both types of malignancy but this was greater in solid tumour patients 4 of whom had markedly increased renal tubular reabsorption of calcium before the start of diphosphonate treatment. However, renal tubular reabsorption of calcium remained markedly increased in only one patient following treatment (Figure 5.6). This man, who had a

Table 5.3 COMPARISON OF HAEMATOLOGICAL MALIGNANCY AND SOLID TUMOURS

	Type of malignancy	Time since start of treatment (days) -2	0	7	14
Serum calcium (mmol/l)	Haem.	3.20 (0.10)	3.21 (0.09)	2.45*** (0.07)	2.70*** (0.21)
	Solid	3.48 (0.09)	3.35 (0.09)	2.37*** (0.10)	2.77*** (0.12)
Serum phosphate (mmol/l)	Haem.	1.32 (0.12)	1.16 (0.10)	1.02 (0.09)	1.13 (0.09)
	Solid	1.01 (0.09)	0.96 (0.09)	0.83 (0.10)	1.00 (0.12)
Alkaline phosphatase (U/l)	Haem.	87 (76-99)	90 (82-99)	102 (93-112)	94 (83-107)
	Solid	158 (137-182)	160 (139-182)	190* (160-227)	212* (180-249)
Serum creatinine (umol/l)	Haem.	208 (55)	183 (38)	149 (33)	115 (17)
	Solid	126 (12)	124 (14)	98 (11)	86 (8)

*p<0.05 **p<0.01 ***p<0.0001 from pretreatment values on paired t-testing.
Patients were treated with either clodronate or AHDP.

(continued over).

Table 5.3 (continued)

	Malignancy type	Time since start of treatment (days) -2	0	7	14
Serum albumin (g/l)	Haem.	35.4** (2.4)	31.1 (1.4)	32.8 (2.0)	34.0 (1.9)
	Solid	33.7* (1.2)	30.4 (1.2)	31.9 (1.4)	33.3 (1.5)
Urinary Ca/Cr (mol/mol)	Haem.	1.76 (0.75)	1.57 (0.22)	0.21** (0.05)	0.54 (0.26)
	Solid	1.14 (0.44)	1.62 (0.24)	0.55 (0.26)	0.56 (0.10)
Urinary OHP/Cr (mmol/mol)	Haem.	76.2 (61-95)	77.6 (68-88)	38.9** (32-47)	70.5 (49-100)
	Solid	59.8 (49-71)	76.6 (69-84)	38.0* (30-49)	51.7* (42-64)

*p<0.05 **p<0.01 ***p<0.0001 from pretreatment values on paired t-testing.
Patients were treated with either clodronate or AHDP.

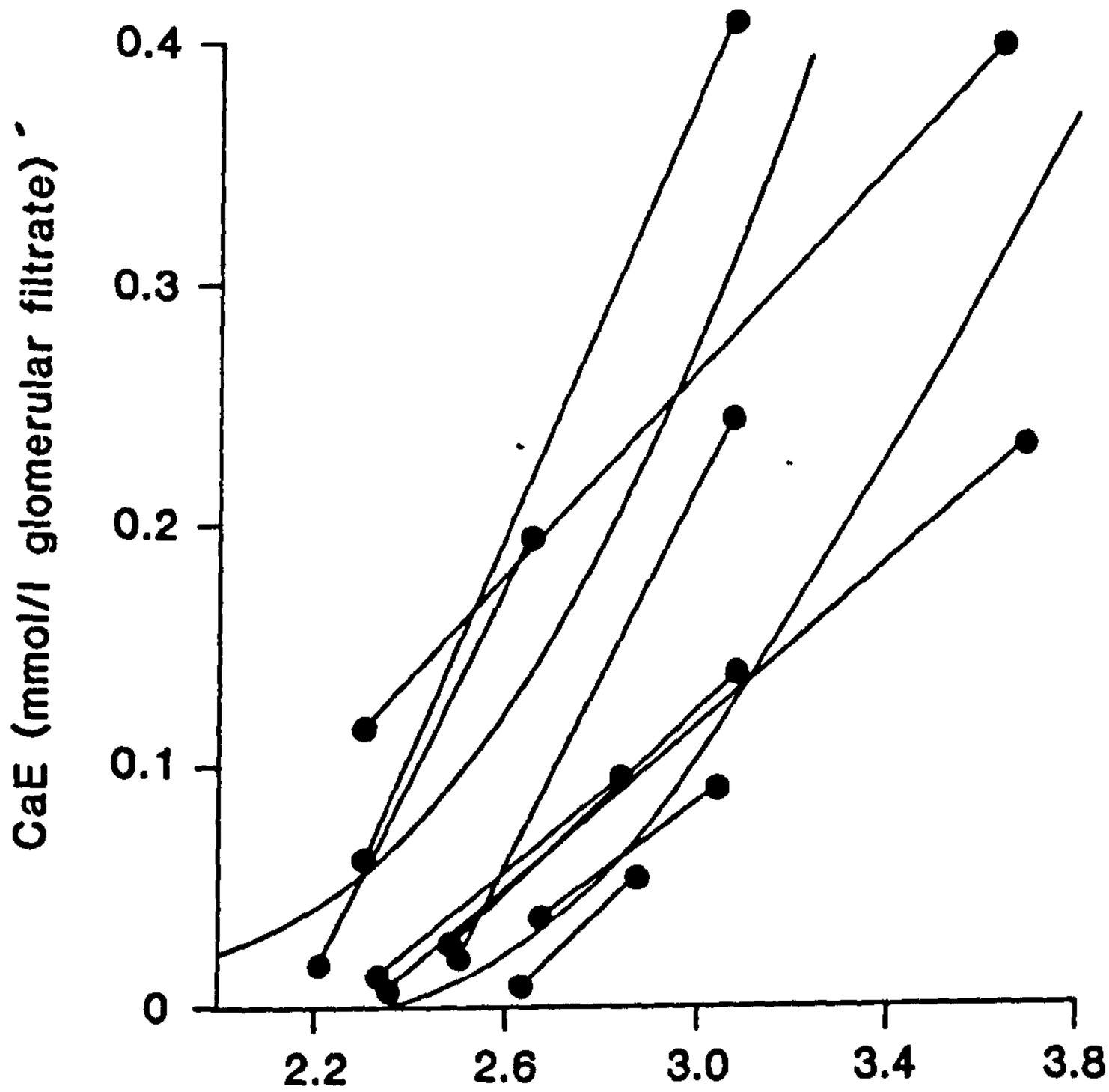


Figure 5.5 Effects of treatment with either clodronate or AHDP on CaE and serum calcium in patients with hypercalcaemia due to haematological malignancy. In the majority of patients the relationship between these measurements was close to normal (denoted by the curved lines) both before and after diphosphonate treatment.

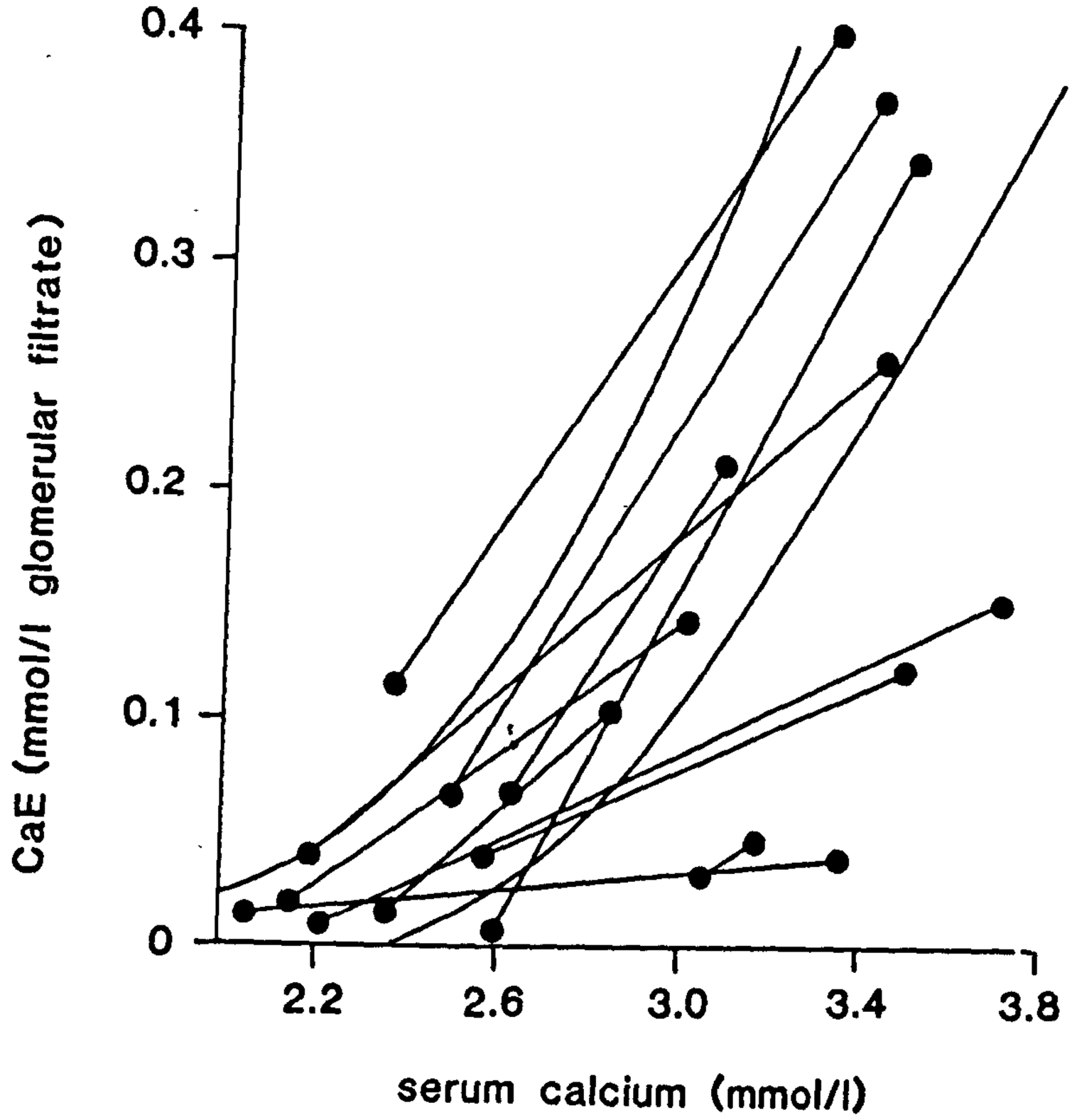


Figure 5.6 Changes in the relationship between CaE and serum calcium in patients with hypercalcaemia due to solid tumours following treatment with clodronate or AHDP. In 3 of 4 patients with increased renal tubular reabsorption of calcium before treatment (indicated by a shift to the right from the normal relationship) this returned to normal following treatment.

parotid carcinoma without evidence of skeletal metastases, had a negligible response to clodronate treatment. Bone resorption in this man, as judged by urinary OHP/Cr, was within the normal range and thus the hypercalcaemia in this case appears to have been entirely due to a PTH-like effect to enhance renal tubular reabsorption of calcium. iPTH was undetecTable and it is thus assumed that his tumour was producing a PTH-like humoral factor.

