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THE PATHOGENESIS AND MANAGEMENT OF  
MALIGNANCY-ASSOCIATED HYPERCALCAEMIA

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## DECLARATION

The work described in this thesis was performed in the University Department of Medicine, Glasgow Royal Infirmary, between 1981 and 1986. All of the studies were conceived, designed and analysed by the author in person and the opinions expressed are therefore those of the author. Some of the studies described in this thesis have already been published, or are about to be published in peer-review journals. They include:-

Ralston SH, Fogelman I, Gardner MD, Boyle IT. Hypercalcaemia and metastatic bone disease: is there a causal link? *Lancet* 1982; i:903-905.

Ralston SH, Fogelman I, Gardner MD, Dryburgh FJ, Boyle IT. Hypercalcaemia of malignancy: evidence for a non-parathyroid humoral agent with an effect on renal tubular handling of calcium. *Clinical Science* 1984; 66:187-191.

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## SUMMARY

Traditionally, accelerated bone resorption has been considered to play the principal pathogenic role in malignancy-associated hypercalcaemia (MH), by one of two basic mechanisms. Hypercalcaemia in patients with metastatic bone disease, is thought to be due to the local release of skeletal calcium at a rate in excess of that which can be excreted by the kidney. In patients without bone metastases, hypercalcaemia is also thought to arise as the result of bone resorption, in this case stimulated by humoral mediators, which are released by the primary tumour. Although both humoral and metastatic mechanisms of hypercalcaemia could in theory coexist, the demonstration of bone metastases has conventionally been considered to provide reason enough for the occurrence of hypercalcaemia without invoking an additional humoral component.

The humoral mediators responsible for MH are at present poorly defined, but have been generally been considered to differ from parathyroid hormone (PTH) both structurally and functionally. Previous investigators have failed to find evidence of increased renal tubular calcium reabsorption or stimulation of 1,25 dihydroxyvitamin D<sub>3</sub> (1,25(OH)<sub>2</sub>D<sub>3</sub>) synthesis in MH. The skeletal effects of these humoral mediators have also been considered to be distinct from PTH, in causing "uncoupling" of bone cell activity, with greatly increased osteoclastic bone resorption and depressed bone formation.

While metastatic bone disease has traditionally been considered to be the commonest cause of MH, a retrospective study of 195 cancer patients undergoing bone scan examinations showed no correlation between the extent of skeletal involvement and serum calcium values.

These observations, indicated that, in most cases, the development of hypercalcaemia could not simply have been explained on the basis of local bone destruction, but rather may have been due to humoral mechanisms (chapter 3.1). A further prospective study of bone scan appearances in 87 consecutive cancer patients confirmed these results but in addition, showed that other biochemical indices of bone resorption were significantly higher in hypercalcaemic when compared with normocalcaemic patients, and were disproportionately raised with respect to the extent of metastatic disease seen on the bone scan. These observations indicated that in MH, bone resorption was largely occurring on a systemic, humorally mediated basis, rather than on a local "metastatic" basis (chapter 3.2).

Further studies compared renal tubular reabsorption of calcium in MH, primary hyperparathyroidism (HPT) and normal subjects undergoing acute calcium infusions. While immunoreactive PTH levels were invariably low or undetectable in the cancer patients, renal tubular reabsorption of calcium was raised to a level comparable with that in HPT. These observations raised the possibility that there frequently may be released a humoral mediator in MH which, although immunologically distinct from PTH, did possess a "PTH-like" effect on renal tubular reabsorption of calcium (chapter 4.1). Studies of renal tubular calcium reabsorption in patients with benign, non-parathyroid hypercalcaemia revealed generally normal results, excluding the possibility that the raised levels of renal tubular calcium reabsorption in malignancy may simply have been the result of chronic hypercalcaemia per se (chapter 4.2). Further studies also excluded the possibility that the raised levels of renal tubular reabsorption of

calcium in malignancy may have been due to sodium depletion, since the renal handling of sodium in relation to renal tubular calcium reabsorption was similar in MH and HPT (chapter 4.3).

Studies performed during the surgical exploration of tumours associated with humoral hypercalcaemia (HHM) demonstrated that a fall in renal tubular calcium reabsorption was the major factor responsible for the normalisation of serum calcium values after surgical resection (chapter 6.1). A comparison of the mechanisms of hypercalcaemia in patients with early and advanced HHM showed that in the early stages, increased renal tubular reabsorption of calcium was the most important pathogenic mechanism, often occurring in the absence of increased bone resorption. With progression of the tumour and increasing immobilisation however, accelerated osteoclastic bone resorption with depressed bone formation became more apparent. This indicated that, the HHM-associated humoral factor, like PTH itself, had more potent effects on the kidney than on osteoclastic bone resorption. A possible explanation for the increased bone resorption and depressed bone formation associated with advanced tumours may however have been due to the synergistic effects of immobilisation and HHM-associated humoral factors on bone cells (chapter 6.2).

In further studies, serum levels of  $1,25(\text{OH})_2 \text{D}$  were found to be inappropriately detectable or raised in about 50% of patients with cancer-associated hypercalcaemia, (chapter 5.1) However, intestinal calcium absorption, was depressed when compared with primary hyperparathyroidism, despite the fact that  $1,25(\text{OH})_2 \text{D}$  levels were raised within the hyperparathyroid range in some cases. This data indicated that there may have been end-organ resistance to the effects



of the vitamin D metabolite in malignancy (chapter 5.2). Other aspects of PTH-like activity such as raised urinary cAMP excretion indicated that the detectable  $1,25(\text{OH})_2 \text{D}$  levels were likely to have been due to a PTH-like effect on renal  $1-\alpha$ -hydroxylase activity. These PTH-like effects were noted less commonly in breast carcinoma, myeloma and other tumours with very extensive metastases however, indicating that in these cases a true local osteolytic mechanism of hypercalcaemia may have been operative (chapters 5.2, 5.3, 6.3)

A randomised study of antihypercalcaemic therapy in malignancy showed that most treated patients improved symptomatically, although survival remained short. The diphosphonate APD was found to be the most effective agent in the longer-term due to potent suppression of bone resorption although some APD-treated patients remained hypercalcaemic after treatment, due to humorally-mediated elevations in renal tubular calcium reabsorption. The combination of corticosteroid and calcitonin was useful because of its rapid effect, but rarely controlled hypercalcaemia in the long term. Mithramycin had intermediate effects due to inhibition of bone resorption and renal tubular calcium reabsorption (chapter 6.1). A further study using a combination of APD and calcitonin gave rapid control of hypercalcaemia, with a sustained duration of action (Chapter 6.2). In analysing the calcium-lowering response to the above agents, it appeared that the most important factor in predicting response was a raised level of renal tubular calcium reabsorption.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

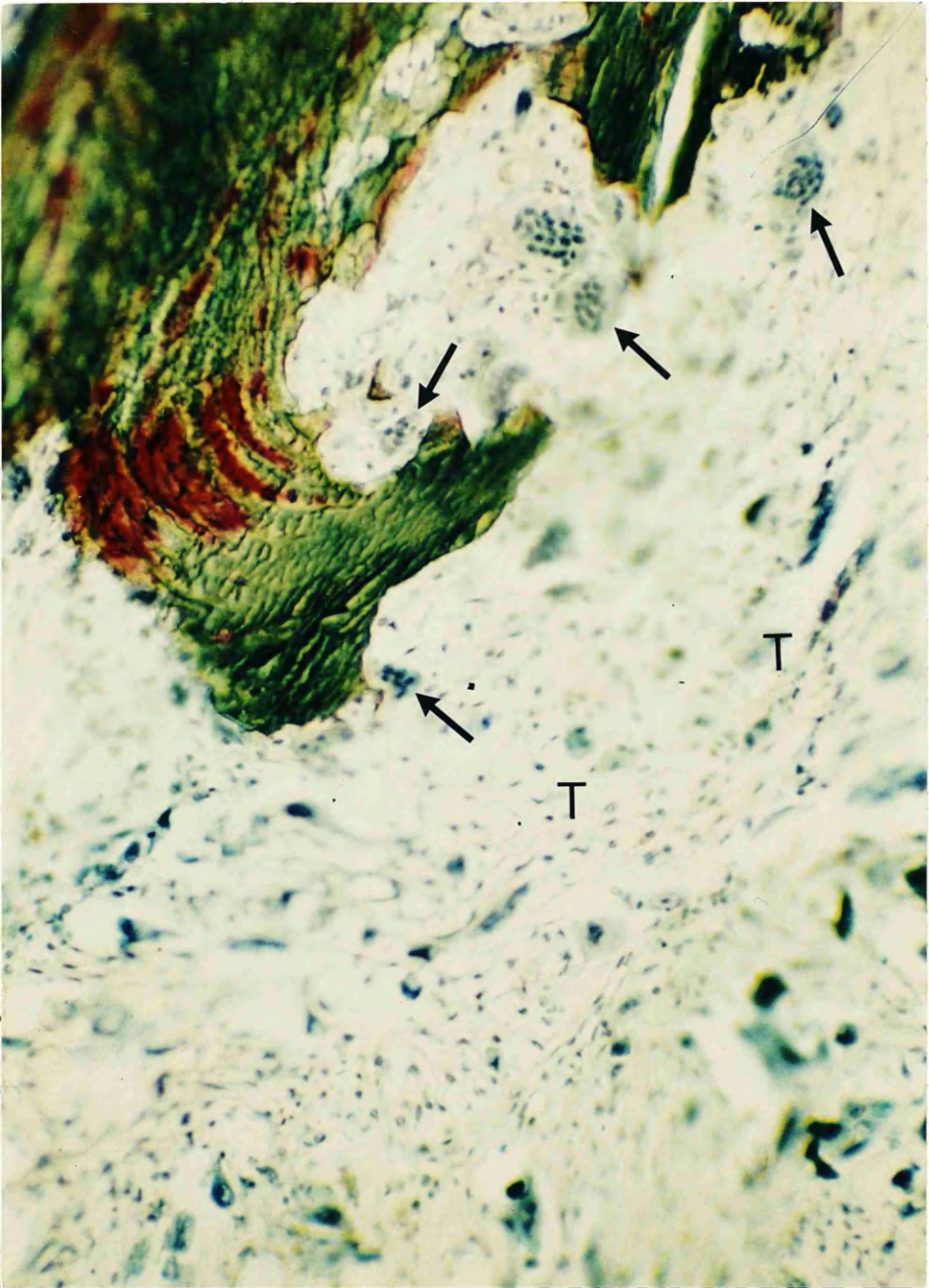
## 1.1 PATHOGENIC MECHANISMS OF HYPERCALCAEMIA

### 1.1.1 INTRODUCTION

Two mechanisms are commonly invoked in the pathogenesis of malignancy associated hypercalcaemia; in patients with metastatic bone disease, hypercalcaemia is usually attributed to local release of skeletal calcium by invading tumour, at a rate exceeding the renal capacity for its excretion (1,2,3,4,5). When hypercalcaemia occurs in patients who do not have bone metastases, the elevation in serum calcium values is also thought to be due to increased bone resorption. In this case however, osteoclastic bone resorption is increased on a systemic basis, stimulated by a circulating osteolytic humoral mediator, which is released by tumour tissue (5,6,7,8,9).

### 1.1.2 HYPERCALCAEMIA AND METASTATIC BONE DISEASE

Although this is currently considered to be the commonest mechanism of hypercalcaemia in malignancy (5,10,11,12), evidence in favour of a causal relationship between hypercalcaemia and metastatic deposits in bone is mainly circumstantial. Early case reports between the 1920's-1930's documented that hypercalcaemia was a relatively common occurrence in patients with tumours such as breast carcinoma and myeloma which were characteristically associated with extensive osteolytic disease (13,14,15,16,17,18,19,20,21). The first substantial review of calcium biochemistry in cancer patients was reported by Gutman (22), who in 1936, noted a 12% incidence of hypercalcaemia in patients with metastatic bone disease overall, compared with a 25% incidence in those with radiologically osteolytic lesions only. Hypercalcaemia subsequently became recognised as a common cause of



**FIGURE 1.1**

Photomicrograph (x343 magnification; Masson stain) of bone biopsy through an osteolytic lesion in a patient with breast carcinoma. Note presence of multinucleated osteoclasts in resorption lacunae (arrowed), interpositioned between tumour cells (T) and bone surface.

morbidity and mortality in patients with breast carcinoma (1,2,3,5,10,22,23,24,25) and myeloma (5,10,11,13,15,16,18,20,22). Indeed, these tumours, when combined accounted for the majority of patients with cancer-associated hypercalcaemia in Myers' large series of 1960 (10). Surprisingly, a rather poor correlation was noted to exist between the radiological extent of skeletal metastases, and the development of hypercalcaemia in both of these tumours (26,27,28) although this was thought to relate to the relative insensitivity of standard radiological methods in detecting occult bone metastases (5). The mechanisms of hypercalcaemia in patients with bone metastases due to tumours other than breast carcinoma and myeloma are rather less well documented. It has been assumed however, that in these tumours also, hypercalcaemia occurs as the result of excessive skeletal calcium release by invading tumour metastases.

At a cellular level, bone resorption in metastatic lesions is thought to be mediated by the osteoclast, rather than by the tumour cells themselves (5,29,30,31). Thus, while breast carcinoma cells have been shown to be capable of directly resorbing bone in vitro(32), two lines of evidence suggest that this mechanism of bone resorption is less important in vivo. These are; the proven efficacy of specific osteoclast inhibitory drugs in the treatment of bone resorption (33,34) and hypercalcaemia (35) associated with metastatic lesions, and histological data which usually show osteoclasts interpositioned between tumour cells and resorption lacunae, in actively osteolytic lesions (29,30,31) (Figure 1,1).

At a subcellular level, the mediators of osteoclast activation in metastatic lesions remains unclear, although a number of substances

have been implicated in this respect. Since many of these have also been implicated as circulating mediators of bone resorption and hypercalcaemia in patients without metastatic bone disease, all will be considered together in a later section.

### 1.1.3 HYPERCALCAEMIA UNASSOCIATED WITH METASTATIC BONE DISEASE

Hypercalcaemia was first noted to occur in a patient without bone involvement by Zondek in 1924 (36), and subsequently in one patient with bronchial carcinoma from Gutman's series of 1936 (22). The mechanism of hypercalcaemia was unclear to these workers however, as the concepts of parathyroid hormone action and ectopic hormone production had not then evolved. In 1941 however, Albright recognised that hypercalcaemia in situations like these may be due to the "ectopic" release of a humoral substance by tumour tissue (6). This postulate was made on the basis of a case report in which hypercalcaemia and hypophosphataemia in a patient with metastatic hypernephroma was corrected by irradiation of a solitary ileal metastasis. Since there was no evidence of primary hyperparathyroidism on post-mortem examination, Albright reasoned that the metabolic abnormalities may have been caused by a parathyroid hormone-like substance released by tumour tissue. Subsequently, further case reports appeared in the literature, documenting hypercalcaemia and hypophosphataemia in cancer patients without metastatic bone disease (37,38,39). Data from 50 such patients were reviewed by Lafferty in 1966 (7) using the term "pseudohyperparathyroidism" previously coined by Fry (39). Lafferty delineated three important features of the syndrome. Firstly, and by definition, Lafferty considered that the syndrome of humorally-

mediated hypercalcaemia could only be made in the absence of metastatic bone disease. Secondly, patients suffering from this syndrome were noted to exhibit certain "hyperparathyroid-like" features, in that the hypercalcaemia was usually associated with hypophosphataemia and renal phosphate wasting. Thirdly, the tumour types associated with this syndrome comprised mainly of squamous or genitourinary cancers. The absence of breast carcinoma as a cause could, of course, have been explained by Lafferty's first stipulation that the presence of bone metastases excluded the diagnosis.

Conclusive evidence that such hypercalcaemia was indeed due to the release of a humoral factor by tumour tissue, was gained by studies which demonstrated that, in patients with localised tumours, surgical resection reversed both the hypercalcaemia and hypophosphataemia (8,9).

## 1.2 PROPOSED MEDIATORS OF HYPERCALCAEMIA IN MALIGNANCY

### 1.2.1 OSTEOCLAST ACTIVATING FACTORS

In 1972, Horton and his colleagues discovered that supernatant fluid from cultures of normal human lymphocytes possessed bone-resorbing activity in vitro (40). This activity was attributed to the release of an osteoclast-activating substance (OAF) by these cells. A variety of cell lines was subsequently demonstrated to exhibit similar activity including; normal and activated human leukocytes (40,41,42,43), B- and T- lymphocytes (44), myeloma cell-lines (31,45), lymphosarcoma cell-lines (46), normal monocytes (47), and possibly some solid-tumour cell lines (48).

Although it was originally thought that OAF was a single substance (40), subsequent data indicated that the in vitro bone resorbing activity referred to as OAF was probably due to the release of a heterogeneous group of mediators (5,49). Thus, recent studies have shown that tumour necrosis factor-alpha ( $TNF\alpha$ ) and tumour necrosis factor-beta ( $TNF\beta$ ) all possess OAF-like bone-resorptive properties in vitro (47,51). Since  $TNF\alpha$  and  $TNF\beta$  are both present in activated leukocyte cultures in vitro, and  $TNF\beta$  is produced by myeloma cell-lines (52), Bertolini (51) has recently suggested that  $TNF$  production may account, in whole or in part, for the bone-resorbing activity previously referred to as OAF. Other cytokines which may also contribute to this activity include interleukin-1 (47) and the prostaglandins (53).

Because of their association with cells of the lymphoid series, OAF's have been implicated predominantly in the pathogenesis of



hypercalcaemia caused by haematological cancers such as myeloma and lymphosarcoma (5,31,46,50,54) While OAF-like material has been detected by radio-immunoassay in the circulating blood of patients with myeloma (55), it is probable that the OAF's bring about their effects by a local, rather than systemic action. Osteoclastic bone resorption has been found to be markedly elevated in areas of the skeleton which are infiltrated by myeloma cells (31), but is normal in uninvolved bone (56). When bone-resorbing doses of OAF's are injected into experimental animals hypercalcaemia does not occur (41), and in man, serum calcium values in patients with myeloma correlate more closely with renal glomerular function than OAF production (28).

With one possible exception (48), OAF's, as defined above, have not been shown to be produced by solid malignant tumours. However, it is possible that, in metastatic bone lesions, the presence of solid tumour cells could lead to release of OAF's by lymphocytes and monocytes, as the result of a cell-mediated immune response, leading to a local increase in bone resorption (57).

### 1.2.2 PROSTAGLANDINS

Prostaglandins were first invoked in the pathogenesis of malignancy-associated hypercalcaemia, shortly after Klein and Raisz, showed that they possessed potent bone-resorbing activity in vitro (53). Tashjian described bone-resorbing activity in extracts of tumour from two animal models of human hypercalcaemia; the HDSM-1 mouse fibrosarcoma and the rabbit VX-2 carcinoma (58,59). Conditioned media from the HDSM-1 tumour cells in culture were shown to contain large amounts of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). The bone resorbing activity normally present in in this medium was abolished by pre-

incubation of the HDSM-1 cells with aspirin or indomethacin - potent inhibitors of prostaglandin synthesis (60). In the rabbit VX-2 carcinoma, tumour-bearing animals were found to have raised circulating PGE<sub>2</sub> levels when compared with normal controls; moreover, PGE<sub>2</sub> levels were particularly high in the venous drainage of the tumours (59). Pre-treatment with indomethacin prevented the development of hypercalcaemia in animals, into whom the VX-2 carcinoma had been transplanted. From these observations, it was postulated that the hypercalcaemia may have been due to the systemic release of PGE<sub>2</sub> by tumour tissue. In a complementary study by Galasko, intense osteoclastic bone-resorption was found in the ipsilateral femur of rabbits, whose adjacent muscle had been injected with VX-2 carcinoma cells (30). In these experiments, the bone-resorbing effects of the tumour were blocked by indomethacin, and the tumour tissue was found to contain PGE<sub>2</sub>-like bioactivity. Nonetheless, Galasko failed to find evidence of significantly increased bone resorption in areas of the skeleton distant from the tumour implants, indicating that the osteolytic mediator had a local, rather than systemic action (30).

Prostaglandins were first invoked in the pathogenesis of human hypercalcaemia of malignancy by Brereton (61) who found raised levels of PGE<sub>2</sub> and prostaglandin F (PGF) in tumour tissue from a patient with hypercalcaemia and renal adenocarcinoma. Although plasma prostaglandins were undetectable in the above patient, the hypercalcaemia seemed to respond to indomethacin therapy, leading the authors to suggest that release of prostaglandins by tumour tissue may have been responsible for the hypercalcaemia. In a similar report by Robertson (62), raised levels of immunoreactive PGE were found in

serum and tumour tissue from a patient with hypercalcaemia and a renal adenocarcinoma. While a striking increase in PGE levels seemed to coincide with worsening of hypercalcaemia in this patient, aspirin therapy failed to correct the hypercalcaemia, despite reducing serum PGE levels to normal. The most extensive studies of prostaglandins in human hypercalcaemia of malignancy were those of Seyberth, who measured the common metabolite of PGE<sub>1</sub> and PGE<sub>2</sub> in the urine of patients with cancer associated hypercalcaemia (63). Levels of the PGE metabolites were found to be substantially raised in hypercalcaemic solid-tumour patients when compared with those in "control" groups of patients with haematological cancers, primary hyperparathyroidism and normocalcaemic solid-tumour patients. In four out of six hypercalcaemic patients who were treated with aspirin or indomethacin, serum calcium values fell, leading the authors to suggest that a PGE - mediated increase in bone resorption may have been responsible for the hypercalcaemia.

Other workers however, have failed to confirm that prostaglandin synthetase inhibitors such as indomethacin and aspirin have a significant calcium-lowering effect in cancer-associated hypercalcaemia (64). They have similarly been found to be ineffective in preventing the progression of metastatic bone disease in clinical practice (5). Additional evidence which weighs against a causal role for PGE as a circulating mediator of hypercalcaemia in malignancy is as follows: studies of plasma immunoreactive PGE levels in cancer patients have shown a substantial overlap between hypercalcaemic and normocalcaemic subjects, and a poor correlation between serum calcium values and circulating PGE levels (65,66). The circulating concentrations of PGE hitherto observed in humans with cancer-

associated hypercalcaemia would in addition, be insufficient to account for a systemic increase in bone resorption (67). Indeed, in vivo, circulating PGE<sub>2</sub> is rapidly broken down in the pulmonary circulation to metabolites which have relatively weak bone-resorbing activity in vitro (67). Finally, infusions of PGE<sub>2</sub> intra-arterially in dogs failed to produce significant hypercalcaemia, except when the doses used were so high as to cause haemoconcentration due to diarrhoea (69). In rats, intravenous PG E<sub>2</sub> infusions at high doses were noted to cause hypercalcaemia in one study (70), although the relevance of this to the in vivo situation in man is doubtful.

From the above data, it is unlikely that the prostaglandins are commonly involved as circulating humoral mediators of bone resorption or hypercalcaemia in human cancers. They may however, be involved as local mediators of increased bone resorption in metastatic lesions (71,72,73,74). Furthermore, it has recently been shown that in vitro the bone-resorbing effects of many factors such as complement-sufficient serum (75), epidermal growth factor (76) and extracts of tumour associated with hypercalcaemia (77,78) are mediated by increased prostaglandin production in bone at a local level

### 1.2.3 VITAMIN D METABOLITES

The metabolites of vitamin D (25-hydroxycholecalciferol, and 1,25-dihydroxycholecalciferol) are potent stimulators of bone resorption in vitro (79) and in vivo (80), and cause enhanced absorption of calcium and phosphate from the intestine (80,81,82). The former effect in particular, prompted Gordan to suggest, in 1966, that hypercalcaemia and bone resorption associated with breast carcinoma was due to a "vitamin-D like" sterol which he and his

colleagues had isolated from breast cancer tissue (83,84). Subsequent investigations by Haddad however, demonstrated that similar sterols were also present in the circulating blood of normal women, lactating women, and normocalcaemic patients with breast carcinoma (85). In addition, the concentrations of these sterols were considered by Haddad to be insufficient to account for significant increases in bone resorption, at least as judged by in vitro evidence (85). At the intestinal level, Coombes (86) found low rates of calcium absorption in both normocalcaemic and hypercalcaemic cancer patients, also implying that vitamin-D like effects were not usually evident in these situations.

In patients with lymphoreticular cancers, the active vitamin D metabolites may play a part in the pathogenesis of hypercalcaemia however. Breslau (87), Rosenthal (88), and Davies (89) all have noted elevated circulating levels of 1,25-dihydroxycholecalciferol ( $1,25(\text{OH})_2\text{D}_3$ ) and raised intestinal absorption of calcium (87) in hypercalcaemic patients with certain types of lymphoma. In this situation, metabolism of vitamin D is abnormal, in that synthesis of  $1,25(\text{OH})_2\text{D}_3$  is directly related to levels of the precursor, 25-hydroxycholecalciferol ( $25(\text{OH})\text{D}_3$ ) (89), reflecting failure of normal feedback control mechanisms (80,81,82). It is currently considered that these abnormalities arise as the result of  $1,25(\text{OH})_2\text{D}_3$  synthesis by tumour tissue itself (90,91). Accordingly, the local and systemic effects of  $1,25(\text{OH})_2\text{D}_3$  may be invoked as a contributory factor in the pathogenesis of hypercalcaemia associated with these tumours (92). In solid tumours, similar mechanisms of hypercalcaemia are not thought to be operative however, as circulating  $1,25(\text{OH})_2\text{D}_3$  levels have been found to be depressed (93).

#### 1.2.4 TUMOUR AND PLATELET-DERIVED GROWTH FACTORS

Three growth factors have so far been implicated in the pathogenesis of malignancy-associated hypercalcaemia; Transforming Growth Factor-alpha ( $TGF\alpha$ ), Transforming Growth Factor-beta ( $TGF\beta$ ), and Platelet-Derived Growth Factor (PDGF) (90). These substances are peptides, which are produced by both normal (94,95,96) and malignant tissues (97,98). In addition to a variety of other biological effects, these growth factors possess, in common, powerful bone-resorbing properties in vitro (90,99,100). While the TGF's share many biological properties in common with Epidermal Growth Factor (EGF) (101,102,103), these substances are clearly different, as antibodies to EGF do not cross-react with TGF's (90). Nonetheless, the bone-resorbing effects of  $TGF\alpha$  at least, appears to be mediated by interaction with EGF receptors since these effects are blocked by antisera directed against the EGF receptor (104).

To-date,  $TGF\alpha$  has been implicated in the pathogenesis of hypercalcaemia in two situations; the rat leydig-cell tumour model of humorally mediated hypercalcaemia in malignancy (104), and the hypercalcaemia associated with one human tumour (105). In both cases, extracts of tumour tissue contained potent bone-resorbing activity which co-eluted with  $TGF\alpha$  bio-activity in gel-filtration columns. Moreover, the bone-resorbing activity was inhibited by antisera to the EGF receptor, lending support to the hypothesis that TGF was the factor responsible (104,105).

$TGF\beta$  has similar biological effects to  $TGF\alpha$  but is an entirely different molecule, encoded by a separate gene (106). While  $TGF\beta$  is

produced by many malignant tissues (106) it is also found in other replicating tissues including normal platelets, fibroblasts, and bone cells (90). Hitherto, TGF $\beta$  has been implicated in the pathogenesis of bone resorption and hypercalcaemia associated with one animal model of cancer associated hypercalcaemia - the rat Walker Carcinosarcoma (107). In this situation, bone resorbing activity was found to co-elute from gel-filtration columns with TGF $\beta$  bio-activity, thus implicating TGF $\beta$  as the cause of hypercalcaemia.

Platelet Derived Growth Factor, in common with the above factors has been implicated as a potential mediator of hypercalcaemia in malignancy (90). It is known to stimulate bone resorption in vitro (100) and it is of interest that many cancers associated with hypercalcaemia in vivo express the v-sis oncogene, which encodes a peptide with considerable homology to PDGF (108,109). However, PDGF has not yet been implicated in the hypercalcaemia associated with any particular human or animal tumour.

In view of their powerful bone-resorbing effects in vitro (90,99,100), and association with cancerous tissues (97,98,104,105,106,107), the above named growth factors may well be involved as local mediators of bone resorption in metastatic lesions. It is currently unclear however, whether these factors can also be implicated as systemically active, humoral mediators of hypercalcaemia. Indeed, while TGF's have been shown to occur in tumour tissue from patients with humorally-mediated hypercalcaemia (104,107) they are also present in tumours unassociated with hypercalcaemia (97,98). Moreover, in order to implicate TGF's as humoral mediators of hypercalcaemia, two major criteria must be

fulfilled: firstly, they must be released into the circulation by tumour tissue in an active form sufficient to cause bone resorption at sites distant from tumour deposits. Secondly, if injected or infused into experimental animals at bone-resorbing doses, these growth factors would be expected to cause hypercalcaemia. Since the effects of the TGF's and PDGF have not yet been studied in vivo, these questions remain unanswered.

#### 1.2.5 PARATHYROID HORMONE

Parathyroid hormone (PTH) excess has long been recognised as a cause of hypercalcaemia and hypophosphataemia (110,111), by virtue of its effects on osteoclastic bone resorption (112,113), renal tubular reabsorption of calcium (114) and phosphate (111), and intestinal absorption of calcium (115) via increased production of  $1,25(\text{OH})_2\text{D}_3$  (116). Since many of the biological effects of PTH are mediated by interaction with an adenylyl-cyclase linked receptor (117), patients with PTH excess also exhibit elevated urinary excretion of cyclic-adenosine 3' 5' monophosphate (cAMP) (118).

For many years, similarities have been noted between primary hyperparathyroidism and "non-metastatic" hypercalcaemia of malignancy (6,7). In both conditions, the elevated serum calcium levels are associated with hypophosphataemia, renal phosphate wasting (9,10), and elevated levels of nephrogenous cyclic AMP (NcAMP) (93). On removal of the malignant tumour (9,10) or parathyroid adenoma (119), these abnormalities are reversed. Because of these similarities, "ectopic" PTH production was one of the first mechanisms postulated to explain the development of hypercalcaemia in patients without metastatic bone lesions (6,7,120,121).



With the advent of immunoassay techniques in the 1960's, many investigators found detectable PTH-activity in serum and/or tumour extracts from patients with "non-metastatic" hypercalcaemia. For example, Tashjian's group, using quantitative complement fixation techniques, found evidence of PTH-like activity in tumour tissue from patients with humorally-mediated hypercalcaemia (122,123). In later studies, using more sensitive radio-immunoassays, Sherwood also detected PTH in tumour tissue from about 50% of patients with "non-metastatic" hypercalcaemia - but at much lower concentrations than in parathyroid adenomata (124). Similar findings were also reported by Knill-Jones (125), Melick (126), Maviligit (127) and Blair (128) in subsequent individual case-reports.

Benson, using a radio-immunoassay with carboxyl-terminal specificity found serum immunoreactive PTH (iPTH) levels to be "inappropriately detectable" in over 90% of unselected patients with cancer-associated hypercalcaemia (129). These findings were interpreted by the authors as indicating that "ectopic" release of PTH was responsible for the hypercalcaemia (129). Other workers, using similar radio-immunoassays, also found detectable levels of circulating iPTH in cancer-associated hypercalcaemia (130,131). A consistent feature in all of the above studies however, was the fact that the iPTH levels observed in hypercalcaemia of malignancy were lower than those in true primary hyperparathyroidism for any given level of serum calcium (129,130,131). Moreover, in other studies, elevated iPTH levels were frequently noted in normocalcaemic cancer patients - particularly those with oat-cell or adenocarcinoma of lung (121,131). In contrast with the above data, Powell, using a different radio-immunoassay system with amino-terminal specificity, failed to

detect iPTH in either serum or tumour tissue from a series of patients with "non-metastatic" hypercalcaemia(132). Recent findings indicate that the differences between Powell's data and those of other workers (129,130,131), were largely due to the different characteristics of the assay systems used. Thus, in recent studies, Stewart (93) has shown that iPTH levels were frequently detectable in the serum of hypercalcaemic cancer patients using carboxyl-terminal specific assays. However, using amino-terminal assays, serum from the same patients usually yielded undetectable iPTH concentrations. Moreover, when compared with true primary hyperparathyroidism, iPTH levels in malignancy-associated hypercalcaemia were invariably low,irrespective of the assay system used.

The probable explanation for these rather paradoxical findings is as follows; normal parathyroid glands, in vivo and in vitro appear to possess an element of "non-suppressible" PTH secretion, in that they continue to release small amounts of intact PTH and its fragments even under hypercalcaemic conditions (133,134). Moreover, under these conditions there is secreted a higher relative proportion of carboxyl-terminal fragments to intact hormone (134). Since these carboxyl-terminal fragments are cleared slowly from the circulation - particularly in the presence of renal impairment (135), it is apparent that the detectable levels of iPTH observed using carboxyl-terminal-specific assays may have derived from normally suppressed parathyroid glands rather than the tumour itself (136). Such a mechanism would certainly be consistent with recent studies which failed to detect messenger RNA, coding for PTH, in tumour tissue derived from human breast cancers and other animal and human tumours associated with hypercalcaemia (137).

From the above data, it is currently considered that true "ectopic" PTH production is a rare cause of hypercalcaemia in malignancy (138). There are, however a few cases documented in whom such a mechanism seems to have been operative (124,125,126,127,128).

#### 1.2.6 PARATHYROID HORMONE-LIKE FACTORS

Reference has been made to the many biochemical similarities which exist between humoral hypercalcaemia of malignancy (HHM) and primary hyperparathyroidism. There are also major differences between these conditions however. These were first noted by Lafferty who commented on the absence of periosteal bone erosions and tendency to hypochloraemic alkalosis in HHM, when compared with primary hyperparathyroidism which is classically associated with subperiosteal bone erosions and a tendency to hyperchloraemic acidosis (7).

In recent years, Stewart and his colleagues have made detailed studies of the biochemical differences between primary hyperparathyroidism and hypercalcaemia of malignancy (93). Stewart divided patients with cancer-associated hypercalcaemia into two groups by the pattern of nephrogenous cyclic AMP (NcAMP) excretion. In patients with high NcAMP values, squamous and genitourinary cancers were the commonest tumours, and bone metastases were infrequent - consistent with previous series' of patients with humorally mediated hypercalcaemia (7,8,10). In contrast, low NcAMP values were usually found in patients with hypercalcaemia due to breast carcinoma and myeloma where skeletal metastases were extensive, suggesting that non-humoral mechanisms of hypercalcaemia were operative (5). In the group of patients with a presumed humoral mechanism of hypercalcaemia, NcAMP excretion and renal tubular phosphate reabsorption were

indistinguishable from patients with primary hyperparathyroidism. Immunoreactive PTH values were, of course low or undetectable in the patients with malignancy, using four separate region-specific immunoassays. In comparison with primary hyperparathyroidism, the levels of urinary calcium excretion in malignant hypercalcaemia were significantly raised, and the levels of  $1,25(\text{OH})_2\text{D}_3$ , significantly depressed, indicating to the authors that PTH-like effects on distal renal tubular reabsorption and renal  $1\alpha$  hydroxylase activity were largely absent.

Subsequent studies by Stewart also demonstrated differences in bone histology between the humoral hypercalcaemia of malignancy (HHM) and primary hyperparathyroidism (139). Examination of undecalcified bone biopsy specimens by quantitative histomorphometry confirmed that in HHM, there was a marked increase in osteoclastic bone resorption in bone at sites distant from areas of tumour involvement. Similar findings had been noted by previous workers in non-histomorphometric studies of patients with HHM (140,141,142). In addition, Stewart noted that there was a striking reduction in osteoblastic activity in HHM when compared with primary hyperparathyroidism, and suggested that the putative humoral mediator responsible for HHM specifically caused "uncoupling" of bone resorption and bone formation (139). These observations were taken as further evidence of a fundamental difference between the functional effects of the HHM-associated mediator and parathyroid hormone.

Subsequent studies aimed at identifying the putative humoral mediator responsible for HHM were based on the in vivo biochemical evidence which suggested that some of its biological effects were

mediated by interaction with a adenylyl-cyclase linked receptor in the kidney (93). In 1980, Goltzman had shown that serum samples from HHM patients possessed glucose-6-phosphate dehydrogenase stimulating activity in a PTH-sensitive bioassay system in vitro (143). Further studies by Stewart (144), Strewler (145) and Rodan (146) demonstrated that extracts of tumours which caused the clinical syndrome of HHM in man contained adenylyl-cyclase stimulating activity in vitro, which was competitively inhibited by synthetic antagonists of PTH. Characterisation of the substances responsible for this activity indicated that they were peptides with molecular-weights ranging between 9000 and 50,000 daltons and were distinct from PTH itself (144,145,146). From these observations it has been considered that in HHM a humoral substance is released which can interact with the PTH receptor in kidney and bone, yet is structurally quite distinct from PTH (144,145,146). Whether this substance is indeed the cause of hypercalcaemia in these patients is as yet unclear; Stewart and other workers have failed to find evidence of increased renal tubular reabsorption of calcium in HHM (93,147,148). Similarly,  $1,25(\text{OH})_2\text{D}_3$  levels and intestinal calcium absorption have been reported to be depressed in HHM (86,93). These data contrast markedly with the analogous situation in primary hyperparathyroidism where increased renal tubular reabsorption of calcium and increased intestinal absorption of calcium make a greater contribution to hypercalcaemia than increased bone resorption (4,114,115,116). Indeed., in most conditions associated with accelerated bone resorption alone, hypercalcaemia is rare since efficient renal homeostatic mechanisms tend to preserve normocalcaemia at the expense of hypercalciuria (4). Moreover, while HHM is, in many cases

associated with release of the adenyl-cyclase stimulating factors discussed above (144,145,146) these have not convincingly been shown to possess bone-resorbing properties in vitro. Although Stewart and his colleagues have identified both bone-resorbing and adenylate cyclase-stimulating activity in the rat Leydig cell tumour model of HHM (149), it has been suggested that the bone-resorbing activity associated with this tumour is due to the effects of  $TGF\alpha$ , rather than the adenylate cyclase stimulating factor (90). Thus, recent data has shown that bone resorption stimulated by extracts of the rat Leydig tumour in vitro is inhibited by antiserum to the EGF receptor but not by synthetic antagonists of PTH (150).

Moreover, while Stewart and his colleagues (93) found a bimodal distribution of NcAMP values in patients with cancer-associated hypercalcaemia, and on this basis were able to separate their patients into "humorally-mediated" and "non-humorally-mediated" subgroups, other workers have failed to find such clear-cut differences in the patterns of NcAMP excretion in patients with malignancy. Rude (151) found a substantial overlap of NcAMP values between patients with "non-metastatic" hypercalcaemia and those with extensive metastatic disease due to myeloma and breast carcinoma, where a local-osteolytic mechanism of hypercalcaemia would have been predicted. In addition, both Rude (151) and Kukreja (152) found that NcAMP values were elevated in many normocalcaemic cancer patients - particularly those with squamous lung carcinoma. From these data it is apparent that elevated levels of NcAMP may be a relatively non-specific feature of some cancers rather than a biochemical marker for PTH-like humoral mediators of hypercalcaemia.

### 1.2.7 SUMMARY

Relevant points with regard to the putative humoral mediators of hypercalcaemia discussed above may be summarised as follows:

- (1) Virtually all of the putative humoral mediators of hypercalcaemia in malignancy are thought to raise serum calcium by stimulating osteoclastic bone resorption (5,6,30,31,51,64,77,78,90,93,139,140,141,142).
- (2) In most cases it is probable that tumour tissues contain more than one substance with in vitro bone resorbing activity. Since many normal tissues also possess such activity [eg platelets (95,100),placenta (94), normal serum (75)], the demonstration of bone-resorbing activity in tumour tissue does not necessarily mean that this is the cause of hypercalcaemia.
- (3) Ectopic production of bioactive PTH by non parathyroid tumours, although documented (124,125,126,127,128), is a rare cause of malignancy-associated hypercalcaemia (137,138).
- (4) In haematological cancers, the mechanisms of hypercalcaemia are best-established; local osteolysis on a multifocal basis, stimulated by mediators which are best characterised - the OAF's (31,40,44,45,46), TNF's (51) Interleukin-1 (47) and  $1,25(\text{OH})_2\text{D}_3$  (87,88,89).
- (5) In solid tumours associated with a heavy skeletal tumour load - breast carcinoma for example, local osteolysis is though to be the principal mechanism of hypercalcaemia

(2,3,5). The mediators of hypercalcaemia are less clear in this situation, although possible candidates include the prostaglandins (71,72), TGF's (97), EGF (103) and others as yet undefined.

- (6) In solid tumours other than breast carcinoma (eg squamous carcinoma of lung) humorally mediated effects on bone and possibly other target organs of calcium homeostasis are the likely cause of hypercalcaemia. The PTH-like factors are released in a high proportion of these tumours (93) but the mechanisms by which they could cause hypercalcaemia are unclear; unlike PTH they are thought not to increase renal tubular calcium reabsorption or  $1,25(\text{OH})_2\text{D}_3$  synthesis (93), nor have they been shown to possess bone resorbing properties in vitro (150). The release of other bone -resorbing factors by these tumours such as the transforming growth factors, for example, may prove to be important in the pathogenesis of hypercalcaemia in these situations (5,90,104).



### 1.3 PATHOPHYSIOLOGY OF HYPERCALCAEMIA

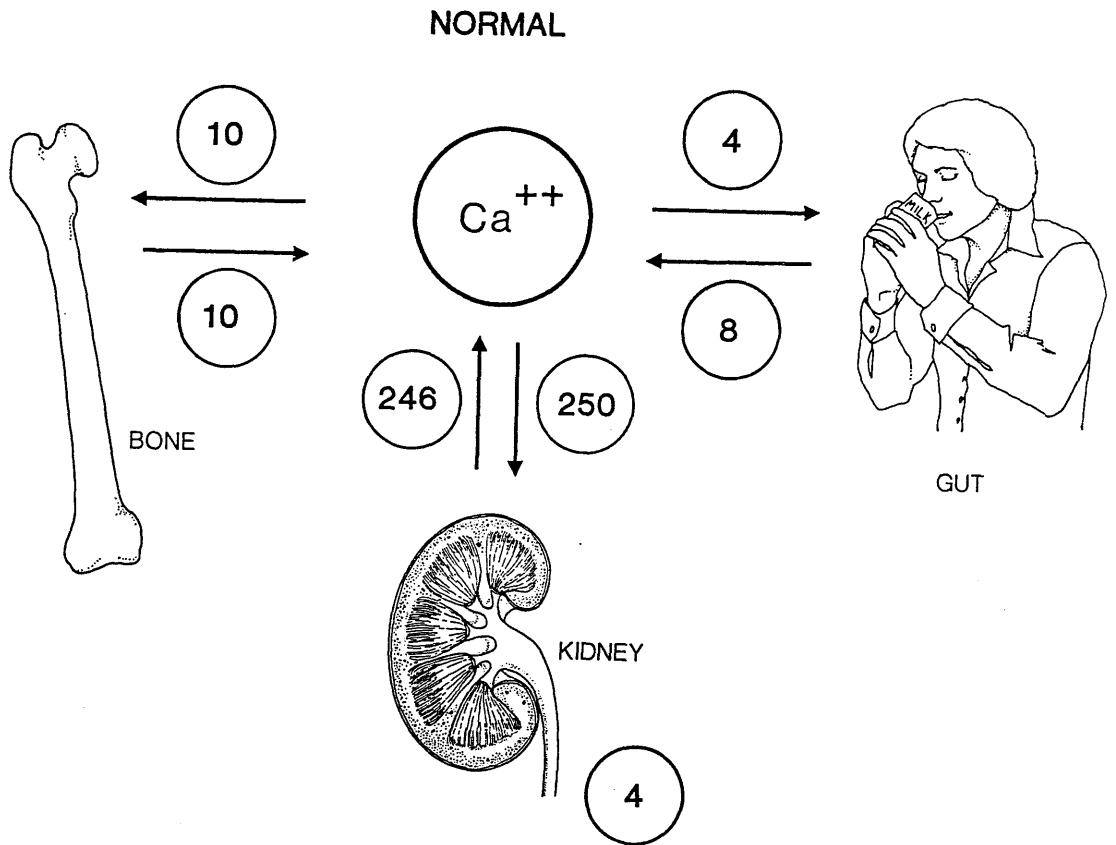
In health, the concentration of ionised calcium in the extracellular fluid (ECF) is maintained within a narrow range by homeostatic mechanisms which control the processes of calcium exchange in three main target tissues; bone, kidney and intestine (153) The most important calcium regulating mechanisms are hormonal in nature and involve, in particular, parathyroid hormone (PTH) and  $1,25(\text{OH})_2\text{D}_3$ . Together, these hormones form a classical endocrine system which serves principally to regulate the ECF calcium concentration (154,155). This system can be regarded as having two main components: a rapidly-acting component of limited capacity which accounts for the minute-to-minute regulation of ECF calcium and a slowly-acting component of almost infinite capacity which is involved in control of calcium balance in the longer term.