Mean responses (+/- SEM) for the two diagnostic groups are shown in Figure 5.7. Note that whereas renal tubular reabsorption of calcium is, on average, higher in the solid tumour group at the start of treatment, this difference is lost by the end of treatment when mean values for both groups fell within the reference limits.

Other biochemical changes are shown in Table 5.3. It is noteworthy that serum alkaline phosphatase increased in the solid tumour group following treatment. However, this is likely to reflect increasing liver dysfunction, rather than an increase in osteoblast activity, as it was accompanied by a rise in gamma-glutamyl transpeptidase from a mean of 115U/l to 172U/l at 14 days.

Clinical observations

No side effects of diphosphonate treatment were

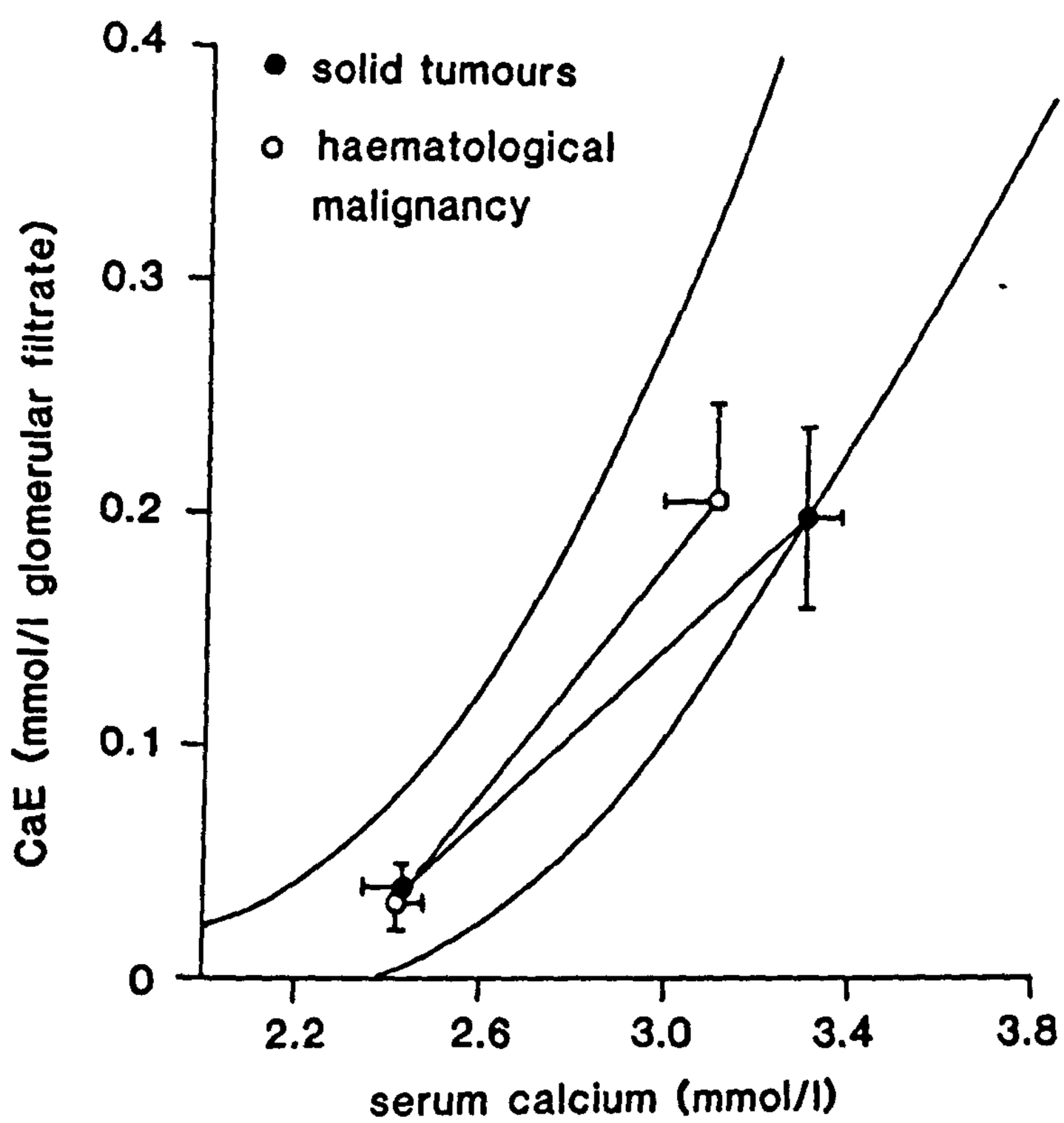


Figure 5.7 Mean values (+/- SEM) for CaE and serum calcium in patients with either solid tumours or haematological malignancy before and after treatment with diphosphonates.

noted in any of the treatment groups. Symptomatic improvement occurred in the majority of patients and was related to the reduction in serum calcium and improved hydration.

Discussion

In this study the efficacies of three diphosphonates were compared directly in well matched groups of patients with malignant hypercalcaemia. By ensuring a minimum period of 48 hours of rehydration and excluding 7 patients who had an excessive subsequent fall in serum albumin, differences between groups due to rehydration effects were minimised. The stable concentrations of serum calcium before the start of diphosphonate treatment and the time course of the hypocalcaemic response, which did not become marked until 2 days after the start of treatment, suggest that the observed effects were predominantly related to the diphosphonate treatment rather than to the effects of saline. However, we have previously found that continuous saline treatment alone may be associated with a progressive fall in serum calcium over several days and thus may have accounted for part of the observed hypocalcaemic responses (Percival et al, 1984).

In the current study the hypocalcaemic effect of intravenous etidronate, either in terms of mean calcium

concentrations or in numbers of patients achieving normocalcaemia, was significantly less marked than that seen with the other two agents. This attenuated response was not due to a lesser effect to inhibit bone resorption as urinary OHP/Cr fell to a similar degree following all three treatments. By analogy to the differential effects of these three diphosphonates given intravenously to patients with Paget's disease reported in Chapter 4, it is likely that the lesser hypocalcaemic response to etidronate is due to the impaired mineralisation and thus impaired entry of calcium into bone induced by this agent. This was not verified directly because of the difficulties in obtaining tetracycline labelled bone biopsies from patients with acute episodes of hypercalcaemia of malignancy. Further evidence that etidronate is a less effective hypocalcaemic agent than clodronate is provided by studies of the oral use of these agents in patients with primary hyperparathyroidism reported in Chapter 6. In this study oral clodronate, but not oral etidronate, induced a significant hypocalcaemic response.

The time course of the hypocalcaemic responses to diphosphonates in the present study, in which little change had occurred at 1 day, contrasts with that observed in the study by Ryzen and colleagues (1985) in which there was an early marked fall in serum calcium following treatment with etidronate. In their study

normocalcaemia was achieved in 19 of the 26 patients studied which compares with only 2 of 13 patients etidronate treated patients in the present study. Such an early fall in calcium in their patients is likely to have been due to the effects of saline infusion rather than of the diphosphonate. Thus inadequate pretreatment with saline may have accounted for the apparent more marked hypocalcaemic response to etidronate in their study than was observed in the present study.

We have recently reported continued increased tubular reabsorption of calcium and a low TmP/GFR in patients with hypercalcaemia due to carcinoma of the breast suggesting that a PTH-like renal effect may be important (Percival et al, 1985b). Other authors have made similar observations (Ralston et al, 1984) suggesting that even in patients with bony metastases, humorally mediated increases in renal tubular reabsorption of calcium may be an important mechanism for producing hypercalcaemia. Furthermore, biochemical studies of patients with sold tumours have noted increased excretion of cAMP in the urine suggesting the possible presence of a PTH-like factor (Rude et al, 1981; Stewart et al, 1980). However, if renal tubular reabsorption of calcium remained elevated serum calcium would be expected to fall only to around the upper end of the normal reference range after diphosphonate treatment as has been observed in patients with primary

hyperparathyroidism (Douglas et al, 1983; also see Chapter 6). In support of this hypothesis, in a separate study in which clodronate was given by mouth we observed only a partial hypocalcaemic response in solid tumour patients (Percival et al, 1985a).

Thus the lack of any solid demonstrable differences in response between patients with haematological malignancies and those with solid tumours and also between patients with metastases and those with no evidence of metastases was unexpected. The reason for this is unclear but may be largely a result of patient selection. The present study demonstrates that patients with solid tumours in particular show highly heterogeneous responses with only a proportion having clearly increased renal tubular bone resorption after 48 hours of rehydration with intravenous saline. In some cases tubular handling of calcium appears to have normalised after normocalcaemia had been induced by diphosphonate treatment. It may be that the nephrogenic diabetes insipidus that accompanies hypercalcaemia results in inadequate rehydration despite the precautions taken to try to ensure this. Following normalisation of serum calcium this tubular defect is likely to improve (Levi et al, 1983) and thus renal handling of calcium might also be expected to return to normal.

Another factor of possible relevence is the tendency for greater impairment of renal glomerular

function in myeloma patients compared with patients with solid tumours. This is reflected by differences in serum creatinine in the two groups (Table 5.3). For a given fluid throughput an increased fluid load per nephron will occur in a patient with a reduced GFR and because of increased sodium delivery this might be expected to cause a relative reduction in renal tubular reabsorption of calcium. Such a mechanism may account, in part, for the observed differences in renal handling of calcium between the two groups before diphosphonate treatment.

Only one of the patients in the present study appeared to have a true PTH-like effect to increase renal calcium reabsorption. The type of solid tumour studied appears to have some bearing on the degree to which renal tubular reabsorption of calcium is increased. Thus in the study of Ralston and colleagues (1984), in which nearly all patients had evidence of increased renal tubular reabsorption of calcium, the majority of tumours were either squamous carcinomata or cancers of the genito-urinary system. By contrast, Hosking and coworkers (1981), whose patient group contained mainly patients with breast cancer or myeloma, found CaE entered the normal range in the majority of cases following rehydration. A relationship between these differing effects on renal calcium transport and the production of factors which bind to PTH receptors (other than PTH) as described by Strewler (1983) would seem likely but

further work in this area is required.

The results of this study suggest that both clodronate and AHDP are highly effective treatments for malignant hypercalcaemia when given intravenously in conjunction with saline rehydration. Their use appears to be problem free and either agent may be conveniently added to the standard intravenous saline rehydration regimen. The short duration of these diphosphonate effects would suggest that longer-term maintenance treatment with oral diphosphonates should be considered, particularly in patients in whom the underlying malignant disease is not amenable to treatment. A recent study has suggested that long-term oral clodronate significantly reduces the development of new metastases and hypercalcaemia in women with carcinoma of the breast (Elomaa et al, 1983). We are currently involved in the design of two multi-centre trials to assess the value of such long-term treatments in patients with myeloma and in those with carcinoma of breast. Using a combination of intravenous diphosphonates for the acute management of hypercalcaemic episodes and long-term oral treatment for prophylaxis of hypercalcaemia and pathological fracture it is hoped to be able to substantially reduce the morbidity due to skeletal involvement in malignancy.

Summary

1) The effects of three diphosphonates given intravenously to patients with hypercalcaemia of malignancy were studied. Comparisons were made between treatment groups and also between patients with haematological malignancies and those with solid tumours.

2) Following at least 48 hours of treatment with intravenous physiological saline, patients were given etidronate (n=13), clodronate (n=27) or AHDP (n=8) by daily slow intravenous infusion for 3 to 10 days.

3) Mean serum calcium fell to normal in both clodronate and AHDP treated groups (2.42mmol/l and 2.39mmol/l respectively at 7 days) but remained elevated in the etidronate treated group (3.00mmol/l) despite comparable suppression of bone resorption, as judged by the fall in urinary OHP/Cr. Only 2 of the 13 etidronate treated patients became normocalcaemic compared with 24 of 27 clodronate treatments and 6 of 8 treatments with AHDP.

4) Changes in urinary Ca/Cr were in the same direction as changes in serum calcium for each group indicating that differences in calcaemic response were unlikely to be due to differential effects of diphosphonates on renal tubular reabsorption of calcium.

5) By analogy with the calcaemic responses in Pagetic

patients, the less marked hypocalcaemic response to etidronate is probably related to the associated impairment of mineralisation and thus of entry of calcium into bone.

6) As was noted in Pagetic patients treated with intravenous diphosphonates, hypocalcaemic responses did not become marked until 2 days from the start of treatment suggesting that these were due to the diphosphonates rather than to the effects of intravenous saline.

7) Before diphosphonate treatment a proportion of patients with solid tumours had increased renal tubular reabsorption of calcium. However, there were no significant differences in response between patients with haematological malignancies and those with solid tumours in the degree of suppression of serum calcium, urinary Ca/Cr or OHP/Cr or in renal tubular reabsorption of calcium following treatment with clodronate or AHDP. Thus a sustained PTH-like effect on renal tubular reabsorption of calcium in a substantial proportion of patients with solid tumours could not be confirmed.

8) Both clodronate and AHDP given intravenously are effective treatments in the management of hypercalcaemia of malignancy.

CHAPTER SIX

COMPARATIVE STUDY OF CLODRONATE AND HIGH OR LOW DOSES OF ETIDRONATE BY MOUTH IN THE TREATMENT OF PRIMARY HYPERPARATHYROIDISM

COMPARATIVE STUDY OF CLODRONATE AND HIGH OR LOW DOSES OF ETIDRONATE BY MOUTH IN THE TREATMENT OF PRIMARY HYPERPARATHYROIDISM

Introduction

Hypercalcaemia in patients with primary hyperparathyroidism is a result of the effect of PTH on the three major organs involved in calcium homeostasis, i.e. bone, gut and kidney. As discussed in Chapter 1, probably the most important mechanism for sustaining an increased serum calcium is the PTH-induced increased renal tubular reabsorption of calcium. Bone turnover is modestly increased in the majority of patients but marked bone loss is less common. Thus treatment with inhibitors of bone resorption might not be expected to completely normalise serum calcium in these patients. This is borne out by studies using oral clodronate in which mean serum calcium values fell close to, but not within, the normal range (Douglas et al, 1983; Shane et al, 1981). Similar effects have been observed with the use of APD (van Breukelen et al, 1982) although mean values did fall into the normal range with this treatment probably reflecting lower pretreatment values (mean calcium before treatment 2.83mmol/l).

In contrast, in a study by Kaplan and colleagues (1977) using etidronate 20mg/kg in 6 hyperparathyroid

patients no significant change in serum calcium was seen despite a significant fall in total urinary hydroxyproline excretion. However, the small number of patients in this study meant that a significant hypocalcaemic effect of etidronate in primary hyperparathyroidism could not be excluded. The lack of hypocalcaemic response to high-dose oral etidronate was ascribed to the likely mineralisation defect although this was not examined directly (Kaplan et al, 1977). In a report of a single case of primary hyperparathyroidism Licata and O´Hanlon (1983) found that low dose etidronate (400mg/day) induced a reproducible hypocalcaemic effect. Such a low dose of etidronate might suppress resorption while having only minimal effects to impair skeletal mineralisation and thus may produce a greater hypocalcaemic effect than higher doses of this agent. In the present study both high and low doses of etidronate by mouth have been used and effects on calcium metabolism compared with those following clodronate treatment. In addition, the effects of long-term treatment with oral clodronate were examined.

Patients and methods

A total of 29 patients with primary hyperparathyroidism were studied. 5 patients received more than one course of treatment. 14 patients were treated

with a total of 15 courses of oral clodronate 0.8-1.6g/day (1 patient was re-treated). 9 patients received etidronate 400mg/day whereas 10 patients were given etidronate 1.0-1.2g/day. Details of patients and treatment are given in Table 6.1. All patients gave informed consent. The study had been approved by the local Ethics Committee. All treatments were given continuously as a single daily dose taken on waking with water only. Routine biochemical measurements on fasting blood and urine samples were carried out as described in Chapter 2.

Results

Treatment with clodronate

Serum calcium fell significantly from a mean of 2.95mmol/l before treatment to 2.77mmol/l at 2 months from the start of clodronate treatment (Figure 6.1). A significant decrease was also observed in fasting urinary Ca/Cr which fell from initially high values (mean 0.55mol/mol) to within the normal range (mean 0.27mol/mol at 2 months: NR 0.1-0.4mol/mol). There was an early fall in urinary OHP/Cr which remained suppressed for the duration of clodronate treatment. In contrast, an apparent attenuation of both the hypocalcaemic and hypocalciuric effects of treatment occurred after 3 to 4

Table 6.1

PATIENTS WITH PRIMARY HYPERPARATHYROIDISM TREATED WITH ORAL CLODRONATE

Treatment number	Initials	Age	Sex	Dose (g)	Duration (months)	Pretreatment values Alk.Phos (U/l)	OHP/Cr (mmol/mol)	calcium (mmol/l)	calcium at 1 month (mmol/l)
1	MC	61	F	1.6	4.5	82	45.3	2.78	2.40
2	AG	44	F	1.2	11	129	25.3	3.11	3.00
3	JO	38	F	0.8	2	50	14.1	2.75	2.62
4	RG	63	M	1.6	2	206	63.2	2.94	3.14
5	SA	61	F	1.6	1.5	83	36.8	2.77	2.62
6	BB	60	F	1.6	1	137	25.0	3.14	3.11
7	BB	73	F	1.0	7	137	25.0	3.14	3.11
8	MT	69	F	1.2	6	77	35.5	2.74	2.63
9	MH	68	F	1.6	5.5	69	33.0	2.82	2.68
10	IG	68	F	0.8	3	105	24.3	2.79	2.67
11	EM	63	F	1.6	2.5	121	54.6	2.64	2.53
12	VP	72	F	1.6	7	76	15.4	3.00	2.75
13	MB	70	F	1.0	2	128	69.3	3.16	2.56
14	EH	66	F	0.8	4	239	65.7	3.31	3.31
15	RG	64	M	1.6	5	193	67.0	3.40	2.89

(continued over)

Table 6.1 (continued)

PATIENTS WITH PRIMARY HYPERPARATHYROIDISM TREATED WITH ETIDRONATE

Treatment number	Patients initials	Age	Sex	Dose (g)	Duration (months)	Pretreatment values Alk.Phos (U/l)	OHP/Cr (mmol/mol)	calcium (mmol/l)	calcium at 1 month (mmol/l)
Low-dose etidronate									
16	MT	62	F	0.4	3.5	89	22.2	2.68	2.72
17	EM	57	F	0.4	8	81	40	2.77	2.62
18	GJ	47	F	0.4	3	120	22.3	3.45	3.49
19	IS	56	F	0.4	1.5	99	28.2	2.67	2.70
20	IB	68	F	0.4	2	109	38.2	3.28	3.22
21	GI	69	F	0.4	4	55	41.2	2.80	2.80
22	CG	78	M	0.4	2	171	45.5	2.98	3.30
23	LH	58	F	0.4	4	169	47.3	2.95	2.94
24	ES	78	F	0.4	3	66	46.4	2.57	2.72
High-dose etidronate									
25	BB	72	F	1.0	6	141	36.0	3.17	3.18
26	NC	69	F	1.0	3	56	23.9	2.87	2.95
27	MC	62	F	1.0	2	79	21.9	2.63	2.60
28	IG	67	F	1.0	6	123	22.8	2.96	2.51
29	EH	65	F	1.0	4	557	113.0	3.30	3.12
30	FJ	52	F	1.0	3	97	39.0	2.72	2.70
31	JO	39	F	1.0	2	77	46.0	2.85	2.76
32	BP	50	F	1.0	3.5	83	35.2	2.90	2.76
33	BP	64	F	1.2	1.5	103	30.6	2.74	2.82
34	EM	64	F	1.2	4	99	22.2	2.81	2.73