The rapidly acting system is largely controlled by PTH, via its effects on renal tubular reabsorption of calcium (114) and non-osteoclast mediated exchange of calcium at the ECF-bone interface (4,156). In the longer term, calcium balance is achieved by the combined effects of both hormones; intestinal absorption of calcium is modulated by circulating  $1,25(\text{OH})_2\text{D}_3$  levels (116), which in turn, are determined mainly by the circulating levels of PTH (157). The body's main reserves of calcium are in bone however, and long-term changes in calcium balance in the adult are usually due to alterations in skeletal remodelling which result from the combined effects of PTH and  $1,25(\text{OH})_2\text{D}_3$  on bone forming (158,159,160) and bone resorbing cells (80,112)

In man, about 50% of the calcium in serum is in the ionised form, a further 10% is ultrafilterable but ligand-bound and the remaining 40% protein bound (153). Since the ionised fraction of serum calcium is that which is biologically active (153,161,162) and under homeostatic control (153,164), hypercalcaemia may be defined to exist when there is an elevation in ionised calcium levels. Over 90% of the protein-bound calcium is bound to albumin (165) by mechanisms which are affected by pH (166). Accordingly plasma or serum ionised calcium measurements may not correspond with measurements of total calcium values in dysalbuminaemic states or where there is a significant disturbance in acid-base status (153). Nonetheless, several nomograms have been devised to "correct" for differences in albumin binding so that it is possible to obtain a "derived" calcium figure from total serum calcium and albumin measurements which approximates to the measured ionised calcium value, provided that there are no major disturbances in acid-base status (153,161,167).

In figure 1.2, the principal elements of calcium homeostasis are shown schematically in a normal individual with a steady state of zero external calcium balance. Although the actual quantities of calcium exchanged at each site are approximate, the figure serves to illustrate that hypercalcaemia may potentially arise when alterations in calcium flux occur at any one of the three regulatory sites in bone kidney or intestine.

Traditionally, most attention has been paid to factors which increase the amount of calcium released from bone in the pathogenesis of malignancy-associated hypercalcaemia (5). However, Parfitt has estimated that, given the normal characteristics of tubular calcium



**FIGURE 1.2**

Schematic representation of calcium homeostasis in a normal adult. Note that a state of zero net balance is maintained by homeostatic mechanisms despite large daily fluxes of calcium at regulatory sites in bone, kidney and intestine. Numbers refer to daily exchanges in mmol.

reabsorption (114) and normal renal glomerular function, 5mmol of calcium would need to be released from bone to account for each 0.1mmol/l rise in serum calcium. Since actual measurements of calcium balance in normal individuals give values of between 1-2mmol/day (168,169), it would be anticipated that a 15-30 fold increase in net bone resorption would be required to raise serum total calcium from 2.50mmol/l to a modestly elevated level of 3.10mmol/l. Relatively few detailed studies of calcium balance have been carried out in patients with malignant disease. However, Laszlo has estimated that, in patients with metastatic bone disease associated with breast carcinoma and myeloma the negative calcium balance varies between 2-10mmol/day in normocalcaemic patients to between 5 and 15mmol/day in hypercalcaemic subjects (23,170). From the data in normal individuals cited above (168,169), negative calcium balances of this magnitude can be calculated to represent a net increase in bone resorption of between 2-15 fold at most. In the presence of normal renal function, such increases in bone resorption would not be expected to cause substantial hypercalcaemia. This is exemplified by the fact that in most benign disorders associated with increased bone resorption, hypercalcaemia is rare because of the efficient renal homeostatic mechanism (4,153).

In malignant disease however, further mechanisms are invoked to explain the development of hypercalcaemia on the basis of a "bone-resorptive" mechanism. Histological evidence has suggested that in HHM, there is "uncoupling" of bone resorption and bone formation leading to a net loss of skeletal calcium far greater than that observed in benign conditions where bone resorption is increased (139). Patients with cancer are also prone to impairment of renal

function because of dehydration, which may develop as the result of nausea, vomiting and anorexia associated with the tumour itself, with a central effect of hypercalcaemia or with chemotherapy and radiotherapy (5). Since the kidney plays a central role in elimination of excess calcium from the ECF, impairment of renal glomerular function (GFR) critically impairs the ability to respond to a calcium load (4,153)

In hypercalcaemia of malignancy the following scheme has been proposed (171); a point is reached where, due to a combination of mild dehydration and increased bone resorption, release of calcium from bone exceeds renal calcium excretion thus causing serum calcium to rise. The resultant hypercalcaemia brings homeostatic mechanisms into play which tend to restore calcium levels toward normal; PTH secretion is suppressed and as a result, renal tubular calcium reabsorption falls (114) and efflux of skeletal calcium at quiescent bone surfaces is reduced (156). In addition, synthesis of  $1,25(\text{OH})_2\text{D}_3$  is suppressed resulting in reduced intestinal absorption of calcium (155). If these homeostatic mechanisms are inadequate however, hypercalcaemia becomes established with deleterious effects on renal function. Severe hypercalcaemia is associated with a direct reduction in renal blood flow and GFR (171,172). Tubular abnormalities also occur, leading to inappropriate losses of sodium chloride (172,173), water (173), potassium (171) and a metabolic alkalosis (174). When these renal tubular defects are combined with decreased fluid intake as the result of nausea, vomiting and anorexia, sodium depletion and dehydration become increasingly severe (175). There then ensues a deteriorating spiral of worsening hypercalcaemia due to increased sodium-linked reabsorption of calcium in the proximal renal tubule (176) and

impairment of GFR (172). Ultimately this sequence of events culminates in hypercalcaemic crisis and death.

Parfitt (4) has drawn attention to the important differences which exist between the stable type of hypercalcaemia generally associated with primary hyperparathyroidism, and the unstable hypercalcaemia described above, which is traditionally associated with malignant disease. In primary hyperparathyroidism hypercalcaemia is largely mediated by increased renal tubular calcium reabsorption which may or may not be accompanied by an increase in net bone resorption. Accordingly, most patients with primary hyperparathyroidism have a steady-state type of hypercalcaemia which changes little with time due to a raised "set-point" for control of serum calcium as the result of an elevated renal tubular threshold for calcium reabsorption (4,114). In contrast, renal tubular calcium reabsorption is generally considered to be depressed in cancer-associated hypercalcaemia provided that dehydration and sodium depletion are not present (4,5,93,147,148). In this situation, hypercalcaemia cannot be due to a stable elevation in the renal tubular calcium threshold by definition. Accordingly, it is currently considered that when established, the hypercalcaemia associated with malignancy always progresses due to the positive feedback system described above (4,5).

Another difference between the hypercalcaemia of malignancy and primary hyperparathyroidism deserves further emphasis. In primary hyperparathyroidism, intestinal calcium absorption is enhanced as the result of increased  $1,25(\text{OH})_2\text{D}_3$  synthesis (115,116,157). Indeed, patients with mild primary hyperparathyroidism may only exhibit

hypercalcaemia after provocative testing with an oral calcium load (177). In malignant disease however, intestinal calcium absorption has been found to be depressed (86), which corresponds with the finding of low  $1,25(\text{OH})_2\text{D}_3$  levels in hypercalcaemic cancer patients (93).

#### 1.4 CLINICAL FEATURES OF CANCER-ASSOCIATED HYPERCALCAEMIA

Cancer-associated hypercalcaemia is important to the clinician for two reasons: it is a common condition, second only to primary hyperparathyroidism as a cause of hypercalcaemia in the general community (178). In hospitalised patients, cancer is the commonest cause of hypercalcaemia (179). Although it has been estimated that about 10% of all cancer patients develop hypercalcaemia (175) the proportion of patients developing hypercalcaemia varies markedly in different tumour types (5). For example, squamous carcinoma of the lung, renal carcinoma, breast carcinoma and myeloma are common causes of hypercalcaemia. In contrast, small-cell lung carcinoma, and adenocarcinoma of colon, although common tumours, are rare causes of hypercalcaemia (5,179,180,181). These differences in the incidence of hypercalcaemia are not wholly related to the pattern of metastatic bone involvement; although bone metastases are a prominent feature of hypercalcaemic patients with myeloma and breast carcinoma, such metastases are also common in normocalcaemic patients with small-cell lung carcinoma. Accordingly, it is probable that the differences in prevalence of hypercalcaemia between different tumour types relates to other factors such as the release of calcium-elevating humoral substances by tumour tissue.

It is important for the clinician to recognise hypercalcaemia in patients with malignancy, principally because of its symptomatic effects. These were first noted by Collip in 1925 (110), when he reported that dogs which were overdosed with parathyroid extract suffered from anorexia, obtundation, coma, asthaenia, hypotension, renal failure and ultimately, death. In man, mild elevations in serum



calcium ( $<3.00\text{mmol/l}$ ) may not be accompanied by obvious symptomatology (178). However, the hypercalcaemia associated with malignancy is often symptomatic. Warwick (175), in a review of the clinical features associated with cancer-associated hypercalcaemia noted gastrointestinal symptoms such as nausea, anorexia, vomiting and constipation in 75% of cases. Disturbances of the central nervous system such as muscular weakness, confusion and obtundation were noted in 85%, thirst and polyuria in 11%, and generalised malaise and fatigue in 5%. Similar findings have also been reported by Myers (10) and Fiske (179) although in the latter study, "non-specific" symptoms such as fatigue and malaise were predominant. A rather unusual feature which has been noted by a number of workers in cancer-associated hypercalcaemia is an apparent lowering of the pain threshold (5,10,175,179). With extreme elevations in serum calcium, cardiovascular effects such as hypotension and arrhythmias may be observed, either as the result of the hypercalcaemia per se (182), or in association with the electrolyte disturbances of acute renal failure (172). It is probable that these cardiac abnormalities are the immediate cause of death in untreated patients dying from hypercalcaemic crisis.

Many of the symptoms and signs of hypercalcaemia are non-specific and may easily be confused with the terminal features of malignant disease itself. However, these features are important to identify, as they may be reversed by appropriate antihypercalcaemic therapy (5). In malignant disease, antihypercalcaemic therapy is probably not given as often as it should be; in one study, less than 50% of patients with severe hypercalcaemia received specific therapy aimed at reducing serum calcium levels (183).

## 1.5 MANAGEMENT OF CANCER-ASSOCIATED HYPERCALCAEMIA

A variety of drugs and other therapeutic manoeuvres have been used in the treatment of malignancy-associated hypercalcaemia. These are discussed below:

### 1.5.1 INORGANIC PHOSPHATE

This was probably the first effective treatment to be widely used in cancer-associated hypercalcaemia following Bulger's demonstration in 1920 that phosphate had a powerful calcium-lowering effect in primary hyperparathyroidism (184). The first large series of phosphate-treated cancer patients with hypercalcaemia was reported by Goldsmith in 1966 (185). In this study, intravenous and oral phosphate were found both to be effective in lowering serum calcium concentrations and improving symptoms in patients with malignancy-associated hypercalcaemia due to a variety of malignant tumours. While similar beneficial effects were noted by other workers using phosphate therapy (186,187,188,189), problems also arose; a number of reports appeared in the literature documenting massive extraskeletal calcification, acute renal failure and hypotension in patients who had been given intravenous phosphate (190,191,192). Oral phosphate, although less likely to cause extraskeletal calcification (193), had other adverse effects including nausea, abdominal discomfort and diarrhoea which limited its use in up to 40% of hypercalcaemic cancer patients (187,188,189). The mechanism by which intravenous phosphate lowers blood calcium has been studied in most detail by Herbert (194). There is an acute calcium-lowering effect which is operative within minutes of starting phosphate infusion, due to the precipitation of insoluble calcium - phosphate salts in bone and soft tissues. After

phosphate infusion is stopped however, there is a delayed return of serum calcium to the pre-treatment level possibly as the result of a more sustained inhibition of osteoclastic bone resorption (195). The mechanisms by which oral phosphate lowers serum calcium in malignancy are less clear but are probably similar to those described above. A further potential mode of action of oral phosphate is inhibition of calcium absorption by the intestine (196). Although this may be relevant in hyperparathyroidism, it is probably of minor importance in cancer-associated hypercalcaemia as in the latter, intestinal calcium absorption is depressed (86).

#### 1.5.2 SODIUM SULPHATE

Sodium sulphate infusions were employed in the clinical treatment of hypercalcaemia following Walser's experiments, which showed that sodium sulphate infusion caused marked hypercalciuria and hypocalcaemia in dogs (197). Although subsequent evidence indicates that this effect may, in part, have been due to a natriuresis (176), the calciuric effect of sodium sulphate was 5-fold greater than that observed with a similar quantity of sodium chloride. Walser postulated that the greater effect of sodium sulphate was due to the formation of ultrafilterable, non-reabsorbable calcium sulphate complexes. Sulphate infusions were first employed in clinical practice by Kenny (198), in the treatment of a hypoparathyroid patient who had been accidentally overdosed with parathyroid extract. Lemann subsequently used sodium sulphate in the treatment of primary hyperparathyroidism (199). These and other studies in cancer-associated hypercalcaemia (186,188,200), confirmed that sodium sulphate had potent calcium-lowering effects in man. Although most

workers found that sodium sulphate had a reliable calcium-lowering effect, problems were encountered in some cases because of hypernatraemia and sodium overload (200,201). Finally, the short duration of sodium sulphate's action and intravenous mode of administration render it impractical as an option for the long-term management of hypercalcaemia (186,188). Accordingly, in recent years, sodium sulphate treatment of hypercalcaemia has fallen out of general usage (189).

### 1.5.3 ETHYLENEDIAMINE TETRA ACETIC ACID (EDTA)

EDTA has the specific effect of chelating calcium in the blood - a process which directly decreases ionised calcium without affecting total calcium concentration. The EDTA-calcium complexes so formed are soluble and ultrafiltrable but are not reabsorbed by the renal tubules (202,203). These combined effects give EDTA potent hypocalcaemic and hypercalciuric effects. EDTA has two major drawbacks however; its effects are transient (204), and with repeated doses, there is a substantial risk of severe renal damage (205,206). For these reasons EDTA is seldom used now in the treatment of hypercalcaemia.

### 1.5.4 CORTICOSTEROIDS

Corticosteroids have been used for many years in the treatment of cancer-associated hypercalcaemia with variable results. In some studies they have been found to be highly effective (207,208), whereas in others they have been relatively ineffective (186,189,209,210). It is probable that some of the early reports of successful "responses" to corticosteroids were accounted for by simultaneous administration of intravenous fluids to steroid-treated patients (207,208).

Accordingly in two recent studies, corticosteroids were strikingly ineffective in the treatment of cancer-associated hypercalcaemia when patients were adequately rehydrated to begin with (209,210). A beneficial response to steroids may be observed however, when the tumour itself is steroid sensitive such as is the case in lymphoreticular neoplasms and some patients with breast or prostatic carcinoma (211).

Glucocorticoids have been shown to inhibit bone resorption stimulated by OAF's released by myeloma and lymphosarcoma cell lines in vitro (46,212). Glucocorticoids have also been shown to inhibit bone resorption stimulated by a number of other substances in vitro such as prostaglandins, vitamin A, and dibutyryl cyclic AMP (213). However, the bone resorbing effects of PTH and the vitamin D metabolites are inhibited little, if at all, by corticosteroids (213). Certainly, in clinical practice, corticosteroids are not effective inhibitors of bone resorption in most patients with solid tumour hypercalcaemia (209,210).

An additional potential mode of action for glucocorticoids would be inhibition of intestinal calcium absorption (214). Indeed, this effect is thought to explain the calcium-lowering response to corticosteroid treatment in hypercalcaemic patients with sarcoidosis and vitamin D intoxication (215,216,217). As stated previously however, intestinal calcium absorption is usually low in cancer-associated hypercalcaemia so rendering this action of minor importance.

### 1.5.5 MITHRAMYCIN

Mithramycin is a cytotoxic agent which has been used in the treatment of a variety of malignant tumours (218). A number of workers have drawn attention to its calcium-lowering effects (218,219,220) which appear to be due to inhibition of bone resorption (220,221,222), as the result of a cytotoxic effect on bone cells (5). Mithramycin, used in doses much lower than those employed for an antitumour effect is an effective treatment for cancer-associated hypercalcaemia (223,224,225). Mithramycin's major disadvantage is toxicity however; specific side-effects include a coagulopathy which may be due to thrombocytopenia, altered platelet function, a vascular defect, or reduced levels of clotting factors (226). Hepatic dysfunction, usually manifest by an elevation in serum transaminases is common although more serious and even fatal hepatic necrosis has been described (218,226). Renal dysfunction may also occur due to direct renal tubular and glomerular damage (227). Despite these adverse effects, it should be emphasised that, in the relatively small doses used to treat hypercalcaemia, mithramycin is usually free of troublesome toxicity. As safer alternatives are available however, some workers advise that mithramycin should only be given as a last resort in patients who have failed to respond to other measures (5). The converse is also true however; because of its reliable and potent calcium-lowering effects, mithramycin is considered by many oncologists to be the drug of first choice in cancer-associated hypercalcaemia.

### 1.5.6 CALCITONIN

Following the postulated existence of a parathyroid gland associated calcium-lowering factor by Copp in 1962 (228), Calcitonin was shown by Mc Intyre's group to be of thyroidal origin (229,230), confirming the data of Hirsch (231). The mechanism by which calcitonin lowers blood calcium concentration was initially thought to depend on inhibition of osteoclastic bone resorption (232,233). Indeed, it is under conditions of most rapid bone turnover that calcitonin has its most potent calcium-lowering effects (234). In addition, however, calcitonin has powerful natriuretic and calciuric effects which probably contribute to its efficacy in the treatment of hypercalcaemia (235,236).

Hitherto, calcitonin has been shown to be an effective treatment for hypercalcaemia of various aetiologies (232,234,236,237,238,239,240,241,242). Advantages of calcitonin therapy include its rapid onset of action and lack of serious toxicity. However, a significant proportion of patients with cancer-associated hypercalcaemia fail to respond favourably to calcitonin, for reasons unknown (236,241,242). In addition, the hypocalcaemic effect may "wear off" despite continued calcitonin administration (237,238,242) due to "down-regulation" of calcitonin receptors in bone (243).

Studies on the effects of calcitonin on bone resorption in tissue culture suggest that glucocorticoids may prevent the "escape" from calcitonin's inhibitory effect (243). Binstock (244) made practical use of these observations by combining calcitonin and corticosteroids in the treatment of cancer-associated hypercalcaemia. Although this

combination of drugs did seem to give better control of hypercalcaemia than either agent used alone, the effects of treatment were only studied for four days. Accordingly, the role of the calcitonin and corticosteroid combination in the long-term management of cancer-associated hypercalcaemia is, as yet, unexplored.

#### 1.5.7 PROSTAGLANDIN SYNTHETASE INHIBITORS

The rationale for using these drugs in the management of hypercalcaemia in malignant disease lies in the fact that prostaglandins were considered by some workers to be of pathogenic importance in this syndrome (58,59,62,63,66). Although patients have been described in whom therapy with indomethacin or aspirin seemed to bring about a reduction in serum calcium values, most workers have been disappointed with the results of prostaglandin synthetase inhibitors in this situation (64,189,245). It is possible that these discrepancies could relate to failure to take account of the hypocalcaemic effect of concomitant rehydration in patients given these drugs, or the fluid-retaining and plasma-volume expanding properties of the drugs themselves (246).

#### 1.5.8 DIPHOSPHONATES

The diphosphonates are a relatively new group of compounds which are synthetic analogues of pyrophosphate. Unlike pyrophosphate however, the diphosphonates are resistant to enzymatic hydrolysis in body fluids (247,248). The diphosphonates possess important effects on bone metabolism; they inhibit bone resorption (248,249) and bone mineralisation (247) , both in vitro and in vivo. However, their relative potency with respect to these processes depends on the



particular diphosphonate being studied. For example, Ethane-1,1-hydroxy-diphosphonate (EHDP), used at doses sufficient to inhibit bone resorption also has substantial inhibitory effects on bone mineralisation (250). On the other hand, 3-amino-1-hydroxypropylidene 1,1-diphosphonate (APD) and dichloromethylene diphosphonate (Cl<sub>2</sub>MDP) have negligible effects on mineralisation at doses which effectively inhibit bone resorption in vivo (251,252).

In view of their potent inhibitory effects on bone resorption, the diphosphonates have been widely used in the treatment of cancer-associated hypercalcaemia. In a number of studies, all three diphosphonates currently available for clinical usage (EHDP, APD, Cl<sub>2</sub>MDP) have been shown to be effective agents in the treatment of cancer-associated hypercalcaemia, when administered intravenously (253,254,255,256,257,258,259). Although Cl<sub>2</sub>MDP (253,260,261) and APD (258) are also effective when given orally, EHDP is not (189). The reasons for this are unclear but may be due to EHDP's poor gastrointestinal absorption, or its inhibitory effect on bone mineralisation (248,250), which by decreasing entry of calcium into bone, may theoretically lessen the calcium-lowering effect of inhibiting osteoclastic bone resorption (260).

The optimum dosage schedules and duration of action of the diphosphonates have yet to be determined in the treatment of cancer-associated hypercalcaemia. Although the diphosphonates' hold great promise because of their lack of obvious side effects and powerful effect on osteoclastic bone resorption, they appear to have a relatively slow onset of action (254,256) and in some cases, fail to return levels of serum calcium to normal (254,255,256,258,259,260).

### 1.5.9 WR-2721

This is a recently-discovered compound which has been employed in oncological patients to protect normal tissues against the toxic effects of radiation and chemotherapy (262). During phase-1 clinical trials it was noted to cause transient hypocalcaemia in a proportion of patients. Subsequent studies by Glover demonstrated that these effects were partly due to inhibition of parathyroid hormone secretion (263). In clinical practice, WR-2721 has been used in the treatment of a hypercalcaemic patient with parathyroid carcinoma, where it lowered both serum calcium and parathyroid hormone levels (264).

The calcium-lowering effect of WR-2721 is not solely due to reduction of circulating PTH levels however; Glover estimated that a marked reduction in renal tubular reabsorption of calcium accounted for between 50%-60% of the fall in serum calcium after administration of WR-2721 (263). In accordance with these findings, Hirshel-Scholz has recently demonstrated that in rats, WR-2721 has an inhibitory effect on the renal tubular reabsorption of calcium, both in thyro-parathyroidectomised and intact animals (265). Although this drug may prove to be of value in the treatment of cancer-associated hypercalcaemia in humans, no systematic studies on the effects of this agent have yet been carried out.

### 1.5.10 INTRAVENOUS FLUIDS

Patients with severe hypercalcaemia of any aetiology are often profoundly dehydrated for the reasons discussed previously (171,172,173). Accordingly, rehydration has long been established as

an important part of routine management in patients with hypercalcaemia (5).

The most appropriate intravenous fluid for rehydration in these circumstances is isotonic (0.9%) sodium chloride solution (saline) although in some cases, hypotonic saline (0.45%) may have to be employed in patients who are hypernatraemic because of impaired urinary concentrating ability (173). The main reason for using sodium-containing intravenous fluids lies in the fact that the renal tubular handling of calcium and sodium are closely related. Accordingly, measures that promote a sodium diuresis also tend to promote a calcium diuresis (176,266,267).

Detailed studies of the effects of rehydration in cancer-associated hypercalcaemia have been carried out by Hosking (147), who estimated that in these circumstances, there was an initial sodium deficit of about 500mmol - equivalent to approximately 4 litres of isotonic saline solution. On replacing this fluid deficit, with 4-8 litres of saline over 24-48 hours, Hosking noted a mean decrement of 0.40mmol/l in serum calcium - comparable to that observed in response to the administration of many pharmacological agents.

Two mechanisms were invoked to explain the calcium-lowering effect; firstly, there was an improvement in renal glomerular function, as manifest by a fall in serum creatinine values; secondly, there was a reduction in renal tubular reabsorption of calcium. Indeed, it was noted that many patients showed a modest increase in renal tubular calcium reabsorption prior to rehydration which fell after sodium repletion. This response was considered to be consistent with correction of a sodium-linked elevation in proximal renal tubular

calcium reabsorption (172,176,268).

Subsequent studies by Sleeboom (259) yielded similar results although the mean fall in serum calcium after saline repletion was rather less impressive than that noted by Hosking (0.15mmol/l vs 0.60mmol/l). This difference was probably due to the fact that lesser quantities of saline (3 litres/24hours) were used in Sleeboom's studies. In agreement with the findings of Hosking however, Sleeboom emphasised that, while saline repletion often improved the general condition of patients, additional drug therapy was usually necessary in the treatment of cancer-associated hypercalcaemia (5,147).

#### 1.5.11 LOOP DIURETICS: FORCED SALINE DIURESIS

This method of treatment was pioneered by Suki (269), who studied the effects of large doses of intravenous frusemide (100mg every 2 hours) in combination with a large intravenous fluid load (10-20 litres daily), in the treatment of severe hypercalcaemia due to malignancy and primary hyperparathyroidism. While substantial falls in serum calcium resulted, this regime has fallen out of favour with clinicians' since it requires the facilities of an intensive care unit. Moreover, even with close monitoring, this procedure carries the risk of serious electrolyte disturbances (notably hypokalaemia and hypomagnesaemia) and haemodynamic problems. There is no indication for the use of loop diuretics alone in the treatment of hypercalcaemia; in the absence of a continued "throughput" of renal sodium excretion stimulated by intravenous fluids, diuretics would tend to make hypercalcaemia worse by causing dehydration (270).

#### 1.5.12 DIALYSIS

Both haemodialysis and peritoneal dialysis are effective methods of transiently reducing serum calcium levels in patients with severe hypercalcaemia (271,272). In the setting of cancer-associated hypercalcaemia however, dialysis should be regarded as a short term measure to buy time for patients with hypercalcaemia and renal failure where other treatments are ineffective and specific antitumour therapy is available.

#### 1.5.13 ANTITUMOUR THERAPY

This is considered to be the only effective means of controlling cancer-associated hypercalcaemia in the long-term (5,273), although in point of fact, no detailed studies of have been carried out on the precise effects of chemotherapy or radiotherapy in malignancy associated hypercalcaemia. It has been established that surgical removal of tumours associated with hypercalcaemia causes serum calcium values to fall to normal (8,9,138,142,274).

#### 1.5.14 SUMMARY

There have been few substantial comparative studies on the relative efficacy of agents used in the treatment of cancer-associated hypercalcaemia. Certainly, none of those agents listed above combine the desirable qualities of a consistent hypocalcaemic response, rapid of action and lack of toxicity. On the basis of current evidence however, intravenous phosphate or calcitonin may be the treatments of choice when a rapid effect is desired. For a consistent and more sustained effect, either mithramycin or one of the diphosphonates would seem to be indicated. In all cases, rehydration with

intravenous fluids should form part of the initial management although, as stated above, treatment with fluids alone would not be expected to give long-term control of hypercalcaemia in this situation. In the long term, effective antitumour therapy is necessary for a sustained reduction in serum calcium values. Failing this, options for control of hypercalcaemia include; repeated administration of one of the diphosphonates, intermittent infusions of mithramycin, continuous therapy with combined corticosteroids and calcitonin or, if tolerated, continuous oral phosphate therapy.

**CHAPTER 2**

**TECHNICAL METHODS**

## 2.1 BIOCHEMICAL TECHNICAL METHODS

### 2.1.1 ROUTINE BIOCHEMICAL ANALYSES

Calcium, phosphate, creatinine, albumin, bilirubin, transaminases, and electrolytes were measured using a standard autoanalyser (Technicon). Serum total calcium was in all cases "adjusted" for albumin concentration using the algorithm:  $\text{Calcium}(\text{adjusted}) = \text{measured calcium}(\text{total}) \pm [47 - \text{measured albumin}(\text{g/l}) \times 0.019]$ . This method has been shown to correlate well with measured ionised calcium values in previous studies (167).

### 2.1.2 PARATHYROID HORMONE

Immunoreactive Parathyroid hormone (iPTH) was measured in plasma by a double antibody radio-immunoassay which employs bovine PTH (bPTH) for standards (MRC 71/324) and radioiodination (MRC 76/568), and a guinea pig anti-bPTH antiserum (Wellcome AS 211/32), which recognises both amino- and carboxyl- terminals of the PTH molecule. The mean between assay coefficient of variation (cv) was 7% and the normal range in our laboratory was from the limit of detection (typically 150ng/l) to 600ng/l.

### 2.1.3 CALCITONIN

Calcitonin (iCT) was measured in plasma by a double antibody radioimmunoassay which employs a human synthetic calcitonin monomer for radioiodination and standards (MRC 70/234). A rabbit anti-human calcitonin serum (MRC 77/660) recognises the carboxyl-terminal of the calcitonin molecule. The mean between assay cv was 7% and the normal range was from the limit of detection (typically 10ng/l) to 45ng/l.



#### 2.1.4 VITAMIN D METABOLITES

25-Hydroxyvitamin D ( $25(\text{OH})\text{D}$ ) was measured using a modification of the method described by Preece et al (275), in which extracts of serum are chromatographed on silicic acid and the vitamin D metabolite is quantitated using human serum as the binding protein and charcoal separation. This method measures the sum total of serum 25-Hydroxy-ergocalciferol ( $25(\text{OH})\text{D}_2$ ) and 25-Hydroxy-cholecalciferol ( $25(\text{OH})\text{D}_3$ ) concentrations. The mean between assay cv was 10% and the normal range for our laboratory was 15-100 nmol/l. The sensitivity of the assay was typically 5nmol/l.

1,25 dihydroxyvitamin D ( $1,25(\text{OH})_2\text{D}$ ) was measured by a method essentially similar to that described by Reinhardt et al (276) with the exception that, in our laboratory, a high performance liquid chromatography (HPLC) step is retained in the purification of serum samples, prior to the protein binding assay. Foetal calf thymus was used as the binding protein, prepared in a manner similar to that described by Reinhardt et al (276)

In some patients with malignant disease where serum concentrations of  $1,25(\text{OH})_2\text{D}$  were detectable or raised, further experiments were carried out to characterise the material recognised as  $1,25(\text{OH})_2\text{D}$  in the routine assay: in all cases, the putative  $1,25(\text{OH})_2\text{D}$  co-chromatographed in an identical fashion to authentic tritium-labelled  $1,25(\text{OH})_2\text{D}_3$  (Amersham International plc), during HPLC on a silica and reverse phase column, and displaced the radioactive ligand from the specific receptor protein in a parallel fashion in the competitive protein binding assay. Repeat analyses of serum from the same patients yielded similar results.

The mean between assay cv for the  $1,25(\text{OH})_2\text{D}_3$  assay was 20% and the reference range lay between 20-100pmol/l. The sensitivity of the assay was typically 15pmol/l.

#### 2.1.5 CYCLIC ADENOSINE 3' 5' MONOPHOSPHATE

In one study (Chapter 6.1), cyclic adenosine 3' 5' monophosphate (cAMP) was measured, after appropriate dilution in plasma and urine samples using a radioimmunoassay kit obtained from RIA (UK) Ltd. The nephrogenous component of urinary cyclic AMP excretion (NcAMP) was derived using the following equation:  $\text{NcAMP} = \text{urine cAMP} \text{ divided by urine creatinine, multiplied by serum creatinine, minus plasma cAMP.}$  (all values in nmol/l). The mean between assay cv was 10% and the reference range lay between 10-35 nmol/l of glomerular filtrate.

In all other studies, urine cAMP was measured, after appropriate dilution using a radioimmunoassay technique as described by O'Reilly et al (277). In these studies, cyclic AMP excretion was expressed as a molar ratio, relative to urinary creatinine (UcAMP). The reference range for UcAMP was 0.10-0.65 nmol/mmol creatinine. Between assay cv was less than 10% over the working range and the limit of detection 2.6nmol/l.

#### 2.1.6 HYDROXYPROLINE

Hydroxyproline was measured in true fasting urine specimens using a colorimetric technique similar to that described by Goverde et al (278) using a kit (Organon Diagnostics Ltd). The mean between assay cv was 8% and the typical sensitivity was 50 $\mu$ mol/l. Urinary hydroxyproline was expressed as a molar ratio, relative to urinary creatinine concentration (OHP/Cr). In the fasting state, OHP/Cr ratio

is thought to be an index of total bone resorptive activity (153). The reference range for OHP/Cr in our laboratory lay between 5-30  $\mu\text{mol}/\text{mmol}$  creatinine.

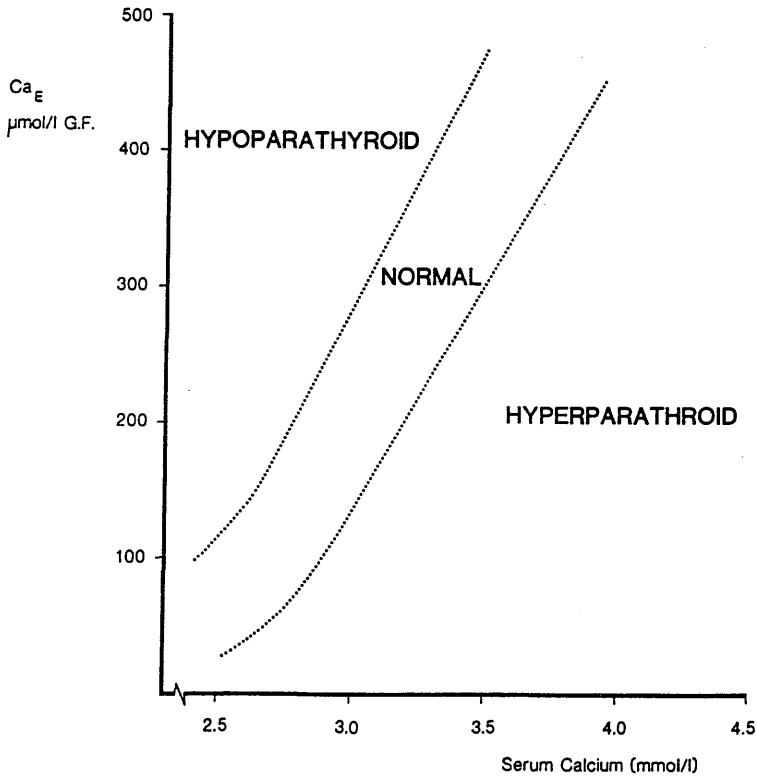
#### 2.1.7 RENAL TUBULAR REABSORPTION OF PHOSPHATE

The notional threshold for renal tubular reabsorption of phosphate ( $\text{TmPO}_4$ ) was determined from fasting serum and urinary measurements using a nomogram (268). The normal range lay between 0.80-1.40 mmol/l of glomerular filtrate.

#### 2.1.8 URINARY CALCIUM EXCRETION

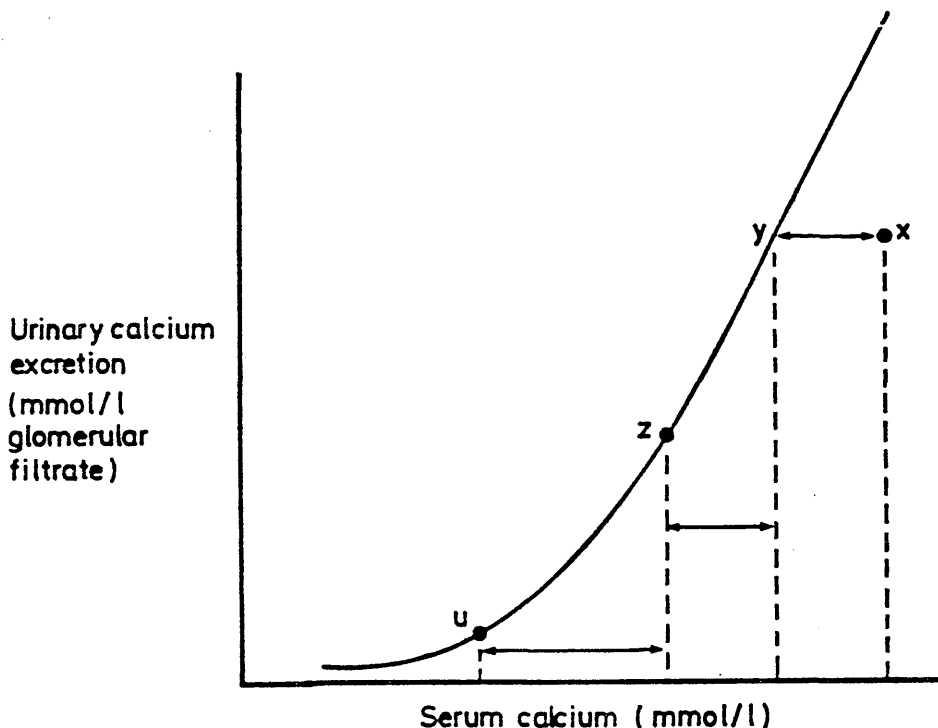
Urinary excretion of calcium was expressed as a molar ratio relative to urinary creatinine ( $\text{Ca}/\text{Cr}$ ; mmol/mmol; reference range less than 0.35). In the fasting state,  $\text{Ca}/\text{Cr}$  ratio is thought to be an index of net bone resorption (153). Calcium excretion was also expressed as a function of glomerular filtration rate ( $\text{Ca}_E$ ), derived from the molar ratio multiplied by the serum creatinine concentration ( $\text{Ca}_E$   $\mu\text{mol}/\text{l}$  Glomerular filtrate; reference range for normocalcaemic subjects less than 50), and as the ratio of calcium clearance to creatinine clearance, derived from the  $\text{Ca}_E$  divided by the serum calcium concentration ( $\text{Cl}_{\text{Ca}}/\text{Cl}_{\text{Cr}}$ )

Renal tubular reabsorption of calcium was assessed by comparing individual serum adjusted calcium and urinary  $\text{Ca}_E$  points with the normal range obtained by calcium infusion studies in healthy subjects (114) (Figure 2.1). The extent to which increased renal tubular reabsorption of calcium, increased calcium "throughput" and reduced glomerular filtration rate contributed to hypercalcaemia were calculated by a graphical method in which the serum and urinary



**FIGURE 2.1**

Relationship between urinary calcium excretion (expressed as  $\mu\text{mol}$  per litre of glomerular filtrate ( $\text{Ca}_E$ )) and serum calcium. The dotted lines indicates 2 standard deviations from the mean values obtained by Peacock et al in normal subjects during calcium infusions (114). Points in patients with primary hyperparathyroidism fall to the right of the expected normal, indicating that renal tubular calcium reabsorption is increased. Conversely, points in patients with hypoparathyroidism fall to the left of normal indicating decreased renal tubular reabsorption of calcium.



**FIGURE 2.2**

Diagram to illustrate method of analysing hypercalcaemia (from reference 279). The solid line indicates the mean of values obtained by calcium infusion studies in normal subjects (114). Lateral deviations from the curvilinear relation between serum calcium and urinary excretion of calcium ( $Ca_E$ ) are attributable to changes in renal tubular reabsorption of calcium. The increment in serum calcium due to increased renal tubular reabsorption in patient X is equivalent to the distance between points x and y. In patients with impaired glomerular function, the contribution of renal glomerular failure is obtained by multiplying the observed fasting urinary  $Ca/Cr$  value by a theoretically "normal" creatinine level of  $80\mu\text{mol/l}$ . The horizontal distance between the observed  $Ca_E$  and the calculated  $Ca_E$  with serum creatinine  $80\mu\text{mol/l}$  is measured as  $y-z$  on the figure. Any residual increment ( $z-u$ ) in serum calcium compared with a control subject (u) with serum calcium  $2.50\text{mmol/l}$  is then attributed to increased calcium "flow" from bone resorption.

calcium points in any given case are compared with the mean values obtained by calcium infusion studies in healthy subjects (279) (Figure 2.2). The notional renal tubular threshold for calcium reabsorption ( $TmCa/GFR$ ) was used as an index of renal tubular calcium reabsorption in one study (Chapter 4.3).  $TmCa/GFR$  was calculated using a nomogram (279).

#### 2.1.9 URINARY SODIUM EXCRETION

Urinary sodium excretion was expressed in two ways; as a function of glomerular filtration rate by multiplying the ratio of urinary sodium to urinary creatinine by serum creatinine (all values in mmol/l), to give  $Na_E$  mmol/l glomerular filtrate; reference range in healthy normocalcaemic subjects 0.10-1.30mmol/l GF. Secondly, as the ratio of sodium excretion to creatinine excretion, obtained by dividing the  $Na_E$  value by serum sodium concentration ( $Cl_{Na}/Cl_{Cr}$ ).

#### 2.2 BONE HISTOMORPHOMETRY

Except where otherwise stated, histomorphometry was performed on bone samples obtained under local anaesthesia from the iliac crest using a trephine biopsy needle (8mm diameter). All bone samples were processed and embedded undecalcified in methyl methacrylate as described by Boyce et al (280). Bone resorption and formation were assessed using a Zeiss II integrating eyepiece by line-intersect measurements. In all cases histomorphometry was assessed by one observer (S.H.Ralston) and the results were checked by a further observer (Dr B.F.Boyce) who was unaware of the individual patient-details.

A number of relevant bone-histomorphometric variables were

derived from line intersect measurements. These were; total osteoid surface (TOS; reference range <24%), active osteoid surface (AOS; reference range <2%), absolute osteoid volume (AOV; reference range <2.4%), total resorption surface (TRS; reference range <7.3%), active resorption surface (ARS; reference range <2.4%) and number of osteoclasts per mm<sup>2</sup> (NO; reference range <0.28)

### 2.3 RADIONUCLIDE BONE SCANS

Radionuclide bone scans were performed using standard techniques, three hours after the intravenous injection of 530 Megabequerels of <sup>99m</sup>Tc labelled methylene diphosphonate.

**EXPERIMENTAL WORK**



CHAPTER 3

ROLE OF METASTATIC BONE DISEASE IN THE

PATHOGENESIS OF HYPERCALCAEMIA ASSOCIATED WITH MALIGNANCY

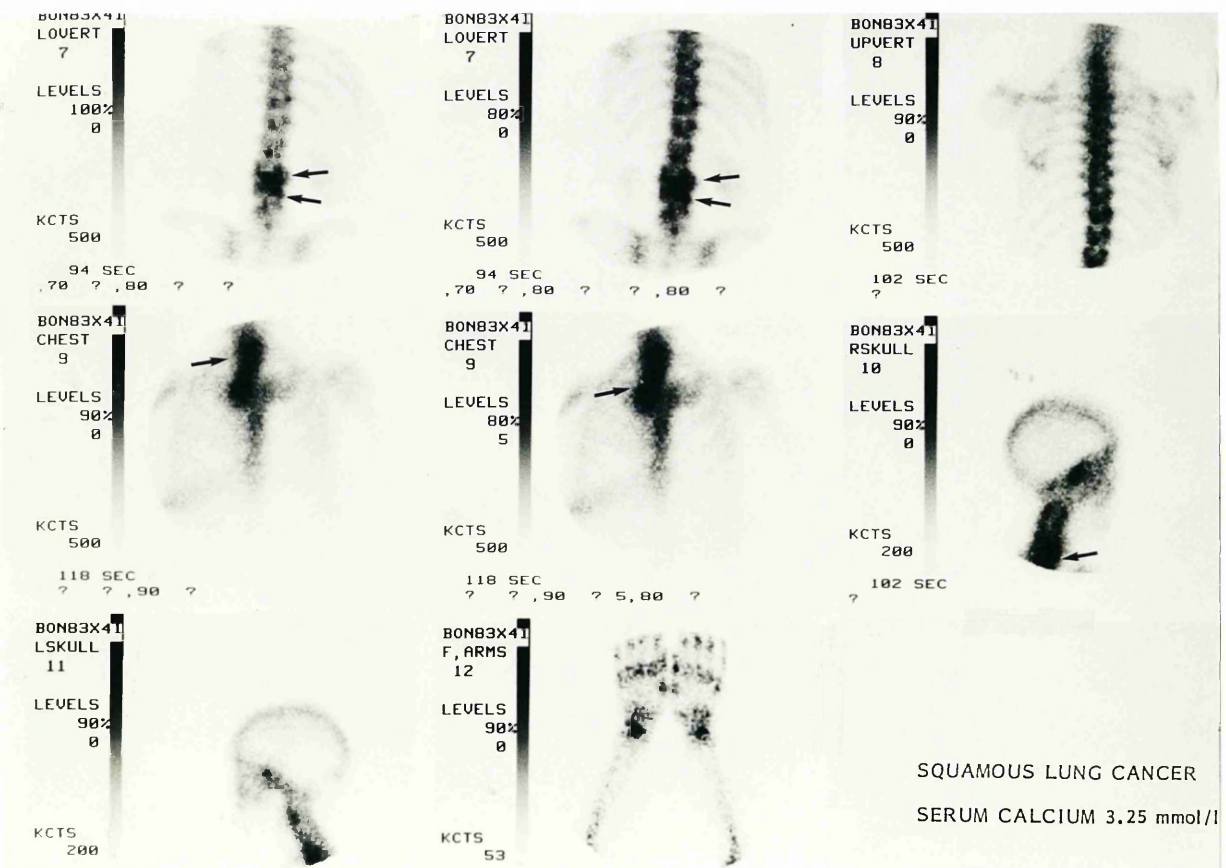
### 3.1 RELATION BETWEEN SERUM CALCIUM VALUES AND EXTENT OF METASTATIC BONE DISEASE IN CANCER PATIENTS

#### 3.1.1 INTRODUCTION

Humoral factors released by tumour tissue are thought to play a role in the pathogenesis of hypercalcaemia in some patients with malignancy (7). However, direct involvement of the skeleton by metastases is generally considered to be the commonest cause of such hypercalcaemia (1,5,10). Although hypercalcaemia and bone metastases commonly coexist (1,2,3,5,10), there is little direct evidence to suggest that such bone lesions are the cause of hypercalcaemia. In this study the relation between serum calcium values and metastatic bone disease was examined in 195 patients with malignant disease, using the radionuclide bone scan as a means of assessment.