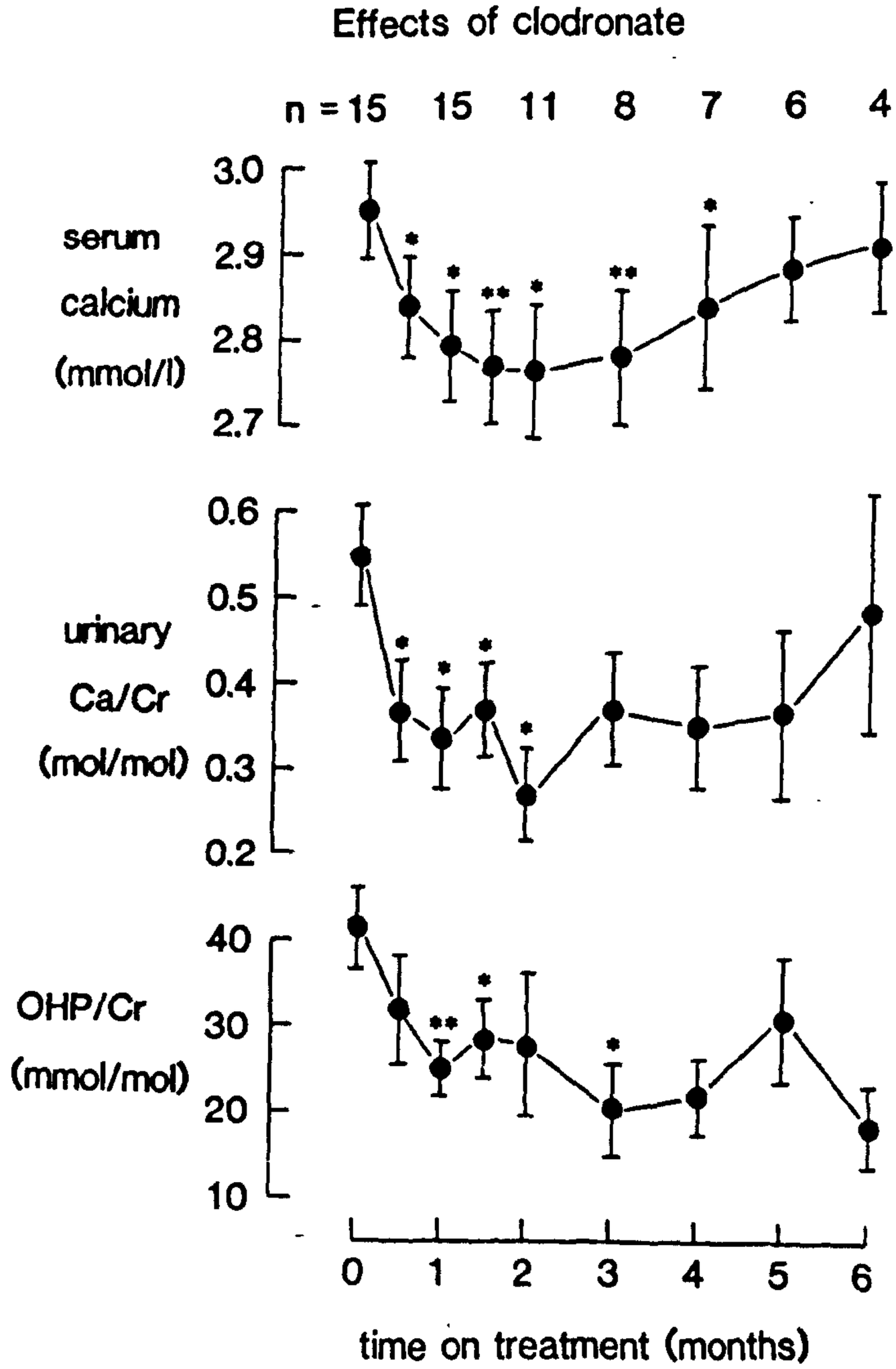


Figure 6.1 Changes in serum calcium, urinary Ca/Cr and OHP/Cr during treatment in patients with primary hyperparathyroidism treated continuously with oral clodronate.

months of treatment (Figure 6.1). This is further demonstrated in Figure 6.2 which shows the individual responses of the 6 patients treated for at least 5 months. In 4 of these 6 patients (patients 8,9,12 and 15 see Table 6.1) the maximum hypocalcaemic effect was achieved within 2 months and this was followed by a steady upward trend in calcium values. However, in the other 2 patients (Nos 2 and 7) serum calcium fell but no secondary increase was observed.

These data would suggest that the hypocalcaemic effect resulted from the inhibition of bone resorption and transient net calcium accretion into bone similar to the effect observed in patients with Paget's disease of bone treated with diphosphonates (Chapter 4). The observed attenuation of the hypocalcaemic response could thus be explained by a "recoupling" of the rates of bone formation and resorption. However, only a modest fall in serum alkaline phosphatase occurred (pretreatment mean 122U/l to 103U/l at 6 months; $p>0.1$) and thus "recoupling" could not be confirmed biochemically. However, in one patient with tertiary hyperparathyroidism due to chronic renal failure who was treated with AHDP for 20 weeks biochemical recoupling was clearly evident (Figure 6.3). In this patient attenuation of the hypocalcaemic response coincided with the secondary fall in serum alkaline phosphatase.

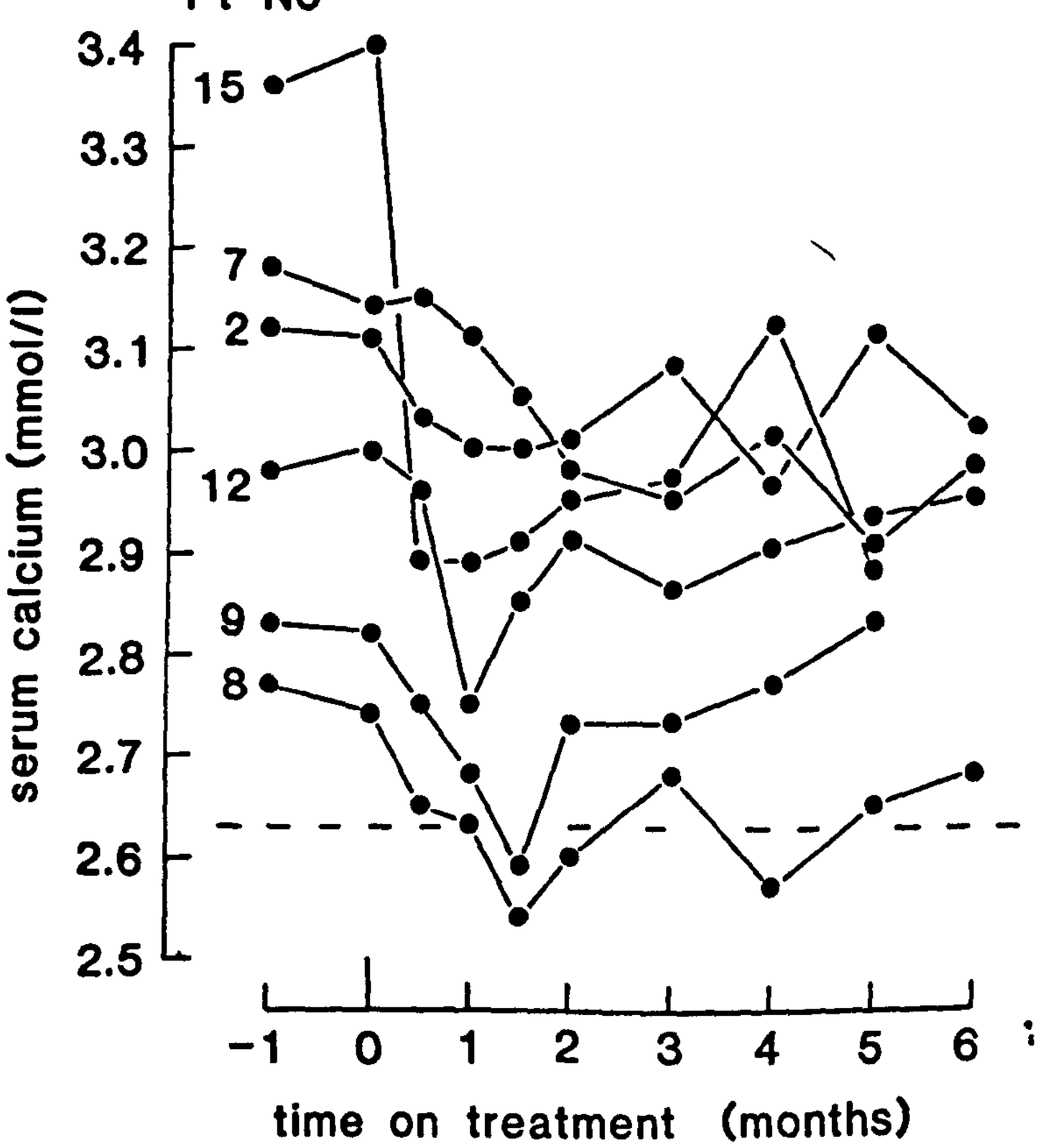


Figure 6.2 Changes in serum calcium in 6 patients with primary hyperparathyroidism treated with oral clodronate for 5 months or longer. Attenuation of the hypocalcaemic response occurred after 2 months in 4 of the 6 patients.

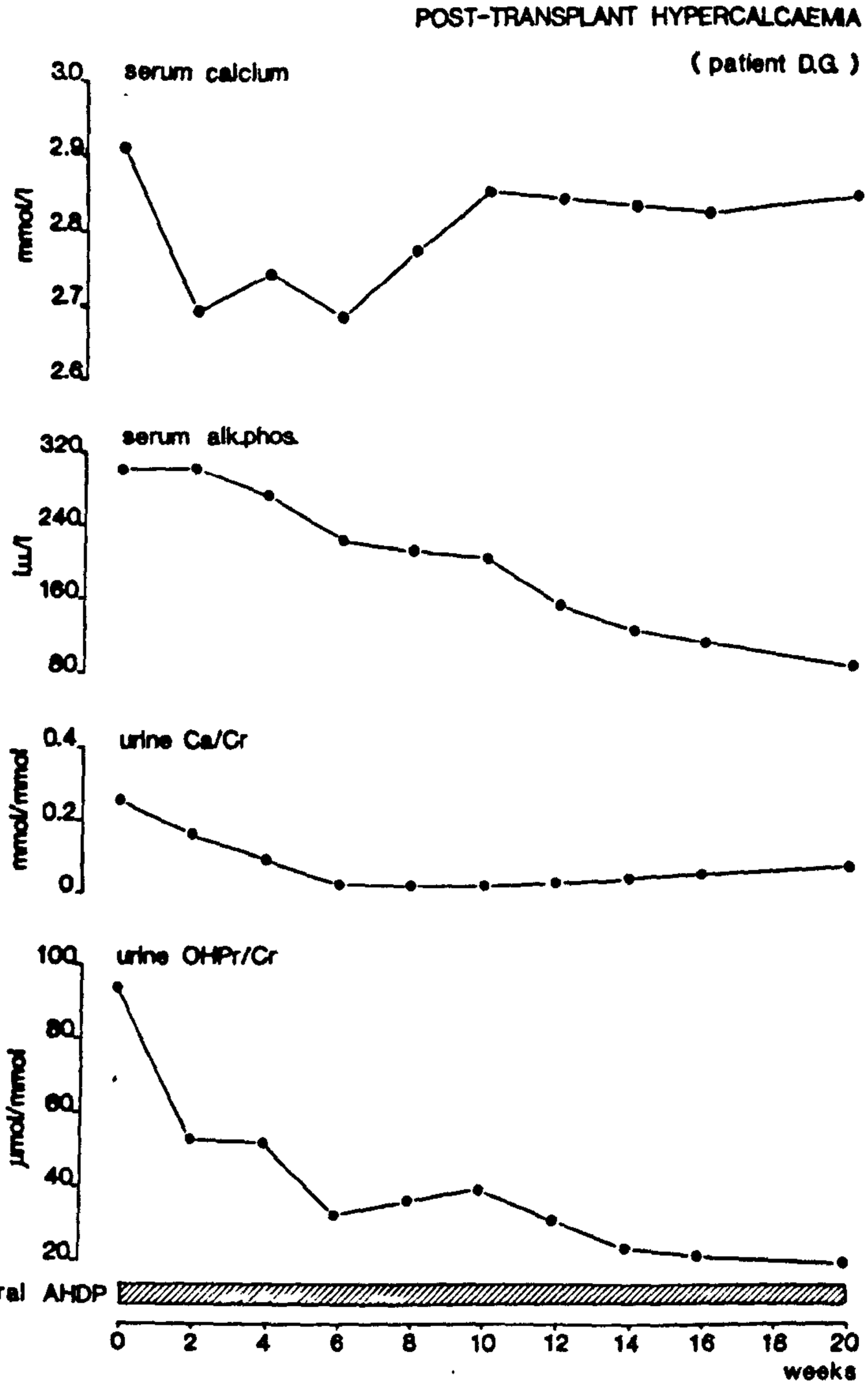


Figure 6.3 Effects of continuous treatment with AHDP 400mg/day by mouth in a patient with tertiary hyperparathyroidism following renal transplantation. Attenuation of the hypocalcaemic response coincides with a fall in alkaline phosphatase and thus with biochemical "recoupling".

Treatment with etidronate

Responses to high-dose and low-dose etidronate treatment are shown in Figure 6.4. Neither dose induced a significant change in serum calcium although a downward trend was apparent with the higher dose. Little overall change in Ca/Cr occurred with the lower dose (although a significant fall was present at 8 weeks) whereas a sustained suppression was observed in the high-dose group. Even so, the absolute values of Ca/Cr remained raised at around the upper end of the normal range (0.4mol/mol). Perhaps surprisingly, OHP/Cr fell significantly and by a similar extent in the two groups. However, it is notable that a marked and significant increase in serum phosphate occurred in the low-dose etidronate group comparable to, or even greater than, that seen with the higher dose (Figure 6.5) suggesting a high drug bioavailability in both groups. Moreover, the hyperphosphataemic responses might indicate that impairment of skeletal mineralisation was occurring with both high and low-dose etidronate treatment. Unfortunately bone histological data was not available to support this hypothesis.

Values before treatment and at 1 month were compared in all three groups (clodronate and high and low-dose etidronate) in Figure 6.6. Thus, despite

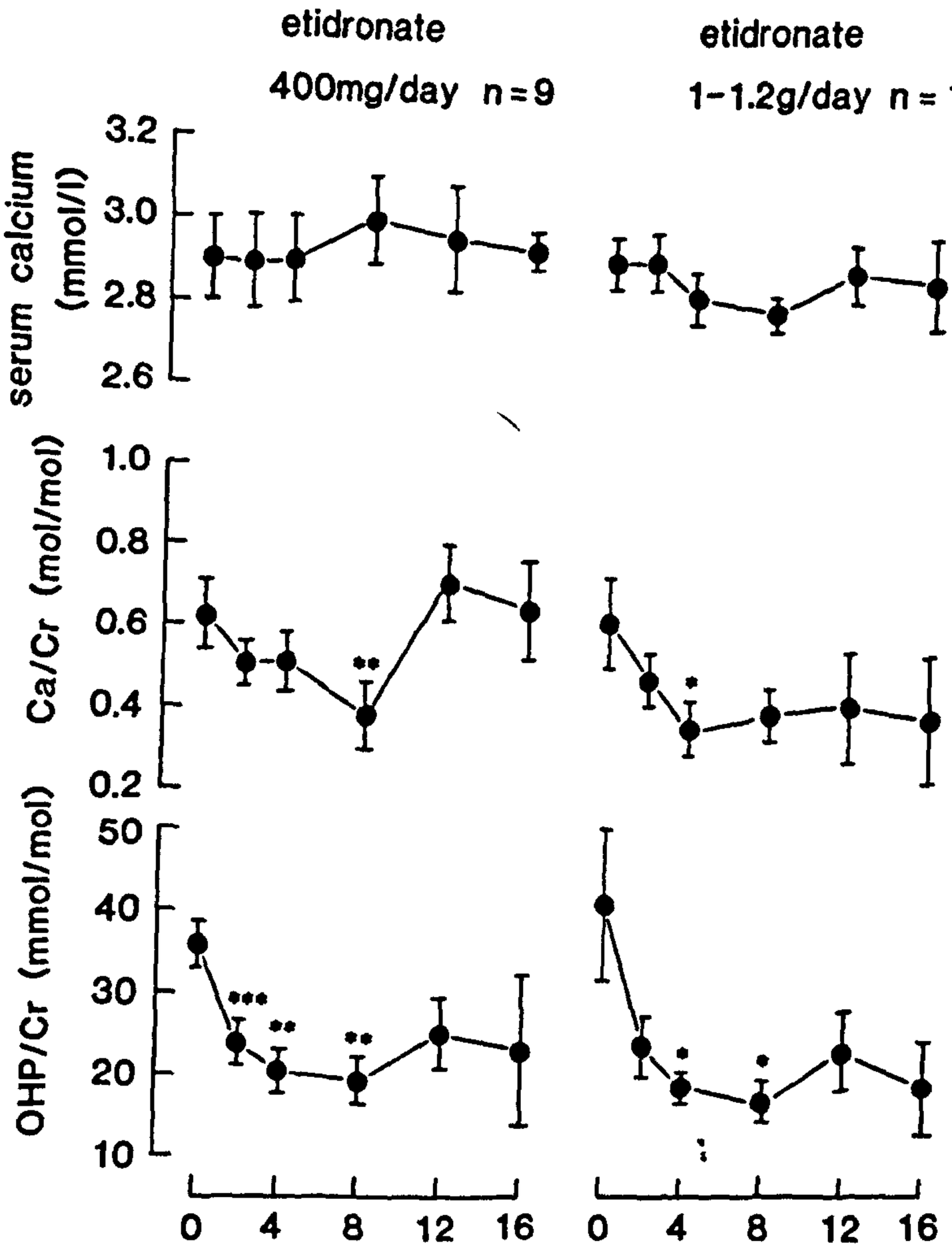


Figure 6.4 Comparison of the effects of low-dose and high-dose oral etidronate treatment in patients with primary hyperparathyroidism.

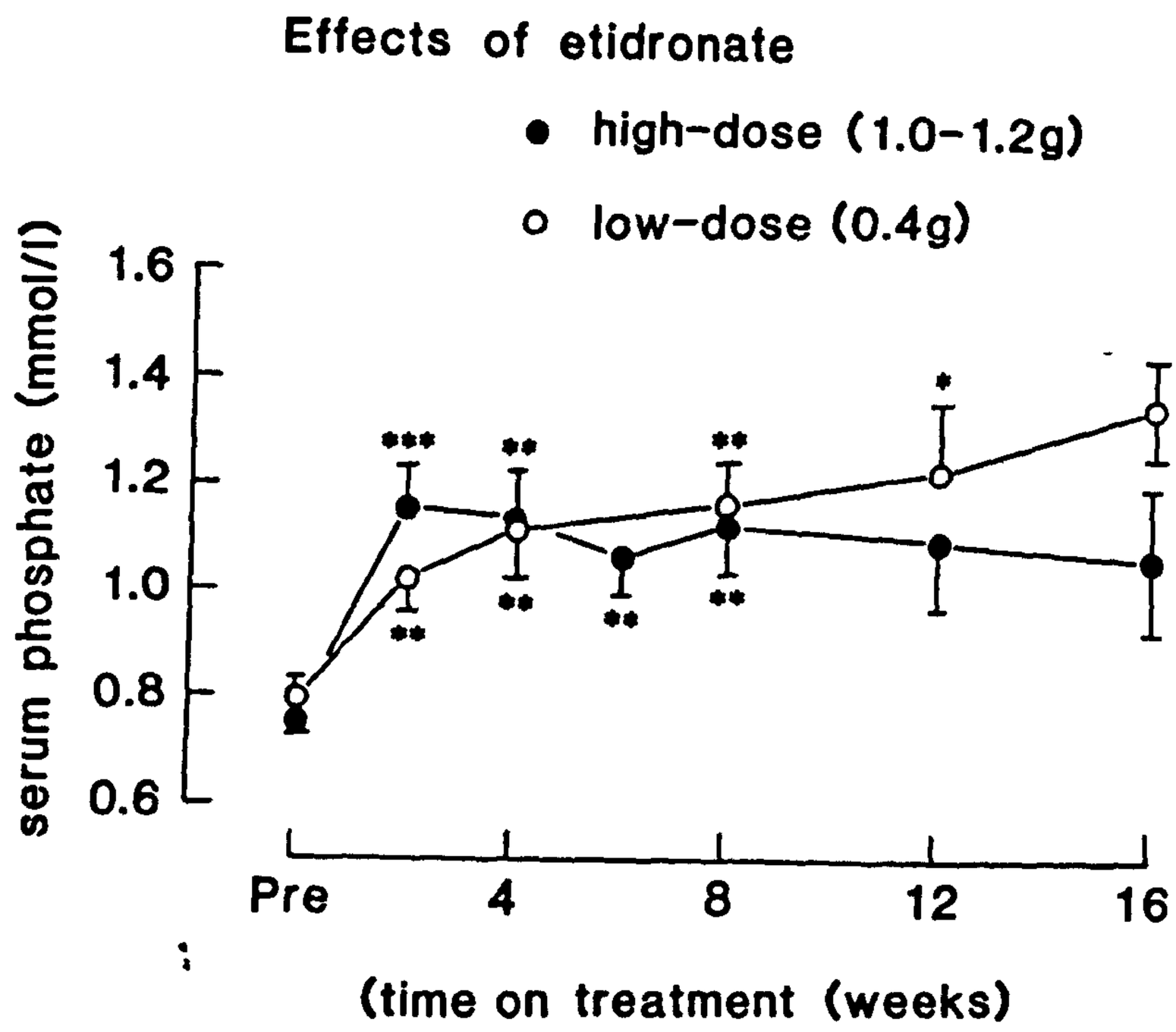


Figure 6.5 Changes in serum phosphate in response to low or high doses of oral etidronate in patients with primary hyperparathyroidism.