#### 3.1.2 PATIENTS AND METHODS

Bone scan reports were scrutinised in 725 patients with malignant disease who attended the nuclear medicine department of Glasgow Royal Infirmary for bone scan examination between January 1980-January 1982. The reports fell into three groups: (1) No evidence of skeletal metastases (n=385 - negative scan). (2) Abnormalities thought to be diagnostic of metastatic bone disease (n=181 - positive scan). (3) Abnormalities of uncertain significance (n=159 - non-diagnostic scan). Biochemical records were traced in the above population and those with hypercalcaemia were identified (n=87). Hypercalcaemic patients plus normocalcaemic patients with positive bone scans were submitted to further study. In the resultant 216 patients, case notes were obtained and clinical data were extracted. A number of patients



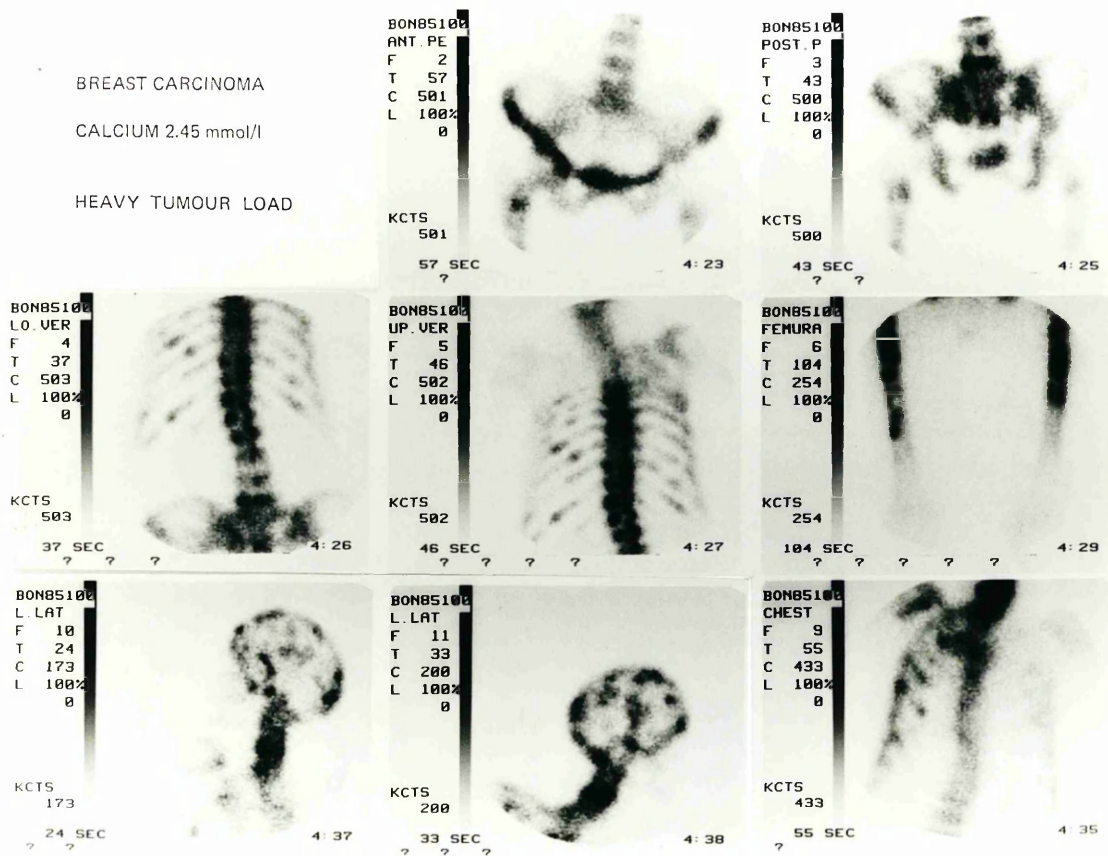
**FIGURE 3.1**

Photograph of a bone scan showing an example of a light skeletal tumour load in a hypercalcaemic patient with squamous carcinoma of bronchus. Metastatic lesions are evident as "hot spots" (arrowed) in the lumbar and cervical spine. Increased uptake in the hands is due to degenerative joint disease.

BREAST CARCINOMA

CALCIUM 2.45 mmol/l

HEAVY TUMOUR LOAD



**FIGURE 3.2**

Photograph of bone scan showing an example of a heavy skeletal tumour load in a normocalcaemic patient with breast carcinoma. Multiple "hot spots" are seen throughout the skeleton.

were excluded because of incomplete biochemical or clinical data, leaving a final study population of 195.

In the final study group of 195 patients, bone scans were reviewed by an experienced observer who was unaware of the individual patient details (Dr I Fogelman). In the 160 cases where the scan was considered to be diagnostic of metastatic bone disease a subdivision into "light tumour load" and "heavy tumour load" categories was made. These corresponded to 6 or less (figure 3.1) and more than 6 (figure 3.2) identifiable lesions respectively. In all cases serum calcium had been measured within 14 days of the bone scan. Hypercalcaemia was defined as an adjusted serum calcium of greater than 2.70 mmol/l. In the event of hypercalcaemia, the adjusted serum calcium used in analysis was that measured before the administration of specific antihypercalcaemic therapy. The statistical method used was the Mann-Whitney "U" test for unpaired samples.

### 3.1.3 RESULTS

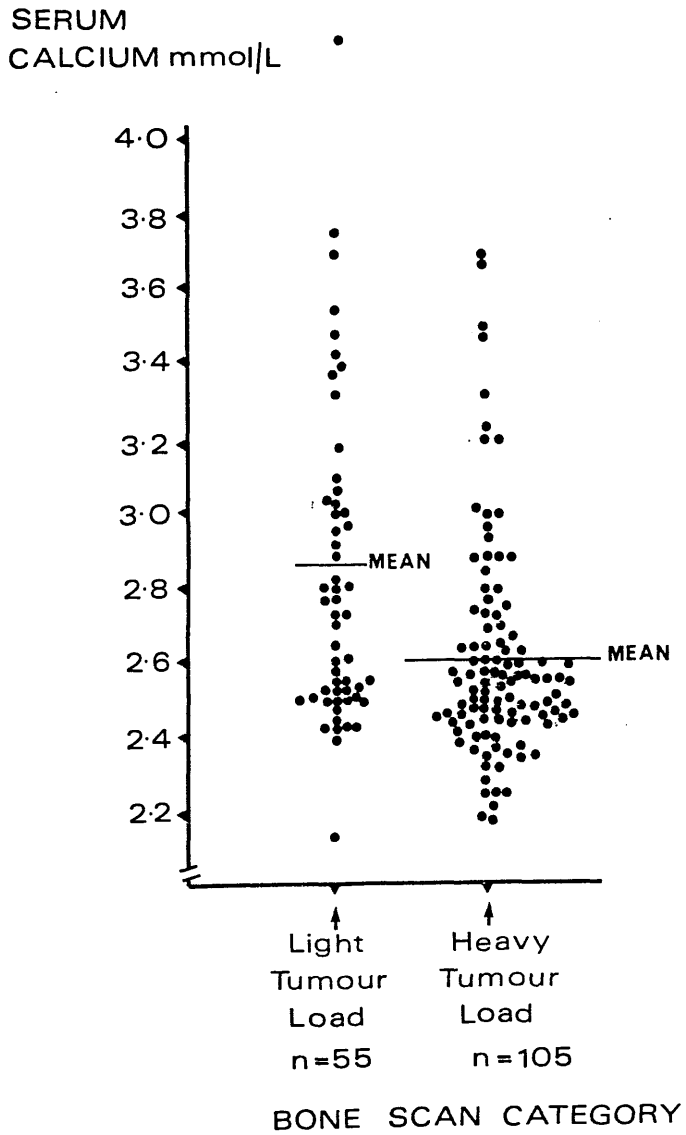
#### Serum Calcium Values in Patients with Metastatic Bone Disease

Bronchial and breast carcinoma were the most common tumours in this group (Table 3.1). In figure 3.3, the distribution of serum calcium values is shown on division of patients into "light tumour load" and "heavy tumour load" categories. Significant hypercalcaemia (>2.70 mmol/l) occurred in 32.5% of patients with bone-scan evidence of skeletal metastases, 16.9% having a light skeletal tumour load and 15.6% a heavy skeletal tumour load. Serum calcium values were significantly higher in patients with a light skeletal tumour load ( $p < 0.001$ ).

TABLE 3.1

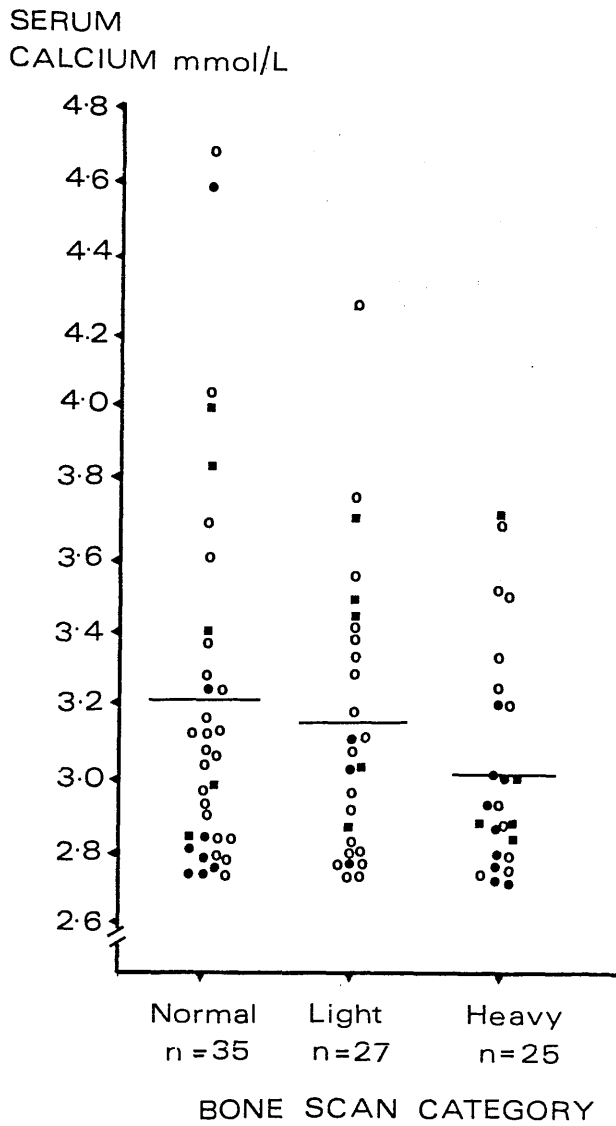
TUMOUR TYPE IN 160 PATIENTS WITH METASTATIC BONE DISEASE

<u>TUMOUR TYPE</u>	<u>NUMBER (%)</u>
BRONCHUS	76 (47.5)
BREAST	50 (31.2)
PROSTATE	11 (6.8)
MYELOMA	5 (3.1)
ANAPLASTIC	5 (3.1)
BLADDER	4 (2.5)
CERVIX	2 (1.0)
BOWEL	2 (1.0)
THYROID	1 (0.5)
LARYNX	1 (0.5)
CARCINOID	1 (0.5)
CHRONIC LYMPHATIC LEUKAEMIA	1 (0.5)
LYMPHOMA	1 (0.5)



**FIGURE 3.3**

Distribution of serum calcium values in patients with metastatic bone disease.



○ - bronchial carcinoma  
 ● - breast carcinoma  
 ■ - other tumours

**FIGURE 3.4**

Bone scan appearances in patients with hypercalcaemia of malignancy. Horizontal line indicates mean.



### Skeletal Tumour Load in Patients with Hypercalcaemia

In this group, breast and bronchial carcinoma were again predominant (figure 3.4). A relatively high proportion of hypercalcaemic patients with breast carcinoma had a heavy skeletal tumour load (47%). In contrast, few patients with bronchial carcinoma and hypercalcaemia had extensive skeletal involvement (21%). Hypercalcaemic patients with other malignancies were distributed evenly among the three bone-scan categories. In the hypercalcaemic group as a whole, 40% had a normal bone scan and mean serum calcium values varied inversely with the extent of skeletal involvement, being highest in the normal bone scan group and lowest in the heavy tumour load group (figure 3.4). These differences were not statistically significant however.

#### 3.1.4 DISCUSSION

It has been considered that the occurrence of hypercalcaemia in malignancy can usually be explained on the basis of local release of skeletal calcium by invading tumour metastases (1,2,3,5,7,10). However, a causal relationship between these factors has not been proven, and indeed, evaluation of the extent of metastatic bone disease has hitherto been made difficult by the relative insensitivity of radiological methods used for the detection of skeletal metastases (26,27). Discrepancies between severity of hypercalcaemia and extent of metastatic bone disease are frequently apparent in clinical practice but have generally been explained on the basis of occult skeletal metastases undetected by standard radiological techniques (7,26).

In recent years, radioisotope scanning with  $^{99m}$  technetium

diphosphonate has emerged as an important means of skeletal imaging. The bone scan, which reflects skeletal metabolic activity is an extremely sensitive means of detecting skeletal metastases and is recognised to be superior to conventional radiology in this respect (281). While a false negative bone scan in malignancy may occur when there is a dissociation of bone formation from bone resorption, this situation is at present recognised to occur commonly only in myeloma (281). Of the 5 patients with myeloma in the present series, all had positive bone scans.

In this study, the bone scan was used to assess the relation between metastatic bone disease and the development of hypercalcaemia in malignancy. In 87 hypercalcaemic patients, no positive correlation was found between the presence or extent of metastatic bone disease and serum calcium values, suggesting that in this group, an elevated serum calcium cannot simply be explained on the basis of local skeletal destruction by metastatic deposits. Among all 725 patients who had bone scans, however, hypercalcaemia occurred in a higher proportion of those with a positive scan than a negative one. This probably reflects the fact that patients with metastatic bone disease have, in general, more advanced malignancies than their counterparts with an uninvolved skeleton, a large proportion of whom are patients with small tumours or carcinomas-in-situ, in whom a bone scan has been requested as a screening procedure prior to curative resection of a primary tumour. It is therefore possible that the higher prevalence of hypercalcaemia in the positive bone scan group reflects a greater total-body tumour load, increased tumour aggressiveness, or other factors as yet uncharacterised, rather than a direct consequence of calcium release from local areas of bone destruction. Accordingly, in

160 patients with bone scan evidence of metastatic disease involving the skeleton, there was no positive correlation between serum calcium values and extent of metastatic bone disease.

A humoral aetiology of hypercalcaemia accompanying malignant disease has been thought to be uncommon (1,5,10,183), and has usually been invoked only when evidence for skeletal metastases was lacking (7). Conversely, the demonstration of skeletal metastases has conventionally been thought to provide reason enough for hypercalcaemia by causing local bone destruction invoking an additional humoral component (5,7,10,90).

This study has shown that, in hypercalcaemic patients, there is no positive correlation between the occurrence or extent of metastatic bone disease and serum calcium values in malignancy. Further, in patients with metastatic involvement of the skeleton, serum calcium values were inversely related to the extent of skeletal involvement by tumour. These data suggest that the development of hypercalcaemia in malignancy may frequently be mediated by an alternative mechanism, such as the production of humoral substances by tumour tissue, having effects on calcium metabolism at sites or organs distant from local areas of tumour involvement.

## 3.2 RELATION BETWEEN EXTENT OF METASTASTIC BONE DISEASE AND BIOCHEMICAL INDICES OF BONE RESORPTION IN HYPERCALCAEMIA OF MALIGNANCY

### 3.2.1 INTRODUCTION

In the previous retrospective study, no positive correlation was found to exist between the extent of metastatic bone disease and the presence or severity of hypercalcaemia in malignancy. In this study, a prospective analysis was made of the relationship between some other biochemical indices of bone resorption and the extent of metastatic bone disease in cancer patients.

### 3.2.2 PATIENTS AND METHODS

A group of 81 patients with proven malignant disease attending the nuclear medicine department of Glasgow Royal Infirmary for routine bone scans were studied. They comprised 27 patients with hypercalcaemia (adjusted calcium  $>2.70\text{mmol/l}$ ), who had presented consecutively over an 18-month period and 54 normocalcaemic patients presenting consecutively over three months. Seven patients with breast carcinoma had been treated with tamoxifen (3-normocalcaemic, 4-hypercalcaemic) and three with prostatic carcinoma had received stilboestrol (1-hypercalcaemic, 2-normocalcaemic) for several months before the study. These drugs were not considered to have exerted a major influence on the variables assessed however, as four patients had developed hypercalcaemia while receiving them. Furthermore, biochemical measurements in the remaining six normocalcaemic subjects were not significantly different from those of their counterparts with similar bone scan appearances who had not received hormone treatment. No patient had received other anticancer chemotherapy,

antihypercalcaemic agents (except fluid), or diuretics at the time of study. Patients with myeloma and other haematological malignancies were excluded.

Hypercalcaemic patients were rehydrated with a standard protocol of intravenous 0.9% sodium chloride solution, 3 litres daily given over a 48 hour period or until a daily urine output of more than 2500ml was achieved. Biochemical assessment was then performed during the continued infusion of 0.9% sodium chloride solution 2 litres daily. Bone scans were assessed and divided into "normal", "light skeletal tumour load", "heavy skeletal tumour load", and "non diagnostic" categories as described in the previous study (Chapter 3.1).

Statistical methods used were the Mann-Whitney test for unpaired samples, the Wilcoxon test and Spearman's rank correlation coefficient.

### 3.2.3 RESULTS

Table 3.2 gives the relevant clinical and biochemical details of the patients studied. Patients with hypercalcaemia of malignancy had uniformly undetectable plasma levels of iPTH whereas in normocalcaemic patients, iPTH values varied from undetectable to levels which lay within the normal range. Serum creatinine concentrations were similar in normocalcaemic and hypercalcaemic subjects. Tumours were mostly breast carcinomas in normocalcaemic patients and squamous bronchial carcinoma in patients with hypercalcaemia.

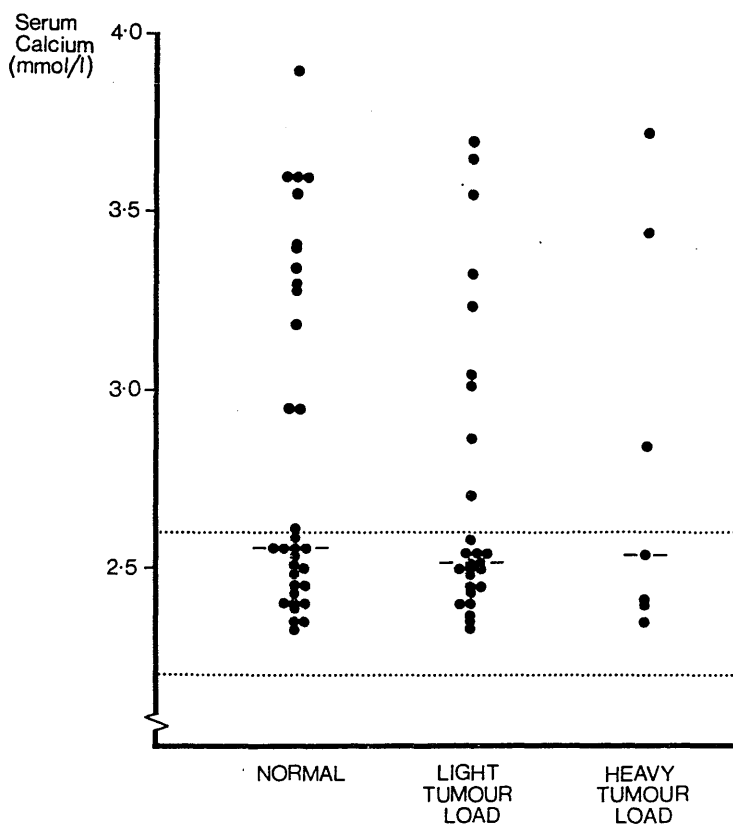
In 6 normocalcaemic patients with a "non diagnostic" bone scan, biochemical values correlated most closely with those who had a negative scan. However, data from these patients and one other with

TABLE 3.2

CLINICAL AND BIOCHEMICAL DETAILS IN PATIENTS WITH MALIGNANCY

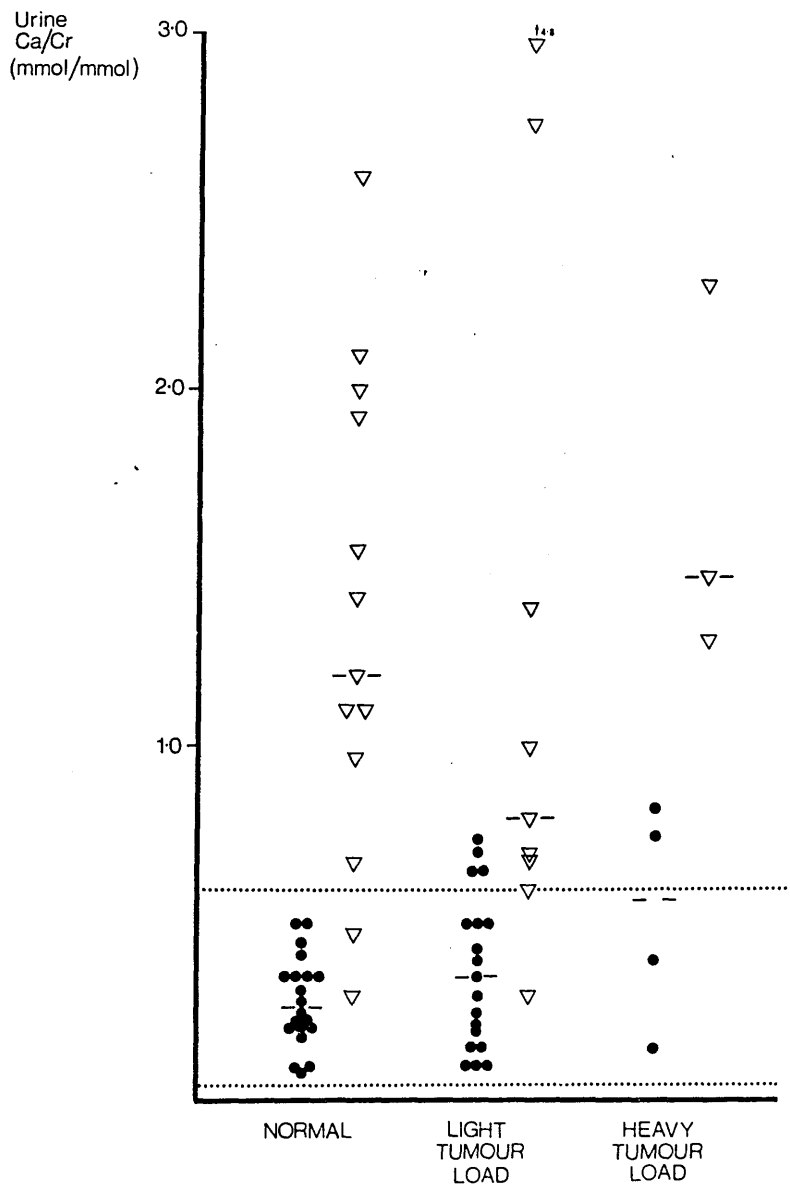
	<u>SERUM CALCIUM</u> (mmol/l)	<u>SERUM PHOSPHATE</u> (mmol/l)	<u>SERUM CREATININE</u> (µmol/l)	<u>SERUM ALBUMIN</u> (g/l)	<u>TmPO<sub>4</sub></u> (mmol/l GF)	<u>MALE/FEMALE</u>	<u>BONE SCAN APPEARANCE</u>		<u>TUMOUR TYPE</u>		
							<u>NORMAL</u>	<u>LIGHT HEAVY</u>		<u>N-D**</u>	
<u>NORMOCALCAEMIC</u>	2.45	1.05	75	39	0.85	9/45	23	19	6	6	BRONCHUS 13 BREAST 32 PROSTATE 3 OTHERS 6+
<u>n=54</u>	(2.30-2.65)	(0.50-1.60)	(50-150)	(22-46)	(0.50-1.80)						
<u>HYPERCALCAEMIC</u>	3.30	0.80	80	33	0.60	19/10	14	9	3	1	BRONCHUS 14 BREAST 4 PROSTATE 1 OTHERS 8*
<u>n=27</u>	(2.72-2.90)	(0.50-1.45)	(50-135)	(22-42)	(0.20-1.20)						
<u>SIGNIFICANCE</u>	<0.001	<0.001	>0.05	<0.001	<0.001						
(p-value)											

+ GI tract-4; bladder-1; thyroid-1.  
 \* Nasopharynx-2; pancreas-1; oesophagus-1; cholangiocarcinoma-2.  
 \*\* N-D = non diagnostic  
 For biochemical variables, values shown are mean (range)



**FIGURE 3.5**

Serum calcium values in patients with malignancy. Dotted lines indicate normal range, Bars are medians, Normal = no evidence of skeletal metastases, Light tumour load =  $\leq 6$  metastases, Heavy tumour load =  $> 6$  metastases.

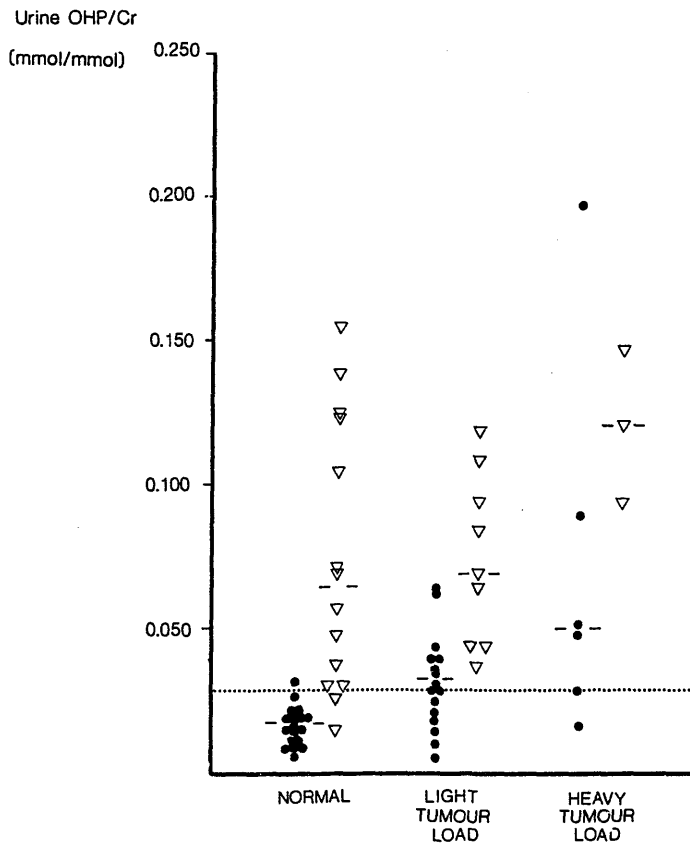


**FIGURE 3.6**

Urinary calcium excretion in patients with malignancy, expressed as the fasting molar ratio of calcium to creatinine (Ca/Cr). Dotted lines indicate normal range. Bars are medians. Normal = No evidence of skeletal metastases, Light tumour load =  $\leq 6$  metastases, Heavy tumour load =  $> 6$  metastases.

● - Normocalcaemic    ▽ - Hypercalcaemic.

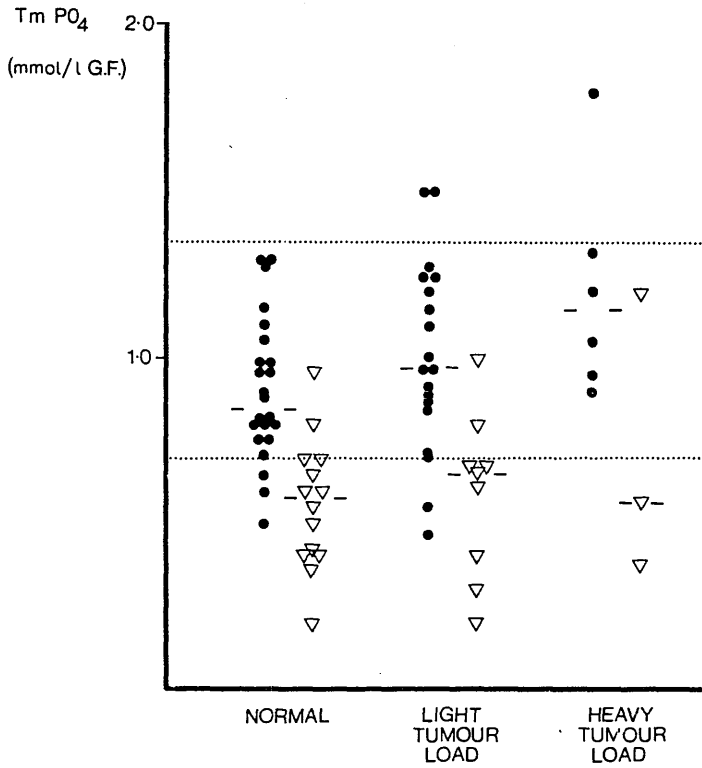




**FIGURE 3.7**

Urinary hydroxyproline excretion in patients with malignancy, expressed as a molar ratio of hydroxyproline relative to creatinine (OHP/Cr). Dotted lines indicate normal range. Bars are medians. Normal = No evidence of skeletal metastases, Light tumour load =  $\leq 6$  metastases, Heavy tumour load =  $> 6$  metastases.

● - Normocalcaemic    ▽ - Hypercalcaemic.



**FIGURE 3.8**

Renal tubular threshold for phosphate reabsorption (TmPO<sub>4</sub>) in mmol/l of glomerular filtrate (GF). Dotted lines indicate normal range. Bars are medians. Normal = No evidence of skeletal metastases, Light tumour load = ≤ 6 metastases, Heavy tumour load > 6 metastases.

● - Normocalcaemic    ▽ - Hypercalcaemic

hypercalcaemia and a non diagnostic scan (who was later found to have no evidence of metastases at necropsy) were excluded from further study.

In the remaining 74 patients (figure 3.5), serum calcium values were related to extent of metastatic bone disease as judged by the bone scan appearance. There was no significant difference in serum calcium values when patients were divided by the extent of metastatic bone disease. In all subsequent analyses, patients were further subdivided on the basis of the adjusted serum calcium value into normocalcaemic (<2.70mmol/l) and hypercalcaemic (>2.70mmol/l) groups.

In figure 3.6, the relation between fasting urinary ratio of calcium to creatinine (Ca/Cr) is shown, in the hypercalcaemic and normocalcaemic groups. Urinary Ca/Cr values were significantly higher in the hypercalcaemic group for any given category of metastatic bone disease ( $p < 0.001$ ). Although Ca/Cr values tended to rise in normocalcaemic patients with increasing extent of metastatic disease, there was no statistically significant correlation between Ca/Cr values and extent of metastatic disease in either normocalcaemic or hypercalcaemic subjects.

Figure 3.7 gives a similar comparison between fasting urinary ratio of hydroxyproline to creatinine (OHP/Cr) and extent of metastatic disease seen in the bone scans. In normocalcaemic patients with bone metastases, OHP/Cr values were significantly raised when compared with the normal bone scan group ( $p < 0.01$ ). While median OHP/Cr values were lowest in those subjects with a normal bone scan and rose with the extent of metastatic bone disease, there was considerable overlap between the three bone scan categories. In

hypercalcaemic patients, OHP/Cr values were significantly higher than in normocalcaemic patients for any given category of metastatic bone disease ( $p < 0.001$ ), and no positive correlation was observed between the extent of metastatic disease and OHP/Cr values, although the numbers were small in the group categorised as heavy tumour load. No positive correlation was observed in normocalcaemic patients between OHP/Cr values and serum calcium concentration ( $r = 0.20$ ; NS) or between OHP/Cr and Ca/Cr values ( $r = 0.08$ ; NS). In hypercalcaemic patients however, OHP/Cr and Ca/Cr values correlated significantly ( $r = 0.42$ ;  $p < 0.05$ ), although OHP/Cr and serum calcium values did not ( $r = 0.29$ ; NS).

In normocalcaemic patients, the renal tubular threshold for phosphate reabsorption ( $TmPO_4$ ) lay within the normal range in most cases (figure 3.8). While values tended to rise progressively with the category of tumour load the differences were not statistically significant. In hypercalcaemic patients,  $TmPO_4$  values were significantly lower ( $p < 0.001$ ) but were similar in each of the three bone scan categories. In the study group as a whole, there were significant inverse correlations between  $TmPO_4$  and OHP/Cr values ( $r = -0.40$ ;  $p < 0.001$ ),  $TmPO_4$  and Ca/Cr values ( $r = -0.46$ ;  $p < 0.001$ ) and  $TmPO_4$  and serum calcium concentration ( $r = -0.56$ ;  $p < 0.001$ ).

#### 3.2.4 DISCUSSION

As in the previous retrospective study, (Chapter 3.1) no correlation was found in this study between the extent of metastatic bone disease and serum calcium values. Moreover, in normocalcaemic patients, both Ca/Cr and OHP/Cr values were much lower than in hypercalcaemic patients who had a similar extent of metastatic bone

disease on the bone scan. In hypercalcaemic patients, Ca/Cr and OHP/Cr values were generally elevated, but to a similar level in the three bone scan groups. Since fasting urinary OHP/Cr and Ca/Cr are considered to reflect bone resorptive activity and net release of skeletal calcium respectively (153), these observations suggest that in many hypercalcaemic patients, bone resorption largely occurred on a systemic, humorally mediated basis, rather than as the result of focal bone destruction by metastatic lesions. Thus, although OHP/Cr and Ca/Cr values were raised in normocalcaemic patients with metastatic disease, they did not reach the order of magnitude associated with hypercalcaemia, even in patients with a heavy skeletal tumour load.

It has been previously suggested that a reduction in the renal threshold for phosphate reabsorption ( $\text{TmPO}_4$ ) is a useful biochemical marker for a humoral mechanism of hypercalcaemia in malignancy (6,7,93). In this study,  $\text{TmPO}_4$  values were noticeably depressed in most of the hypercalcaemic patients and in the study group as a whole, significant inverse correlations were observed between  $\text{TmPO}_4$  and OHP/Cr, Ca/Cr and serum calcium values, respectively. These observations support the concept that hypercalcaemia was caused by a single humoral mediator with effects both on bone and on the renal tubule.

In summary, this study has shown that biochemical indices of bone resorption in hypercalcaemic cancer patients were disproportionately increased, relative to the extent of metastatic disease seen in the bone scans. Furthermore, the renal threshold for phosphate reabsorption was significantly reduced in hypercalcaemic patients when compared with those who were normocalcaemic. This suggests that in many hypercalcaemic patients with metastatic bone disease, a humoral

factor is released by tumour tissue which not only stimulates bone resorption on a systemic basis, but which also possesses a PTH-like effect on the renal tubular reabsorption of phosphate. The release of such a humoral factor could partially explain the occurrence of hypercalcaemia in patients with a limited extent of metastatic bone involvement where release of calcium from metastatic lesions alone would not be expected to cause hypercalcaemia on a theoretical basis. These data do not exclude the possibility that hypercalcaemia may arise as the result of extensive metastatic bone destruction alone in some circumstances. However, this mechanism appears to be less common in the present study population, where squamous carcinoma of the bronchus was the commonest tumour associated with hypercalcaemia.

CHAPTER 4

ROLE OF THE KIDNEY IN THE PATHOGENESIS

OF HYPERCALCAEMIA ASSOCIATED WITH MALIGNANCY

4.1 RENAL TUBULAR REABSORPTION OF CALCIUM IN MALIGNANCY ASSOCIATED  
HYPERCALCAEMIA: EVIDENCE FOR A NON-PARATHYROID HUMORAL AGENT  
WITH AN EFFECT ON RENAL TUBULAR HANDLING OF CALCIUM

4.1.1 INTRODUCTION

Reference was made in chapter 3.2 to the importance of bone resorption in the pathogenesis of cancer-associated hypercalcaemia. Indeed, it has been considered that a "bone-resorptive" mechanism of hypercalcaemia prevails, both in patients with hypercalcaemia due to metastatic bone disease (2,3,5,7,10), and in patients who do not have metastatic bone lesions, due to the release of a systemically active bone-resorbing factor by tumour tissue (5,6,8,9,93,139,140,141,142). Nonetheless, normocalcaemia is maintained in many conditions associated with increased bone resorption, due to the large renal reserve for excretion of calcium (4).

An exception is primary hyperparathyroidism, but here, increased renal tubular reabsorption of calcium plays a major role in the pathogenesis of hypercalcaemia (114). In this study, the role of increased renal tubular reabsorption of calcium was assessed in the pathogenesis of hypercalcaemia associated with malignancy.

4.1.2 PATIENTS AND METHODS

Thirty one patients presenting consecutively to Glasgow Royal Infirmary with hypercalcaemia complicating solid malignant tumours were studied. A patient was selected for study on the basis that the clinician in charge had considered rehydration an appropriate form of management. At the time of study, no patient received diuretics, vitamin D analogues, or other medication known significantly to effect



calcium homeostasis. Patients were rehydrated with intravenous infusions of 0.9% saline, 3 litres daily for a minimum of 48 hours and/or until a daily urine output of more than 2500ml was achieved. After initial fluid repletion, hydration was maintained by the continued infusion of 0.9% saline 2 litres daily. At the time of study serum creatinine and urea concentrations had stabilised on consecutive days to within  $\pm 10 \mu\text{mol/l}$  and 2 mmol/l respectively.

The presence and extent of metastatic bone disease was assessed by radionuclide bone scan examination, as described in chapter 3.1.

Results from patients with malignancy were compared with similar data in 19 patients with primary hyperparathyroidism and a control group of 12 normal subjects rendered acutely hypercalcaemic by calcium infusion, using the methods described by Peacock et al (282). Briefly, each calcium infusion study began with the collection of a timed fasting urine specimen with a mid-point blood sample. Infusion of calcium gluconate 10% was then commenced with the aid of an infusion pump at the rate of about 10 mmol/hr. Consecutive timed urine samples were then collected over the next 4-6 hours and a blood sample was taken at the mid-point of each urine sample. In patients with malignancy and primary hyperparathyroidism biochemical analyses were made on blood and untimed true fasting urine samples.

Statistical methods used were linear regression analysis, and the Mann-Whitney test for unpaired samples.

#### 4.1.3 RESULTS

Relevant clinical and biochemical details in the three groups of patients studied are shown in table 4.1 Patients with primary

TABLE 4.1

CLINICAL AND BIOCHEMICAL DETAILS IN PATIENTS WITH MALIGNANCY  
PRIMARY HYPERPARATHYROIDISM AND NORMALS UNDERGOING CALCIUM INFUSIONS

	<u>SERUM CREATININE (<math>\mu\text{mol/l}</math>)</u>	<u>SERUM UREA (<math>\text{mmol/l}</math>)</u>	<u>SERUM PTH (<math>\text{ng/l}</math>)</u>	<u>SERUM BICARBONATE (<math>\text{mmol/l}</math>)</u>	<u>SERUM ALBUMIN (<math>\text{g/l}</math>)</u>	<u>TmPO<sub>4</sub> (<math>\text{mmol/l GF}</math>)</u>	<u>THEORETICAL CALCIUM THRESHOLD</u>	<u>MALE/ FEMALE</u>	<u>AGE (years)</u>
<u>MALIGNANT HYPERCALCAEMIA (n=31)</u>	73 (4.3) (30-125)	6.5 (0.5) (2.9-14.9)	UD (UD)	27 (0.5) (23-33)	32 (0.8) (22-42)	0.56 (0.04) (0.26-1.20)	2.89 (0.08) -	20/11	66 (5.4) (50-87)
<u>PRIMARY HYPERPARATHYROIDISM (n=19)</u>	79 (5.0) (40-130)	4.9 (0.2) (3.6-6.6)	1100 (134) (290-2500)	25 (0.3) (21-29)	40 (2.7) (36-44)	0.57 (0.02) (0.23-0.81)	2.76 (0.06) -	1/18	62 (7.6) (21-73)
<u>NORMAL CONTROLS* (n=12)</u>	87 (1.4) (50-100)	5.5 (0.9) (4.5-6.3)	UD (UD)	27 (0.2) (24-31)	47 (0.2) (45-51)	1.20 (0.03) (1.00-1.40)	2.41 (0.03) -	8/2	24 (2.3) (21-40)
<u>SIGNIFICANCE (p-value) **</u>	>0.05	>0.05	<0.001	<0.001	<0.001	<0.001	<0.001		<0.001

values are mean (SEM) and (range) UD = undetectable

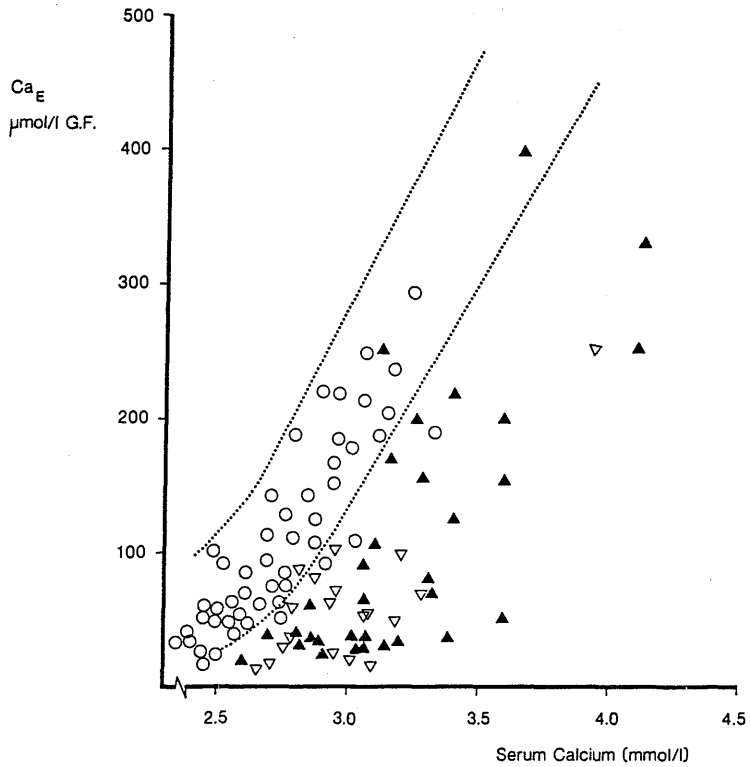
\*values in normal controls refer to data between 3-4 hours of calcium infusion, except theoretical calcium threshold, which was calculated from all data points.

\*\* significant differences between normal controls and other groups. Patients with malignancy and primary hyperparathyroidism differed only with respect to serum albumin and PTH values (p<0.001).

hyperparathyroidism had significantly higher iPTH levels when compared with both other groups. Serum albumin levels were significantly depressed in patients with malignancy-associated hypercalcaemia when compared with primary hyperparathyroidism, which in turn were lower than in normals. Renal tubular threshold for phosphate reabsorption ( $TmPO_4$ ) was significantly higher in normals when compared with both other groups. Tumour types in malignant hypercalcaemia were: squamous bronchial carcinoma-18; other squamous carcinomas-5; genito-urinary cancers-4; cholangiocarcinoma-2; breast carcinoma-2.

Values relating the urinary excretion of calcium (expressed as  $Ca_E$ ) to serum calcium in normal controls rendered hypercalcaemic by calcium infusion were in keeping with those previously described by Peacock et al (114) (figure 4.1). In primary hyperparathyroidism the points fell to the right of the expected normal indicating that renal tubular reabsorption of calcium was increased (114). In patients with malignancy associated hypercalcaemia, the points also fell to the right of the expected normal, reflecting a similar increase in renal tubular reabsorption of calcium. There were no significant differences in the gradients of the lines of linear regression relating serum calcium to urinary  $Ca_E$  values between normal subjects ( $Y = 0.32X - 0.51$ ), patients with primary hyperparathyroidism ( $Y = 0.42X - 0.65$ ) and those with malignant hypercalcaemia ( $Y = 0.25X - 0.71$ ). The calculated theoretical threshold for tubular calcium reabsorption (i.e. intercept on the X-axis) was significantly higher in primary hyperparathyroidism and hypercalcaemia of malignancy when compared with normals (Table 4.1)

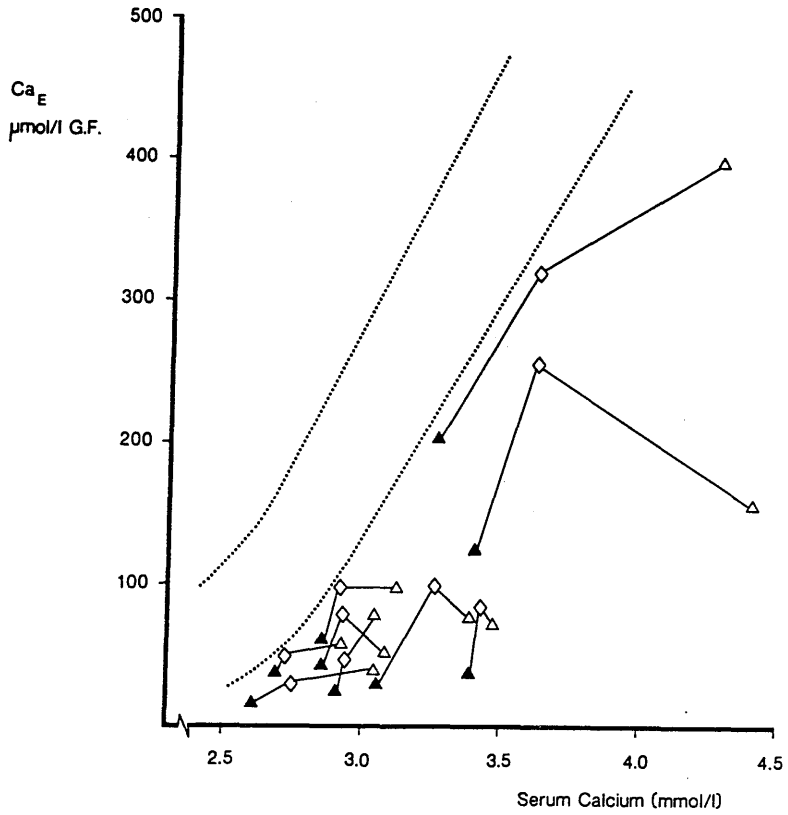
Sequential data on renal calcium handling throughout sodium



**FIGURE 4.1**

Relation between adjusted serum calcium values and urinary calcium excretion expressed as  $Ca_E$  in  $\mu\text{mol/l}$  of glomerular filtrate (GF) in the study group. Dotted lines indicate normal range as defined by Peacock (114).

- - Normal controls undergoing calcium infusions.
- ▽ - Patients with primary hyperparathyroidism.
- ▲ - Patients with malignant hypercalcaemia.



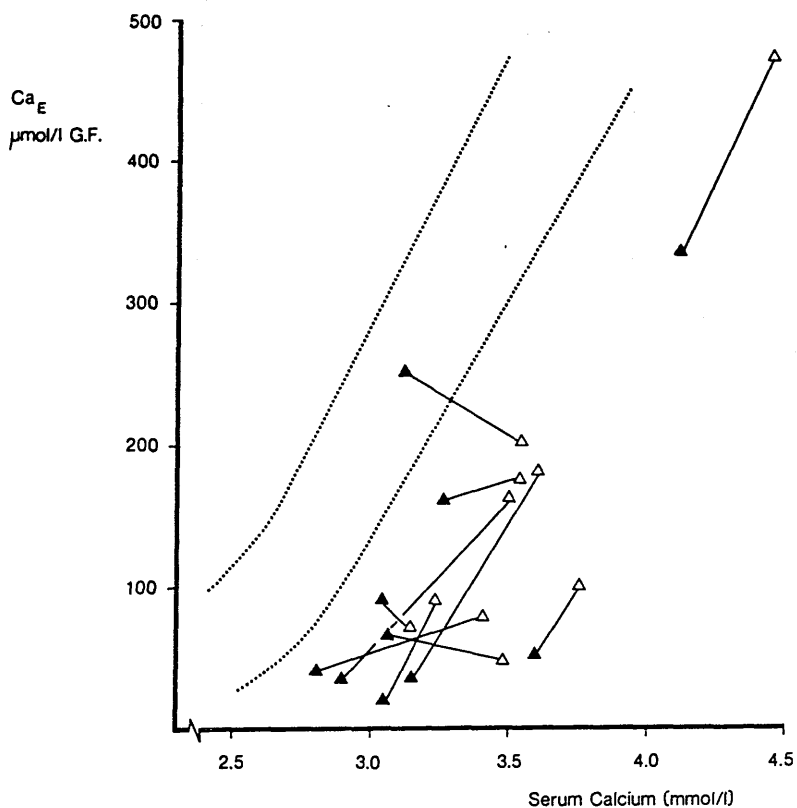
**FIGURE 4.2**

Relation between serum adjusted calcium values and  $Ca_E$  values during sodium repletion of patients with malignant hypercalcaemia. Dotted lines indicate normal range defined by Peacock (114).

△ - initial values

◇ - values after 24-48 hours of sodium repletion.

▲ - values after > 96 hours of sodium repletion.

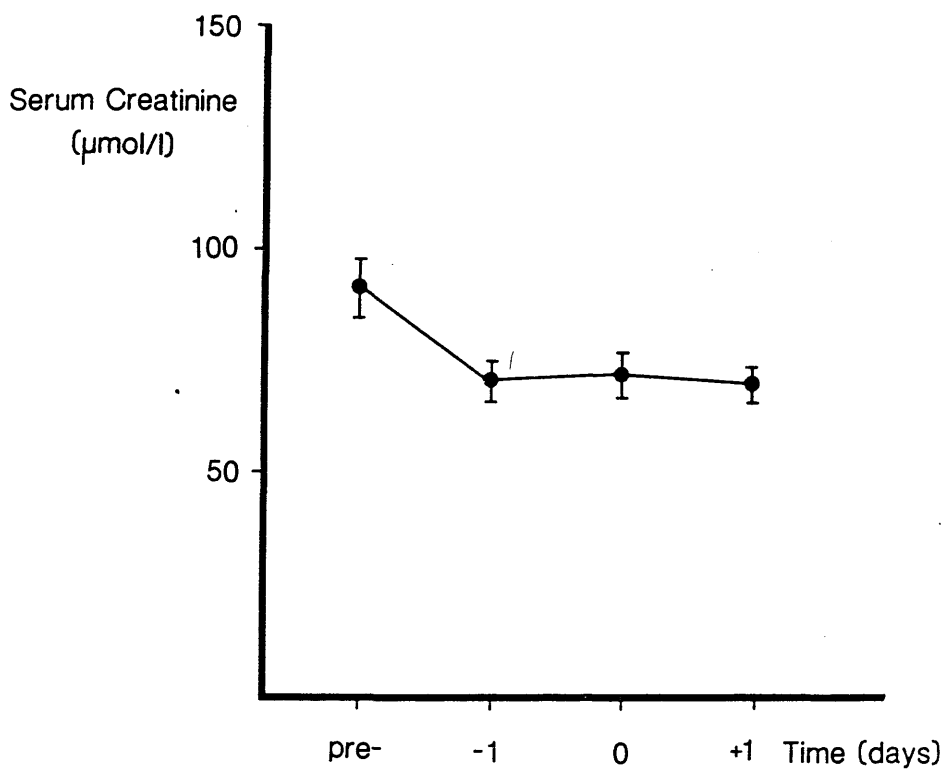


**FIGURE 4.3**

Relation between serum adjusted calcium values and  $Ca_E$  values during sodium repletion in patients with malignant hypercalcaemia. Dotted lines indicate normal range defined by Peacock (114).

$\triangle$  - Initial values.

$\blacktriangle$  - values after > 48 hours of sodium repletion.



**FIGURE 4.4**

Serum creatinine values during sodium repletion in patients with malignant hypercalcaemia. Points are means, bars are SEM.

PRE - At presentation.

-1 - 24 hours before assessment.

0 - After > 48 hours of sodium repletion

+1 - After > 96 hours of sodium repletion.

repletion were available in 19 patients. These subjects were divided into two groups for reasons of clarity and to some extent, pattern of response (figures 4.2 and 4.3). In nine patients, renal calcium handling was assessed at three points during the saline infusions (figure 4.2). This shows that a leftward shift of the serum calcium vs  $Ca_E$  points generally occurred within the first 24-48 hours, indicating that renal tubular calcium reabsorption had fallen. However, there was little further change on continuing saline infusions 2 litres daily for 96 hours or longer. The reductions in serum calcium which occurred in these subjects after 48 hrs were largely due to an improvement in GFR as manifest by a fall in serum creatinine (figure 4.4). Values before and after rehydration in a further 10 patients are shown in figure 4.3. In some patients a reduction in the setting of renal tubular calcium reabsorption occurred, often in combination with a reduced calcium load. Others, however showed a load dependent reduction in calcium alone, which again probably reflected the improvement of GFR on fluid repletion (4.3). Bone scans were performed in 17 patients with malignant disease. On division of these subjects into categories of "normal", "light tumour load" and "heavy tumour load", there was no significant correlation between extent of metastatic bone disease and urinary Ca/Cr values after rehydration. (Ca/Cr mean(SD) 1.05(0.91) vs 1.30(0.66) vs 2.07(1.71) respectively).

#### 4.1.4 DISCUSSION

In this study, rehydrated patients with malignancy-associated hypercalcaemia showed evidence of increased renal tubular reabsorption of calcium, of a degree similar to that observed in primary



hyperparathyroidism.

Several factors may influence the renal handling of calcium including acid-base disturbances, renal impairment, dehydration and sodium depletion (268). Although detailed acid-base studies and measurements of glomerular filtration rate were not performed, there was no significant difference in serum bicarbonate levels and serum creatinine levels between the three groups. Careful efforts were also made to ensure that all patients with malignant hypercalcaemia were replete in fluids and sodium at the time of study; all had received a minimum of 6 litres of 0.9% saline solution containing 900mmol of sodium. Thereafter sodium repletion was maintained by the continued infusion of 0.9% saline 2 litres daily. At the time of final assessment, serum creatinine and urea concentrations had stabilised to within fairly narrow limits and a high urine output was achieved in all cases. Previous workers who measured sodium balance during rehydration of severely hypercalcaemic cancer patients found the average deficit to be in the order of 200-600 mmol (147). This is considerably less than was received by all of the patients with malignant hypercalcaemia in this study. Sequential studies of renal calcium handling in a number of our patients revealed that, while renal tubular reabsorption of calcium often fell during the first 24-48 hours of saline repletion, it remained relatively static thereafter. This suggests that, after the initial sodium deficit is replaced, continued sodium delivery at a moderate level does not alter the renal tubular reabsorption of calcium further. It must be conceded that the levels of urinary calcium excretion in normal subjects rendered acutely hypercalcaemic by calcium infusion may not strictly be comparable with those in more chronically hypercalcaemic

patients with malignancy and primary hyperparathyroidism. However, experience of other workers who have studied renal calcium handling in malignancy-associated hypercalcaemia suggests that urinary  $Ca_E$  vs serum calcium points may often fall within the normal range (147,148), indicating that elevations in renal tubular calcium reabsorption are not simply a feature of chronic hypercalcaemia per se.

Notwithstanding the difficulties which are inherent in the clinical measurement of tubular calcium reabsorption (268), renal tubular reabsorption of calcium appeared to be increased in the majority of patients with malignant hypercalcaemia in this study. This finding contrasts with most previous work which has generally concluded that renal tubular reabsorption is depressed in cancer associated hypercalcaemia (3,23,27,93,147,148). There is relatively little information on the levels of urinary calcium excretion in relation to serum calcium in this situation however. In two studies where the above information is available,  $Ca_E$  vs serum calcium points have generally fallen within (147) or to the left of the normal range (148), indicating that renal tubular calcium reabsorption was normal or depressed in cancer-associated hypercalcaemia. The discrepancy between our findings and those cited above may be partially due to differences in tumour type, which in both previous studies comprised mainly of breast and haematological cancers. Further, in Hosking's series, many patients had a substantial degree of renal impairment, and this may also have caused lower levels of tubular calcium reabsorption (153). Van Breukelen and his colleagues (148) in another study appear to have made no adjustment for serum albumin concentrations which are usually low in patients with cancer-associated hypercalcaemia. Finally, individual variations in renal

tubular calcium reabsorption may have been overlooked in the latter study (148) since  $Ca_E$  vs serum calcium points were expressed as an average value for the study group as a whole, rather than individually.

In agreement with the previous study (chapter 3.2) and other published work (93), the renal tubular threshold for phosphate reabsorption was generally low in this group of patients with malignancy-associated hypercalcaemia, who had predominantly squamous and genito-urinary tumours.

The current data indicate that, in patients with malignancy associated hypercalcaemia, renal tubular calcium reabsorption is increased to a similar level as that in primary hyperparathyroidism. In the absence of sodium depletion and dehydration, this suggests that a humoral mediator is released in many patients with malignant hypercalcaemia which possesses a "PTH-like" effect on the renal tubular handling of both calcium and phosphate. By impairing the renal excretion of calcium, such a humoral mediator may explain the frequent occurrence of hypercalcaemia in malignant disease, when compared with benign conditions associated with increased bone resorption.

## 4.2 RENAL TUBULAR REABSORPTION OF CALCIUM IN PATIENTS WITH NON-PARATHYROID HYPERCALCAEMIA OF BENIGN AETIOLOGY AND IN NORMOCALCAEMIC AND HYPERCALCAEMIC CANCER PATIENTS WITH AND WITHOUT METASTATIC BONE DISEASE

### 4.2.1 INTRODUCTION

In the previous study, reference was made to the paucity of data concerning the renal tubular reabsorption of calcium in patients with hypercalcaemia of benign, non-parathyroid aetiology. There is, in addition, little information available on renal tubular calcium reabsorption in normocalcaemic patients with malignant disease. In this study, renal tubular handling of calcium was assessed in both hypercalcaemic and normocalcaemic patients with malignancy and in a small group of patients with hypercalcaemia of benign, non-parathyroid aetiology.

### 4.2.2 PATIENTS AND METHODS

The patients with malignancy who were assessed comprised the 74 subjects described in chapter 4.1, who had presented consecutively to Glasgow Royal Infirmary for bone scan examinations and thirteen patients with non-parathyroid hypercalcaemia of benign aetiology who had presented consecutively to Glasgow Royal Infirmary over a 5-year period. Hypercalcaemic patients with malignancy were rehydrated with intravenous 0.9% saline as previously described (chapter 4.1). Patients with benign non-parathyroid hypercalcaemia with serum calcium values of greater than 3.10 mmol/l were rehydrated using a similar protocol. Patients with milder degrees of hypercalcaemia were generally well-hydrated on a clinical basis and were not routinely

given intravenous fluids. Biochemical analyses were made on blood samples and true fasting urine samples generally obtained after an overnight fast.

#### 4.2.2 RESULTS

The aetiology of hypercalcaemia in patients with benign, non-parathyroid hypercalcaemia was as follows: hypoparathyroidism and vitamin D toxicity-3 (23%), osteoporosis and vitamin D toxicity-2 (15%), hyperthyroidism-3 (23%), immobilisation-5 (33%). Relevant clinical and biochemical details are shown in table 4.2. There were no significant differences in levels of serum calcium and serum albumin levels in patients with benign non-parathyroid hypercalcaemia compared with those who had malignant hypercalcaemia. Levels of serum phosphate and  $TmPO_4$  were significantly higher in the patients with benign hypercalcaemia, however.

The relation between urinary excretion of calcium expressed as  $Ca_E$  and serum calcium in the three groups of patients is shown in figure 4.5. With the exception of 3 out of the 4 hypercalcaemic breast carcinoma patients who were studied, the urine  $Ca_E$  vs serum calcium points in malignant hypercalcaemia generally fell to the right of the expected normal, indicating that renal tubular reabsorption of calcium was increased. In normocalcaemic cancer patients, and in patients with hypercalcaemia of benign, non-parathyroid aetiology, the points generally fell within the normal range indicating that renal tubular calcium reabsorption was normal. Although renal tubular reabsorption of calcium was slightly increased in two patients with benign, non-parathyroid hypercalcaemia, the renal tubular contribution to hypercalcaemia, was significantly lower than in malignant

TABLE 4.2

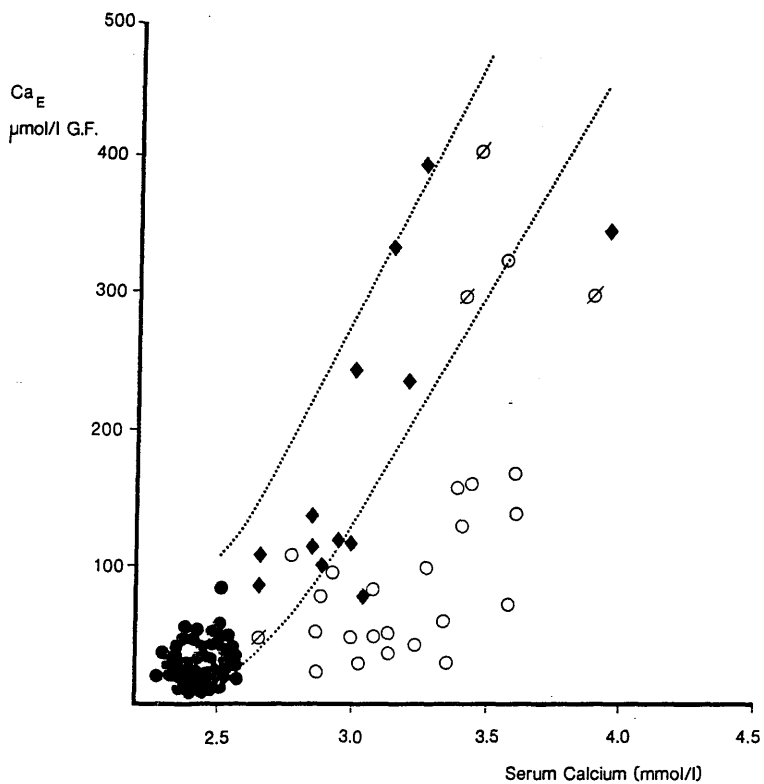
CLINICAL AND BIOCHEMICAL DETAILS IN PATIENTS WITH BENIGN NON-PARATHYROID HYPERCALCAEMIA

	<u>SERUM</u> <u>CALCIUM</u> <u>(mmol/l)</u>	<u>SERUM</u> <u>PHOSPHATE</u> <u>(mmol/l)</u>	<u>SERUM</u> <u>CREATININE</u> <u>(μmol/l)</u>	<u>SERUM</u> <u>ALBUMIN</u> <u>(g/l)</u>	<u>URINE</u> <u>Ca/Cr</u> <u>(mmol)</u>	<u>TmPO<sub>4</sub></u> <u>(mmol/l GF)</u>	<u>MALE/</u> <u>FEMALE</u>	<u>AGE</u> <u>(years)</u>
NON-MALIGNANT	3.03 (0.09)	1.18 (0.09) *	107 (18.6) *	33 (6.7)	1.92 (0.34)	0.96 (0.08) *	3/9	49 (5.7)
NON-PARATHYROID HYPERCALCAEMIA	(2.65-3.95)	(0.65-1.60)	(30-230)	(18-46)	(0.75-5.20)	(0.35-1.40)		(22-78)

(n=13)

values are mean (SEM)  
(range)

\* significantly different (p<0.05) from hypercalcaemic patients with malignancy - (table 3.2).



**FIGURE 4.5**

Relation between serum adjusted calcium values and  $Ca_E$  values in the study group. Dotted lines indicate normal range defined by Peacock (114).

- - Normocalcaemic patients with malignancy.
- - Hypercalcaemic patients with malignancies other than breast carcinoma.
- ⊘ - Hypercalcaemic patients with breast carcinoma.
- ◆ - Benign non parathyroid hypercalcaemia.

hypercalcaemia overall; renal tubular component of hypercalcaemia (mean(SEM)) = +0.54(0.05) mmol/l (malignancy) vs +0.09(0.06) mmol/l (benign hypercalcaemia), ( $p < 0.001$ )

#### 4.2.4 DISCUSSION

These data indicate that renal tubular reabsorption of calcium is generally normal in normocalcaemic cancer patients both with and without bone metastases. In combination with the previous data concerning biochemical indices of bone resorption in normocalcaemic patients with metastatic bone disease (chapter 4.1), this finding helps to explain why such patients can maintain normocalcaemia in the presence of increased bone resorption; not only is the severity of bone resorption insufficient to cause hypercalcaemia, but also, the renal tubular threshold for excretion of calcium is normal, thus permitting elimination of excess calcium from the ECF.

In agreement with the previous study (chapter 4.1) renal tubular calcium was found to be elevated in most of the hypercalcaemic cancer patients, consistent with a parathyroid-hormone like effect on renal tubular calcium reabsorption. However, 3/4 of the the breast cancer patients - all of whom had a heavy skeletal tumour load - had normal renal tubular reabsorption of calcium. This suggests that a "local osteolytic" mechanism of hypercalcaemia may be more prevalent with this tumour type.

In patients with benign hypercalcaemia of non-parathyroid origin, renal tubular reabsorption of calcium was generally normal and was significantly lower than in patients with malignant hypercalcaemia. This indicates that the elevations in renal tubular calcium



reabsorption noted previously (chapter 4.2) were not simply due to a non-specific effect of chronic hypercalcaemia per se, but rather, were the result of a "PTH-like" effect on renal tubular calcium reabsorption.

### 4.3 RELATION BETWEEN RENAL SODIUM HANDLING AND RENAL CALCIUM HANDLING IN HYPERCALCAEMIA OF MALIGNANCY AND PRIMARY HYPERPARATHYROIDISM

#### 4.3.1 INTRODUCTION

Reference has been made to the fact that renal tubular reabsorption of calcium may be elevated in patients who are sodium depleted (268). In this situation, it is thought that increased sodium reabsorption in the proximal renal tubule is accompanied by a concomitant increase in proximal renal tubular calcium reabsorption (176,267,268). In malignancy associated hypercalcaemia, normalisation of renal tubular calcium reabsorption after sodium repletion has previously been interpreted as evidence against a "PTH-like" effect of the putative humoral mediators of hypercalcaemia (147,148,259). In this study, the relation between urinary sodium excretion and renal tubular calcium reabsorption has been examined in patients with hypercalcaemia of malignancy. These data were compared with those in primary hyperparathyroidism, where renal tubular calcium reabsorption is known to be increased, due to the effects of PTH on the distal renal tubule.

#### 4.3.2 PATIENTS AND METHODS

Data were obtained from two groups of patients; 31 with primary hyperparathyroidism and 36 with hypercalcaemia of malignancy, all of whom had presented consecutively to Glasgow Royal Infirmary.

All biochemical analyses were made on true fasting urine and blood samples, obtained after an overnight fast. Patients with primary hyperparathyroidism fell into two groups; 30 patients were normally

hydrated on a clinical basis, and were receiving oral fluids only at the time of study. One patient who had presented with hypercalcaemia and severe dehydration was studied prior to receiving intravenous fluids. In seven patients with primary hyperparathyroidism, further studies were performed during acute saline infusions (see below). Twenty seven patients with malignancy-associated hypercalcaemia were studied after rehydration with intravenous saline; a minimum of 6 litres had been given initially, followed by 2 litres daily for 24 hours prior to and during the study. Four patients with malignancy who were clinically dehydrated at presentation were studied before the administration of intravenous fluids. The remaining 5 patients had presented with stable hypercalcaemia and renal function documented for a period of at least 4 weeks prior to the study. These patients were normally hydrated on a clinical basis and were receiving oral fluids only prior to the study. In these patients, further studies were performed during acute saline infusions.

Saline infusion studies were performed as follows; in hyperparathyroidism, infusions of saline (0.9%) were commenced shortly after collection of baseline samples and were continued for 48 hours. The rate of infusion was 500 ml every 4 hours (450 mmol sodium chloride daily). In patients with malignancy, saline infusions were commenced after collection of baseline samples and were also continued, 500ml every four hours for 48 hours.

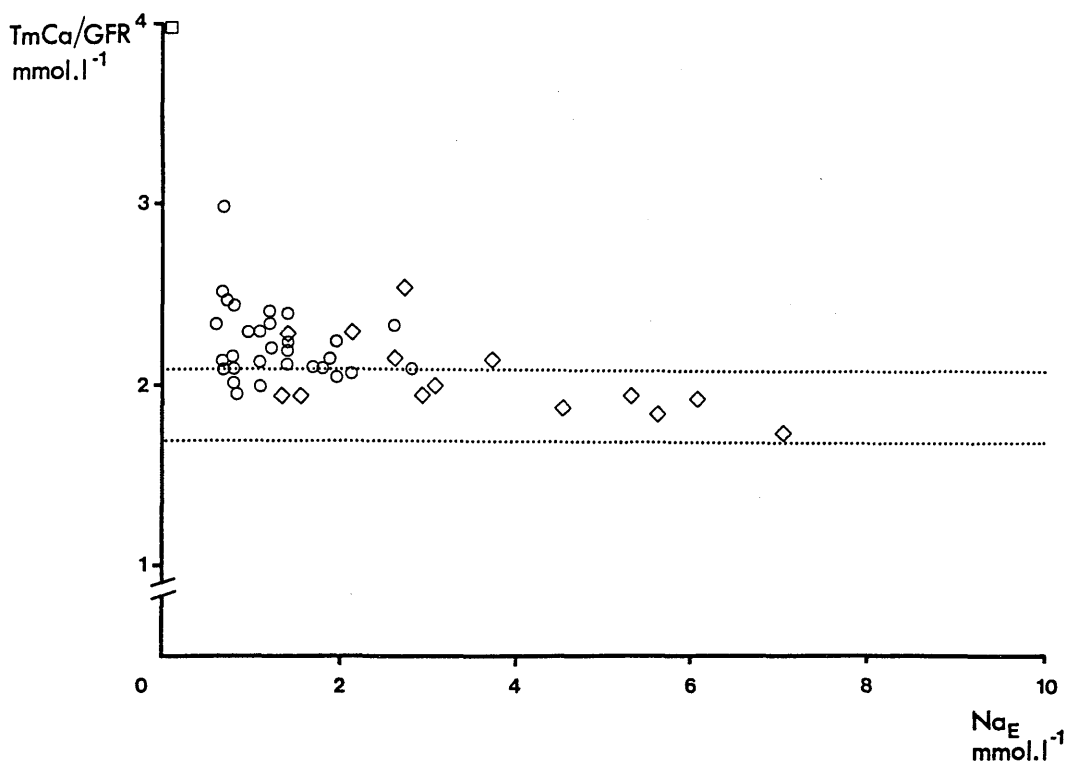
Statistical methods used were the Wilcoxon test for paired samples and Spearman's rank correlation coefficient.

#### 4.3.2 RESULTS

All 31 patients with primary hyperparathyroidism were known to have sustained hypercalcaemia [mean (SEM) serum calcium = 2.89 (0.02) mmol/l], with "inappropriately" detectable plasma iPTH values, in the absence of another identifiable cause of hypercalcaemia. In 20 patients, the diagnosis was subsequently proven by surgical exploration of the neck. Serum creatinine values were within the normal range in most cases (serum creatinine; mean (SEM) = 81.8 (5.9)  $\mu\text{mol/l}$ ). Two subjects with renal stone disease had elevated creatinine values however (230, 150  $\mu\text{mol/l}$ , respectively). Serum albumin values were within the normal range in most cases (serum albumin; mean (SEM) = 39.5 (1.1) g/l).

Serum calcium values in patients with malignancy were; mean (SEM) = 3.21 (0.06) mmol/l, significantly higher than in the hyperparathyroid group ( $p < 0.01$ ). In all of these patients plasma iPTH values were low or undetectable. Serum creatinine concentrations were normal in most cases and were comparable with those in patients with primary hyperparathyroidism (serum creatinine mean (SEM) = 81.9 (4.9)  $\mu\text{mol/l}$ . Creatinine concentrations lay outwith the normal range in 3 cases; (150, 150, 190  $\mu\text{mol/l}$ , respectively). Serum albumin values in patients with malignancy were generally reduced when compared to those in patients with primary hyperparathyroidism (serum albumin; mean (SEM) = 29.6 (0.7) g/l;  $p < 0.001$ ).

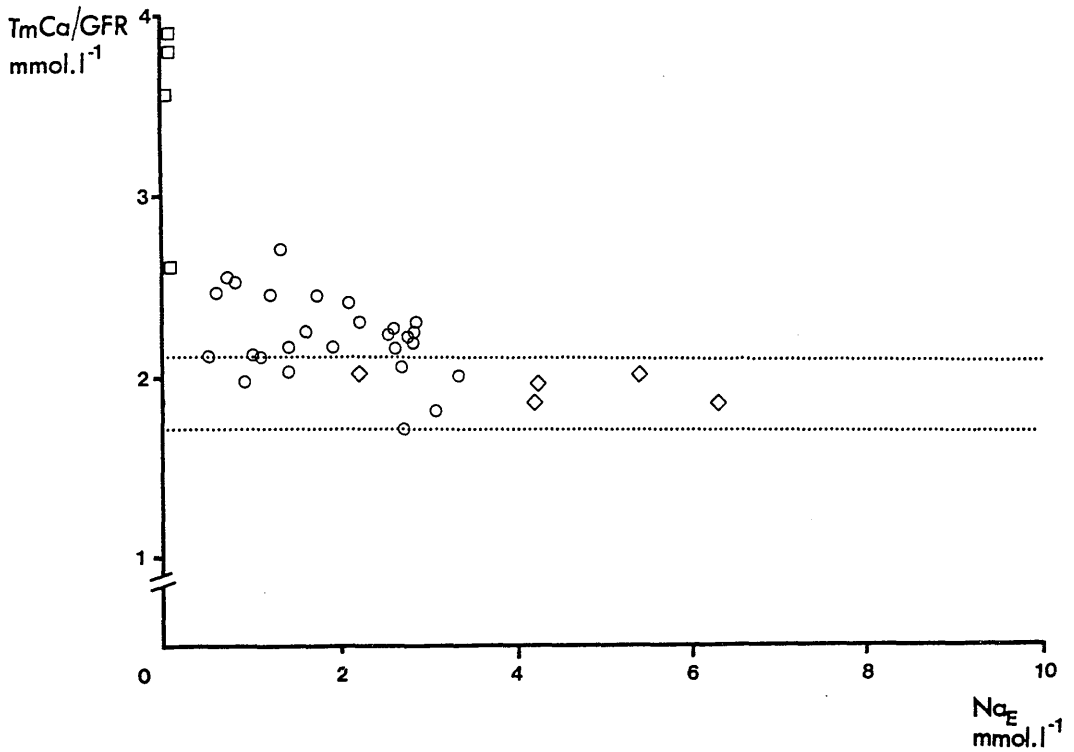
In figure 4.6, the relation between the notional renal tubular threshold for calcium reabsorption ( $T_m\text{Ca}/\text{GFR}$ ) and urinary sodium excretion ( $\text{Na}_E$ ) is shown in patients with primary hyperparathyroidism. When data from the 30 normally hydrated patients only was considered,



**FIGURE 4.6**

Relation between urinary sodium excretion ( $Na_E$ ) and renal tubular calcium reabsorption ( $TmCa/GFR$ ) in patients with primary hyperparathyroidism.

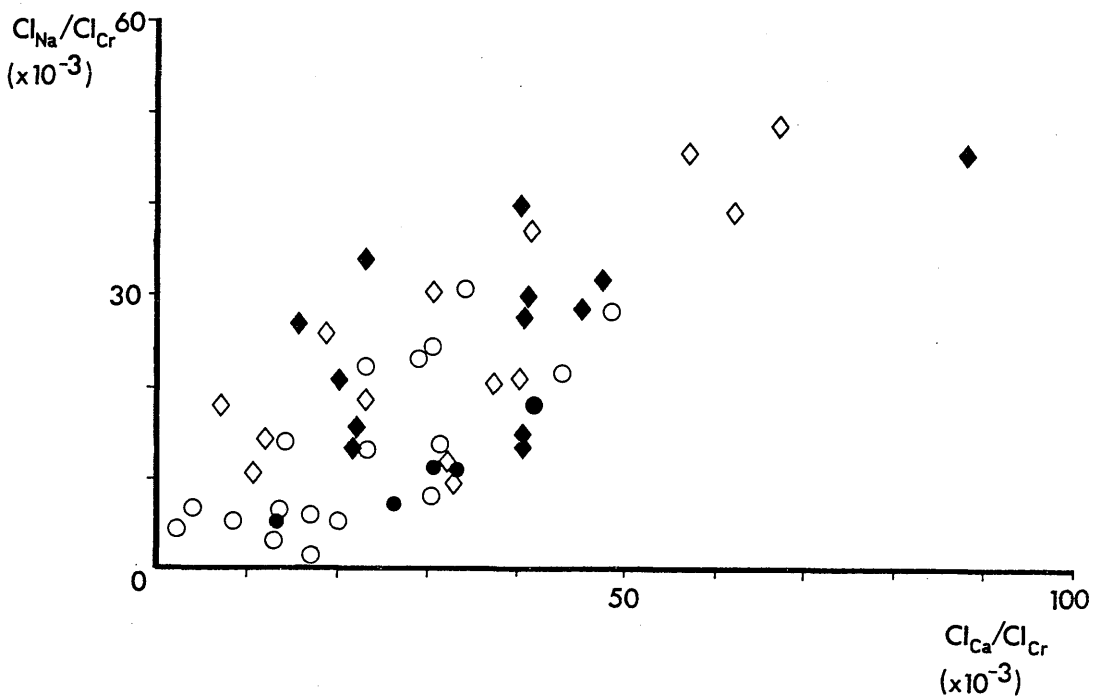
- - Clinically dehydrated.
- - normally hydrated receiving oral fluids.
- ◇ - during saline infusions.



**FIGURE 4.7**

Relation between urinary sodium excretion ( $Na_E$ ) and renal tubular calcium reabsorption ( $TmCa/GFR$ ) in patients with malignant hypercalcaemia.

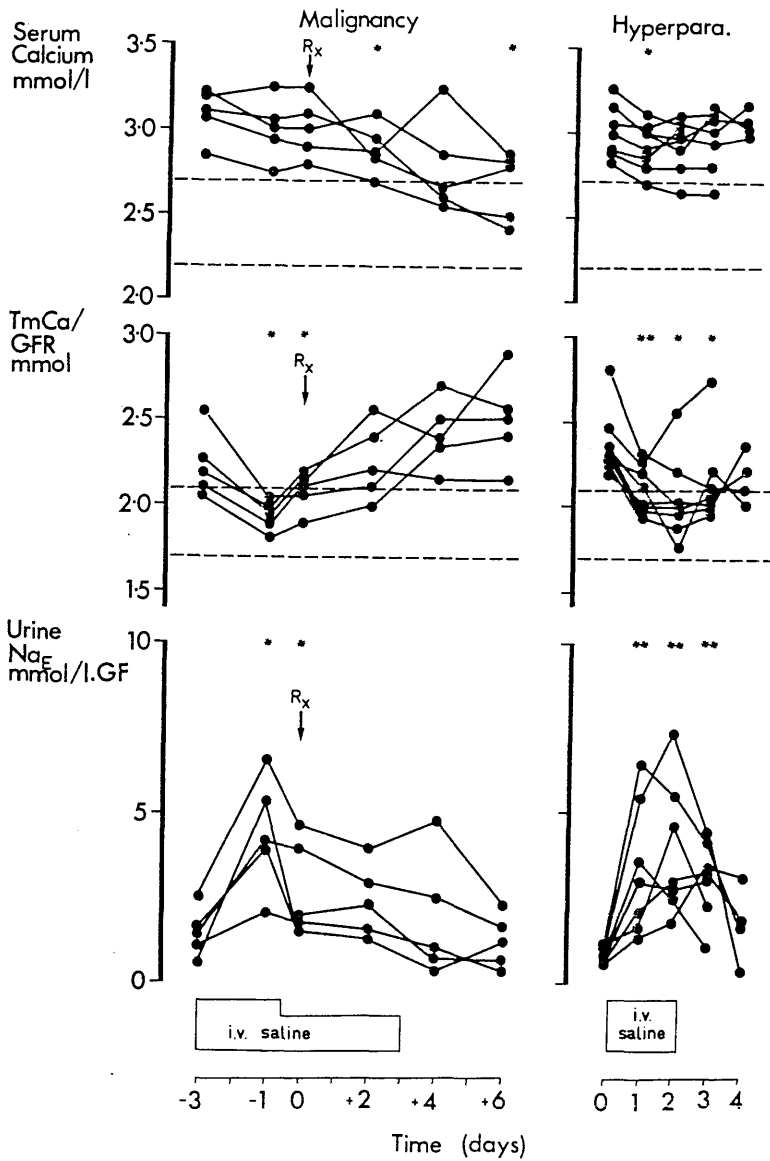
- $\square$  - Clinically dehydrated
- $\circ$  - Normally hydrated, receiving I.V. saline 2 litres daily.
- $\diamond$  - During acute saline loading, receiving I.V. saline 3 litres daily.



**FIGURE 4.8**

Relation between renal sodium clearance ( $Cl^{Na}/Cl^{Cr}$ ) and renal calcium clearance ( $Cl^{Ca}/Cl^{Cr}$ ) during acute sodium loading of patients with malignancy and hyperparathyroidism.

	Hyperparathyroidism	Malignancy
Before/after I.V. saline	○	●
During I.V. saline	◇	◆



**FIGURE 4.9**

Response of serum calcium, TmCa/GFR and  $\text{Na}_E$  in normally hydrated patients with primary hyperparathyroidism and malignant hypercalcaemia during saline infusions. Interrupted lines indicate normal ranges. Significant change from initial values:  $*=p<0.05$ ,  $**=p<0.02$ .  $R_x$  = Start of antihypercalcaemic drug therapy in patients with malignancy (not referred to in text).



there was no significant correlation between TmCa/GFR and Na<sub>E</sub> ( $r = 0.27$ ; NS). However, when data were included from the patients who was dehydrated and from the seven patients who were studied during acute saline infusions (2 values in each patient) there was a significant inverse correlation between TmCa/GFR and Na<sub>E</sub> ( $r = -0.49$   $p < 0.002$ ).

In figure 4.7, similar data is shown from the patients with malignancy associated hypercalcaemia. In the 27 patients who were studied after rehydration with intravenous saline, TmCa/GFR and Na<sub>E</sub> were not significantly correlated ( $r = -0.16$ , NS). When data were included from 4 dehydrated patients and from the seven normally hydrated patients undergoing acute saline infusion studies, there was a significant inverse correlation ( $r = 0.60$ ,  $p < 0.001$ ).

In the 12 patients (7-hyperparathyroid, 5-malignancy) undergoing detailed studies prior to and during acute saline infusion studies, TmCa/GFR and Na<sub>E</sub> were not significantly correlated before saline infusions were commenced, either in primary hyperparathyroidism ( $n = 7$ ,  $r = -0.23$ , NS) or in malignancy ( $n = 5$ ,  $r = -0.17$ , NS). When all the data were included from measurements made prior to, during and after saline infusions however, a highly significant inverse correlation was observed between TmCa/GFR and Na<sub>E</sub> in primary hyperparathyroidism ( $r = -0.72$ ,  $p < 0.001$ ) and in malignancy ( $r = 0.70$ ,  $p < 0.01$ ). In both conditions there was a positive correlation between the ratio of sodium to creatinine clearance and calcium to creatinine clearance; (hyperparathyroidism;  $r = 0.73$ ,  $p < 0.005$ ), malignancy  $r = 0.77$ ,  $p < 0.005$ ) (figure 4.8).

The response of serum calcium, Na<sub>E</sub>, and TmCa/GFR to the acute

saline infusions in hyperparathyroidism and malignancy is shown in figure 4.9. In primary hyperparathyroidism, there was a significant rise in  $\text{Na}_E$  and a significant fall in both serum calcium and  $\text{TmCa}/\text{GFR}$  during the saline infusions. These changes were reversed when saline infusions were stopped. In malignancy, a similar pattern of response was observed; a significant elevation in  $\text{Na}_E$  during saline infusion was accompanied by a significant fall in  $\text{TmCa}/\text{GFR}$ . Serum creatinine concentrations were not altered significantly by saline infusions in either group of patients; serum creatinine concentrations before and after saline infusions in primary hyperparathyroidism were (mean(SEM)) 73.5 (4.9) and 73.5 (8.5)  $\mu\text{mol}/\text{l}$  respectively. In malignancy the corresponding values were 86 (14.4) and 79 (15.5)  $\mu\text{mol}/\text{l}$  respectively.

#### 4.3.5 DISCUSSION

In this study, a close correlation was observed between urinary sodium excretion and renal tubular calcium reabsorption in patients with malignancy associated hypercalcaemia and primary hyperparathyroidism who had been sodium loaded. These findings concur with previous data: in various experimental circumstances, proximal renal tubular reabsorption of calcium has been shown to be correlated with that of sodium (176,266,267). Nonetheless, other data have suggested that proximal renal tubular reabsorption of calcium is relatively stable under normal conditions, and is a function of the filtered calcium load (268), whereas changes in net renal tubular calcium reabsorption are determined mainly by the effects of parathyroid hormone on the distal tubule (114). In clinical practice however, measurements of renal tubular calcium reabsorption reflect

the sum of changes in both proximal and distal tubular reabsorptive processes, and as such, do not distinguish between their relative contribution to  $TmCa/GFR$ . Interestingly, there was no significant correlation between  $TmCa/GFR$  and urinary  $Na_E$  values either in malignancy or primary hyperparathyroidism where patients were studied in a "steady state"- either receiving oral fluids or constant amounts of intravenous saline. This suggests that, in this situation, sodium-independent and probably humoral changes in distal renal tubular calcium reabsorption were of primary importance in determining  $TmCa/GFR$ .

In clinically dehydrated patients,  $TmCa/GFR$  was invariably raised presumably as the result of increased sodium-linked calcium reabsorption in the proximal renal tubule (268). Conversely, under conditions of acute sodium loading, there was a significant fall in  $TmCa/GFR$  suggesting that decreased sodium-linked calcium reabsorption in the proximal renal tubule was the major factor responsible for the fall in  $TmCa/GFR$  (176,266,267,283). Taken together, these findings suggest that sodium-related changes in renal tubular calcium reabsorption are of relatively minor importance under normal conditions, but may become increasingly important at the upper and lower extremes of urinary sodium excretion.

In two previous studies of renal tubular calcium reabsorption in hypercalcaemia of malignancy, tubular calcium reabsorption was raised prior to sodium repletion but generally fell thereafter (147,148). These findings were taken as evidence against a renal tubular action of putative humoral mediators of hypercalcaemia. In the present study, however,  $TmCa/GFR$  values fell towards normal in patients with primary hyperparathyroidism who were saline loaded, suggesting that renal

tubular reabsorption of calcium may be reduced by a sodium load, even in the presence of a PTH-mediated increase in distal renal tubular calcium reabsorption (114). A further relevant observation was that the relation between urinary sodium excretion and renal tubular calcium reabsorption was similar in patients with primary hyperparathyroidism and hypercalcaemia of malignancy. This lends support to the suggestion, made in chapter 4.1, that in hypercalcaemia of malignancy, there is exhibited a humoral mediator with a PTH-like effect on the renal tubular reabsorption of calcium. In two previous studies (147,148), renal tubular calcium reabsorption was assessed at relatively high rates of urinary sodium excretion ( $\text{Na}_E$  (median) = 4.9mmol/l GF and 5.8mmol/l GF respectively); this suggests that the "normal" renal tubular reabsorption of calcium observed in some cases may, in fact have been the result of a sodium-linked reduction in proximal renal tubular calcium reabsorption, and as such, did not exclude a concomitant increase in distal renal tubular calcium reabsorption which was humorally-mediated.

These data emphasise the importance of measuring sodium excretion in any investigation of renal tubular calcium reabsorption. Caution must be exercised in the interpretation of data relating to renal tubular reabsorption of calcium in patients who are sodium-loaded or sodium depleted.