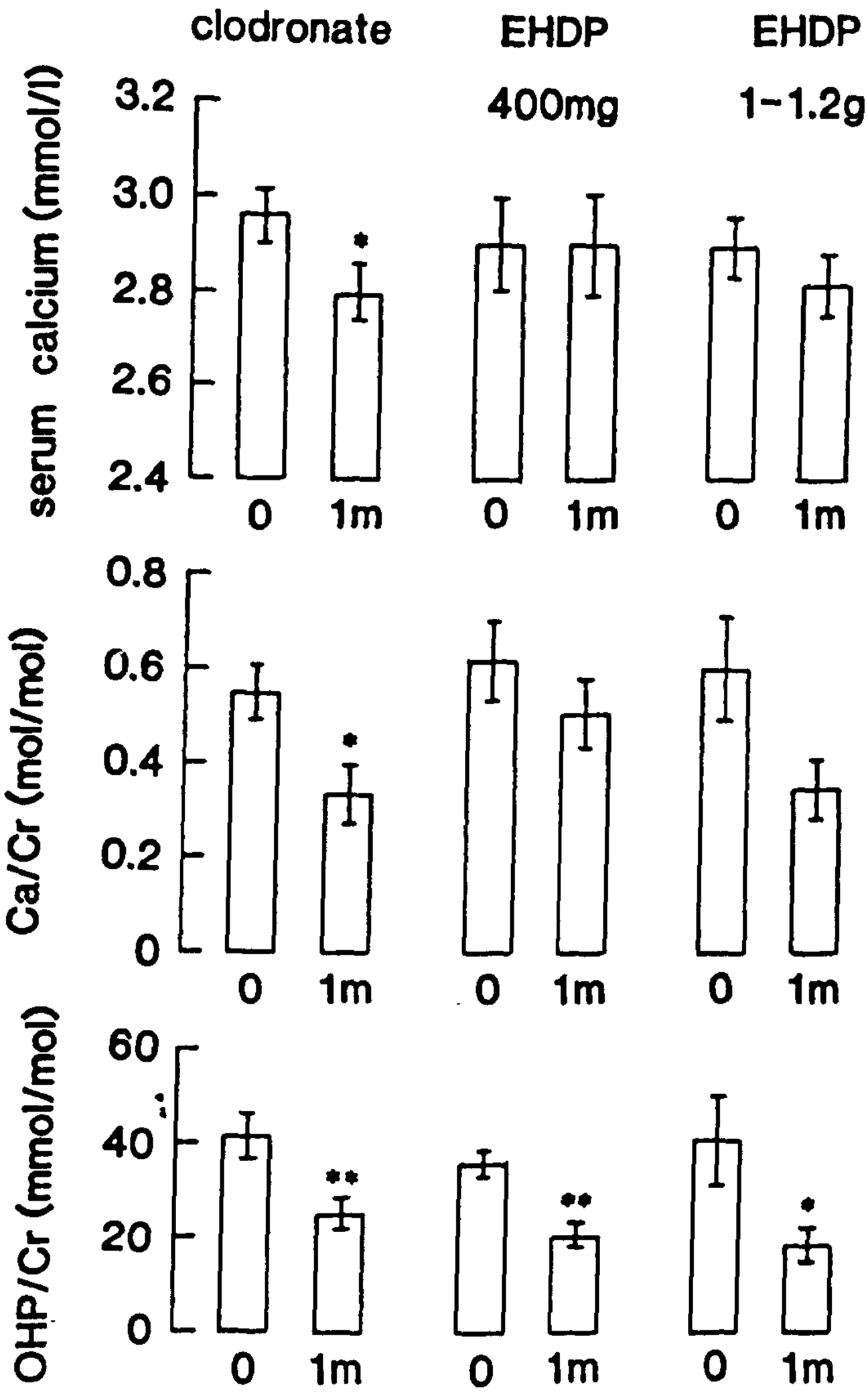


Figure 6.6 Comparison of effects of three diphosphonate regimes (clodronate and high-dose or low-dose etidronate by mouth) after 1 month of treatment in patients with primary hyperparathyroidism.

comparable suppression of bone resorption, as judged by urinary OHP/Cr, in all three groups, a significant hypocalcaemic effect occurred only in the clodronate treated group.

Discussion

The experience with clodronate treatment of primary hyperparathyroidism reported here is similar to that of previous authors (Douglas et al, 1983; Shane et al, 1981) in that mean serum calcium fell significantly, but remained above normal. However, in the present study the lowest mean values achieved were still substantially above normal (2.77mmol/l) whereas these more closely approximated to normal in the previous studies (2.63mmol/l and 2.70mmol/l). In part this may be due to more marked initial hypercalcaemia in the present study (2.95mmol/l compared with 2.88mmol/l and 2.87mmol/l respectively). In the study by Douglas and colleagues an attenuation of the hypocalcaemic response was documented in one patient given prolonged treatment. This effect has been confirmed in a larger number of patients in the present study and is likely to be a consequence of recoupling of bone formation and resorption at a reduced rate of bone turnover.

The present study also confirms the relative lack of hypocalcaemic response to high-dose etidronate

treatment (Kaplan et al, 1977). Although mineralisation was not studied directly this lack of effect probably results from the mineralisation defect associated with these high oral doses (Meunier et al, 1975). The lack of hypocalcaemic effect of the lower dose of etidronate was surprising in view of the marked fall in bone resorption in this group. However, the hyperphosphataemic response in these patients was much larger than that expected with this low dose and suggests increased bioavailability. That such an effect may occur is suggested by the findings of Boyce and colleagues (1984) of hyperphosphataemia and focal osteomalacia in pagetic patients treated with low-dose etidronate. Thus, markedly impaired mineralisation may have occurred in both low-dose and high-dose etidronate treated groups to account for the observed responses. It is possible that even lower doses, such as 200mg of etidronate, might have had a lesser effect to impair mineralisation and thus might have induced a greater hypocalcaemic effect.

Parathyroidectomy remains the mainstay for treatment of symptomatic hyperparathyroidism. The indications for the medical management of primary hyperparathyroidism (discussed in Chapter 1) appear to be relatively few, particularly if, as suggested by the current study, the effects of treatments are incompletely effective in reducing calcium and these effects are only short-lived. Nonetheless under certain circumstances

diphosphonate treatment may be potentially useful. Thus a temporary reduction in serum calcium may prove useful in assessing the potential benefits of parathyroidectomy in patients with non-specific symptoms. In patients with chronic renal failure with either secondary or tertiary hyperparathyroidism diphosphonates might have a long term use in preventing or treating hyperparathyroid bone disease. In patients with marked hyperparathyroid bone disease severe hypocalcaemia may follow parathyroidectomy due to a sudden suppression of PTH-induced bone resorption and net entry of calcium into bone (so-called "hungry bone" syndrome). Pretreatment of these patients with diphosphonates might reduce the prevailing rate of bone turnover and thus reduce postoperative hypocalcaemia due to this mechanism. Primary hyperparathyroidism is commonly found in of postmenopausal women. There is concern that the accelerated rate of bone turnover in these patients may be associated with a rapid loss of bone to precipitate or exacerbate osteoporosis (Nagant de Deuxchaisnes et al, 1985; Seeman et al, 1982). Long-term treatment with diphosphonates, by reducing the rate of bone turnover, may be beneficial in preventing this complication. There are, however, no published studies available on these putative roles of diphosphonate treatment.

From the present study, neither high-dose nor low-dose etidronate treatment appears to have any role in

the treatment of this disorder. However, it is possible that if our patients had been given even lower doses of etidronate a more marked hypocalcaemic response would have occurred. The overall findings concur with the results of intravenous diphosphonates in Paget's disease and in hypercalcaemia of malignancy discussed in Chapters 4 and 5 in which the hypocalcaemic effects of etidronate were attenuated due to the associated impairment of mineralisation.

Summary

1) In previous studies both clodronate and APD have been shown to produce a hypocalcaemic effect in patients with primary hyperparathyroidism. In other studies high dose oral etidronate had no significant effect on calcium but there is an anecdotal suggestion that low dose treatment may be more effective.

2) A direct comparison of three regimens was made in the present study. 15 hyperparathyroid patients were given clodronate 0.8-1.2g/day and 19 received etidronate at either 400mg/day (n=9) or 1-1.2g/day (n=10).

3) Mean concentrations of serum calcium fell significantly (but not into the normal range) in the clodronate treated group only, despite marked falls in

urinary OHP/Cr in all three groups.

4) An attenuation of the hypocalcaemic and hypocalciuric responses to clodronate occurred after 3 months of continuous treatment possibly due to "recoupling" of bone formation and resorption.

5) Clodronate, but neither high-dose nor low-dose etidronate, may have a useful role in the medical management of patients with primary hyperparathyroidism.

CHAPTER SEVEN

THE USE OF CLODRONATE IN THE TREATMENT OF IMMOBILISATION HYPERCALCAEMIA

THE USE OF CLODRONATE IN TREATMENT OF IMMOBILISATION HYPERCALCAEMIA

Introduction

Hypercalcaemia is a well recognised, albeit uncommon, complication of prolonged immobilisation. It occurs in patients with high rates of bone turnover, including children and adolescents (Claus-Walker et al, 1975; Lerman et al, 1977; Van Zuiden et al, 1982) and in patients with Paget's disease of bone (Reifenstein & Albright, 1944). In adults with previously normal bones hypercalcaemia is rare (Mason, 1957), but may be more common than hitherto recognised (Heath et al, 1972). The mechanism of hypercalcaemia is thought to be an increase in net bone resorption due to both a suppression of bone formation and an increase in osteoclastic bone resorption (Minaire et al, 1974, 1982).

Two cases of immobilisation hypercalcaemia in adults with previously normal bone turnover and their responses to treatment with clodronate are reported here. At the time of this study there were no previous reports of the use of diphosphonates in the treatment of this disorder. These cases have previously been the subject of a short report (Yates et al, 1984).

Case reports

Two female patients, aged 43 years (Patient 1) and 32 years (Patient 2), developed severe hypercalcaemia following a prolonged period of immobilisation. Blood and urine collections and biochemical measurements were performed as described in chapter 2.

Patient 1 developed severe widespread flaccid paralysis over 36 hours in March 1981 due to acute fulminating post-infective polyneuritis. Assisted ventilation was required for 23 weeks, this period being complicated by autonomic dysfunction, chest infection and thrombo-embolic episodes. By February 1982 she had regained grade 3 power in the proximal leg muscles, although power of the distal muscle groups was still very poor and she was unable to stand unaided. At this time, 11 months after the onset of her illness, she developed malaise, nausea, vomiting and constipation and became clinically dehydrated. The serum calcium concentration was markedly increased (3.92mmol/l). Treatment with continuous intravenous saline 3l/day for 19 days resulted in a small and transient fall in plasma calcium followed by an increase to 3.66mmol/l despite continued treatment. During this period serum iPTH was undetectable and $1,25(OH)_2D_3$ concentrations were low (Table 7.1). Serum creatinine was normal although renal function (endogenous

Table 7.1: Biochemical measurements before and 5 days after the start of treatment of immobilisation hypercalcaemia with clodronate.

	Patient 1		Patient 2		Normal
Serum measurement	Before	After	Before	After	range
Calcium (mmol/l)	3.55	2.51	3.53	2.40	2.12-2.63
Phosphate (mmol/l)	1.09	0.81	1.35	0.76	0.7-1.5
Creatinine (umol/l)	71	55	49	47	60-120
Alkaline phosphatase (u/l)	98	99	120	97	35-105
iPTH (pg/ml)	<40	-	<40	<40	<130
$1,25(OH)_2D_3$ (pg/ml)	8.2	7.7	16.9	14.8	20-60
Urine measurement					
Ca/Cr (mol/mol)	7.25	0.63	2.10	0.06	<0.5
OHP/Cr (mmol/mol)	375	403	83.3	100	<27

creatinine clearance) was reduced at 41 ml/min.

Patient 2 underwent renal transplantation for end-stage renal failure 3 months before presentation. At that time she had no evidence of bone disease as judged by a radiographic skeletal survey and serum activity of alkaline phosphatase. However, 14 months previously she had been treated with alphacalcidol (1-alpha $OH-D_3$) for hyperparathyroid bone disease. Apart from one rejection episode, renal function was stable (creatinine clearance = 38ml/min) and immunosuppression was maintained with azathioprine and prednisolone. She was discharged home but readmitted 3 days later with cough and dyspnoea subsequently attributed to a cytomegaloviral infection. She became increasingly hypoxic and required tracheostomy and assisted ventilation for 4 weeks. For the following 2 weeks she was unable to move her limbs due to a peripheral motor neuropathy which was confirmed by electromyography and nerve conduction studies. Power returned gradually over the subsequent 2 months but was associated with a progressive rise in the serum calcium (to 3.54mmol/l) despite treatment with prednisolone. Serum iPTH was undetectable and the concentration of $1,25(OH)_2D_3$ was low (Table 7.1)

Skeletal radiographs were normal in both patients and in the one patient examined (Patient 1) a bone scan showed no evidence of extraskeletal calcification. Both patients had biochemical evidence for increased bone resorption as judged by urinary Ca/Cr and OHP/Cr (Table 7.1)

Both patients were treated with clodronate (Figure 7.1). Patient 1 received 100mg intravenously daily for 3 days followed by 13 days of treatment by mouth (1.6g/day). Patient 2 was initially given 5 days of oral clodronate 1.6g/day. Both patients became normocalcaemic within 5 days (Figure 7.1). There were similar, though less marked, falls in serum phosphate. When treatment was stopped after 5 days in Patient 2, hypercalcaemia recurred, but responded to further treatment. Fasting urinary Ca/Cr, initially high in both patients, fell to normal within 2 weeks from the start of treatment. In Patient 1 there was a transient rise in urinary Ca/Cr when clodronate was stopped after 1 month of treatment although hypercalcaemia did not recur. Urinary OHP/Cr was little changed initially in either patient but subsequently decreased gradually over 4 to 6 months.

Discussion

These two patients were unusual in that they

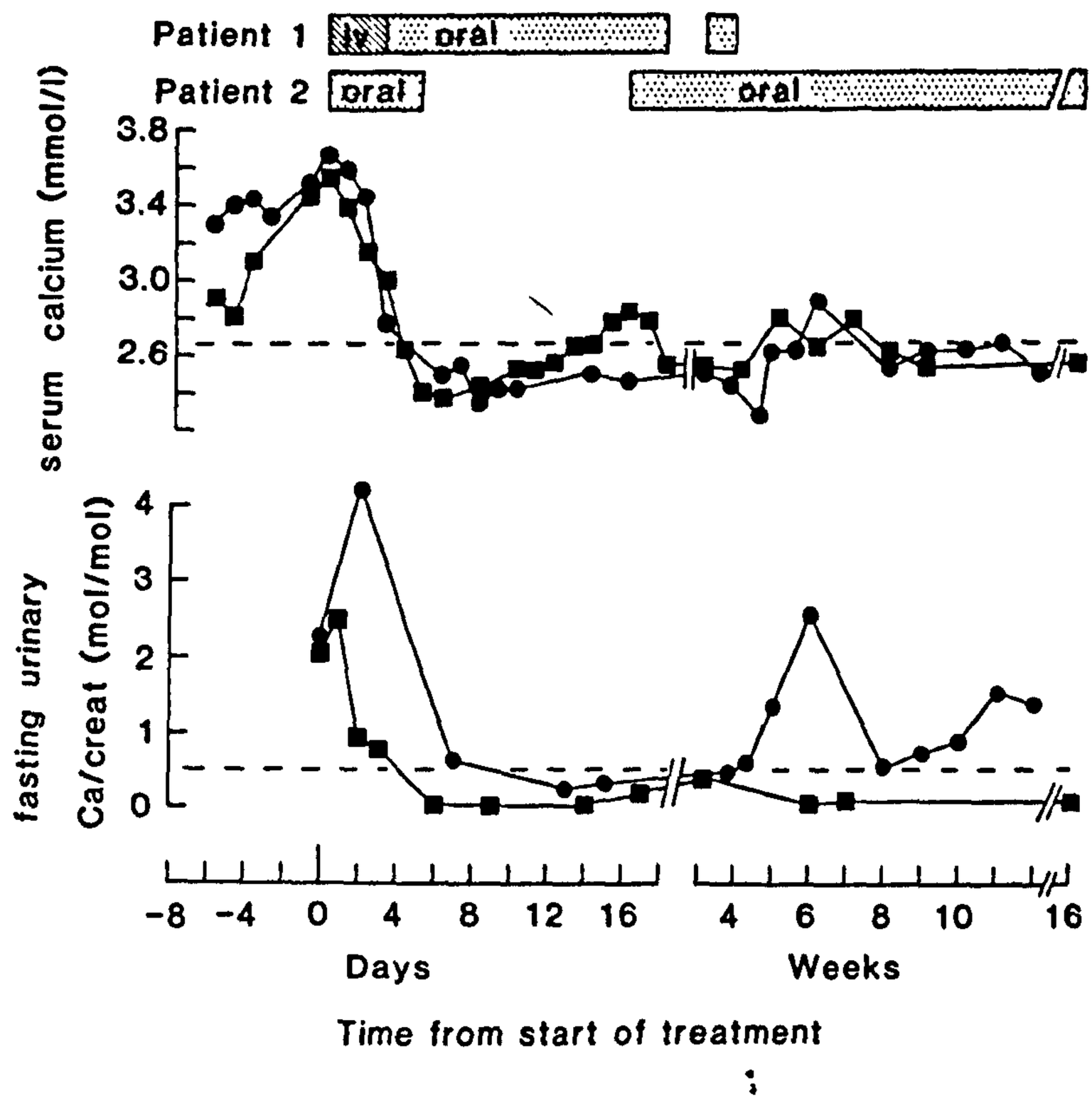


Figure 7.1 Effects of treatment with clodronate on serum calcium and urinary Ca/Cr in 2 adults with immobilisation hypercalcaemia. Patient 1 is denoted by circles and Patient 2 by squares.

were adults with severe hypercalcaemia due to immobilisation but without fracture, coexisting Paget's disease or marked renal failure (Drivas et al, 1975). In common with adolescents, bone resorption, as judged by OHP/Cr, was markedly increased. This notion is supported by the finding of hypercalciuria, in common with the general experience of others in immobilised adults (Minaire et al, 1974; Whedon & Shorr, 1957). Increased intestinal absorption of calcium might also augment urinary calcium excretion. However, the measurement of fasting Ca/Cr, which eliminates the effects of gut derived calcium, was increased in both our patients. Moreover, the dietary intake of calcium was low and serum concentration of $1,25(OH)_2D_3$ were low, suggesting that intestinal absorption of calcium was also likely to be low. The mechanism for the low concentrations of $1,25(OH)_2D_3$ was probably related to low PTH concentrations and high plasma calcium, both factors which reduce the activity of the 1-alpha hydroxylase enzyme in man (Norman et al, 1982). Whereas hypercalciuria is common in adults following immobilisation (Whedon & Shorr, 1957; Lutwak et al, 1969), significant hypercalcaemia is extremely rare. This raises the possibility that increased delivery of calcium to the ECF from sources other than bone, or its reduced clearance from the circulation, may have contributed to hypercalcaemia. Both patients were

recovering from muscle paralysis, so that a high calcium content of damaged muscle fibres could have contributed to hypercalcaemia. However, no evidence for extraskeletal calcification was found in either patient as judged by radiographic criteria or the more sensitive index of scintigraphy. Creatinine clearance was impaired in both patients even though serum creatinine concentrations were normal. Impairment of renal function would not cause, but is likely to have contributed to, hypercalcaemia by decreasing the ability to clear large fluxes of calcium from the ECF. Serum iPTH was low in our patients, in common with the experience of others (Rosen et al, 1978; Stewart et al, 1982; Claus-Walker et al, 1977) indicating that hyperparathyroidism did not account for the hypercalcaemia. In both cases, therefore, marked bone resorption appeared to be the major mechanism for hypercalcaemia.

These conclusion are supported by the response of these patients to suppression of bone resorption with clodronate. The effects of clodronate on immobilisation hypercalcaemia have not been previously reported, although this agent has been shown to inhibit bone loss in experimental animals (Fleisch et al, 1969b) and in paraplegia (Minaire et al, 1982). Etidronate has been used successfully to treat immobilisation hypercalcaemia (Hagg et al, 1984; Merli et al, 1984) but its effect on mineralisation may limit the hypocalcaemic action.

Although severe hypercalcaemia following immobilisation is rare, accelerated bone loss appears to be universal. Thus, treatment with diphosphonates may be potentially useful, not only for immobilisation hypercalcaemia, but also for the prevention of the progressive osteoporosis associated with prolonged immobilisation.

Summary

1) Two adult patients developed marked hypercalcaemia associated with prolonged immobilisation.

2) In both patients hypercalcaemia resulted from increased bone resorption in association with impaired renal glomerular function, and thus impaired calcium excretion.

3) Treatment with clodronate decreased bone resorption and thereby reduced serum calcium in both patients. This agent may also be useful in preventing osteoporosis associated with immobilisation.

CONCLUDING REMARKS

CONCLUDING REMARKS

These studies have provided new information on several aspects of the effects of diphosphonates in disorders of the skeleton and of calcium metabolism. Many of the objectives stated earlier in this work have been at least partly met. To summarise these:-

1) The effects of different diphosphonates were directly compared, particularly in patients with Paget´s disease of bone. All three diphosphonates investigated, including the hitherto clinically untested diphosphonate, AHDP, effectively inhibited bone resorption when given in short intravenous courses. AHDP appears to be a more potent inhibitor of bone resorption than either clodronate or etidronate and is likely to be a useful agent in the treatment of both Paget´s disease and hypercalcaemia of malignancy. Although all three intravenously-administerd diphosphonates tested were shown to inhibit the uptake of mineral into bone, this effect was only partial and short-lived following treatment with either clodronate or AHDP whereas a sustained and complete block of mineralisation occurred following treatment with etidronate. The effects of these agents on serum calcium are explicable in terms of their effects on the influx and efflux of calcium between

bone and the ECF compartment. Because etidronate completely inhibiting skeletal mineralisation, and thus calcium entry into bone, this agent has no acute hypocalcaemic effect in Paget´s disease. For this reason, and as confirmed by the clinical studies reported herein, etidronate appears to be less suitable than either clodronate or AHDP in the treatment of hypercalcaemic disorders associated with increased bone resorption.