CHAPTER 5

CALCIUM REGULATING HORMONES AND INTESTINAL CALCIUM

ABSORPTION IN MALIGNANCY ASSOCIATED HYPERCALCAEMIA

5.1 CIRCULATING LEVELS OF VITAMIN D METABOLITES IN NORMOCALCAEMIC  
AND HYPERCALCAEMIC PATIENTS WITH MALIGNANCY

5.1.1 INTRODUCTION

The data presented in chapters 3 and 4 suggest that in most patients with malignancy associated hypercalcaemia, evidence can be found for a humoral aetiology, whether or not bone metastases are present. It has been considered that the putative humoral mediator causing such hypercalcaemia possesses PTH-like properties with regard to renal tubular phosphate handling (6,7,8,9,93,259), nephrogenous cyclic AMP production (93), and renal tubular calcium handling (Chapter 4). Nevertheless, it is immunologically distinct from PTH or its fragments, such as circulate in primary hyperparathyroidism (93,130,131,132). Circulating levels of  $1,25(\text{OH})_2\text{D}_3$  have previously been found to be reduced in hypercalcaemia of malignancy when compared with primary hyperparathyroidism (93). It has therefore been considered unlikely that  $1,25(\text{OH})_2\text{D}_3$  plays a significant role in the pathogenesis of hypercalcaemia in malignancy. Moreover, measurement of  $1,25(\text{OH})_2\text{D}_3$  levels has been proposed as a means by which the hypercalcaemia of malignancy may be differentiated from that of primary hyperparathyroidism (93).

In this study, levels of circulating vitamin D metabolites were measured in relation to other hormonal regulators of calcium metabolism in normocalcaemic and hypercalcaemic patients with malignancy.

### 5.1.2 PATIENTS AND METHODS

The study group comprised 88 patients with solid malignant tumours; 44 were hypercalcaemic (serum calcium >3.00 mmol/l at presentation and 44 were normocalcaemic (serum calcium <2.60 mmol/l). Four normocalcaemic and 3 hypercalcaemic patients with breast carcinoma received Tamoxifen therapy and one normocalcaemic patient with prostatic carcinoma received stilboestrol therapy at the time of study. No patients were receiving vitamin D analogues or other medication known significantly to affect calcium homeostasis.

Prior to study, all hypercalcaemic patients were rehydrated with either intravenous or oral fluids for periods of between 24-72hrs. Biochemical analyses were made on true fasting urine samples and blood samples obtained after an overnight fast.

Statistical methods used were the Wilcoxon test for unpaired samples and Linear Regression Analysis.

### 5.1.3 RESULTS

Only two patients with hypercalcaemia of malignancy had detectable plasma iPTH levels (450, 680 ng/l, respectively). Data from these patients was excluded, as these patients may have had co-existing primary hyperparathyroidism.

Plasma iPTH levels were undetectable in the remaining 42 patients with malignancy associated hypercalcaemia. In normocalcaemic cancer patients, iPTH levels lay within the normal range, appropriate to their normal serum calcium concentrations.

The commonest tumour type in hypercalcaemic patients was squamous

TABLE 5.1

CLINICAL AND BIOCHEMICAL DETAILS IN PATIENTS WITH MALIGNANCY

	<u>SERUM CALCIUM (mmol/l)</u>	<u>SERUM PHOSPHATE (mmol/l)</u>	<u>SERUM CREATININE (µmol/l)</u>	<u>SERUM ALBUMIN (g/l)</u>	<u>MALE/ FEMALE</u>	<u>AGE (years)</u>	<u>TUMOUR TYPE</u>
<u>NORMOCAEEMIC</u>	2.45 (2.30-2.65)	1.05 (0.50-1.60)	75 (50-150)	39 (22-46)	9/35	60 (35-76)	BRONCHUS 12 BREAST 26 OTHER 6*
<u>HYPERCALCAEMIC</u>	3.41 (2.81-4.48)	0.85 (0.40-1.25)	80 (50-135)	33 (30-36)	25/17	68 (44-78)	BRONCHUS 20 BREAST 7 OTHER 15+
<u>SIGNIFICANCE p-value</u>	p<0.001	p<0.001	p>0.05	p<0.001		p<0.05	

\*prostate-3; thyroid-1; bowel-2.  
 †genitourinary-6; larynx-3; cholangiocarcinoma-3; oesophagus-1; pancreas-1.

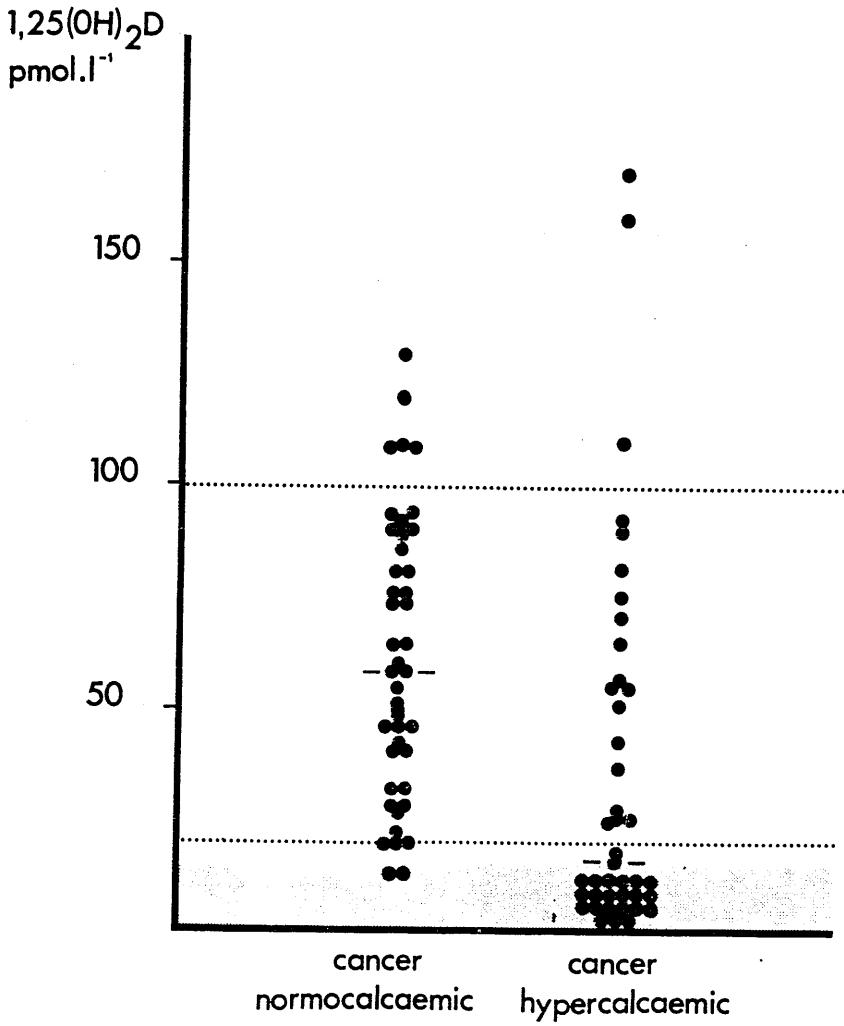
biochemical values are median (range)



TABLE 5.2  
SERUM CALCITONIN AND 25-HYDROXYVITAMIN D LEVELS  
IN PATIENTS WITH MALIGNANT DISEASE

	<u>SERUM</u> <u>CALCITONIN</u> <u>(ng/l)</u>	<u>SERUM</u> <u>25 (OH) D</u> <u>(nmol/l)</u>
<u>NORMOCALCAEMIC</u>	36	27.5
<u>(n=44)</u>	26-48 (15-1700)	11-48 (<5-98)
<u>HYPERCALCAEMIC</u>	86	13.5
<u>(n=42)</u>	66-110 (21-320)	5-23 (<5-100)
<u>SIGNIFICANCE</u>		
<u>(p-value)</u>	<0.001	<0.01

Values are            median  
interquartile range  
(range)



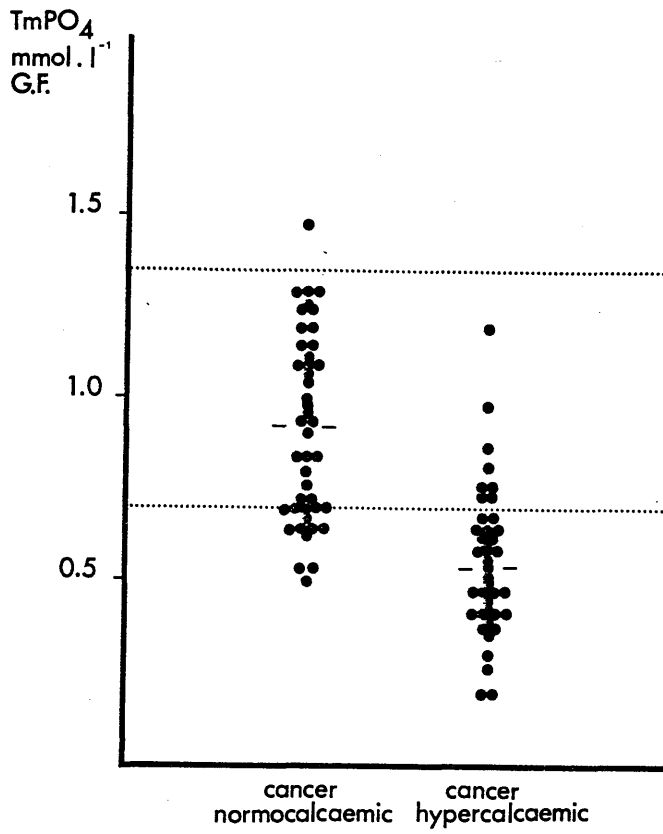
**FIGURE 5.1**

Serum levels of 1,25 (OH)<sub>2</sub> D in normocalcaemic and hypercalcaemic patients with malignancy. Dotted lines indicate normal range. Shaded zone indicates assay's limit of detection.

bronchial carcinoma, compared with breast carcinoma in the normocalcaemic group (Table 5.1). Hypercalcaemic patients were slightly, but significantly older than those who were normocalcaemic ( $p < 0.05$ ), and had significantly lower serum albumin levels ( $p < 0.001$ ). Serum inorganic phosphate levels generally lay within the normal range, although serum phosphate was significantly lower in the hypercalcaemic group ( $p < 0.001$ ).

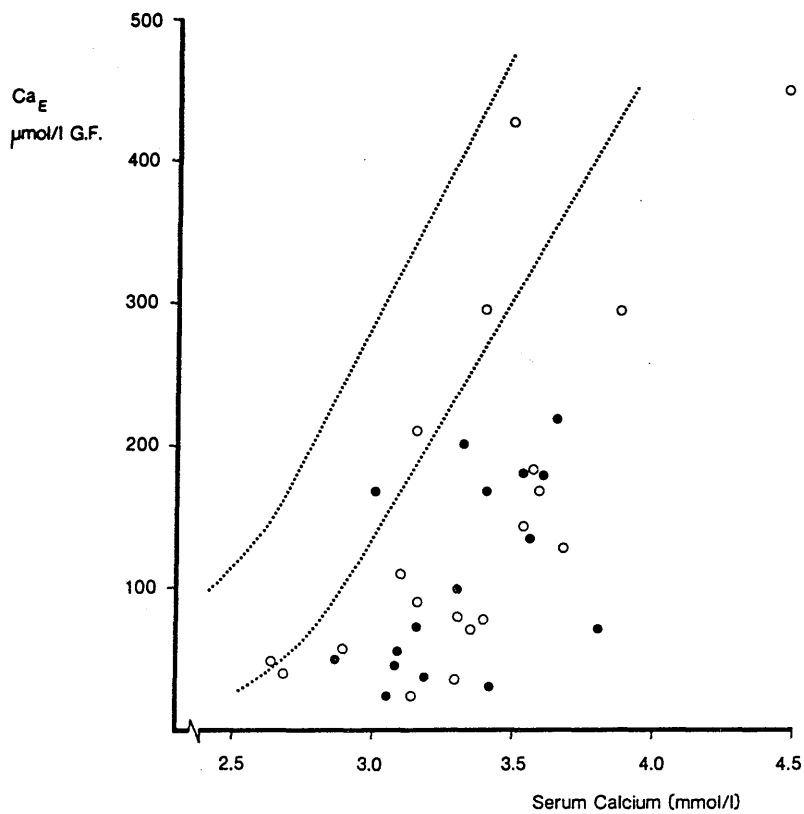
Patients with hypercalcaemia had significantly higher plasma calcitonin concentrations than those who were normocalcaemic ( $p < 0.001$ ). In the latter group, calcitonin levels generally lay within the reference range (Table 5.2). There was no significant correlation between plasma calcitonin level and serum calcium values in patients who were hypercalcaemic however ( $r = 0.18$ , NS). Overall, calcitonin did not correlate with either serum creatinine ( $r = 0.17$ , NS) or serum  $1,25(\text{OH})_2 \text{D}$  levels ( $r = 0.09$ , NS).

Concentrations of  $25(\text{OH}) \text{D}$  were low in both groups of patients with malignancy when compared with our reference range (Table 5.2). Circulating levels of  $1,25(\text{OH})_2 \text{D}_3$  in hypercalcaemic and normocalcaemic patients with malignancy are shown in figure 5.1. Serum  $1,25(\text{OH})_2 \text{D}$  levels were significantly lower in the hypercalcaemic group ( $p < 0.01$ ), when compared with the normocalcaemic group, where plasma  $1,25(\text{OH})_2 \text{D}$  levels generally lay within the reference range. A substantial proportion (18/42) of the hypercalcaemic patients had clearly detectable  $1,25(\text{OH})_2 \text{D}$  levels however, ( $> 24 \text{pmol/l}$ ) and this was considered to be an "inappropriate" response in patients with hypercalcaemia of non-parathyroid origin. In this subgroup of patients, the levels of  $1,25(\text{OH})_2 \text{D}$  were similar to those in a group



**FIGURE 5.2**

Renal tubular threshold for phosphate reabsorption ( $TmPO_4$ ) in normocalcaemic and hypercalcaemic patients with malignancy. Dotted lines indicate normal range. Bars are medians.



**FIGURE 5.3**

Relation between serum adjusted calcium values and  $Ca_E$  in patients with hypercalcaemia of malignancy. Dotted lines indicate normal range defined by Peacock (114).

● -  $1,25(\text{OH})_2 \text{D} < 24 \text{ pmol/l}$

○ -  $1,25(\text{OH})_2 \text{D} \geq 24 \text{ pmol/l}$

of 18 patients with primary hyperparathyroidism: serum  $1,25(\text{OH})_2 \text{D}$  median; interquartile range; range = 69; 42-124; 31-240 pmol/l (primary hyperparathyroidism) vs 53; 37-82; 24-170 pmol/l (malignancy).

Serum concentrations of  $1,25(\text{OH})_2 \text{D}$  in patients with hypercalcaemia of malignancy did not correlate significantly with levels of precursor  $25(\text{OH}) \text{D}$  ( $r = 0.14$ , NS), or with serum calcium ( $r = 0.19$ , NS) or creatinine levels ( $r = -0.23$ , NS).

The renal tubular threshold for phosphate reabsorption ( $\text{TmPO}_4$ ) was significantly depressed in hypercalcaemic when compared with normocalcaemic patients with malignancy ( $p < 0.001$ ) (figure 5.2). There was no significant correlation between serum inorganic phosphate levels or  $\text{TmPO}_4$  levels and  $1,25(\text{OH})_2 \text{D}$  levels in hypercalcaemic patients with malignancy ( $r = 0.21$ , NS;  $r = 0.18$ , NS, respectively).

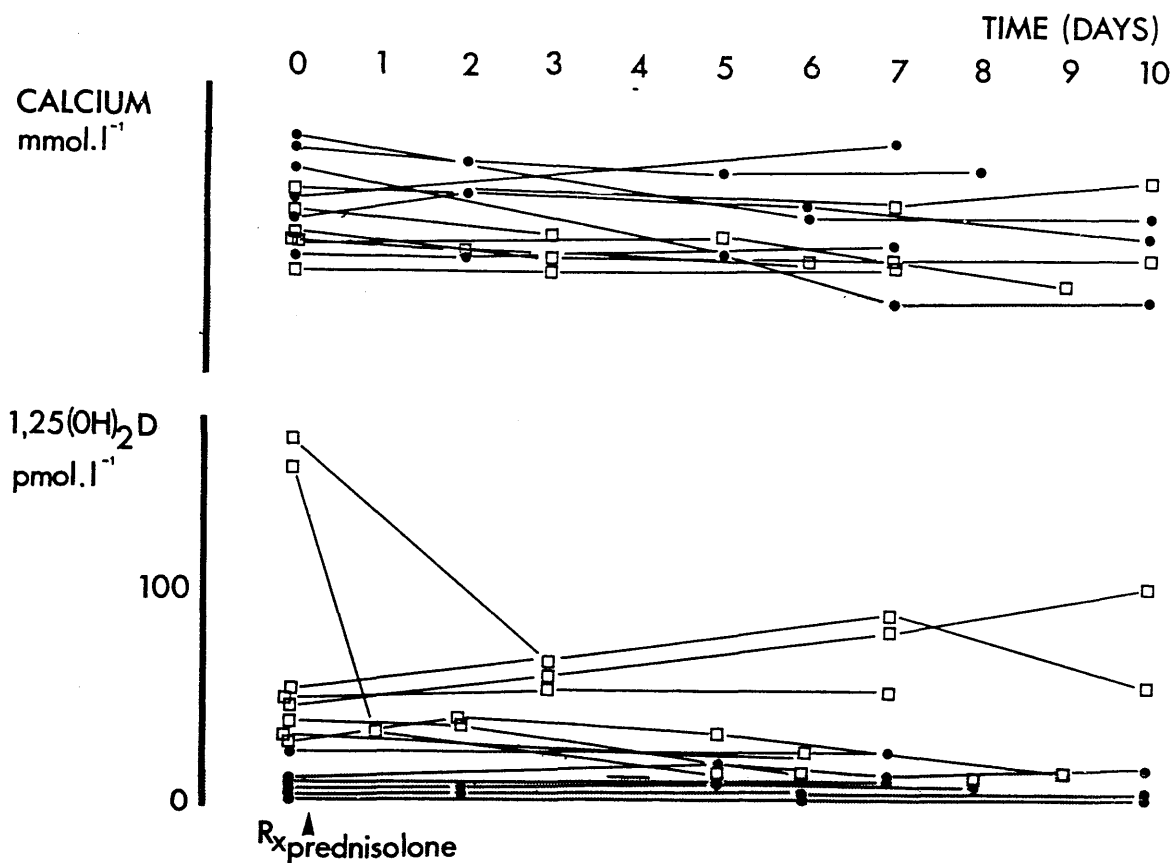
Data on the renal tubular reabsorption of calcium was available in 35 patients with hypercalcaemia who had been rehydrated with intravenous saline, using the protocol described in chapter 4.1 (figure 5.3). In the majority, renal tubular reabsorption of calcium was elevated, consistent with a PTH-like humoral effect on the kidney. It should be noted however, that  $1,25(\text{OH})_2 \text{D}$  levels were frequently undetectable in patients who had increased renal tubular calcium reabsorption and vice versa.

Serum calcium and  $1,25(\text{OH})_2 \text{D}$  levels were followed in 14 patients during therapy with corticosteroids (figure 5.4). Concentrations of  $1,25(\text{OH})_2 \text{D}$  were low or undetectable at the outset in 6 cases and did not change substantially during treatment. In 2 patients, an initially raised level of  $1,25(\text{OH})_2$  fell during treatment, whereas in one case

TABLE 5.3

RELATION BETWEEN TUMOUR TYPE AND 1,25(OH)<sub>2</sub>D  
LEVELS IN HYPERCALCAEMIC PATIENTS

<u>TUMOUR TYPE</u>	<u>HYPERCALCAEMIC PATIENTS</u>		<u>PROPORTION OF</u> <u>PATIENTS WITH</u> <u>1,25(OH)<sub>2</sub>D ≥ 24pmol/l</u>
	<u>WITH 1,25(OH)<sub>2</sub>D</u> <u>&lt; 24pmol/l</u>	<u>WITH 1,25(OH)<sub>2</sub>D</u> <u>≥ 24pmol/l</u>	
BRONCHUS	6	14	30%
BREAST	2	5	28%
GENITOURINARY	3	6	33%
NASOPHARYNX	3	1	75%
CHOLANGIOCARCINOMA	3	0	100%
OTHERS	2	1	66%



**FIGURE 5.4**

Response of serum calcium and 1,25(OH)<sub>2</sub>D levels in patients with malignant hypercalcaemia who were treated with prednisolone.

□ - initial 1,25(OH)<sub>2</sub>D ≥ 24pmol/l

● - initial 1,25(OH)<sub>2</sub>D < 24pmol/l



the  $1,25(\text{OH})_2 \text{D}$  level rose. In the remaining 5 cases, detectable  $1,25(\text{OH})_2 \text{D}$  levels were observed initially but remained substantially unchanged throughout treatment. Although serum calcium values fell on treatment in both patients who had raised levels of  $1,25(\text{OH})_2 \text{D}$  initially, serum calcium also fell in others who had undetectable levels of  $1,25(\text{OH})_2 \text{D}$ . Thus a raised  $1,25(\text{OH})_2 \text{D}$  level did not accurately predict which patients would respond to corticosteroid therapy.

The finding of a detectable  $1,25(\text{OH})_2 \text{D}$  level was not confined to any particular tumour type (table 5.3).

#### 5.1.4 DISCUSSION

In malignancy-associated hypercalcaemia, endogenous parathyroid hormone secretion is suppressed (93,130,131,132). Since PTH is the principal factor which stimulates  $1,25(\text{OH})_2 \text{D}$  synthesis in renal cells (80,81,157,284), one would expect  $1,25(\text{OH})_2 \text{D}$  levels to be low in malignancy-associated hypercalcaemia. Indeed, in a previous study, Stewart et al (93) drew attention to the fact that  $1,25(\text{OH})_2 \text{D}$  levels were generally depressed in malignant hypercalcaemia when compared to primary hyperparathyroidism. In concurrence with these data, circulating levels of  $1,25(\text{OH})_2 \text{D}$  in this study were undetectable in many patients with malignancy associated hypercalcaemia. However, in a substantial proportion of cases,  $1,25(\text{OH})_2 \text{D}$  levels were "inappropriately" detectable and in three cases raised.

The reason for continued synthesis of  $1,25(\text{OH})_2 \text{D}$  in this situation is unclear; while hypophosphataemia may directly stimulate renal  $1-\alpha$ -hydroxylase activity in the absence of PTH (285), this is an

unlikely explanation for the current findings as serum phosphate levels did not correlate with  $1,25(\text{OH})_2 \text{D}$  levels. Although it is possible that intracellular phosphate may have played a role in modulating the levels of  $1,25(\text{OH})_2 \text{D}$  it was not possible to measure intracellular phosphate concentrations in this study. Other hormones such as prolactin, oestrogen, cortisol, androgens, insulin, calcitonin and growth hormone have all been shown to contribute to the regulation of  $1,25(\text{OH})_2 \text{D}$  synthesis in some circumstances (286,287). However, these hormones are unlikely to stimulate significant production of  $1,25(\text{OH})_2 \text{D}$  in the absence of PTH (285). Certainly, pharmacological doses of corticosteroids did not alter  $1,25(\text{OH})_2 \text{D}$  levels substantially in this study and there was no significant correlation between calcitonin levels and  $1,25(\text{OH})_2 \text{D}$  levels.

In hypoparathyroidism, the low levels of  $1,25(\text{OH})_2 \text{D}$  which are observed correlate with the levels of precursor  $25(\text{OH}) \text{D}$  (288). No such correlation was observed here, however, even though plasma levels of PTH were invariably undetectable in patients with malignant hypercalcaemia.

Although documented, ectopic production of PTH is a rare cause of hypercalcaemia in malignancy (90). However, in many patients, a humoral factor is released, which mimics the effects of PTH on renal tubular phosphate reabsorption (6,7,8,9,93,259), nephrogenous cyclic AMP production (93) and renal tubular calcium reabsorption (Chapter 4). The "inappropriately" detectable or raised levels of  $1,25(\text{OH})_2 \text{D}$  observed in some hypercalcaemic cancer patients in this study suggests that the PTH-like effect of the putative humoral mediator may, in some cases, extend to stimulation of renal  $1-\alpha$ -hydroxylase activity.

Our interpretation of the significance of detectable  $1,25(\text{OH})_2 \text{D}$  levels in this situation differs from that of Stewart et al (93), who concluded that the humoral mediator involved in malignancy-associated hypercalcaemia differed from PTH in failing to stimulate renal  $1-\alpha$ -hydroxylase activity. However, their results clearly show that in a proportion of cases,  $1,25(\text{OH})_2 \text{D}$  levels were detectable, thus indicating a possible PTH-like effect on  $1,25(\text{OH})_2 \text{D}$  synthesis. In a previous study, Coombes et al (86) noted that the intestinal absorption of calcium was generally depressed in both hypercalcaemic and normocalcaemic patients with malignant disease. Nonetheless, intestinal calcium absorption was raised in at least one of Coombes' hypercalcaemic patients. Although serum  $1,25(\text{OH})_2 \text{D}$  levels were not measured by Coombes et al, this finding would concur with the current observation that  $1,25(\text{OH})_2 \text{D}$  levels are raised in only a small proportion of patients with malignancy-associated hypercalcaemia.

It has been considered that in clinical practice, suppressed levels of  $1,25(\text{OH})_2 \text{D}$  may provide a means by which the hypercalcaemia of malignancy could be distinguished from primary hyperparathyroidism (5,93). While this appears to hold true in many cases, the current data suggest that, in a proportion of cases,  $1,25(\text{OH})_2 \text{D}$  levels may be raised to within the hyperparathyroid range. Thus it is evident that assessment of  $1,25(\text{OH})_2 \text{D}$  status would be of limited value in the differential diagnosis of these conditions.

Although it is clear that the kidney is the main site of synthesis for  $1,25(\text{OH})_2$ , cell lines derived from bone (289), malignant melanomas (290) and malignant lymphomas (91) have demonstrable activities of the 25 hydroxy-vitamin-D  $1-\alpha$ -hydroxylase enzyme in

vitro. Moreover, low levels of  $1,25(\text{OH})_2 \text{D}$  have been measured in the serum of anephric patients with sarcoidosis, suggesting that granulomatous tissue may also be a site of  $1,25(\text{OH})_2 \text{D}$  synthesis (291,292). From these data, it is apparent that the detectable levels of  $1,25(\text{OH})_2$  observed in this study could have derived from tissues other than the kidney.

While the source of the  $1,25(\text{OH})_2 \text{D}$  is unclear, the presence of the active vitamin D metabolite in the serum of hypercalcaemic cancer patients could contribute to the pathogenesis of hypercalcaemia, by enhancing bone resorption (79,80), or by increasing intestinal calcium absorption (80,81,82). The latter effect, if confirmed, would be of therapeutic importance, as hypercalcaemia in patients with elevated intestinal calcium absorption may respond to restriction of dietary calcium.

## 5.2 INTESTINAL CALCIUM ABSORPTION AND CIRCULATING 1,25 DIHYDROXY-VITAMIN D LEVELS IN MALIGNANCY ASSOCIATED HYPERCALCAEMIA.

### 5.2.1 INTRODUCTION

In the previous study, attention was drawn to the fact that circulating levels of the active vitamin D metabolite  $1,25(\text{OH})_2 \text{D}$  may be "inappropriately" detectable or raised in some patients with hypercalcaemia due to solid malignant tumours. Previous studies have shown however, that intestinal calcium absorption is generally depressed in cancer patients (86). In primary hyperparathyroidism, where  $1,25(\text{OH})_2 \text{D}$  levels are generally raised, intestinal calcium absorption is elevated (116). In this study circulating levels of  $1,25(\text{OH})_2 \text{D}$  in malignancy-associated hypercalcaemia were related to intestinal calcium absorption.

### 5.2.2 PATIENTS AND METHODS

Sixteen patients with hypercalcaemia of malignancy and sixteen with primary hyperparathyroidism were studied. Patients were selected consecutively on the basis that their clinical condition was stable, they were able to take oral fluids and food, and that they had given informed consent to entering the study.

Patients with malignancy had generally been rehydrated with a minimum of 6 litres of intravenous saline prior to the study. During the study, these patients had stable hypercalcaemia and renal function, but in most cases, continued to receive saline infusions of 2 litres daily to prevent possible dehydration. Patients with primary hyperparathyroidism had stable hypercalcaemia and renal function prior to the study and received oral fluids throughout.

Intestinal calcium absorption in both groups of patients was assessed by means of an oral calcium load test essentially as described by Broadus et al (177). Briefly, this test entails measuring the urinary Ca/Cr ratio in a 2-hour urine specimen obtained after an overnight fast, with collection of a mid-point blood sample. A second 2-hour urine specimen and mid-point blood sample were obtained between 2-4 hours after the oral administration of a calcium load, given as effervescent calcium gluconate tablets dissolved in water (total dose of elemental calcium = 20 mmol). Prior to and during the test, patients were encouraged to drink 200 ml of water per hour to sustain an adequate diuresis.

The presence and extent of metastatic bone disease was assessed by standard radionuclide bone scan examination as described in chapter 3.1.

Statistical methods used were the paired and unpaired Wilcoxon tests and Spearman's rank correlation coefficient.

### 5.2.3 RESULTS

Relevant biochemical data prior to the oral calcium load are shown in Table 5.4. Serum albumin, iPTH and  $1,25(\text{OH})_2 \text{D}$  levels were significantly higher, and fasting Ca/Cr ratio significantly lower in primary hyperparathyroidism when compared with hypercalcaemia of malignancy. While serum calcium values tended to be lower in the hyperparathyroid group, the difference was not statistically significant.

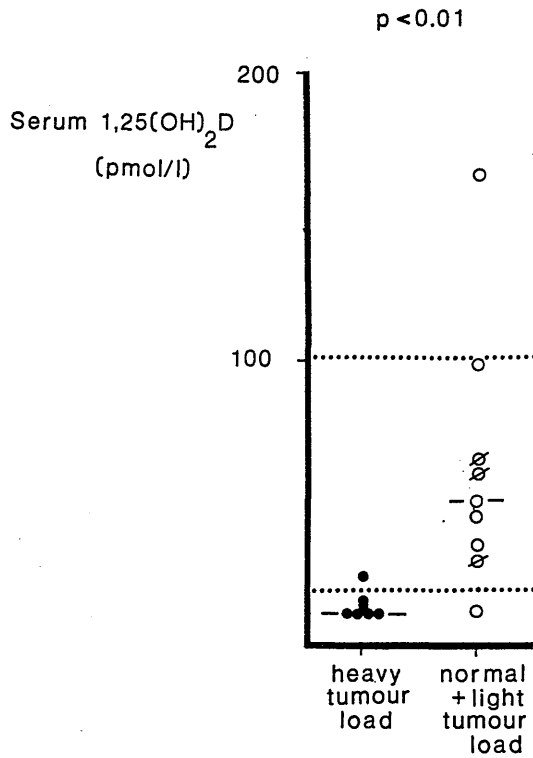
In figure 5.5, serum  $1,25(\text{OH})_2 \text{D}$  levels in patients with malignancy are shown in relation to the extent of metastatic bone

TABLE 5.4

CLINICAL AND BIOCHEMICAL VARIABLES IN PATIENTS WITH  
PRIMARY HYPERPARATHYROIDISM AND HYPERCALCAEMIA OF MALIGNANCY

	<u>SERUM CALCIUM (mmol/L)</u>	<u>SERUM PHOSPHATE (mmol/L)</u>	<u>SERUM CREATININE (µmol/L)</u>	<u>SERUM ALBUMIN (mmol/L)</u>	<u>SERUM PTH (pg/L)</u>	<u>SERUM 1,25(OH)<sub>2</sub>D (pmol/L)</u>	<u>TmPO<sub>4</sub> (mmol/L GF)</u>	<u>URINE Ca/Cr (mmol)</u>	<u>AGE (years)</u>
<u>MALIGNANT HYPERCALCAEMIA (n=16)</u>	3.02 (2.60-3.40)	0.92 (0.55-1.80)	87 (50-150)	31.8 (22-42)	109 (UD-850)	39 (UD-162)	0.70 (0.30-2.10)	1.33 (0.11-2.74)	57 (42-78)
<u>PRIMARY HYPERPARATHYROIDISM (n=16)</u>	2.88 (2.60-3.40)	0.90 (0.70-1.45)	95 (55-260)	40.6 (34-46)	775 (340-3000)	85 (UD-226)	0.68 (0.30-0.95)	0.67 (0.22-1.16)	57 (18-75)
<u>SIGNIFICANCE (p-value)</u>	>0.05	>0.05	>0.05	<0.001	<0.001	<0.001	>0.05	<0.01	>0.05

values are median  
(range)



**FIGURE 5.5**

Serum 1,25(OH)<sub>2</sub> D levels in patients with hypercalcaemia of malignancy. Dotted lines indicate normal range. Bars are medians.

● - Heavy skeletal tumour load.

○ - Light skeletal tumour load.

○ - No evidence of bone metastases.

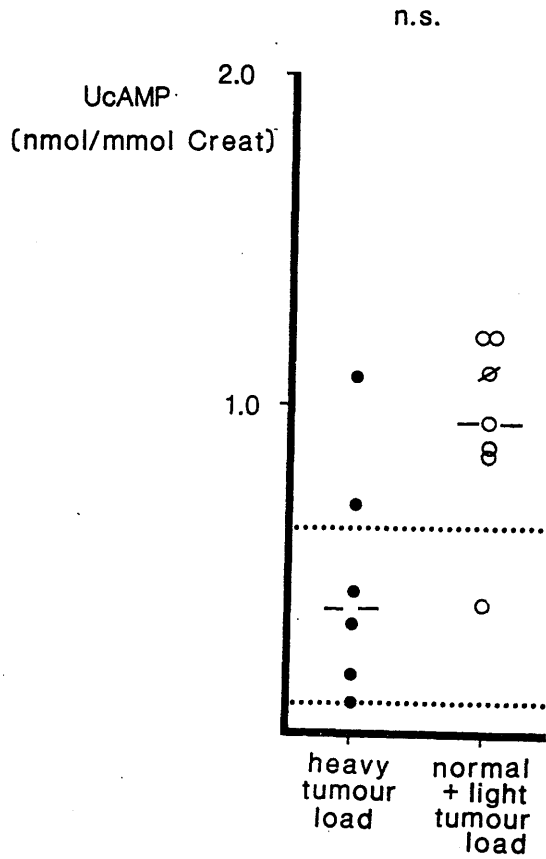


disease;  $1,25(\text{OH})_2 \text{D}$  levels were significantly lower in patients with extensive bone involvement, when compared with those who had few or no metastases.

In figure 5.6, a similar relationship between urinary cyclic AMP levels and metastatic bone disease is shown. Although UcAMP levels tended to be higher in patients with few or no bone metastases, the difference between the groups was not statistically significant. Nonetheless,  $1,25(\text{OH})_2 \text{D}$  levels and UcAMP levels were significantly correlated in patients with hypercalcaemia of malignancy overall ( $r = 0.60$ ,  $p < 0.05$ ). While  $1,25(\text{OH})_2 \text{D}$  levels correlated significantly with  $\text{TmPO}_4$  values in patients with primary hyperparathyroidism ( $r = 0.68$ ,  $p < 0.02$ ), there was no significant correlation between these variables in hypercalcaemia of malignancy. Serum  $1,25(\text{OH})_2 \text{D}$  levels failed to correlate with serum phosphate in either group of patients.

The response of serum calcium values to the oral calcium load is shown in figure 5.7. In hyperparathyroidism, serum calcium values rose in all but one patient, who had chronic renal failure (serum creatinine =  $260 \text{ } \mu\text{mol/l}$ ), nephrocalcinosis and an undetectable  $1,25(\text{OH})_2 \text{D}$  value. The rise was statistically significant in the hyperparathyroid group. In malignancy associated hypercalcaemia, serum calcium values did not change significantly. The mean (SEM) rise in serum calcium was significantly greater in patients with hyperparathyroidism ( $+0.19 (0.03) \text{ mmol/l}$ ) when compared with malignant hypercalcaemia ( $+0.03 (0.02) \text{ mmol/l}$ ) ( $p < 0.01$ ).

The response of urinary Ca/Cr values was similar; in primary hyperparathyroidism, Ca/Cr rose significantly from (mean (SEM))  $0.67 (0.07) \text{ mmol/l}$  (basal) to  $0.97 (0.10) \text{ mmol/l}$  (post calcium load)

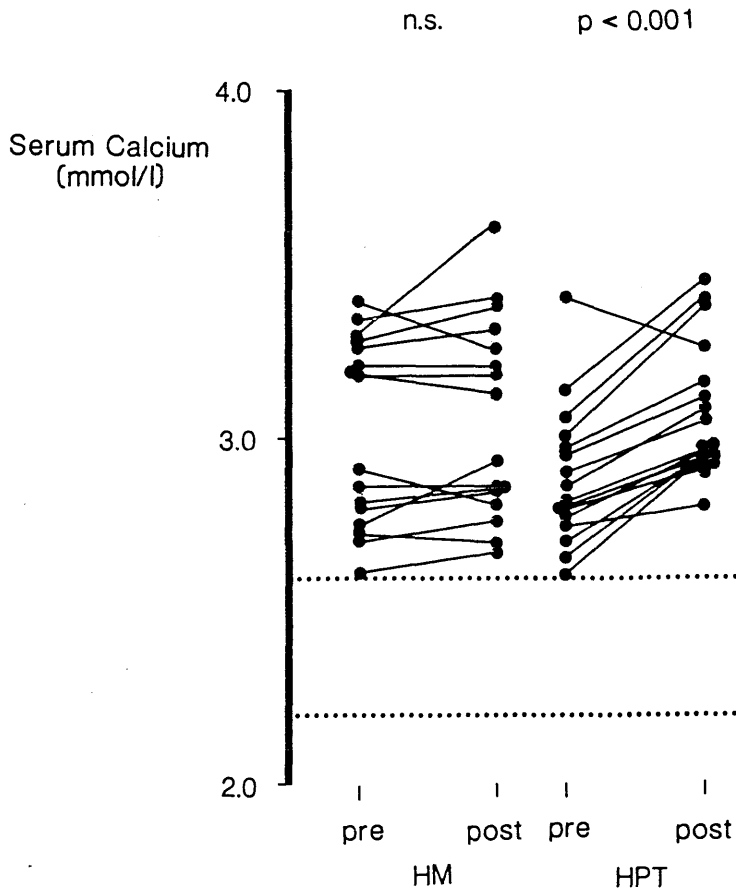


**FIGURE 5.6**

Urinary cyclic AMP excretion in patients with malignancy, expressed as a molar ratio of cyclic AMP to creatinine (UcAMP).

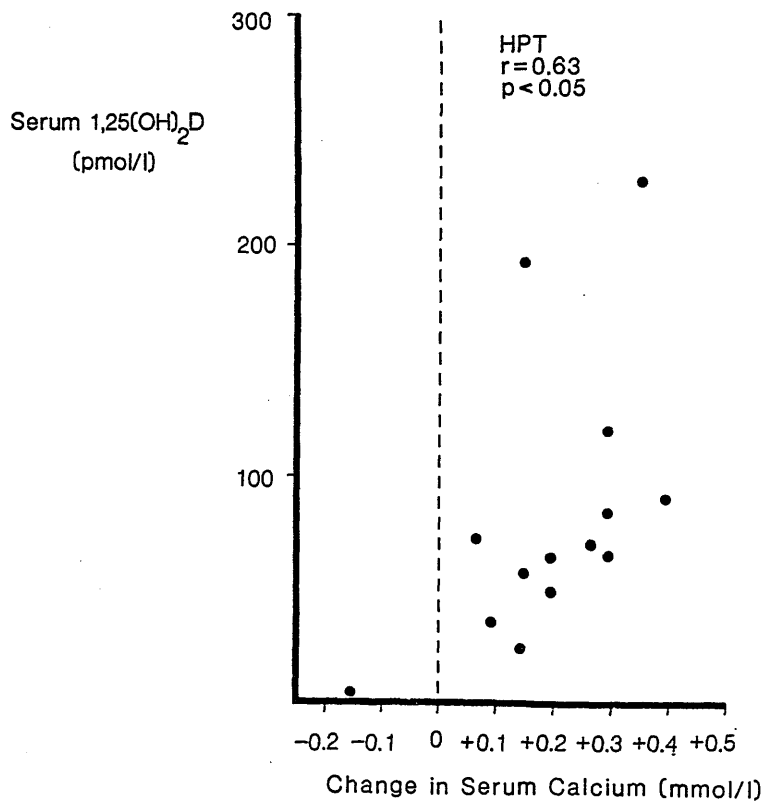
Dotted lines indicate normal range. Bars are medians.

- - Heavy skeletal tumour load
- Ø - Light skeletal tumour load
- - No evidence of bone metastases.



**FIGURE 5.7**

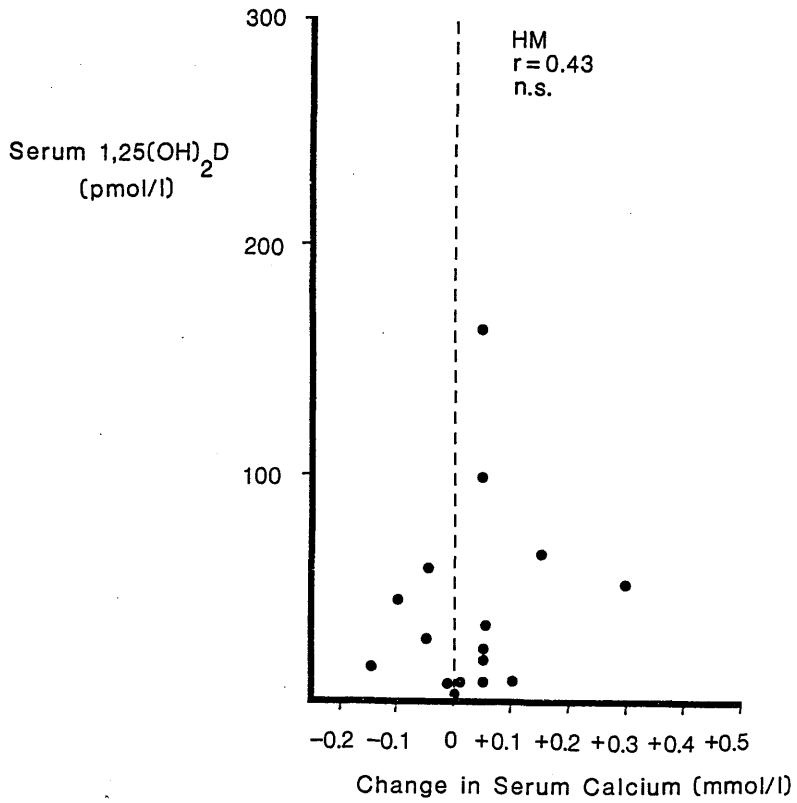
Serum adjusted calcium values before (PRE) and after (POST) oral calcium load in hypercalcaemia of malignancy (HM) and primary hyperparathyroidism (HPT). Dotted lines indicate normal range.



**FIGURE 5.8**

Relation between change in serum calcium values after the oral calcium load and serum 1,25(OH)<sub>2</sub> D levels in primary hyperparathyroidism (HPT)\*

\*1,25(OH)<sub>2</sub> D levels were unavailable in 2 patients.



**FIGURE 5.9**

Relation between change in serum calcium values after the oral calcium load and 1,25(OH)<sub>2</sub>D levels in hypercalcaemia of malignancy (HM).

TABLE 5.5

CLINICAL FEATURES IN RELATION TO TUMOUR TYPE AND EXTENT OF  
METASTATIC BONE DISEASE IN HYPERCALCAEMIA OF MALIGNANCY

<u>TUMOUR TYPE</u>	<u>BONE METASTASES</u>	<u>SERUM PTH</u>	<u>SERUM 1,25 (OH)<sub>2</sub>D</u>	<u>URINE cAMP/CREATININE</u>	<u>TmPO<sub>4</sub></u>	<u>RENAL TUBULAR CONTRIBUTION TO HYPERCALCAEMIA*</u>
		(ng/l)	(pmol/l)	(nmol/nmol)	(nmol/lGF)	(nmol/l)
SQUAMOUS	+	UD	27	1.10	0.30	+0.39
SQUAMOUS	o	UD	97	1.22	0.70	+0.28
SQUAMOUS	o	UD	50	1.21	0.70	+0.69
SQUAMOUS	+	250	62	ND	0.55	-0.01
SQUAMOUS	o	UD	UD	0.81	0.60	+0.01
SQUAMOUS	o	UD	162	0.90	0.70	+0.52
SQUAMOUS	+	850	58	ND	0.60	+0.35
HEPATOMA	o	UD	33	0.80	0.60	+1.09
A.C.U.P	o	UD	45	0.45	0.60	-0.03
BREAST	++	UD	23	0.35	0.95	-0.09
BREAST	++	UD	UD	1.10	0.90	-0.27
BREAST	++	UD	UD	0.10	0.30	-0.05
BREAST	++	260	UD	ND	0.50	+0.44
MYELOMA	++	390	16	0.19	0.40	+0.49
A.C.U.P	++	UD	UD	0.67	2.10	-0.02
SQUAMOUS	++	UD	18	0.40	0.60	-0.13

o - no evidence of bone metastases

+ - light skeletal tumour load

++ - heavy skeletal tumour load

ND - not measured

UD - undetectable

\* - calculated by the method described  
in table 2.2.