TmP/GFR and serum phosphate increased significantly in response to all three intravenously administered diphosphonates. However, the extent and time course of these phosphate responses appeared to be related to the effects of these agents on mineralisation indicating a possible causal relationship. Further studies of the interrelationship between impaired mineralisation and renal tubular transport of phosphate, which might involve a humoral signal between bone and kidney, are required.

2) The effects of diphosphonates in Paget´s disease, hypercalcaemia of malignancy and primary hyperparathyroidism were compared. Despite differences in underlying mechanisms of disease it was found that effects of diphosphonates on bone turnover and calcium metabolism in one disorder, such as Paget´s disease, were similar to those observed in the other disorders. Thus,

by studying the effects of diphosphonates in several disorders a more complete picture of diphosphonate effects and of relative differences between diphosphonates was obtained.

3) Previously untried treatment regimens with short intravenous courses of diphosphonates were studied and compared with a long-term oral treatment of established efficacy. The suppression of biochemical markers of bone turnover following short intravenous courses of diphosphonate treatment in previously untreated patients with Paget´s disease was similar to that during prolonged oral therapy and was sustained for approximately one year in the case of treatment with intravenous clodronate. Such responses have not previously been described and may help to shed further light on the mechanism of action of diphosphonates. These new treatment regimens offer an attractive alternative mode of treatment for this disorder.

4) The initial degree of pagetic activity was found to be an important consideration in the assessment of biochemical response in individual patients with Paget´s disease. Although, previous treatment with diphosphonates appeared to be associated with an impaired response to further treatment with diphosphonates, on closer examination this effect could be entirely

attributed to the effect of incomplete relapse before re-treatment. If allowance for these effects is not made they may result in considerable bias between treatment groups which are not well matched for their degree of pagetic activity and the proportion of re-treated patients. As very many diphosphonate treatment options exist it is important to be able to evaluate these economically using relatively small numbers of patients and also to be able to compare results from different studies. Some methods to facilitate such comparisons have been presented in this work.

5) A dose-response relationship was established for intravenous clodronate in treatment of Paget's disease of bone.

6) The use of clodronate in the treatment of immobilisation hypercalcaemia had not previously been described and was studied in two adult patients reported in this thesis. This form of treatment was found to be effective and may, by implication, also have a role in the prevention of the bone loss that is otherwise an inevitable consequence of prolonged immobility or weightlessness in space.

Although these various findings represent a considerable advance in the knowledge of the effects of

diphosphonates in skeletal disease, the studies were of necessity limited and there remain many unanswered questions. For example, it is possible that prolonged suppression of Pagetic activity, perhaps with continuous or intermittant diphosphonate therapy, might eradicate the pool of pagetic osteoclasts and thus "cure" of the disease may be obtained. Certainly some patients remain in biochemical remission 5 years or more after a single course of treatment. However, others relapse even after such a prolonged interval and thus it is not yet possible to determine whether cure of the disease, as opposed to its long-term control, is a real prospect. Neither is it known whether true resistance to diphosphonate treatment might develop in some patients after prolonged repeated exposure. When the answers to such questions become available the way in which diphosphonates are used to treat disorders of bone turnover may be further rationalised.

Patients with Paget´s disease treated with intravenous clodronate

Clodronate 300mg : Previously untreated

No.	Initials	Age	Sex	Alkaline phosphatase			OHP/Cr (mmol/mol)		
				Original	Pre	3 months	Original	Pre	3 months
1	CR	74	M	*	1015	271	*	113.5	45.5
2	AM	64	F	*	486	200	*	65.2	40.9
3	LR	89	F	*	492	122	*	65.7	26.8
4	AF	67	F	*	323	99	*	80.3	31.8
5	EH	74	M	*	455	285	*	94.7	47.0
6	RC	69	F	*	130	64	*	71.4	34.7
7	AM	71	F	*	373	140	*	95.3	41.3
8	RB	61	M	*	281	128	*	31.8	18.1
9	CH	61	F	*	1326	281	*	230	68.8
10	GD	72	F	*	1217	594	*	147	94.5
11	MP	82	F	*	171	87	*	48.5	31.9
12	PH	66	F	*	100	88	*	33.0	41.0
13	CC	74	F	*	118	62	*	62.0	80.0
14	TS	71	M	*	113	90	*	36.9	71.0
15	WP	75	M	*	915	218	*	112	20.5
16	AC	73	M	*	202	102	*	45.6	28.0

* denotes that original pretreatment alkaline phosphatase is the same as the immediate pretreatment value (i.e. patient previously untreated)

Clodronate 300mg : Previously treated

No.	Initials	Age	Sex	Alkaline phosphatase			OHP/Cr (mmol/mol)		
				Original	Pre	3 months	Original	Pre	3 months
17	MC	71	F	1150	511	162	350	129	51.6
18	JC	63	M	510	230	98	58.6	30	27.9
19	MB	72	F	83	84	65	23.0	28.1	14.8
20	MP	77	F	156	132	134	80	57.2	48.0
21	EP	68	M	4000	2305	2190	822	660	598
22	JS	73	M	1200	279	255	182	32.9	23.4
23	EB	84	F	950	280	287	300	111	80
24	GC	64	M	168	154	147	24.0	23.4	36.0
25	DH	53	M	800	347	371	143	120	62.2
26	AH	72	M	1140	502	341	108	79.5	51.6
27	MM	85	F	920	564	172	320	193	51.5
28	EH	75	M	455	281	210	94.7	54.7	38.9
29	SR	66	M	3400	351	296	210	47.0	38.4
30	FR	76	M	700	120	112	30.1	19.1	14.9
31	RW	51	M	1000	422	231	165	92.0	64.0

Original values are those before the first course of diphosphonates, which in previously untreated patients (denoted by *) are the same as immediate pretreatment (Pre).

Clodronate 100mg

No.	Initials	Age	Sex	Alkaline phosphatase			OHP/Cr (mmol/mol)		
				Original	Pre	3 months	Original	Pre	3 months
1	FO	75	M	*	165	139	*	52.2	36.5
2	AF	76	F	400	239	203	140	62.5	54.6
3	EB	70	F	950	260	279	300	127	104
4	CW	74	M	220	135	127	52	7.8	10.0
5	GC	65	M	170	148	147	24	23.4	36.0
6	EM	76	F	2000	690	626	380	134	123
7	TC	69	M	*	111	98	*	52.0	22.2

* denotes that original pretreatment alkaline phosphatase is the same as the immediate pretreatment value (i.e. patient previously untreated)

Clodronate 600mg

No.	Initials	Age	Sex	Alkaline phosphatase			OHP/Cr (mmol/mol)		
				Original	Pre	3 months	Original	Pre	3 months
1	CD	78	M	*	200	98	*	44.0	21.9
2	VJ	83	M	*	425	128	*	55.0	31.6
3	AG	63	M	*	240	89	*	30.0	21.4
4	TW	84	M	172	172	83	57.0	57.1	25.9
5	MC	72	F	1250	490	191	350	123	61.0
6	JH	48	M	3000	523	204	620	135	86.0
7	AF	68	F	486	287	152	140	55.0	31.8
8	HC	69	F	970	237	97	340	78.0	47.4
9	AM	74	M	2250	1000	495	1000	525	294
10	AP	71	F	609	602	288	230	240	91.8
11	GD	73	F	1100	495	306	140	111	100
12	EM	75	F	2000	975	392	380	140	57.7
13	JS	74	M	1430	350	278	152	30.0	24.7
14	EH	74	M	462	330	178	82.6	60.0	37.5
15	AH	73	M	1140	517	323	108	77	59.5
16	WP	76	M	885	245	139	112.5	48.1	25.2

Patients with Paget's treated with intravenous etidronate

No.	Ins.	Age	Sex	Dose (mg)	Alkaline phosphatase Original	Alkaline phosphatase Pre	Alkaline phosphatase 3 mths	OHP/Cr (mmol/mol) Original	OHP/Cr (mmol/mol) Pre	OHP/Cr (mmol/mol) 3 months
1	NW	81	F	300	*	1065	576	*	206	86
2	EF	79	F	300	*	181	119	*	69	71
3	CB	69	M	300	*	186	147	*	33.0	26.6
4	AA	65	F	300	*	223	196	*	64.0	49.0
5	MR	71	F	300	*	100	86	*	50	23.3
6	PW	76	F	300	*	207	186	*	42.0	28.0
7	EH	68	F	300	*	252	203	*	78	48
8	ES	88	F	420	*	308	-	*	159	-
9	EC	83	F	700	1845	268	86	450	45.5	19.6
10	EC	74	F	410	130	103	46	90.0	80.0	45.6
11	EB	74	F	440	*	152	116	*	52.0	26.9
12	EE	81	F	490	160	147	98	55.0	70.0	35.0
13	AF	68	F	570	313	208	101	82	65.5	-
14	EP	68	M	530	2500	623	523	600	179	71.3
15	PW	78	F	420	245	199	244	52.2	58.3	27.0
16	DW	71	M	660	467	363	269	78.0	59.0	51.8
17	MH	64	F	500	*	446	126	*	91.3	22.6
18	MB	78	F	475	*	198	88	*	53.4	26.7
19	FW	53	M	580	*	156	95	*	36.8	17.7
20	RB	68	M	700	*	557	496	*	107	62.8
21	KT	58	F	430	*	449	326	*	190	59.0

Paget's patients treated with intravenous AHDP 25mg/day

No.	Ins.	Age	Sex	Alkaline phosphatase			OHP/Cr (mmol/mol)		
				Original	Pre	3 months	Original	Pre	3 months
1	IN	62	F	384	266	94	102	102	52.8
2	JB	66	M	161	147	103	41.7	32.9	14.4
3	GN	78	F	*	260	114	*	60.5	70.0
4	FH	83	F	203	197	132	42.4	40.2	30.0
5	WM	77	M	*	236	115	*	58.5	34.2
6	AK	83	F	133	103	87	43.7	41.8	55.8
7	LU	79	M	1960	1888	1595	214	178	156
8	AK	69	M	324	350	178	56	58.9	30.1
9	AK	54	F	*	524	364	*	354	170
10	LB	61	M	*	422	290	*	93	57.0
11	EE	81	F	*	1082	268	*	420	57.1
12	MR	58	F	*	181	80	*	65.0	37.0
13	HH	69	M	*	1370	397	*	220	98.6
14	CF	63	M	*	455	299	*	182	100
15	MC	73	F	*	305	108	*	100	46.5
16	JC	64	M	472	175	76	54.0	41.1	11.9
17	NN	77	F	325	150	125	123	70.3	38.6

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