A.C.U.P = adenocarcinoma unknown primary  
Seven squamous carcinomas were of bronchial  
origin, one squamous carcinoma of prostate.

( $p < 0.005$ ). In malignant hypercalcaemia there was no significant change; 1.33 (0.19) mmol/l (basal) vs 1.42 (0.17) mmol/l (post calcium load) (individual data points for Ca/Cr values are not shown).

The relation between serum levels of  $1,25(\text{OH})_2 \text{D}$  and the change in serum calcium after the oral calcium load is shown in patients with primary hyperparathyroidism and malignancy in figures 5.8 and 5.9 respectively. In hyperparathyroidism, there was a significant correlation between these variables ( $r = 0.63$ ,  $p < 0.05$ ), but in malignant hypercalcaemia, no significant correlation was observed ( $r = 0.43$ , NS).

Selected biochemical variables reflecting PTH-like activity are related to clinical features such as tumour type and extent of metastatic bone disease in table 5.5. If expression of PTH-like activity is arbitrarily defined as:  $1,25(\text{OH})_2 \text{D} > 20 \text{ pmol/l}$ ;  $\text{UcAMP} > 0.10 \text{ nmol/mmol creatinine}$ ;  $\text{TmPO}_4 > 0.80 \text{ mmol/l GF}$ ; and renal tubular contribution to hypercalcaemia of  $> +0.10 \text{ mmol/l}$ , PTH-like activity was expressed to some extent in all the patients' studied, irrespective of the tumour type or extent of metastatic bone disease. However, those with a heavy skeletal tumour load exhibited significantly fewer PTH-like features, when compared with those with few or no bone metastases; 11/27 (40%) vs 30/34 (88%) ( $\chi^2 = 9.6$   $p < 0.01$ ).

#### 5.2.4 DISCUSSION

These findings confirm that serum levels of  $1,25(\text{OH})_2 \text{D}$  -or a  $1,25(\text{OH})_2 \text{D}$  like substance - are detectable or elevated in a proportion of patients with malignancy - associated hypercalcaemia (93, Chapter 5.1). In this study, serum  $1,25(\text{OH})_2 \text{D}$  levels were

highest in patients with squamous carcinoma, a tumour which is usually associated with a humoral mechanism of hypercalcaemia (5,6,7,8,9,93). Conversely, in patients with breast carcinoma, and other tumours with extensive bone metastases,  $1,25(\text{OH})_2 \text{D}$  levels were generally suppressed, consistent with a "local-osteolytic" mechanism of hypercalcaemia (5). Although levels of precursor  $25(\text{OH}) \text{D}$  were not measured in this study, previous data (Chapter 5.1) indicate that the levels of this metabolite are generally low in cancer associated hypercalcaemia and do not correlate with levels of  $1,25(\text{OH})_2 \text{D}$ . As in the previous study (Chapter 5.1), hypophosphataemia could not be implicated as the cause of the elevation in  $1,25(\text{OH})_2 \text{D}$  levels, as there was no correlation between serum phosphate and  $1,25(\text{OH})_2 \text{D}$  concentrations.

Urinary cyclic AMP levels in our patients with malignant hypercalcaemia were generally elevated, but unlike  $1,25(\text{OH})_2 \text{D}$  levels were not significantly different in patients with a heavy skeletal tumour load, when compared with those who had few or no metastases. These findings differ from those of Stewart et al (93) who were able to completely separate patients with "humoral" and "local osteolytic" mechanisms of hypercalcaemia by the pattern of nephrogenous cyclic AMP excretion. Although it is possible that calculation of nephrogenous cyclic AMP (NcAMP) in this study may have yielded different results, it was not possible to measure plasma cyclic AMP and hence calculate the NcAMP component in this study. Nonetheless, Rude et al (151), who measure both urinary cyclic AMP and NcAMP in hypercalcaemic and normocalcaemic cancer patients, found that levels of both may be increased in patients with a variety of tumour types, irrespective of the extent of metastatic bone disease.



It was of interest that the detectable or raised levels of  $1,25(\text{OH})_2 \text{D}$  in some cancer patients were not associated with a rise in serum calcium values after an oral calcium load. While the oral calcium load test is a rather crude index of intestinal calcium absorption, serum calcium values did rise significantly in primary hyperparathyroidism as would have been expected (116), and here, the increment in serum calcium correlated significantly with the circulating level of  $1,25(\text{OH})_2 \text{D}$ .

The reason for the poor correlation which was observed between these variables in the malignancy group is unclear. It is possible that the levels of vitamin D binding globulin may have been altered in the patients with malignancy, leading to lower levels of "free"  $1,25(\text{OH})_2 \text{D}$ . This seems unlikely however, as in other conditions associated with hypoalbuminaemia, hepatic cirrhosis for example, total  $1,25(\text{OH})_2 \text{D}$  and vitamin D binding globulin levels are both reduced, leading to normal concentrations of "free"  $1,25(\text{OH})_2 \text{D}$  (293). A further possibility would be that the substance recognised as  $1,25(\text{OH})_2 \text{D}$  in patients with malignancy may have been a different metabolite which was devoid of biological activity such as 19-nor-10-keto-25-hydroxy vitamin D (294). Although there is no evidence to suggest that such a metabolite is produced in vivo it is difficult to exclude this possibility using standard methods of  $1,25(\text{OH})_2 \text{D}$  measurement, as used in this and other studies (93). While Shigeno et al (295) have recently described excessive production of  $1,24(\text{R})$ -hydroxyvitamin D in a hypercalcaemic patient with oat cell carcinoma of the lung, this metabolite is known to be biologically active with respect to intestinal calcium absorption, having a potency similar to that of  $1,25(\text{OH})_2 \text{D}_3$ . Moreover, the vitamin D metabolite present in

the serum of our patients co-eluted with authentic  $1,25(\text{OH})_2 \text{D}$  on standard and reverse phase HPLC; in contrast,  $1,24(\text{R})$ -dihydroxyvitamin D may be distinguished from  $1,25(\text{OH})_2 \text{D}_3$  by these procedures (294).

It is therefore possible that the intestinal effects of the active vitamin D metabolite were impaired in patients with malignant disease, due to "end-organ" resistance to the effects of  $1,25(\text{OH})_2 \text{D}$  at the intestinal level. Indeed, it is recognised that a generalised malabsorptive state may occur in cancer patients with non-gastrointestinal tumours (296). With regard to this possibility, it is relevant to note that, in one patient with hypercalcaemia of malignancy who may have had coexistent hyperparathyroidism (PTH=850ng/l; patient 7, Table 5.5) serum calcium failed to rise after the oral calcium load.

From the current study, it is apparent that hyperabsorption of dietary calcium contributes little to the pathogenesis of cancer-associated hypercalcaemia even when circulating levels of  $1,25(\text{OH})_2 \text{D}$  are raised. However, the coexistence of other PTH-like biochemical features in patients with "inappropriately" detectable or raised  $1,25(\text{OH})_2 \text{D}$  levels, would be in keeping with the action of a humoral mediator with a PTH-like effect on renal  $1-\alpha$ -hydroxylase activity. These observations corroborate those in the rat Leydig cell tumour model of humoral hypercalcaemia where  $1,25(\text{OH})_2 \text{D}$  levels are raised (149). Together they provide further evidence to suggest that in human hypercalcaemia of malignancy, a humoral mediator is released, which is distinct from PTH, but which can interact with the PTH receptor in renal and skeletal tissues (144,145,146).

### 5.3 URINARY CYCLIC ADENOSINE MONOPHOSPHATE EXCRETION IN HYPERCALCAEMIC AND NORMOCALCAEMIC PATIENTS WITH MALIGNANCY: CORRELATION WITH CLINICAL FEATURES AND OTHER ASPECTS OF PTH-LIKE ACTIVITY

#### 5.3.1 INTRODUCTION

Reference has been made to the importance of urinary cyclic AMP excretion as a biochemical "marker" of a PTH-like humoral mechanism of hypercalcaemia in malignancy (93). In the previous study (Chapter 5.2) levels of urinary cyclic AMP excretion were generally raised in patients with malignant hypercalcaemia, with little difference between subgroups of patients who had extensive, when compared with little or no, evidence of metastatic bone disease.

In this study the levels of urinary cyclic AMP excretion have been assessed in a larger group of normocalcaemic and hypercalcaemic patients with malignancy, in relation to other relevant clinical and biochemical variables.

#### 5.3.2 PATIENTS AND METHODS

The study group comprised 47 hypercalcaemic and 12 normocalcaemic patients with malignant disease, who had presented consecutively to Glasgow Royal Infirmary. The hypercalcaemic patients with malignancy were rehydrated with a minimum of 6 litres intravenous 0.9% saline solution prior to the study. During the study, most hypercalcaemic patients continued to receive 2 litres of intravenous saline 0.9% daily to prevent possible dehydration. The urinary cyclic AMP data in these patients were compared with those in "control" groups of 13 patients with primary hyperparathyroidism, and 26 healthy volunteers.

The presence and extent of metastatic bone disease in patients

with malignancy was assessed by standard radionuclide bone scan examination as described previously (chapter 3.1).

Statistical methods used were the Wilcoxon test for unpaired data and Spearman's rank correlation coefficient.

### 5.3.3 RESULTS

In table 5.6, relevant biochemical data are shown in patients with malignant disease, on division into four categories; normocalcaemic (n=12), hypercalcaemic; heavy skeletal tumour load (n=15), hypercalcaemic; light skeletal tumour load (n=9) and hypercalcaemic; no evidence of skeletal metastases (n=21). In the patients with a heavy skeletal tumour load the tumour types were; breast carcinoma-7 (46%), myeloma-3 (20%), and one patient each (6%) with prostatic carcinoma, squamous lung carcinoma, renal carcinoma, oat cell carcinoma of lung and adenocarcinoma, unknown primary. In the patients with a light skeletal tumour load, tumour types were; squamous lung carcinoma-7 (77%) and genitourinary tumours-2 (23%). In those with no evidence of bone metastases, tumour types were; squamous lung carcinoma-12 (57%), genitourinary tumours 6-(28%), primary liver cancers-4 (19%) and adenocarcinoma, unknown primary-1 (4%). Of the normocalcaemic cancer patients, 9 (75%) had breast carcinoma and 3 (25%) had squamous lung carcinoma. Four of the breast carcinoma patients had a heavy skeletal tumour load, two had a light skeletal tumour load and two had no detectable bone metastases. Two of the patients with bronchial carcinoma had a light skeletal tumour load and one had no detectable metastases.

From table 5.6, it can be seen that, in the normocalcaemic

TABLE 5.6

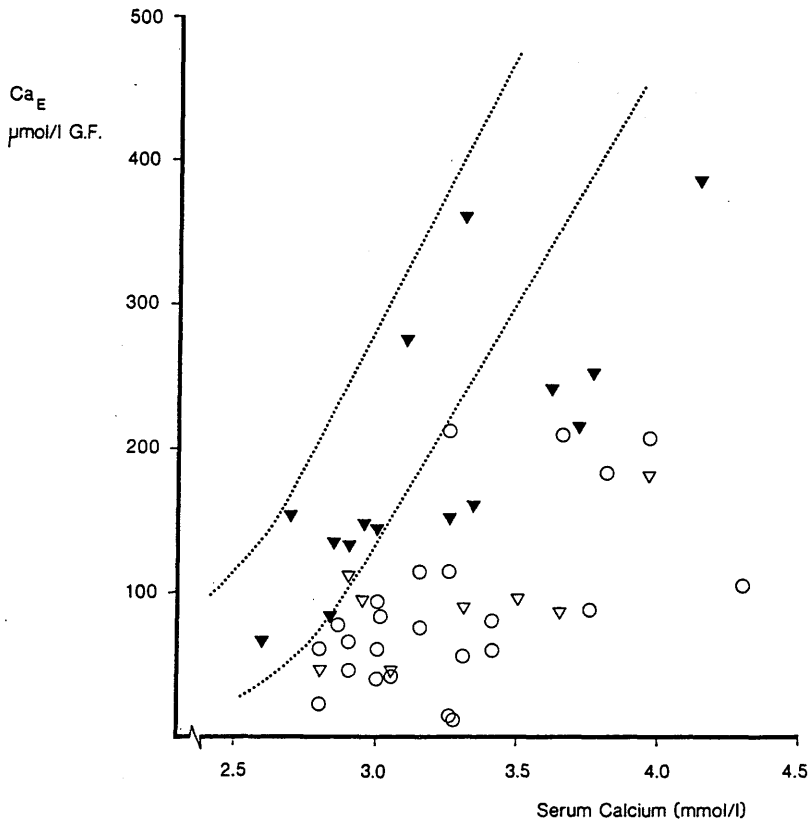
RELEVANT CLINICAL AND BIOCHEMICAL VARIABLES IN PATIENTS WITH MALIGNANT DISEASE

	SERUM CALCIUM (mmol/l)	SERUM PHOSPHATE (mmol/l)	SERUM CREATININE (μmol/l)	SERUM ALBUMIN (g/l)	TmPO <sub>4</sub> (mmol/lGF)	URINE Ca/Cr (mmol)	URINE Ca <sub>E</sub> (mmol)	AGE (years)	MALE/ FEMALE
<u>NORMOCALCAEMIC</u>	2.39 (0.01) <sup>a</sup>	1.05 (0.03)	76 (3.6)	36 (1.1) <sup>a</sup>	0.95 (0.03) <sup>a</sup>	0.29 (0.05) <sup>a</sup>	22 (3.8) <sup>a</sup>	55 (8.1)	2/10
n=12	(2.30-2.50)	(0.85-1.20)	(50-100)	(27-44)	(0.80-1.20)	(0.03-0.75)	(3-56)	(43-67)	
<u>HYPERCALCAEMIC</u>	3.19 (0.11)	1.09 (0.09)	111 (9.0) <sup>d</sup>	30 (1.0)	0.79 (0.11)	1.74 (0.17) <sup>b</sup>	192 (24) <sup>c</sup>	63 (3.3)	6/9
<u>HEAVY TUMOUR</u>	(2.60-4.15)	(0.75-1.80)	(55-190)	(24-41)	(0.30-2.10)	(0.76-3.20)	(64-348)	(40-79)	
<u>LOAD n=15</u>									
<u>HYPERCALCAEMIC</u>	3.26 (0.12)	0.77 (0.04) <sup>e</sup>	92 (14.0)	31 (1.8)	0.51 (0.04)	1.36 (0.27)	89 (13)	62 (4.2)	6/3
<u>LIGHT TUMOUR</u>	(2.80-3.95)	(0.55-0.95)	(45-190)	(25-40)	(0.30-0.75)	(0.47-2.46)	(46-180)	(44-81)	
<u>LOAD n=9</u>									
<u>HYPERCALCAEMIC</u>	3.23 (0.08)	0.85 (0.05) <sup>e</sup>	85 (8.9)	28 (0.9)	0.68 (0.04)	1.09 (0.11)	88 (11)	61 (2.3)	16/5
<u>NO METASTASES</u>	(2.80-4.30)	(0.50-1.55)	(45-240)	(22-41)	(0.35-1.10)	(0.22-2.23)	(13-212)	(43-79)	
n=21									

a p<0.001 - from hypercalcaemic patients  
b p<0.05 - { from hypercalcaemic patients with a  
c p<0.001 - { light tumour load and no metastases  
d p<0.05 - from hypercalcaemic patients with no metastases  
e p<0.05 - { from normocalcaemic patients and hypercalcaemic  
patients with a heavy tumour load.

patients, serum albumin, serum calcium, urinary Ca/Cr and urinary OHP/Cr values were significantly lower than in those with hypercalcaemia. Conversely,  $\text{TmPO}_4$  and serum phosphate values were significantly higher in the normocalcaemic group, when compared to the hypercalcaemic patients without bone metastases. Serum creatinine values were similar in the normocalcaemic group with respect to the hypercalcaemic patients with a light skeletal tumour load or no metastases. Serum creatinine values in the heavy tumour load group were, however, significantly higher than those in the normocalcaemic group and in the patients with a light tumour load or no metastases. In the heavy tumour load group, serum phosphate, urinary Ca/Cr and  $\text{Ca}_E$  values tended to be higher than those in patients with a light tumour load or no metastases, although the differences in Ca/Cr and  $\text{Ca}_E$  were significant, only with respect to the patients who had no metastases, due to the small number of patients having a light skeletal tumour load. Levels of  $\text{TmPO}_4$  were similar in the three groups of patients with hypercalcaemia.

In figure 5.10, the relationship between serum calcium and urinary calcium excretion expressed as  $\text{Ca}_E$  is shown. As in previous studies, most hypercalcaemic patients showed evidence of increased renal tubular reabsorption of calcium. In patients with a heavy skeletal tumour load however, the renal tubular contribution to hypercalcaemia (279), was significantly less than in patients with a light skeletal tumour load or no metastases; (mean (SEM)) increase in renal tubular calcium reabsorption; = +0.11 (0.09) mmol/l; heavy tumour load, n=15) vs +0.50 (0.06) mmol/l; no metastases and light tumour load, n=23) ( $p < 0.002$ ).



**FIGURE 5.10**

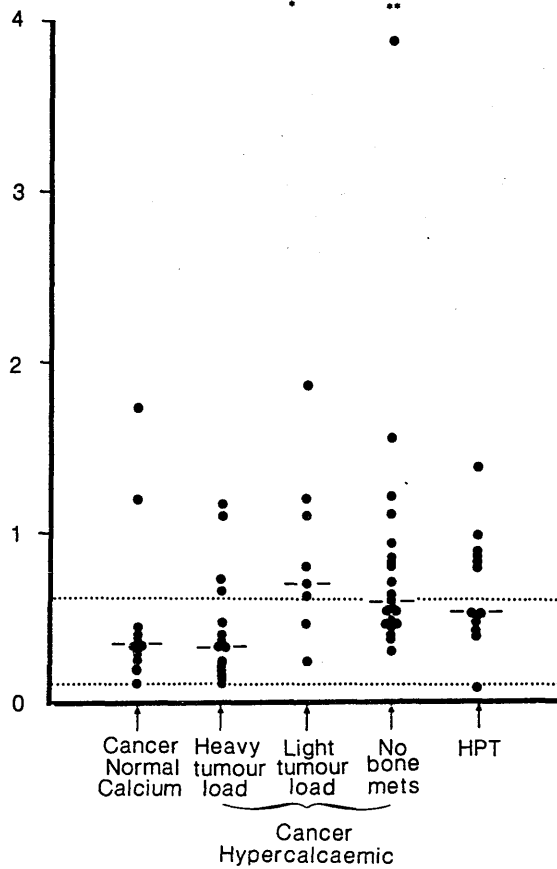
Relation between serum adjusted calcium values and urinary  $Ca_E$  values in patients with malignant hypercalcaemia. Dotted lines indicate normal range defined by Peacock (114).

▼ - Heavy skeletal tumour load.

▽ - Light skeletal tumour load.

○ - No evidence of bone metastases.

UcAMP  
(nmol/mmol creat)



**FIGURE 5.11**

Urinary excretion of cyclic AMP, expressed as a molar ratio, relative to creatinine (UcAMP) in patients with malignancy and primary hyperparathyroidism. Dotted lines indicate reference range, derived from mean  $\pm$  2 SD UcAMP values in 26 healthy volunteers. Bars are medians. \* =  $p < 0.05$  \*\* =  $P < 0.02$ ; from hypercalcaemic patients with a heavy tumour load and normocalcaemic cancer patients.



Urinary excretion of cyclic AMP, expressed as a molar ratio relative to urinary creatinine in the study group is shown in figure 5.11. Urinary cAMP values in the normocalcaemic patients and the hypercalcaemic patients with a heavy skeletal tumour load were significantly lower than those in patients with a light skeletal tumour load ( $p < 0.05$ ) or no metastases ( $p < 0.02$ ). Urinary cAMP values in the patients with primary hyperparathyroidism were significantly higher than in patients with a heavy skeletal tumour load ( $p < 0.05$ ) but were not significantly different from those in patients with a light skeletal tumour load or no evidence of bone metastases.

In patients with hypercalcaemia of malignancy, significant correlations were observed between the following variables; serum calcium vs  $\text{TmPO}_4$  ( $r = -0.56$ ,  $n = 47$ ,  $p < 0.001$ ), serum creatinine vs serum phosphate ( $r = 0.52$ ,  $n = 47$ ,  $p < 0.001$ ), serum phosphate vs UcAMP ( $r = -0.55$ ,  $n = 47$ ,  $p < 0.001$ ).

#### 5.3.4 DISCUSSION

In this study, raised levels of urinary cAMP were frequently noted in patients with malignant hypercalcaemia. Moreover, urinary cAMP levels were significantly higher in the patients with no evidence of bone metastases and a light skeletal tumour load when compared with those who had a heavy skeletal tumour load. These findings are in broad agreement with those of Stewart and his colleagues (93) who, using nephrogenous cAMP (NcAMP) excretion as a biochemical "marker" of humoral hypercalcaemia, found generally elevated NcAMP values in patients with squamous and genitourinary cancers and low levels in patients with haematological tumours and breast carcinoma. In this study, however, the levels of urinary cAMP

did not clearly distinguish between "local osteolytic" and "humoral" mechanisms of hypercalcaemia in malignancy, at least, as judged by bone scan extent of metastatic disease. In contrast, Stewart (93) was able to completely separate "humoral" from "local osteolytic" subgroups, by the pattern of NcAMP excretion. These differences may partly be due to Stewart's use of NcAMP values rather than the ratio of urinary cyclic AMP to creatinine, since the latter fails to take account of an elevation in plasma cAMP levels as a possible explanation for an elevated urinary excretion of cAMP (118). Nonetheless, Rude and his colleagues, who measured both NcAMP and urinary cAMP/creatinine levels in hypercalcaemia of malignancy also failed to obtain a clear distinction between subgroups of patients, on the basis of cAMP measurements (151).

The rather poor specificity of urinary cAMP levels as a marker of humoral hypercalcaemia is exemplified by the current finding (figure 5.11) of grossly elevated UcAMP levels in two normocalcaemic patients with lung cancer and indeed, Kukreja and his colleagues (152) have also found elevated levels of NcAMP in many normocalcaemic patients with squamous lung carcinoma.

The differences the levels of UcAMP excretion in different subgroups of patients with malignancy were accompanied by other biochemical differences; hypercalcaemic patients in the heavy skeletal tumour load group differed from those with few or no bone metastases with respect to other biochemical variables such as urinary Ca/Cr,  $Ca_E$ , serum creatinine and serum phosphate values, suggesting that the mechanisms of hypercalcaemia may have been different in these patients, depending more on the combination of renal glomerular

failure and increased bone resorption, rather than increased renal tubular calcium reabsorption. Nonetheless, renal tubular calcium reabsorption did appear to be elevated and  $\text{TmPO}_4$  values were depressed in many patients with a heavy skeletal tumour load consistent with a "PTH-like" effect on the kidney. These features may have been partly attributable however, to the effects of chronic, severe hypercalcaemia per se (259), since levels of  $\text{TmPO}_4$  correlated inversely with the severity of hypercalcaemia and since the elevation in renal tubular calcium reabsorption was significantly lower in patients with a heavy skeletal tumour load, when compared with those who had few or no metastases.

These observations confirm that, in patients with squamous carcinomas or genitourinary tumours, who characteristically have few, or no metastases, the mechanisms of hypercalcaemia may largely depend on a humoral mechanism, with biochemical evidence of "PTH-like" effects on renal tubular calcium and phosphate handling and cAMP excretion (5,7,8,9,93). In patients with breast carcinoma or myeloma, however, where metastases are usually extensive, humoral effects on renal calcium handling and cAMP excretion are less prominent (93) although reduced levels of renal tubular phosphate reabsorption do occur (259).

From a practical point of view, this study has shown that assessment of UcAMP excretion does not give a clear distinction between primary hyperparathyroidism and malignant hypercalcaemia, nor does it clearly define subgroups within a population of patients with cancer-associated hypercalcaemia. The cause of the elevation in UcAMP levels in some normocalcaemic cancer patients, and the relatively low

levels which were noted in some patients without bone metastases is unclear at present. However these features probably indicate that the humoral mechanisms of hypercalcaemia in malignancy are heterogeneous, and that adenylyl-cyclase stimulating factors may be released by tumours not associated with hypercalcaemia.

CHAPTER 6

RELATIVE CONTRIBUTION OF RENAL TUBULAR AND BONE RESORPTIVE  
MECHANISMS IN HUMORALLY-MEDIATED HYPERCALCAEMIA OF MALIGNANCY

6.1 HUMORAL HYPERCALCAEMIA OF MALIGNANCY: METABOLIC AND HISTOMORPHOMETRIC STUDIES DURING SURGICAL MANAGEMENT OF THE PRIMARY TUMOUR

6.1.1 INTRODUCTION

In patients with "non-metastatic" hypercalcaemia, resection of the primary tumour results in a return of serum calcium levels to normal (7,8,9,138). Although there have been no detailed studies of calcium metabolism in this situation, it has generally been assumed that the fall of serum calcium is due to the removal of the source of a circulating osteoclast-activating substance (5,90,132). While accelerated osteoclastic bone resorption undoubtedly makes a major contribution to the pathogenesis of hypercalcaemia in many cases (139,140,141,142), the data presented previously in this thesis (chapter 4) suggest that a humorally-mediated increase in renal tubular reabsorption of calcium may also play an important pathogenic role.

In the present study the relative contribution of these factors to the pathogenesis of hypercalcaemia were assessed in 10 patients who underwent surgical exploration of tumours associated with humorally-mediated hypercalcaemia.

6.1.2 PATIENTS AND METHODS

Ten patients with solid malignant tumours who had presented with hypercalcaemia were studied. In all cases, clinical radiological and scintigraphic evaluation had shown no definite evidence of tumour metastases, and curative surgical resection of the tumour was

contemplated. In six cases, "curative" surgical resection of the tumour was possible, in a macroscopic sense. In one patient, resection of the tumour was incomplete, leaving a substantial mass of tumour cells in situ. In the remaining three cases, surgical resection of the tumour was precluded by local tumour spread discovered during surgery. Three patients had received previous antihypercalcaemic therapy with the diphosphonate inhibitor of bone resorption aminohydroxypropylidene diphosphonate (APD). In one, (patient 7), APD had been given 25 days before the study. In the others (patients 4 and 5), therapy had been given between 6 and 9 days pre-operatively.

Biochemical analyses were made on blood and true fasting urine samples, obtained after an overnight fast, except on the day of surgery, when timed urine collections with mid-point blood samples were obtained.

Bone histomorphometry was performed on trephine biopsies (8mm diameter), obtained from the iliac crest in eight patients. In two others, bone samples were obtained from sections of rib, which were removed at thoracotomy. In two patients who were treated with APD, (patients 4 and 5), the bone biopsies had been obtained before therapy was commenced. In the other APD-treated patient (patient 7), the bone biopsy was obtained on post-operative day 8, by which time serum calcium values had exceeded those observed prior to APD treatment.

The statistical test used in analysis of data was the paired Wilcoxon test.

TABLE 6.1

CLINICAL AND BIOCHEMICAL DETAILS OF PATIENTS UNDERGOING  
SURGICAL TREATMENT IN HUMORAL HYPERCALCAEMIA OF MALIGNANCY

<u>PATIENT</u>	<u>AGE</u>	<u>SEX</u>	<u>TUMOUR</u>	<u>SERUM CALCIUM (mmol/l)</u>	<u>URINE OHP/Cr (mmol)</u>	<u>PRE-OP</u>	<u>DAY OF SURGERY</u>	<u>POST-OP</u>	<u>DRUG TREATMENT</u>
1	69	F	LUNG	2.95	0.048	SALINE 500ml/ DEXITROSE 1000ml on day -1/ day 0	RINGER LACTATE 1000ml/ BLOOD 750ml	SALINE 500ml/ DEXITROSE 1000ml on days +1/ +2	NIL
2	55	F	LUNG	2.80	0.045	SALINE 1000ml on day -1, BLOOD 1200ml on day -2	RINGER LACTATE 1000ml/ HARTMANN'S 500ml/PLASMA 400ml BLOOD 700ml	SALINE 2000ml on day +1	NIL
3	42	M	KIDNEY	3.55	0.042	SALINE 2000ml on days -4 to -1, BLOOD 800ml on day -2	RINGER LACTATE 1000ml/ DEXITROSE 500ml/ BLOOD 700ml	SALINE 1000ml/ DEXITROSE 2000ml on days +1 to +3	PREDNISOLONE 40mg daily throughout study
4	44	M	LUNG	3.25	0.070	SALINE 2000ml on days -5 to -1	HARTMANN'S 1000ml BLOOD 800ml/ SALINE 500ml	SALINE 1000ml/ DEXITROSE 1000ml on days +1 to +2	APD 15mg daily on pre-op days -7 to -3
5	49	F	LUNG	3.25	0.040	SALINE 2000ml on days -3 to -1	RINGER LACTATE 1300ml/ PLASMA 800ml / BLOOD 1200ml	SALINE 1000ml/ DEXITROSE 1000ml on day +1	APD 15mg daily on pre-op days -10 to -6
6	66	F	KIDNEY	2.95	0.036	BLOOD 1500ml on day -5/ SALINE 2000ml on days -2 to -1	HARTMANN'S 1500ml / BLOOD 800ml	SALINE 1000ml/ DEXITROSE 500ml on days +1 to +2 / PLASMA 800ml day +4	NIL



TABLE 6.1 (CONTINUED)

<u>PATIENT</u>	<u>AGE</u>	<u>SEX</u>	<u>TUMOUR TYPE</u>	<u>SERUM CALCIUM (mmol/l)</u>	<u>URINE OHP/Cr (mmol)</u>	<u>PRE-OP</u>	<u>DAY OF SURGERY</u>	<u>POST-OP</u>	<u>DRUG THERAPY</u>
7	54	F	LUNG	3.10	0.080	SALINE 2000ml on day -1	RINGER LACTATE 500ml/ DEXTROSE 500ml	DEXTROSE 1000ml / SALINE 500ml on day +1	APD 15mg on pre-op days -28 to -25
8	44	M	LIVER*	3.35	0.035	SALINE 1000ml on day -1	SALINE 1000ml/ RINGER LACTATE 100ml	SALINE 4500ml on days +1 to +3	FLUDROCORTISONE 0.1mg and HYDROCORTISONE 30mg daily. HYDROCORTISONE 400mg on day 0 to +3 **
9	59	M	KIDNEY	2.95	0.034	SALINE 1000ml/ DEXTROSE 1000ml on day -1	HARTMANN'S 500ml / SALINE 500ml/ DEXTROSE 500ml/ PLASMA 800ml	DEXTROSE 1000ml / SALINE 1000ml on days +1 to +3	NIL
10	66	M	LUNG	3.15	0.100	SALINE 1000ml/ DEXTROSE 1000ml days -5 to -1/ PLASMA 800ml day -4	SALINE 1300ml/ PLASMA 1000ml/ BLOOD 500ml	SALINE 1000ml/ DEXTROSE 1000ml/ PLASMA 800ml on days +1 to +4	FRUSEMIDE 80mg on day 0 and days +1 to +4 DOPAMINE 5ug/Kg days +2 to +4

\*Primary intrahepatic cholangiocarcinoma

The serum calcium values given refer to those at presentation, after correction of dehydration  
The urinary OHP/Cr values refer to those at the time of bone biopsy.

\*\*As replacement therapy for Addison's disease.

### 6.1.3 RESULTS

#### (a) Clinical details

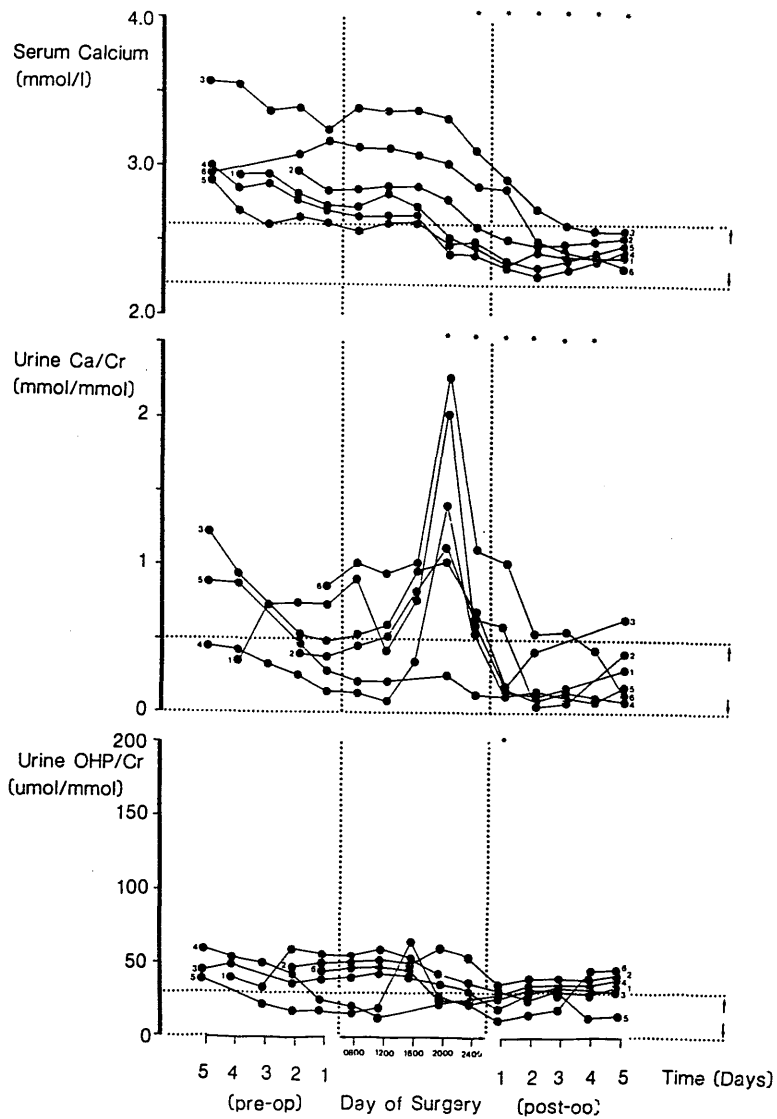
In Table 6.1, relevant clinical details including tumour type, age, and intravenous fluid and drug therapy are shown. In agreement with previous series' of humoral hypercalcaemia (7,93,138), squamous carcinoma of the bronchus was the commonest tumour.

#### (b) Serum calcium

Changes in serum calcium are shown in figures 6.1 and 6.2. All patients were hypercalcaemic prior to surgery, but in 2 cases, (patients 4 and 5), serum calcium values lay at the upper limit of normal on the day of operation. These patients had both received APD therapy between days 6 and 9 preoperatively. In patients 1-6, where the tumour was resected, serum calcium values fell significantly within about 8-10 hours after resection of the tumour. In patients 7-9, where the tumour was not resected, there was no significant change. While serum calcium fell in the patient 10, who had a partial resection, normal levels of serum calcium were not attained.

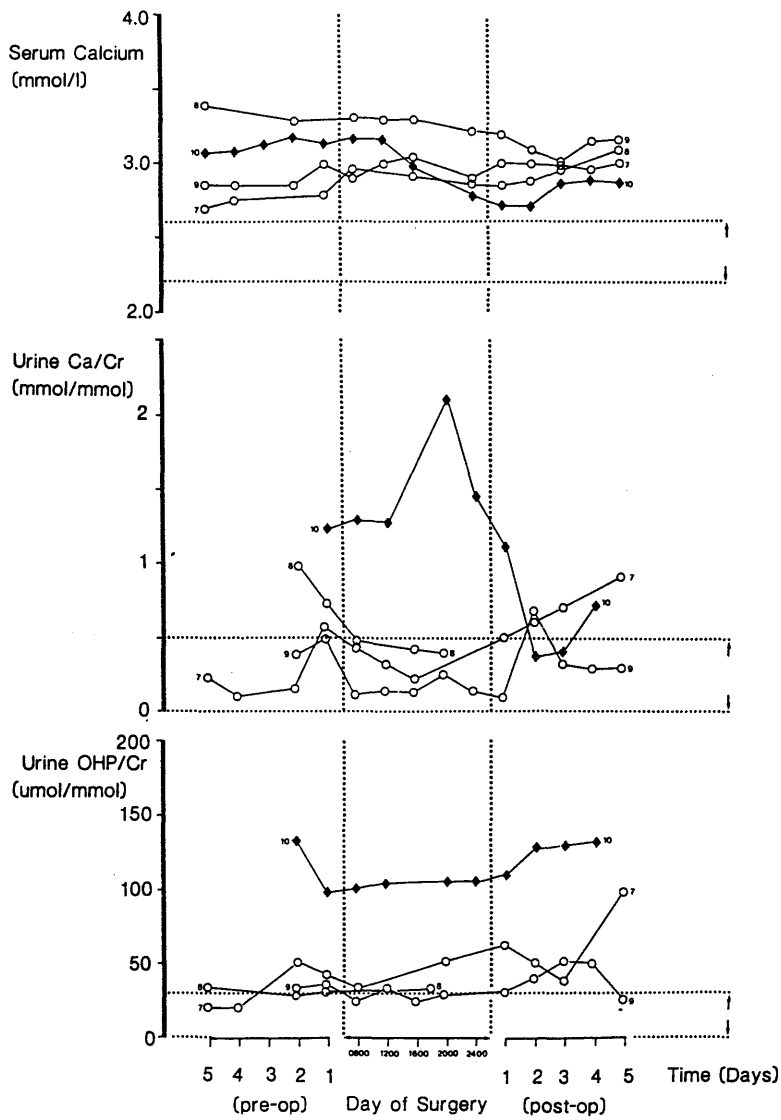
#### (c) Serum phosphate

Changes in serum phosphate are shown in figures 6.3 and 6.4. Serum phosphate values were low or low normal in all cases preoperatively. In patients 1-6, serum phosphate rose significantly from about 4-6 hours after tumour removal, whereas no significant change was observed in patients 7-9, whose tumours were not resected. In patient 10, when the tumour was partially resected, a transient rise in phosphate occurred, although the post-operative level was similar to the pre-operative level.



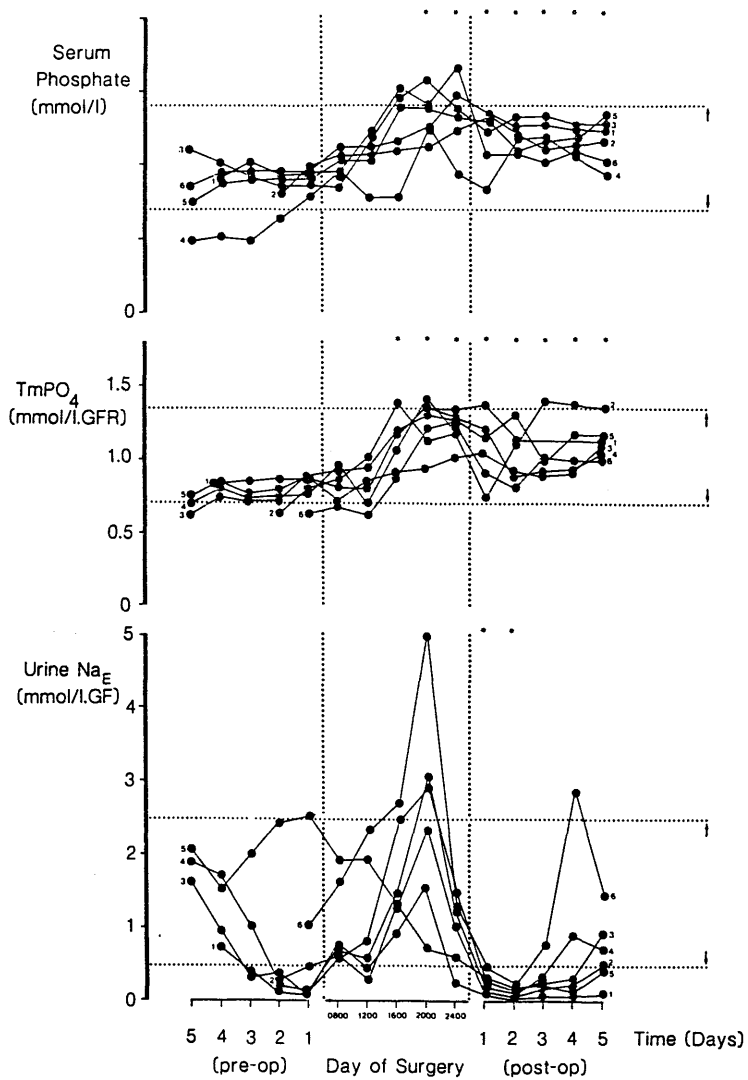
**FIGURE 6.1**

Biochemical response to surgical management in patients (1-6), where the tumour was resected; serum calcium (adjusted for albumin), urinary calcium/creatinine (Ca/Cr), urinary hydroxyproline/creatinine (OHP/Cr). Dotted lines indicate normal ranges. Significant difference from day before surgery indicated as  $*=p<0.05$ .



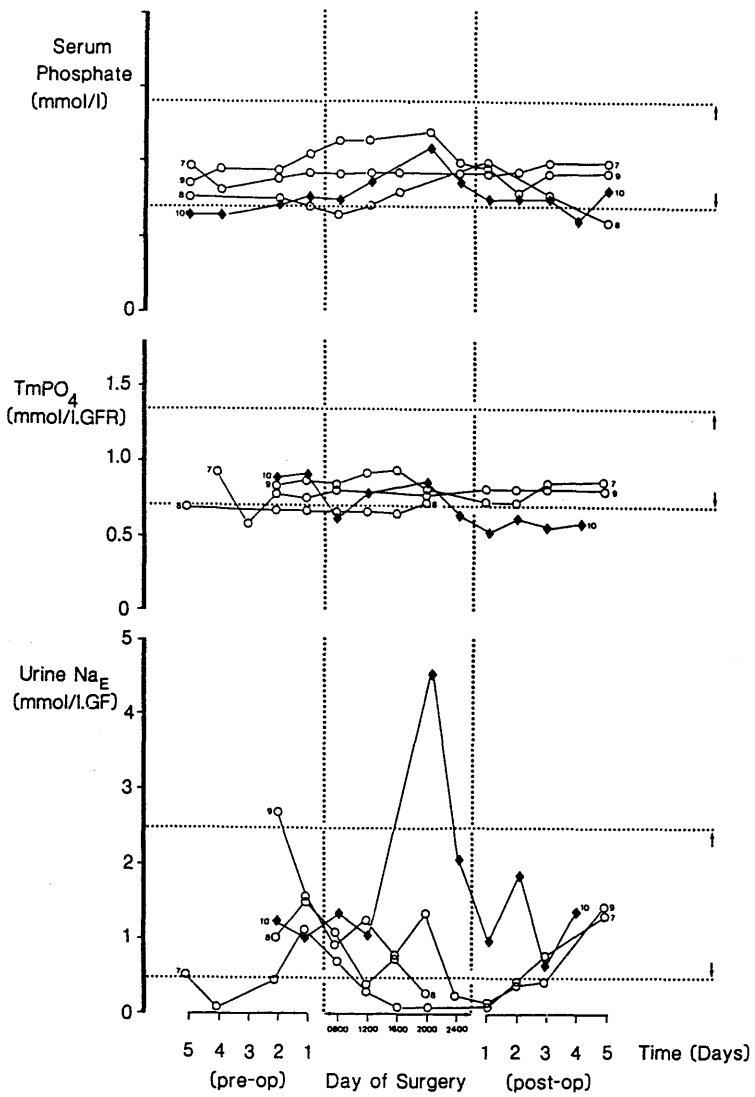
**FIGURE 6.2**

Biochemical response to surgical management in patients where the tumour was not resected (7-9) or partially resected (10); serum calcium (adjusted for albumin), urinary calcium/creatinine (Ca/Cr), urinary hydroxyproline/creatinine (OHP/Cr). Dotted lines indicate normal ranges. Significant difference from preoperative day indicated by  $*=p<0.05$ .



**FIGURE 6.3**

Biochemical response to surgical management in patients (1-6) where the tumour was resected; serum inorganic phosphate, renal tubular threshold for phosphate reabsorption (TmPO<sub>4</sub>) and urinary sodium excretion (Na<sub>E</sub>). Dotted lines indicate normal ranges. Significant difference from day before surgery indicated by \*= $p < 0.05$ .



**FIGURE 6.4**

Biochemical response to surgical management in patients where the tumour was not resected (7-9) or partially resected (10); serum inorganic phosphate, renal tubular threshold for phosphate reabsorption ( $TmPO_4$ ) and urinary sodium excretion ( $Na_E$ ). Dotted lines indicate normal ranges. Significant difference from preoperative day is indicated by  $*=p<0.05$ .

(d) Serum creatinine

Serum creatinine values were within the normal range in all patients pre-operatively; (median (range) = 72.5 (55-110)  $\mu\text{mol/l}$  and did not change significantly after surgery (80 (50-135)  $\mu\text{mol/l}$ ). In two patients who underwent nephrectomy however, creatinine rose from 80 $\mu\text{mol/l}$  to 135 $\mu\text{mol/l}$  (patient 3) and 85 $\mu\text{mol/l}$  to 100 $\mu\text{mol/l}$  (patient 6).

(e) Serum albumin

Serum albumin values tended to be low pre-operatively; median (range) = 29.5 (26-42) g/l and fell slightly after surgery to 28 (24-45) g/l, although the change was not statistically significant (Table 6.2).

(f) Serum bicarbonate and chloride

Serum bicarbonate concentrations were within the normal range in all patients pre-operatively; (median (range) = 28 (25-31) mmol/l and did not change significantly thereafter; 27.5 (23-29) mmol/l. Serum chloride concentrations were reduced in three cases preoperatively (patient 4; 95 mmol/l, patient 8; 94 mmol/l, patient 9; 92 mmol/l), but were normal in the remainder; median (range) = 99 (97-104) mmol/l. and did not change significantly after surgery 99 (95-104) mmol/l.

(g) Plasma parathyroid hormone (iPTH)

Before operation, iPTH levels were undetectable in all cases. In one patient (patient 2) iPTH rose to 490 ng/l on the fourth post operative day. In the remainder, iPTH levels remained undetectable throughout the study period.

(h) Nephrogenous cyclic AMP (NcAMP)

Levels of NcAMP were measured pre-operatively in three cases (patients 2,3,4). Values were elevated at 50, 60 and 42 nmol/l GF respectively.

(i) Urinary calcium/creatinine ratio (Ca/Cr)

Changes in Ca/Cr values are shown in figures 6.1 and 6.2. In patients 1-6, whose tumours' were resected, there was a significant elevation in Ca/Cr values between 4-8 hours after resection of the tumour. A similar elevation in Ca/Cr values was noted in the patient 10, whose tumour was partially resected. There was no significant change in Ca/Cr values in patients 7-9, whose tumours were not resected.

(j) Urinary sodium excretion ( $\text{Na}_E$ )

The response of urinary  $\text{Na}_E$  values are shown in figures 6.3 and 6.4. Changes in urinary  $\text{Na}_E$  values were similar to those of Ca/Cr values, in that there was a substantial rise in  $\text{Na}_E$  between 4-8 hours after resection of the tumour in patients 1-4, 6, and 10. In patient 5 however, urinary  $\text{Na}_E$  values fell on the day of surgery despite the fact that Ca/Cr values in this patient rose slightly. In patients 7-9, there was no consistent pattern of change in  $\text{Na}_E$  values on the day of surgery.

Urinary  $\text{Na}_E$  values were generally low on post-operative days 1 and 2.

(k) Urinary hydroxyproline/ creatinine ratio (OHP/Cr)

Urinary OHP/Cr values are shown in figures 6.1 and 6.2. Urinary OHP/Cr values were normal or moderately elevated in most patients preoperatively, except in patient 10 where OHP/Cr values were markedly



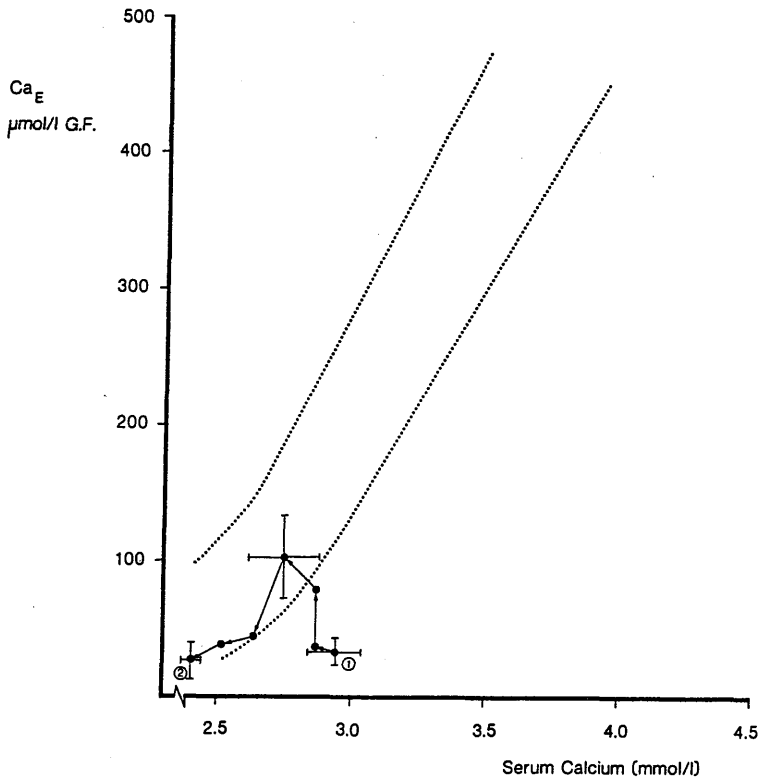
raised. In patients 1-6, OHP/Cr values were transiently reduced on post-operative day 1 from the pre-operative level. In patient 7, OHP/Cr levels rose gradually throughout the study period, reaching a markedly elevated level by the fifth post-operative day. This patient also had increased resorption surfaces on bone biopsy (table 6.2)

(l) Serum calcium vs urinary calcium excretion ( $Ca_E$ )

The response of serum calcium vs urinary  $Ca_E$  values are shown in figures 6.5 and 6.6. In all patients, urinary excretion of calcium was reduced relative to serum calcium preoperatively, indicating that renal tubular calcium reabsorption was increased. Following resection of the tumour in patients 1-6, there was a rightward shift in the serum calcium vs  $Ca_E$  points to within the normal range, indicating that renal tubular calcium reabsorption had fallen. In patients 7-9, where the tumour was not resected, there was no significant movement of the points indicating that renal tubular calcium reabsorption was unchanged. In patient 10, there was a transient leftward shift of the points, indicating that renal tubular reabsorption had fallen during the immediate 4-8 hours after surgery but the points still lay to the right of the expected normal indicating that renal tubular calcium reabsorption had remained elevated above normal.

(m) Renal tubular threshold for phosphate reabsorption ( $TmPO_4$ )

The response of  $TmPO_4$  is shown in figures 6.3 and 6.4. In all patients,  $TmPO_4$  values were low, or low-normal pre-operatively. In patients 1-6 there was a significant elevation on  $TmPO_4$  from within 2 hours of tumour resection and this was sustained thereafter.  $TmPO_4$  values did not change significantly in patients 7-9 where the tumour was not resected, whereas in patient 10,  $TmPO_4$  rose transiently.

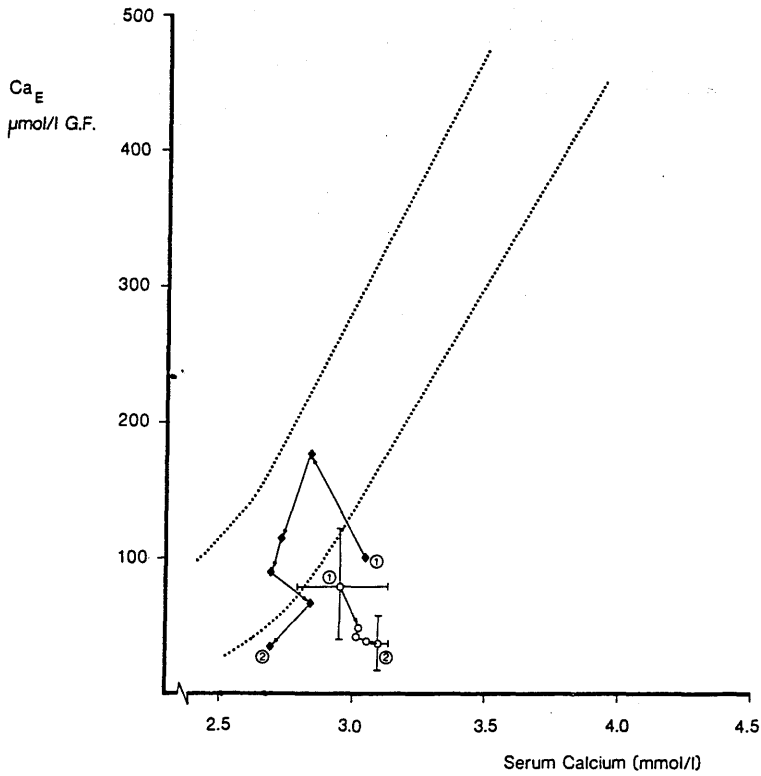


**FIGURE 6.5**

Response of serum adjusted calcium vs urinary  $Ca_E$  values to surgical management, in patients (1-6) where the tumour was resected. Values are mean (SEM). Dotted lines indicate normal range defined by Peacock (114).

① - preoperative value.

② - postoperative value.



**FIGURE 6.6**

Response of serum adjusted calcium vs  $Ca_E$  values to surgical management in patients (7-9) ○ where the tumour was not resected and in patient (10) ◆ where the tumour was partially resected. Values are mean (SEM) in patients (7-9). Dotted lines indicate normal range defined by Peacock (114).

① - preoperative value.

② - postoperative value.

TABLE 6.2

HISTOMORPHOMETRIC DATA IN PATIENTS UNDERGOING SURGICAL TREATMENT  
OF TUMOURS ASSOCIATED WITH HUMORAL HYPERCALCAEMIA OF MALIGNANCY

<u>PATIENT</u>	<u>TOTAL</u> <u>RESORPTION</u> <u>SURFACE</u> (%)	<u>ACTIVE</u> <u>RESORPTION</u> <u>SURFACE</u> (%)	<u>NUMBER OF</u> <u>OSTEOCLASTS</u> <u>PER mm<sup>2</sup></u>	<u>TOTAL</u> <u>OSTEOID</u> <u>SURFACE</u> (%)	<u>ACTIVE</u> <u>OSTEOID</u> <u>SURFACE</u> (%)
1	0.70	0.00	0.03	23.5	1.25
2	-	-	-	-	-
3	3.20	0.40	0.07	12.8	1.20
4	11.7	5.29	0.86	14.5	2.50
5	-	-	-	-	-
6	4.06	1.92	0.37	9.19	0.36
7	8.00	4.00	0.97	21.2	2.40
8	3.20	1.10	0.15	3.20	1.10
9	3.20	0.40	0.07	12.8	1.20
10	12.1	5.10	0.66	26.7	0.39
Reference Range	(< 7.3)	(< 2.4)	(< 0.28)	(< 24)	(< 2.0)

#### (n) Bone Histomorphometry

Histomorphometric data are shown in table 6.2. For technical reasons, histomorphometry could not be performed in two cases; patient 2 (poor staining) and patient 5 (fragmented biopsy). There was no evidence of secondary tumour in any of the specimens. In most cases, indices of bone resorption and bone formation were within the normal range. The only significant abnormal findings were in patients 4, 7 and 10 where total and active resorption surfaces and number of osteoclasts per  $\text{mm}^2$  were increased.

#### 6.1.4 DISCUSSION

In humorally-mediated hypercalcaemia of malignancy, the elevation in serum calcium is attributed to excessive osteoclastic bone resorption, stimulated by an osteoclast activating substance released by tumour cells (5,6,7,8,138). On removal of the primary tumour, serum calcium returns to normal (7,8,9,138), by mechanisms which have hitherto been ill-defined.

If hypercalcaemia were indeed solely due to increased osteoclastic bone resorption, one would expect that the fall in serum calcium would be accompanied by a fall in urinary calcium excretion and hydroxyproline excretion, as the source of the putative osteoclast activating substance was removed. In this study, however, urinary calcium excretion was only slightly elevated prior to surgery, reflecting the importance of increased renal tubular reabsorption of calcium as a factor in the pathogenesis of hypercalcaemia. On removal of the primary tumour in six patients, there was a significant rise in the urinary excretion of calcium, a significant fall in renal tubular reabsorption of calcium and a rise in the renal tubular threshold for

phosphate reabsorption. Subsequently, there was an elevation in serum phosphate and a reduction in serum calcium, both of which were sustained throughout the study. These changes are similar to those observed after parathyroidectomy in patients with primary hyperparathyroidism (297) and suggest that a principal effect of the putative humoral mediator had been PTH-like activity with regard to the renal handling of calcium and phosphate. The changes observed are unlikely to have resulted from the effect of a surgical operation per se, since they did not occur in the patients whose tumours were not resected. It must be acknowledged that blood transfusions which were given to the patients undergoing "curative" resection may have contributed to these changes. However, this effect did not appear to have been of major significance as blood transfusions given to a number of patients pre-operatively had little apparent effect on the biochemical parameters observed.

The rise in urinary sodium excretion which was observed after removal of the tumour is of interest, as elevated renal tubular reabsorption of calcium may occur as the result of sodium depletion (268). Our patients were not dehydrated or sodium depleted on a clinical basis however, and all had received intravenous infusions of saline in the immediate 24 hours before surgery. Moreover, in most cases, urinary  $\text{Na}_E$  values fell within the normal reference range pre-operatively. Although the mechanism of the rise in urinary sodium excretion is unclear, it may have been due to the calciuresis which was also noted (298). The natriuresis is unlikely to have been due to the effects of the intravenous fluids given during surgery, as no rise in  $\text{Na}_E$  values occurred in the patients whose tumours were not resected.

The bone histology and urinary hydroxyproline measurements also support a predominantly renal tubular, rather than bone resorptive mechanism of hypercalcaemia; in the eight patients where histological data was available, bone resorption was normal in four cases and only slightly elevated in the remainder. Although urinary hydroxyproline levels were slightly elevated in some patients preoperatively, there was little change in hydroxyproline after successful surgical resection of the primary tumour, apart from a minor reduction on postoperative day 1.

The biochemical findings in isolation would be insufficient evidence for a predominantly renal tubular action of the humoral mediator causing hypercalcaemia, due to the difficulties which are inherent in the interpretation of data in patients who are undergoing major surgical procedures. However, when taken in conjunction with the bone histomorphometric data, they indicate that hypercalcaemia in these patients was corrected mainly by a fall in the renal tubular calcium threshold, rather than a reduction in bone resorption.

These data differ considerably from those of previous workers, who have considered that humoral hypercalcaemia in malignancy is largely the result of accelerated bone resorption (5,7,8,9,93), but concur with the evidence presented in chapter 4, suggesting that the putative humoral mediator may have an additional site of action on the renal tubule. The absence of increased osteoclastic bone resorption which was observed in some patients is the most surprising finding to emerge from this study and contrasts with the marked increase in osteoclastic bone resorption and depressed osteoblastic activity reported by Stewart and his colleagues (139). In that study, however,

four of the seven bone biopsies were taken from patients who were immobile with advanced malignant disease, and a further three had been obtained post-mortem. Bone samples were also obtained after death in two non-histomorphometric studies, where increased osteoclastic bone resorption was observed (140,141). It should be emphasised however, that bone resorption may be increased in bedridden patients dying from cancer who do not have hypercalcaemia (299), and in general terms, immobilisation may cause marked uncoupling of bone resorption and bone formation (300), sufficient to precipitate severe hypercalcaemic crises in patients with mild hyperparathyroidism who had been confined to bed after fractures (301).

The differences in bone histology between this study and that of Stewart and his colleagues (139) is unlikely to have been due to differences in the nature of the humoral mediators involved, in view of the similarities in terms of tumour type, and biochemical features. The patients in our study were, however, a selected group in whom bone biopsies were obtained ante-mortem, in association with cancers at a less advanced stage than those studied previously (139,140,141,299). A possible explanation would be that in the earlier stages, humoral hypercalcaemia is mediated by a renal tubular mechanism, whereas on progression of the disease, osteoclastic resorption plays a more important role, as the degree of immobility increases.



## 6.2 DIFFERING MECHANISMS OF HYPERCALCAEMIA IN PATIENTS WITH EARLY AND ADVANCED HUMORAL HYPERCALCAEMIA OF MALIGNANCY

### 6.2.1 INTRODUCTION

In the previous study (6.1), it was found that an elevated renal tubular threshold for calcium reabsorption, rather than an elevation in osteoclastic bone resorption was the predominant mechanism of hypercalcaemia in some patients with early malignant tumours. From these observations, it was speculated that the previously noted "uncoupling" of bone resorption and bone formation in humoral hypercalcaemia (139) may have been partly due to the immobilisation of advanced malignant disease.

In this study, histomorphometry and biochemical characteristics were assessed in a larger group of patients with humorally mediated hypercalcaemia, with particular reference to the effects of immobilisation. These data were compared with those in a group of patients with primary hyperparathyroidism, who were matched for the severity of hypercalcaemia.

### 6.2.2 PATIENTS AND METHODS

The study group comprised 15 patients with cancer-associated hypercalcaemia, in whom a humoral aetiology was suspected either because bone metastases were excluded by standard radiological and radionuclide bone scan examination (10 cases), or because the severity of metastatic disease thus demonstrated (<4 lesions) was thought to be insufficient to account for the hypercalcaemia (5 cases). In 8 patients (described in chapter 6.1), bone biopsies were taken prior

to, or during surgical exploration of the primary malignant tumour. In the remaining 7 cases, 5 bone biopsies were obtained ante-mortem, and 2 were obtained post-mortem.

All patients with malignancy were rehydrated with intravenous 0.9% saline solution prior to the study. Three had received previous antihypercalcaemic therapy (2-APD, 1-mithramycin). In all three cases, however, serum calcium values had exceeded those observed before antihypercalcaemic therapy, suggesting that any suppressive effects on bone resorption had disappeared by the time of study. No patient had received previous anti-cancer chemotherapy, or other medication which may have significantly affected calcium homeostasis.

Data from the patients with malignancy were compared with those in a group of patients with primary hyperparathyroidism, who were selected on the basis that they had hypercalcaemia of similar severity. One patient with primary hyperparathyroidism had been bed-bound for some weeks prior to study in another hospital with severe, undiagnosed hypercalcaemia. In this patient, the bone biopsy was obtained immediately prior to emergency neck exploration. The remaining 16 hyperparathyroid patients were normally hydrated on a clinical basis with stable hypercalcaemia and renal function. All were ambulant and attending hospital on a "day patient" basis.

Biochemical analyses were performed on blood and true fasting urine specimens, obtained after an overnight fast.

In all of the patients with hyperparathyroidism and 13 with malignancy, histomorphometry was performed on transiliac anterior iliac crest bone biopsies. In the two remaining patients,

histomorphometry was carried out on a section of rib removed at thoracotomy in one, and on a vertebral body removed at post mortem in the other.

The degree of immobility in patients with malignancy was assessed using a four point scale as follows:

- (1) In patient, completely bed bound.
- (2) In patient, limited mobility in hospital ward.
- (3) Out patient, ambulant, but with restricted activity
- (4) Out patient, fully ambulant, undertaking normal activity.

The stage of tumour advancement at the time of biopsy was assessed simply by recording the interval between the time of death and the time of study.

Statistical methods used were the Wilcoxon test for paired and unpaired samples, and Spearman's rank correlation coefficient.

### 6.2.3 RESULTS

Relevant clinical and biochemical variables in patients with humoral hypercalcaemia of malignancy and primary hyperparathyroidism are compared in table 6.3. Serum albumin and iPTH levels were significantly lower, and urinary hydroxyproline levels significantly higher in the malignant group, although in other respects, the two groups were well matched. One patient with malignancy had a raised iPTH value; 1600ng/l. In this patient, post-mortem examination failed to reveal evidence of a co-incidental parathyroid adenoma. A sample of tumour tissue obtained at post-mortem examination was subsequently found to stain positively for PTH, using immunocytochemical methods, consistent with the rare syndrome of true ectopic PTH production.

TABLE 6.3

CLINICAL AND BIOCHEMICAL DETAILS IN PATIENTS WITH HUMORAL HYPERCALCAEMIA  
OF MALIGNANCY AND PRIMARY HYPERPARATHYROIDISM

	<u>HUMORAL HYPERCALCAEMIA OF MALIGNANCY</u> (n=15)	<u>PRIMARY HYPERPARATHYROIDISM</u> (n=17)	<u>STATISTICAL SIGNIFICANCE</u>
<u>MALE/FEMALE</u>	9/6	5/12	NS
<u>SERUM CALCIUM</u> (mmol/l)	3.31 (0.13) (2.80->4.50)	3.17 (0.11) (2.70->4.50)	NS
<u>SERUM PHOSPHATE</u> (mmol/l)	0.80 (0.02) (0.70-0.95)	0.80 (0.06) (0.90-1.65)	NS
<u>PLASMA iPTH</u> (ng/l)	106 (106) <sup>*</sup> (UD-1600)	1351 (247) (390-3600)	p<0.001
<u>SERUM CREATININE</u> (µmol/l)	92 (5.5) (55-180)	104 (11.8) (55-230)	NS
<u>SERUM ALBUMIN</u> (g/l)	29 (1.5) (23-44)	39 (1.8) (30-47)	p<0.001
<u>URINE Ca/Cr</u> (mmol/mmol)	1.02 (0.17) (0.35-2.40)	0.80 (0.06) (0.34-1.25)	NS
<u>URINE OHP/Cr</u> (µmol/mmol)	55 (6) (28-98)	36 (5) (17-74)	p<0.02
<u>URINE Na<sub>E</sub></u> (mmol/lGF)	2.00 (0.14) (0.90-2.74)	1.70 (0.22) (0.50-3.20)	NS
<u>URINE cAMP</u> (nmol/lGF)	72 (13) (24-230)	53 (8) (8-133)	NS
<u>1mPO<sub>4</sub></u> (mmol/lGF)	0.63 (0.04) (0.45-0.95)	0.56 (0.03) (0.30-0.90)	NS
<u>RENAL TUBULAR CALCIUM REABSORPTION<sup>+</sup></u> (mmol/l)	+0.46 (0.07) (+0.01-+1.02)	+0.40 (0.08) (-0.04-+0.88)	NS

values are mean(SEM)  
(range)

\*one patient with malignancy had a detectable iPTH value (1600ng/l)

NS - no significant difference

+ - calculated by the method described in figure 2.2

Tumour types in patients with malignancy were: squamous carcinoma of the lung 9 (58.5%); squamous carcinoma of gallbladder 1 (6.5%); renal adenocarcinoma 3 (19.5%); cholangiocarcinoma 1 (6.5%); adenocarcinoma, primary unknown 1 (6.5%). In table 6.4, selected biochemical variables from the malignancy group are shown on further division by the clinical circumstances of the patients at the time of study. Serum calcium and creatinine levels were greatly elevated prior to death in two patients where post-mortem biopsies were obtained. Although no urinary measurements were possible immediately prior to death in these patients, data from studies performed at an earlier stage in the patients' illnesses were similar to those in the other patients with malignancy. Indeed, no significant difference in biochemical variables was observed between the subgroups of patients with malignancy, except in 5 patients where serum calcium values fell and serum phosphate values rose significantly after resection of the primary tumour, mainly due to changes in the levels of renal tubular calcium reabsorption and phosphate reabsorption.

Relevant histomorphometric data are shown in figures 6.7 and 6.8, grouped to indicate indices of bone formation and bone resorption. All indices of bone formation (TOS, AOS, AOV), were significantly lower in the malignancy group. In contrast, indices of bone resorption (TRS, ARS, NO), were similar in both. In the malignancy group, histological indices of active bone resorption (ARS, NO) correlated significantly with the calcium "flow" component of hypercalcaemia, as calculated from the biochemical data ( $r=0.65$ ,  $p<0.05$ );  $r=0.62$ ,  $p<0.05$ , respectively). In the hyperparathyroid group, calcium flow did not correlate significantly with resorption indices, but TRS correlated significantly with serum calcium ( $r=0.62$ ,  $p<0.02$ ).

**TABLE 6.4**

**BIOCHEMICAL DATA IN PATIENTS WITH HEMORAL HYPERCALCAEMIA  
OF MALIGNANCY IN RELATION TO STAGE OF TUMOUR PROGRESSION**

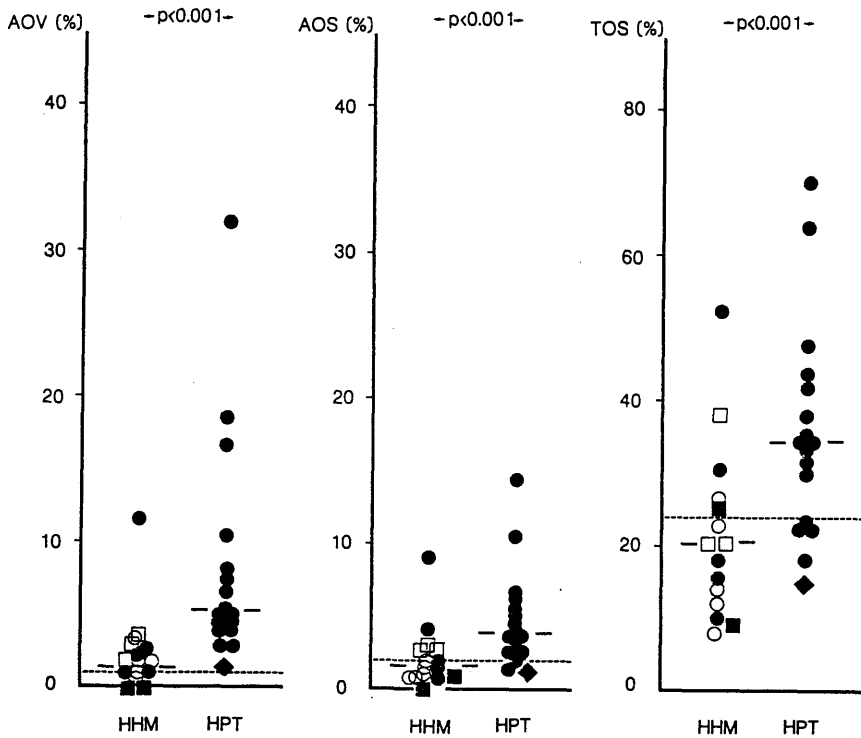
	SURGICALLY TREATED PATIENTS		NON-SURGICALLY TREATED PATIENTS	
	TUMOUR RESECTED PRE-OP	POST-OP	TUMOUR NOT SURGICALLY RESECTABLE	ANTE-MORTEM BONE BIOPSY
<b>NUMBER</b>	5	5	3	5
<b>SERUM CALCIUM</b> (mmol/l)	3.11 (0.08) (2.80-3.50)	2.49 (0.08)* (2.30-2.80)	3.10 (0.14) (2.85-3.35)	3.19 (0.14) (2.90-3.70)
<b>SERUM PHOSPHATE</b> (mmol/l)	0.80 (0.05) (0.70-0.95)	1.04 (0.08)* (0.80-1.15)	0.81 (0.06) (0.75-0.95)	0.78 (0.04) (0.70-0.95)
<b>SERUM CREATININE</b> (µmol/l)	72 (5) (60-85)	90 (14) (55-135)	76 (12) (55-95)	89 (12) (55-120)
<b>URINE cAMP</b> (nmol/lGF)	78 (38) (30-230)	NM	71 (12) (52-96)	54 (15) (24-97)
<b>TiPO<sub>4</sub></b> (mmol/lGF)	0.67 (0.08) (0.45-0.95)	1.01 (0.05) (0.80-1.10)	0.68 (0.06) (0.60-0.80)	0.54 (0.04) (0.48-0.60)
<b>CALCIUM FLOW<sup>a</sup></b> (mmol/l)	+0.20 (0.06) (+0.06-+0.39)	-0.02 (0.05) (-0.16-+0.12)	+0.03 (0.04) (-0.01-+0.11)	+0.25 (0.09) (+0.08-+0.49)
<b>RENAL TUBULAR<sup>a</sup> REABSORPTION</b> (mmol/l)	+0.48 (0.05) (+0.39-+0.66)	-0.03 (0.06)* (+0.22-+0.01)	+0.53 (0.10) (+0.35-+0.71)	+0.42 (0.22) (+0.01-+1.02)
				NM
				NM
				0.87 (0.03) (0.85-0.90)
				172 (7.5) (165-180)
				90 (16) <sup>b</sup> (78-102)
				0.55 (0.14) <sup>b</sup> (0.40-0.70)

values are mean (SEM) and (range)

\* - significant p<0.05 from preoperative data NM - not measured UD - undetectable

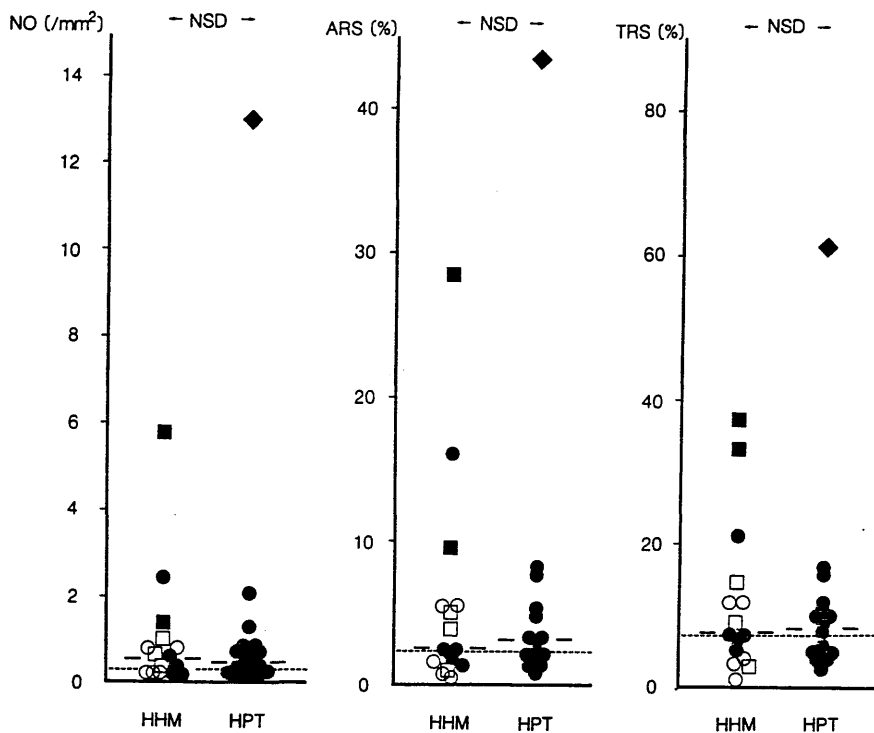
b - values recorded at an earlier stage in the patients' illness.

a - calculated by the method described in figure 2.2



**FIGURE 6.7**

Indices of bone formation in patients with primary hyperparathyroidism (HPT) and humoral hypercalcaemia of malignancy (HHM). AOV = absolute osteoid volume; AOS = active osteoid surface; TOS = total osteoid surface. Dotted lines indicate normal ranges. Bars are medians. In HHM group, symbols denote; surgically treated (tumour resected = ○), (tumour not resected = □); surgery not considered (antemortem biopsy = ●), (postmortem biopsy = ■). In HPT group, symbols denote; (mobile = ●), (immobile = ◆).



**FIGURE 6.8**

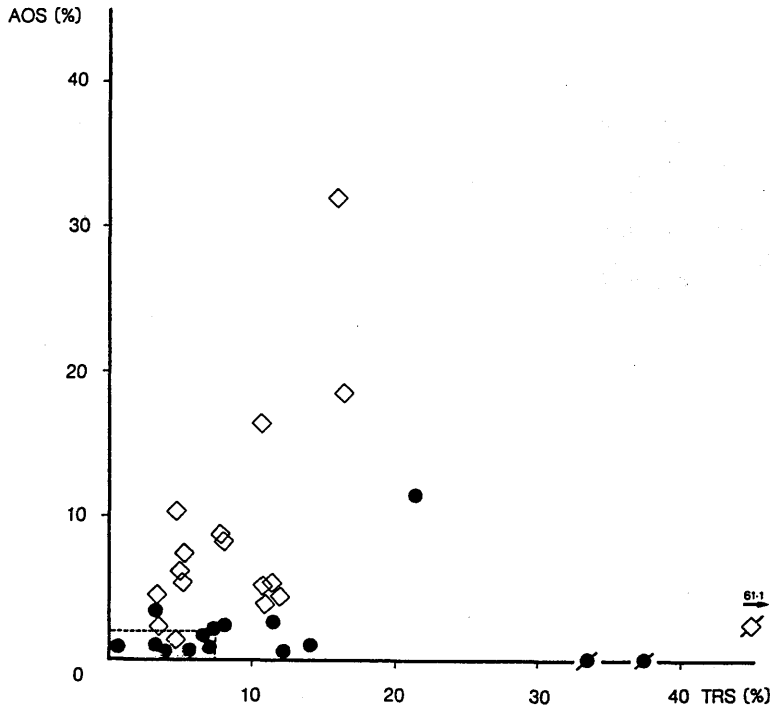
Indices of bone resorption in patients with primary hyperparathyroidism (HPT) and hypercalcaemia of malignancy (HHM). TRS = total resorption surface, ARS = active resorption surface, NO = number of osteoclasts per mm<sup>2</sup>. Dotted lines indicate normal range. Bars are medians. In the HHM group, symbols denote; surgically treated (tumour resected = ○), (tumour not resected = □); surgery not considered (antemortem biopsy = ●), (postmortem biopsy = ■). In the HPT group, symbols denote; (mobile = ●), (immobile = ◆).



The relationship between TRS and AOS in hyperparathyroidism and malignancy is shown in figure 6.9. In both malignancy and hyperparathyroidism, there was no significant correlation between these variables when all patients were considered together. However, when the immobilised patients were excluded, there were positive correlations between AOS and TRS ( $r=0.59$ ,  $p<0.05$ ), TOS and TRS ( $r=0.57$ ,  $p<0.05$ ) in the hyperparathyroid patients, but not in those with hypercalcaemia of malignancy. It is relevant to note that indices of bone resorption and formation were normal in a proportion of patients with both conditions. Conversely, in patients who were immobilised with severe hypercalcaemia, there was marked "uncoupling" of bone resorption and bone formation in both conditions.

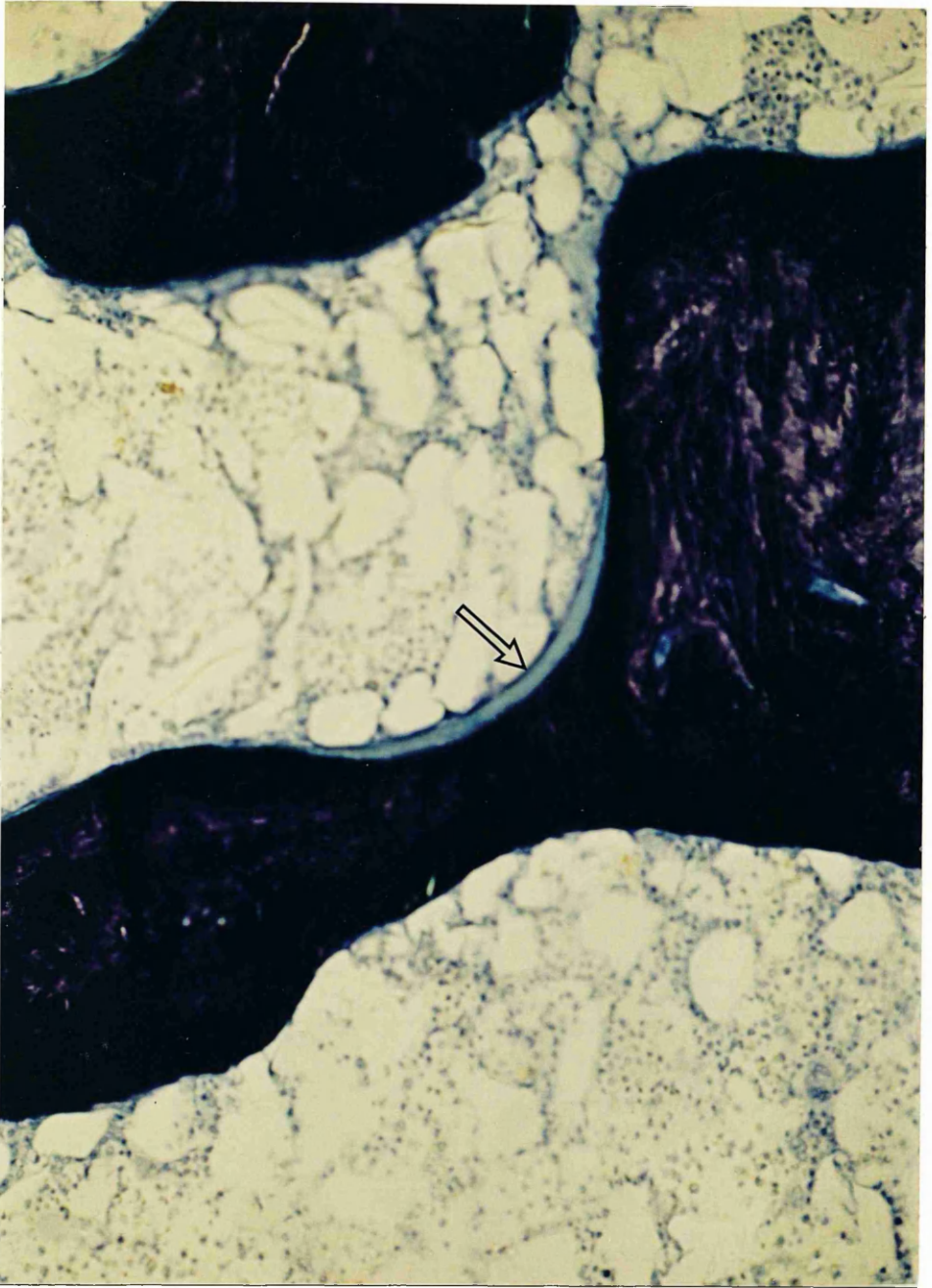
Representative photomicrographs from 6 bone biopsies are shown in figures 6.10 - 6.15, demonstrating that in both conditions, the histological appearance of bone may be normal (figures 6.10, 6.11), show increased bone resorption and formation (figures 6.12, 6.13), or show "uncoupling" of bone resorption and bone formation (figures 6.14, 6.15) depending on the clinical circumstances.

In patients with hypercalcaemia of malignancy, there were significant correlations between the stage of tumour advancement, degree of immobility and severity of bone resorption. Thus, the correlation between TRS and degree of immobility was;  $r=0.75$ ,  $p<0.01$ , and the correlation between TRS and stage of tumour progression was  $r=0.87$ ,  $p<0.01$  (figure 6.16 and inset). Similar correlations were observed between both other histological indices of bone resorption (ARS and NO) and these clinical variables (data not shown). The degree of immobility and stage of tumour advancement were inter-



**FIGURE 6.9**

Relation between histological indices of bone resorption and bone formation in primary hyperparathyroidism (HPT) ◇ and hypercalcaemia of malignancy (HHM) ● . TRS - total resorption surface, AOS - active osteoid surface. ● - immobilised patients with HHM; ◇ - immobilised patient with HPT.



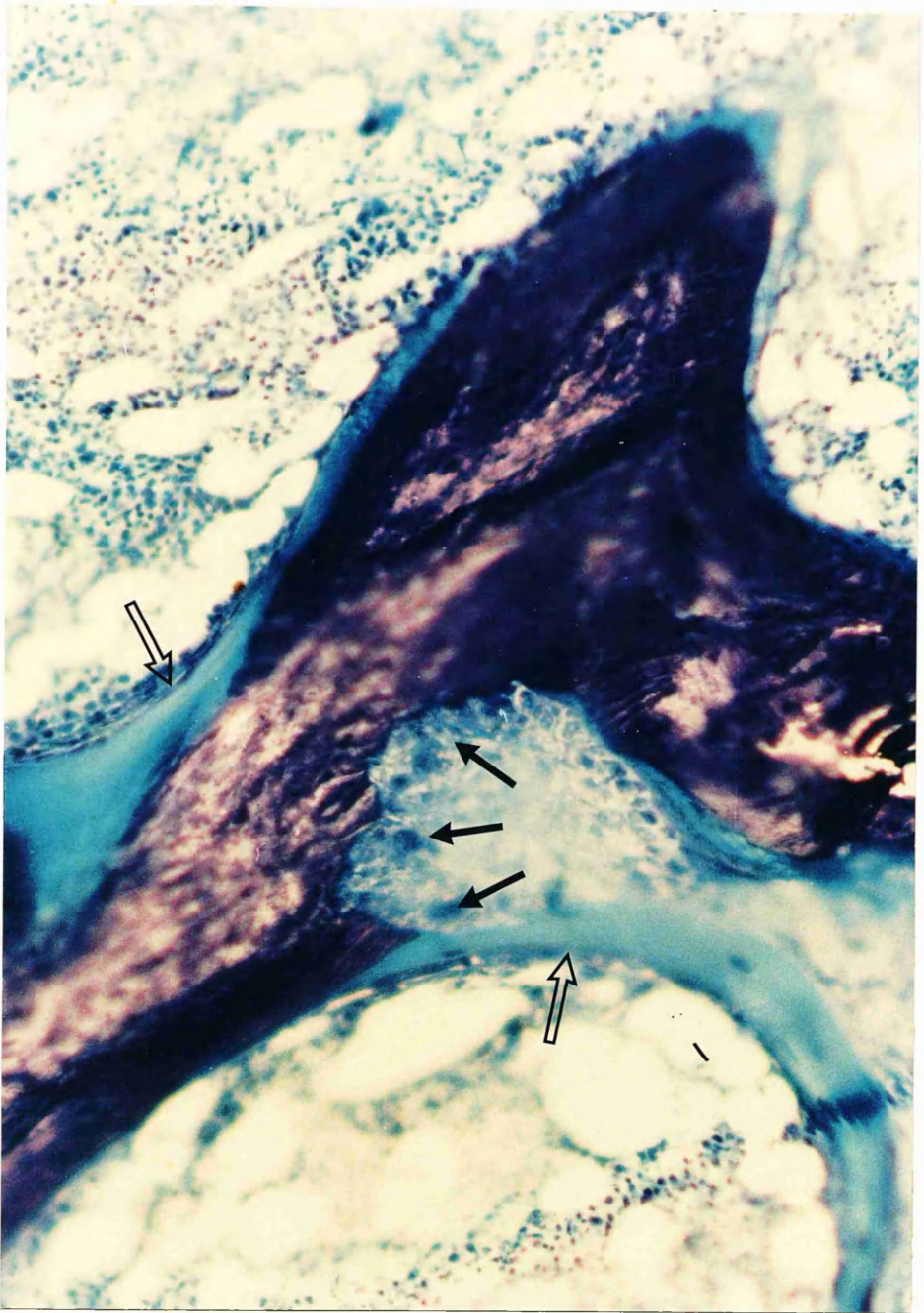
**FIGURE 6.10**

Photomicrograph (xl34 magnification; toluidine blue stain) of bone from patient with primary hyperparathyroidism and mild hypercalcaemia (2.75 mmol/l) showing normal bone architecture. Note smooth bone surfaces, with no evidence of increased resorption. A seam of osteoid is arrowed.



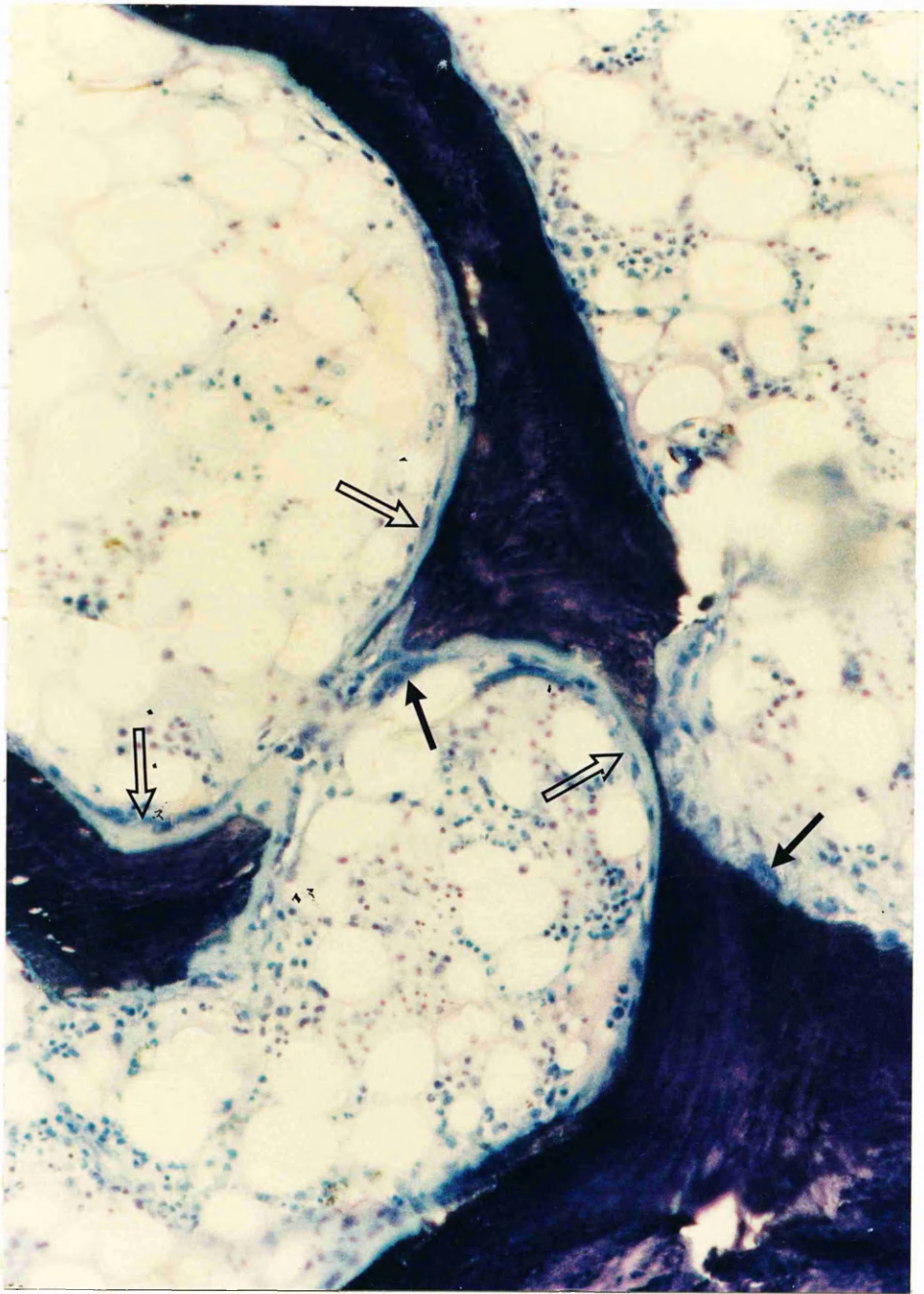
FIGURE 6.11

Photomicrograph (x134 magnification; toluidine blue stain) of bone from an ambulant patient with HHM who had mild hypercalcaemia (3.10 mmol/l), showing normal bone architecture. Note smooth bone surfaces with no evidence of osteoclastic resorption.



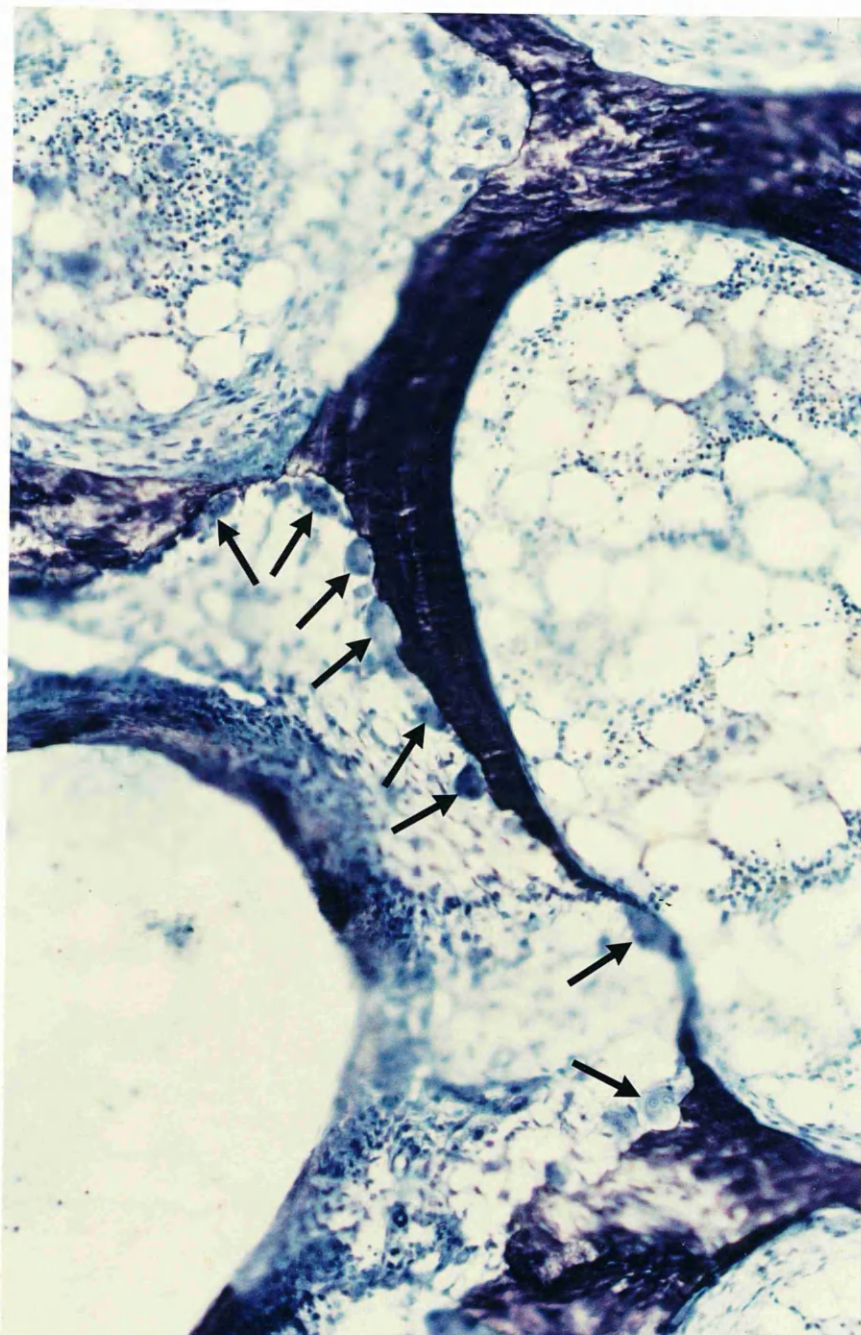
**FIGURE 6.12**

Photomicrograph (x134 magnification; toluidine blue stain) of bone from patient with HPT showing evidence of increased bone turnover. Note areas of osteoclastic bone resorption (closed arrows) in close proximity to osteoid seams (open arrows) Patient fully mobile with moderate hypercalcaemia (3.25 mmol/l).



**FIGURE 6.13**

Photomicrograph (x134 magnification; toluidine blue stain) of bone from a patient with HHM showing evidence of increased bone turnover. Patient had limited mobility around hospital ward and moderate hypercalcaemia (3.25 mmol/l). Note evidence of osteoblastic activity and extensive osteoid seams (open arrows), in close proximity to osteoclastic bone resorption (closed arrows).



**FIGURE 6.14**

Photomicrograph (x134 magnification; toluidine blue stain) of bone from a patient with HPT who had severe hypercalcaemia (serum calcium >4.50 mmol/l) and who was immobilised before the biopsy was taken. Note florid osteoclastic bone resorption (closed arrows). No osteoid is visible.

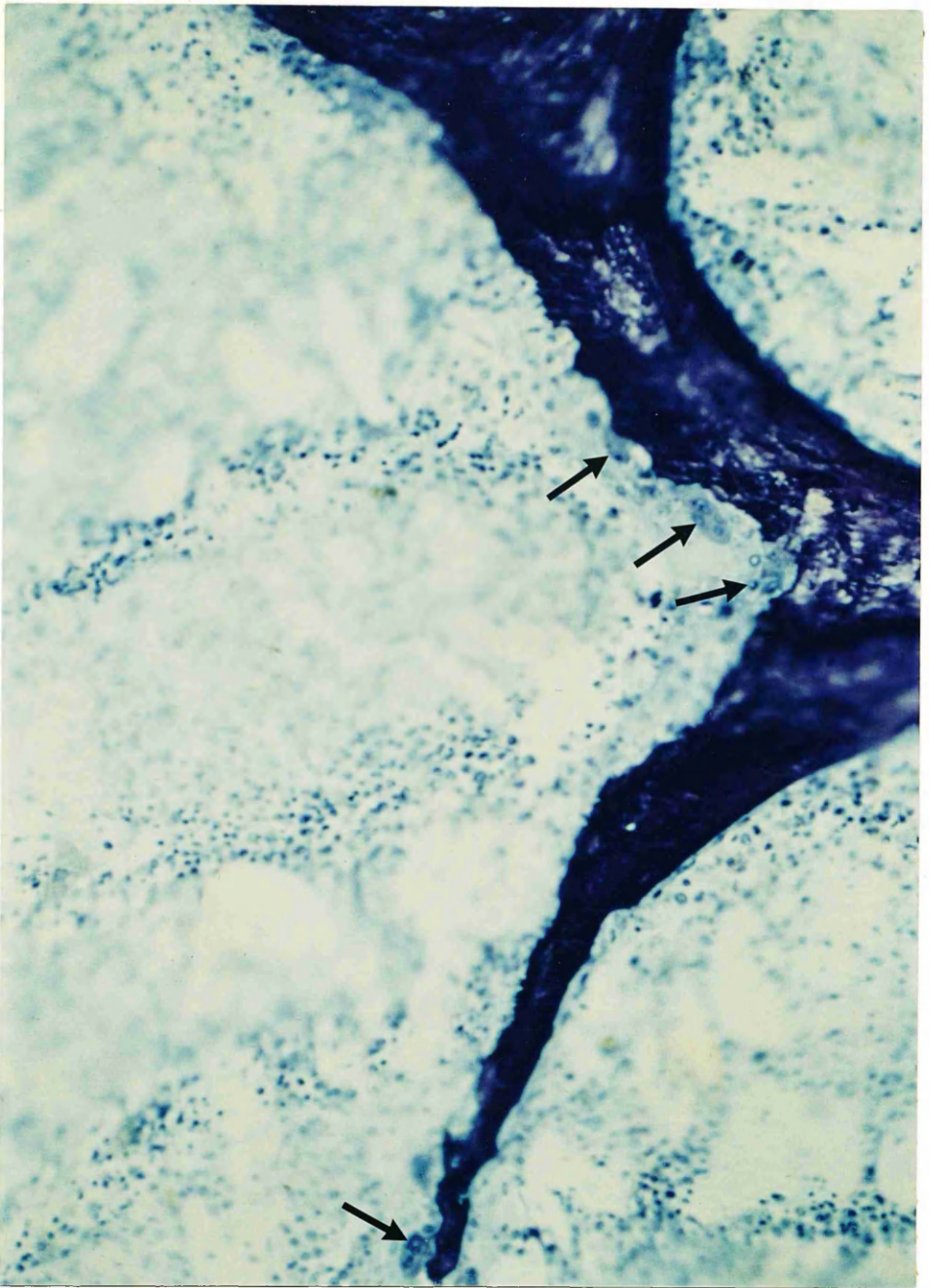
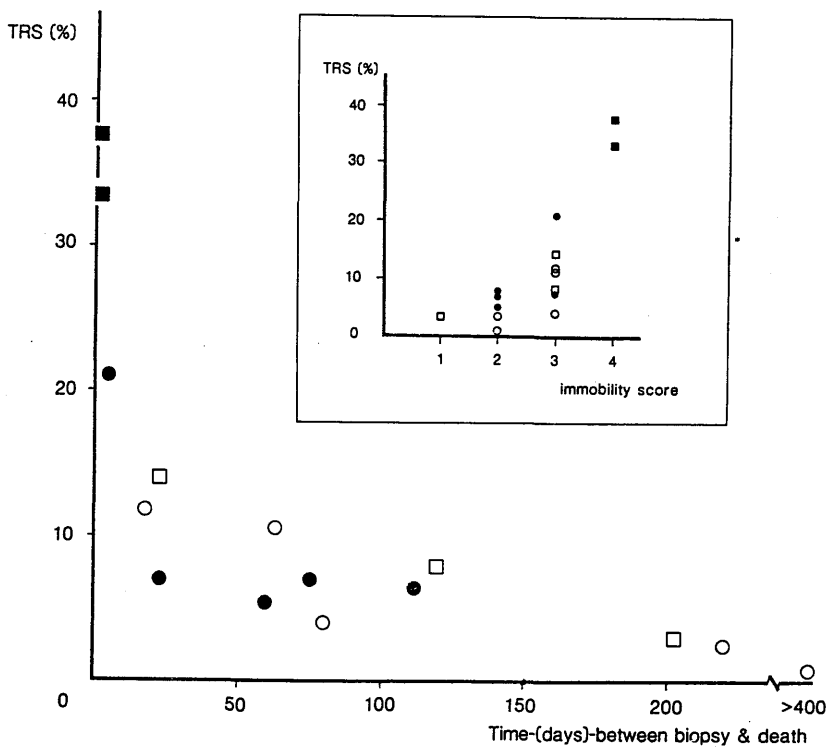


FIGURE 6.15

Photomicrograph (x134 magnification; toluidine blue stain) of bone from a patient with HHM. The bone biopsy was obtained post-mortem in a patient who had been immobilised for some time prior to death with severe hypercalcaemia (serum calcium >4.5 mmol/l). Note florid osteoclastic bone resorption (closed arrows). No osteoid is visible.





**FIGURE 6.16**

Relation between stage of tumour advancement in HHM and extent of bone resorption, represented by total resorption surface (TRS). Inset shows the relation between TRS and degree of immobility. Symbols represent; surgically treated (tumour resected =  $\circ$ ), (tumour not resected =  $\square$ ), not surgically treated (antemortem biopsy =  $\bullet$ ), (postmortem biopsy =  $\blacksquare$ ).

related ( $r=0.86$ ,  $p<0.01$ ).

#### 6.2.4 DISCUSSION

It is generally considered that increased osteoclastic bone resorption alone plays the major role in the pathogenesis of humoral hypercalcaemia of malignancy, due to the osteoclast activating effect of humoral substances released by tumour tissue (5,7,8,9,77,78,93,105). Hitherto, it has been proposed that the putative humoral mediators of hypercalcaemia in malignancy differ from PTH both structurally (93,130,144,145,146) and functionally, in being devoid of PTH-like effects on renal 1- $\alpha$ -hydroxylase activity and renal tubular reabsorption of calcium (93). Moreover, in a previous histomorphometric study of patients with humoral hypercalcaemia of malignancy (139), marked increases in osteoclastic bone resorption were observed in the presence of depressed bone formation, suggesting that the skeletal effects of the putative humoral mediator were also quite distinct from those of PTH.

In this study, wide variability in bone histology was found in patients with both primary hyperparathyroidism and malignant hypercalcaemia, depending on the clinical circumstances; osteoclastic bone resorption was normal in bone biopsy specimens from the majority of patients with early tumours, but was greatly increased in post-mortem biopsies from patients who had been immobilised in the terminal stages of their illness.

These differences in bone histology did not appear to be due to differences in the nature of the humoral mediator causing hypercalcaemia as clinical and biochemical characteristics were

fairly uniform in patients with humorally-mediated hypercalcaemia; the majority had squamous or genitourinary cancers (6,7,8,93), and with one exception iPTH levels were depressed (93,130,132). Despite this, levels of urinary cyclic AMP were raised (93,151) and  $TmPO_4$  values were low or low/normal (6,7,93,148). While most patients with malignancy were hypercalciuric in absolute terms (93), the levels of urinary calcium excretion were not significantly different from those in hyperparathyroidism, reflected by a similar elevation of renal tubular calcium reabsorption was in both conditions. Since levels of serum calcium, serum creatinine, and urinary sodium excretion were similar in both conditions, the elevation in renal tubular calcium reabsorption in malignancy is unlikely to have been due to factors such as renal failure, dehydration or sodium depletion. Rather, these observations suggest that there was a PTH-like effect on renal tubular calcium reabsorption in the patients with malignancy (Chapter 4).

The importance of these renal tubular effects in the pathogenesis of hypercalcaemia is demonstrated by the biochemical response observed in the 5 patients with malignancy who had resectable (n=4) or partially resectable (n=1) tumours. As alluded to in the previous study (Chapter 6.1), biochemical and histomorphometric indices showed that osteoclastic bone resorption was normal or only slightly elevated prior to surgical resection, and that the fall in serum calcium values post-operatively was due mainly to a reduction in renal tubular calcium reabsorption.

An intriguing feature in this study was the fact that, in many cases, these PTH-like effects on the kidney occurred in the absence of osteoclastic bone resorption. These findings concur, with the data

of Caverzasio and his colleagues however, who found that in the rat Leydig cell tumour model of humoral hypercalcaemia of malignancy (HHM), elevations in renal tubular calcium reabsorption occurred before osteoclastic bone resorption became raised (302). In other studies, D'Souza was unable to inhibit bone resorption stimulated by extracts of the rat Leydig cell tumour in vitro, using synthetic analogues of PTH at concentrations which completely abolished their adenylate cyclase stimulating activity (150). Thus, from the current findings and those referred to above, it would appear that, in the early stages of HHM, the adenylate cyclase stimulating factors possess potent PTH-like effects on the kidney. However, at these concentrations, the adenylate-cyclase stimulating factors do not appear to possess significant stimulatory effects on osteoclastic bone resorption. These observations are of interest since PTH itself is recognised to cause hypercalcaemia in most patients with primary hyperparathyroidism by increasing renal tubular reabsorption of calcium, rather than by increasing bone resorption (114)

On the basis of D'Souza's work, referred to above (150), Mundy has suggested that the accelerated bone resorption in HHM may be largely due to release of the transforming growth factors, rather than the PTH-like factors (90). A further possibility however, raised by the present study, is that the combined effects of immobilisation and the adenylate cyclase stimulating factors may have acted synergistically on the skeleton to increase osteoclastic bone resorption in patients with more advanced tumours.

We were able to confirm Stewart's observation that indices of bone formation are generally lower in HHM than in primary

hyperparathyroidism (139). However, greatly increased bone resorption with depressed bone formation were also noted in one immobilised patient with hyperparathyroidism, suggesting that "uncoupling" of bone cell activity is not a specific feature of HHM, as suggested by Stewart (139), but rather, may occur when a humorally-mediated increase in bone resorption occurs in combination with immobilisation. Although indices of bone formation did not correlate significantly with degree of immobilisation or stage of tumour progression in patients with HHM, these subjects were less physically active, more cachectic and more unwell than those with hyperparathyroidism. It is possible therefore, that the combination of these factors may have resulted in lower rates of active bone formation. An alternative explanation would be the release of substances such as the tumour necrosis factors, which have been shown to directly inhibit bone formation in vitro and are thought to be partly responsible for the cachexia associated with cancer (51).

The present studies confirm that the humoral mediators in HHM are similar to PTH with regard to their effects on the kidney (93). They are also analogous to PTH with respect to their skeletal effects, in that enhanced renal tubular reabsorption of calcium was a more important mechanism of hypercalcaemia than increased bone resorption in patients with early tumours (114). While we confirmed that osteoclastic bone resorption is "uncoupled" from bone formation in patients with advanced HHM (139,140,141), these abnormalities may have been partly explained by the effects of immobilisation. From our data, it is unclear whether the florid increases in osteoclastic bone resorption associated with advanced HHM are wholly explained by the

combined effects of the adenylate-cyclase stimulating factors and immobilisation or whether other tumour-associated bone resorbing substances also play a pathogenic role at this stage (78,104,51).

CHAPTER 7

MANAGEMENT OF MALIGNANCY ASSOCIATED HYPERCALCAEMIA

7.1 COMPARISON OF AMINOHYDROXYPROPYLIDENE DIPHOSPHONATE, MITHRAMYCIN  
AND CORTICOSTEROIDS/CALCITONIN IN THE TREATMENT OF MALIGNANCY  
ASSOCIATED HYPERCALCAEMIA

7.1.1 INTRODUCTION

Hypercalcaemia associated with malignant disease is probably the most common indication for the use of medical antihypercalcaemic therapy in hospital practice (179). Clinicians are faced with a variety of drugs for such treatment including glucocorticoids, calcitonin, phosphate, mithramycin, diphosphonates and prostaglandin synthetase inhibitors. There is no uniformly effective antihypercalcaemic treatment available which is also well tolerated however. In view of this, it is surprising that only two substantial comparative studies of antihypercalcaemic therapy have been reported (186,189). In this study, the effects of aminohydroxypropylidene diphosphonate (APD), mithramycin and the combination of corticosteroids and calcitonin - three of the most powerful antihypercalcaemic regimens available (189,244) - have been compared in the treatment of cancer-associated hypercalcaemia.

7.1.2 PATIENTS AND METHODS

Thirty-nine patients who presented consecutively to Glasgow Royal Infirmary with cancer-associated hypercalcaemia - defined as serum calcium (adjusted for albumin) greater than 2.80 mmol/l - were studied. A patient was selected for study if the clinician in charge of the patient had considered that antihypercalcaemic therapy was an appropriate form of management, usually because of symptoms attributable to the hypercalcaemia. All patients or their families



gave informed consent to entering the study.

The patients were initially rehydrated with intravenous 0.9% saline, 500ml every 4 hours for a minimum of 48 hours, then 500ml every 6 hours for 12 hours before administration of drug therapy. During treatment, saline infusions of 2 litres daily were continued until serum adjusted calcium had fallen below 2.80 mmol/l and/or until the patients' clinical state of hydration was judged adequate and a liberal fluid intake (>2 litres daily) was established. APD was given as an intravenous infusion of 15mg in 250ml saline each day until serum calcium was normal (2.60 mmol/l), or a nadir was reached. Mithramycin was given as an infusion of 25mcg/kg body weight in 500 ml 5% dextrose and repeated after 2 days if serum calcium remained above 2.90 mmol/l. Corticosteroids (prednisolone) 40mg daily were given orally in divided doses (or an equivalent parenterally in patients unable to swallow), combined with salmon calcitonin 400 IU every 8 hours by subcutaneous injection, both agents being continued throughout the 9 days of the study.

Biochemical analyses were performed on true fasting urine samples and blood samples obtained after an overnight fast.

The presence of metastatic bone disease was determined by a combination of standard radionuclide bone scans and skeletal radiographs.

The Wilcoxon tests for paired and unpaired data and the Chi-square tests with Yates' correction were used in statistical analyses, as was appropriate.

Before antihypercalcaemic therapy and throughout the study patients were interviewed and symptoms recorded. Symptoms were categorised into one of five groups:

- (1) Central Nervous System - confusion, obtundation, psychosis, depression.
- (2) Gastrointestinal - nausea, vomiting, anorexia, constipation.
- (3) Renal - polyuria, thirst.
- (4) General - malaise, fatigue.
- (5) Pain - usually due to bone or visceral involvement by tumour.

To assess the symptomatic response, individual patient-symptoms were summed and the total numbers compared before and after antihypercalcaemic treatment in each group.

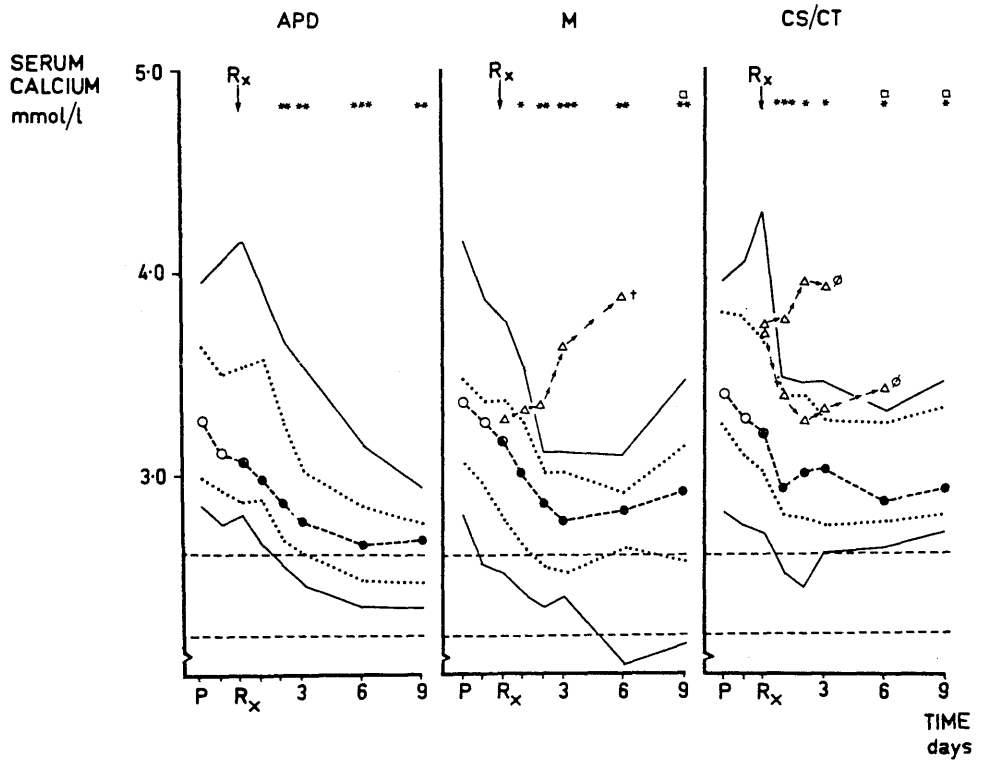
Statistical methods used were the Wilcoxon test for paired and unpaired samples and the Chi Squared test with Yates' correction.

### 7.1.3 RESULTS

Squamous carcinoma of lung and breast cancer were the commonest tumours, accounting for 48% and 17%, respectively of the whole study group; the remainder comprised various other solid tumours and one myeloma. There was no significant difference in the proportions of tumour types between the three treatment groups. Skeletal radiographs and bone scans showed no evidence of metastases in six of the APD treated patients compared with four in each of the other treatment groups. Serum albumin levels were generally low (30.4 (5.8) g/l; mean (SD)) and did not differ between the groups. Plasma iPTH levels were low (<400 ng/l) or undetectable in all patients.

Anti-cancer therapy given during the trial included local radiotherapy for bone metastases (one patient in each group) and continuous hormone therapy (stilboestrol - one APD-treated patient; tamoxifen - one patient each in the mithramycin and corticosteroid/calcitonin groups). Two patients in the APD group had surgical resection of the primary tumour on day seven. Biochemical data from these subjects were not included in statistical analysis at day 9, although serum calcium values were normal at this point in both cases (2.35, 2.45 mmol/l respectively). One patient each in the corticosteroid/calcitonin and mithramycin groups and two in the APD group died of complications of the tumour on days 1, 7, 2, and 4 of the study respectively. Two patients in the corticosteroid/calcitonin group were withdrawn from the study because of clinical deterioration (figure 7.1) and were successfully treated with mithramycin. One mithramycin-treated patient died of hypercalcaemic crisis despite repeated mithramycin administration and aggressive saline diuresis (fig 7.1).

During rehydration, most patients' serum calcium values fell but in some, hypercalcaemia grew worse (figure 7.1). Serum calcium fell significantly by day 1 of antihypercalcaemic therapy in the corticosteroid/calcitonin and mithramycin groups and by day 2 in the APD group. The fall in serum calcium was most rapid in the corticosteroid/calcitonin group; a median fall of 0.35 mmol/l took 24 hours in this group compared with 48 hours in the mithramycin group and 72 hours in the APD group. Although APD had the slowest onset of action, its effect was better sustained; serum calcium values were significantly lower in APD-treated patients at both 6 and 9 days in the corticosteroid/calcitonin-treated patients and mithramycin-treated



**FIGURE 7.1**

Response of serum calcium to antihypercalcaemic therapy. Interrupted line are medians, dotted lines are interquartile ranges, and solid lines are ranges. P - presentation  $R_x$  - at start of treatment. Open triangles show response of individual patients who either died † or were withdrawn ∅ due to clinical deterioration. Normal range indicated between horizontal interrupted lines.

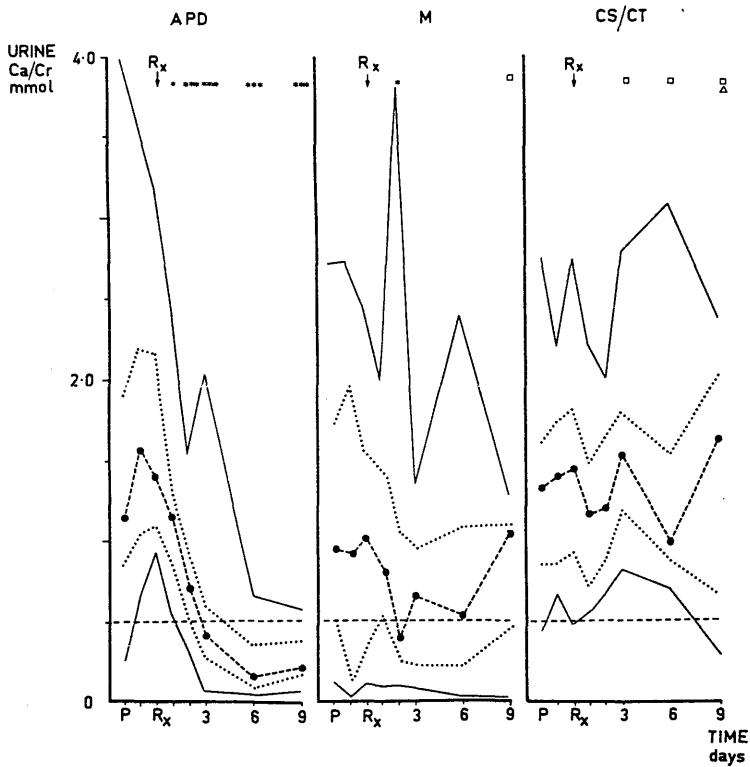
Significant difference from  $R_x$  \*= $p < 0.05$ ; \*\*= $p < 0.01$ ; \*\*\*= $p < 0.005$ .  
 Significant difference from APD group □= $p < 0.05$ .

patients at 9 days.

Figures 7.2 and 7.3 show the changes in fasting urinary Ca/Cr and OHP/Cr ratios, which in this situation, are thought to reflect changes in bone-resorption (153). APD caused a progressive reduction in Ca/Cr, significant from day 1, and in OHP/Cr significant from day 2. In the mithramycin group, Ca/Cr was significantly reduced on day 2 only, but OHP/Cr was significantly reduced on days 2 and 3. In the corticosteroid/calcitonin group there was no significant change in Ca/Cr, but OHP/Cr fell significantly on day 1.

When all 39 patients were considered together, there was a significant fall in the median serum creatinine level during initial rehydration from 80  $\mu\text{mol/l}$  (range 50-350  $\mu\text{mol/l}$ ) to 75  $\mu\text{mol/l}$  (range 45-210  $\mu\text{mol/l}$ ), just before treatment ( $p < 0.01$ ). After antihypercalcaemic treatment, serum creatinine fell further in the corticosteoid/calcitonin group from day 2 onwards to a nadir of 60  $\mu\text{mol/l}$  (range 35-100  $\mu\text{mol/l}$ ) on day 9 ( $p < 0.05$ ). Serum creatinine did not change significantly from the pre-treatment level in the other groups.

In figure 7.4, the components of the calcium-lowering response are depicted in terms of the "normal" relation between urinary calcium excretion ( $\text{Ca}_E$ ) and serum calcium (114), using the median values for each treatment group at each point in time. Changes in renal tubular calcium reabsorption result in a horizontal movement of the points, whereas changes in filtered load cause a diagonal shift, parallel to the slopes indicating the normal range (279). Changes in filtered load may either result from changes in glomerular filtration rate or net bone resorption (279). The initial fall in serum calcium after



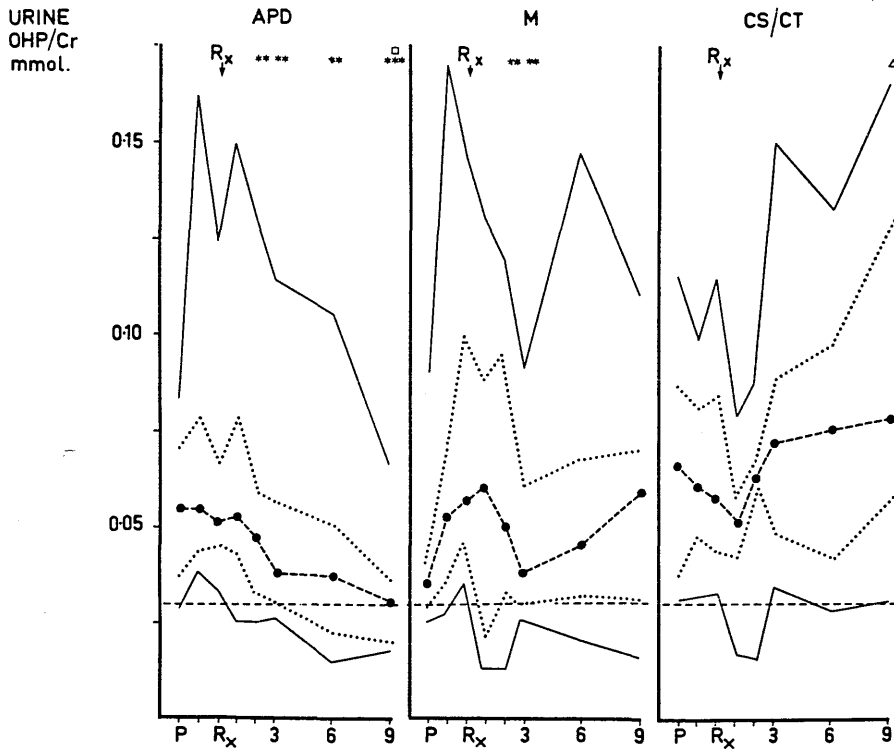
**FIGURE 7.2**

Response of fasting urinary calcium/creatinine (Ca/Cr) values to antihypercalcaemic therapy. Interrupted lines are medians; dotted lines are interquartile ranges; solid lines are ranges. Normal range is indicated by horizontal interrupted lines.

P - presentation,  $R_x$  - at start of treatment.

Significant difference from day  $R_x$  \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ ; \*\*\* =  $p < 0.005$ .

Significant difference from APD group  $\square$  =  $p < 0.05$ ; from mithramycin group  $\Delta$  =  $p < 0.05$ ;



**FIGURE 7.3**

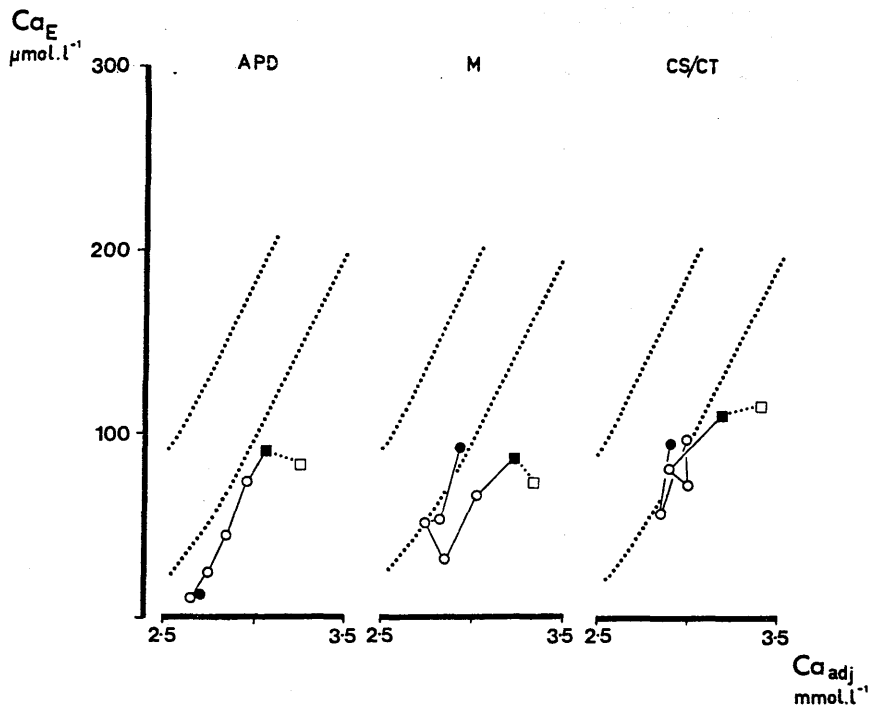
Response of fasting urinary hydroxyproline/creatinine (OHP/Cr) values to antihypercalcaemic therapy. Interrupted lines are medians, dotted lines are interquartile ranges, solid lines are ranges. Horizontal interrupted line indicates normal range. P-presentation; Rx - at start of treatment.

Significantly different from day Rx \*= $p < 0.05$ ; \*\*= $p < 0.01$ ; \*\*\*= $p < 0.005$ . Significantly different from mithramycin and corticosteroid/calcitonin groups □= $p < 0.05$ ; Significantly different from mithramycin group Δ= $p < 0.05$ .

rehydration was due to a reduction in renal tubular calcium reabsorption, as reflected by a general shift in the points to the left. In the APD group, subsequent falls in serum calcium were due to a lower filtered calcium load, which was in turn due to a fall in bone resorption (figures 7.2 and 7.3). In the corticosteroid/calcitonin group, an important component of the early fall in calcium was a reduction in renal tubular calcium reabsorption, reflected by a shift in the points on figure 7.4 further to the left of those after rehydration. There was a subsequent load-dependant reduction in serum calcium in this group, due mainly to a fall in glomerular filtration rate, although there was also a transient reduction in bone resorption (figure 7.3). In the mithramycin group, the early load-dependant reductions in serum calcium were due to a fall in bone resorption (figures 7.2, 7.3). Subsequently, the renal tubular reabsorption of calcium also appeared to fall.

There were symptomatic improvements in each treatment group, particularly in the manifestations of hypercalcaemia on the central nervous system, gastro-intestinal tract and kidney ("specific" symptoms). Thus, for all treated patients, 34 of 61 "specific" symptoms (55%) improved, compared with 7 out of 42 (16%) "non-specific" symptoms such as malaise, fatigue and pain ( $\chi^2=11.2$ ,  $p<0.001$ ). A better response was observed in patients whose post-treatment serum calcium values remained below 2.80 mmol/l on three or more consecutive days; 27 out of 38 (71%) "specific" symptoms improved compared with 7 out of 23 (30%) in those with poor hypercalcaemic control ( $\chi^2=11.2$ ,  $p<0.001$ ). This was reflected by a slightly better response in the APD group where control of hypercalcaemia was best in the long term, but the differences in symptomatic improvements between





**FIGURE 7.4**

Relation between serum adjusted calcium and urinary calcium excretion (as  $Ca_E$ ) during antihypercalcaemic therapy. Data points are medians. Dotted lines indicate normal range defined by Peacock (114).

- - Presentation
- - After rehydration
- - During treatment (days 1-6)
- - Day 9 of antihypercalcaemic therapy

the APD group (58% "specific", 31%, "non-specific") and the other groups (mithramycin 42% and 8%, corticosteroid/calcitonin 50% and 7%) did not reach statistical significance. Side-effects of mithramycin included nausea, vomiting and malaise in two cases, mild thrombocytopenia in one and rises in serum aminotransferases and gamma-glutamyl transpeptidase in eleven. Transient pyrexia occurred in four of the APD group and local thrombophlebitis at the infusion site in two. Surprisingly, none of the corticosteroid/calcitonin group complained of nausea or vasomotor symptoms, but many found the frequent injections of calcitonin uncomfortable.

In the whole study group, the median overall survival time was short (39 days; range 3-420 days); it did not differ significantly between the groups. Only six patients survived for more than six months (three - APD; 2 -mithramycin; 1 - corticosteroid/calcitonin) and the common factor in these patients was administration of additional anticancer therapy which induced a remission in the primary tumour.

#### 7.1.4 DISCUSSION

In agreement with previous studies the hypercalcaemia we observed was due to a combination of increased bone resorption (148,236,253,256,259) and raised renal tubular reabsorption of calcium (236, Chapter 4), with impaired glomerular filtration in some cases (147,236,259). As was noted in chapter 4, volume re-expansion with intravenous saline only partially corrected the abnormal renal handling of calcium, slightly reduced serum calcium and serum creatinine and had no effect on the increased calcium load derived from bone resorption, confirming that rehydration alone is an

unsatisfactory treatment for cancer-associated hypercalcaemia (147,259).

All three drug regimens produced a significant fall in serum calcium from the levels achieved with rehydration. Although the corticosteroid/calcitonin combination reduced serum calcium levels most rapidly, it seldom reduced them to normal. Nonetheless, the response was well-sustained throughout the 9 days of study in all but two cases. The slow onset of action of APD was compensated for by progressive and consistent control of hypercalcaemia later. About half of the APD-treated patients remained mildly hypercalcaemic throughout the study, despite almost complete inhibition of bone resorption (as reflected by the normal urinary Ca/Cr values) in all patients by day 9. This phenomenon reflects the contribution of a hormone-mediated rise in renal tubular calcium reabsorption to the pathogenesis of hypercalcaemia (253), which in this situation, is postulated to be due to the effects of a "PTH-like" mediator (chapter 4). Hydroxyproline excretion remained high in some patients after APD treatment, probably owing to soft tissue catabolism, rather than continued bone resorption (248). Although some of the lowest post-treatment serum calcium levels were recorded in the mithramycin group, about half of the patients had started to relapse by day 9. The antihypercalcaemic effect of mithramycin could probably have been enhanced by repeated daily doses, but this was not justifiable in view of the potential toxicity (218).

Our results show that the three therapeutic regimens bring about their calcium-lowering effects in different ways. In agreement with previous studies (148,259), APD progressively reduced bone resorption

with little change in renal tubular calcium handling. In contrast, the rapid effect of the corticosteroid/calcitonin regime was mainly due to a reduction in renal tubular calcium reabsorption. The reduction in calcium load with continued administration of these agents could largely be explained by the improvement in glomerular filtration rate rather than a substantial fall in bone resorption. These findings contrast with the generally held view that both corticosteroids and calcitonin act principally by inhibiting bone resorption (207,240,241). In a recent study of calcitonin alone, Hosking and Gilson noted that the renal tubular rather than skeletal effects of calcitonin were most important in determining the early calcium-lowering response in malignant hypercalcaemia (236). We have confirmed these observations and shown that, in the longer term also, it is the renal rather than skeletal effect of the corticosteroid/calcitonin combination which sustains the reduction in serum calcium.

Surprisingly, mithramycin appeared to have effects on both bone resorption and renal tubular handling of calcium. Although, as found previously (220,221) the initial fall in serum calcium was due to a reduction in bone resorption, there was also a reduction in renal tubular calcium reabsorption from day 3 onwards. Possible mechanisms for this action include direct tubular damage (218), an anti-tumour effect (219) or inhibition of release by the tumour of putative humoral mediators affecting renal calcium handling.

The very limited duration of survival confirms the poor prognosis of cancer-associated hypercalcaemia, and emphasises that its treatment should probably be reserved for patients who have troublesome symptoms. The finding that "non-specific" symptoms such

as malaise, fatigue and pain responded less well than "specific" symptoms of hypercalcaemia referable to the central nervous system, gastrointestinal tract and kidney, probably reflects that the former were due to the underlying malignant process, rather than the high serum calcium levels. The better symptomatic response in patients with good control of hypercalcaemia indicates that it is more important to control serum calcium levels in this syndrome, than to give less effective antihypercalcaemic agents such as corticosteroids (209,210), in the hope that they will bring about a general improvement in well-being. There was no evidence to suggest that patients who received corticosteroids improved more in terms of symptoms such as malaise, fatigue or anorexia.

In this study, APD was the most effective treatment for malignancy associated hypercalcaemia in the medium-term. For rapid control of severe hypercalcaemia however, APD was less suitable because of its slow onset of action. In this situation, calcitonin (236,240,241), mithramycin (223,224) or intravenous phosphate (185,186) may be more appropriate.

## 7.2 TREATMENT OF CANCER-ASSOCIATED HYPERCALCAEMIA WITH COMBINED AMINOHYDROXYPROPYLIDENE DIPHOSPHONATE AND CALCITONIN

### 7.2.1 INTRODUCTION

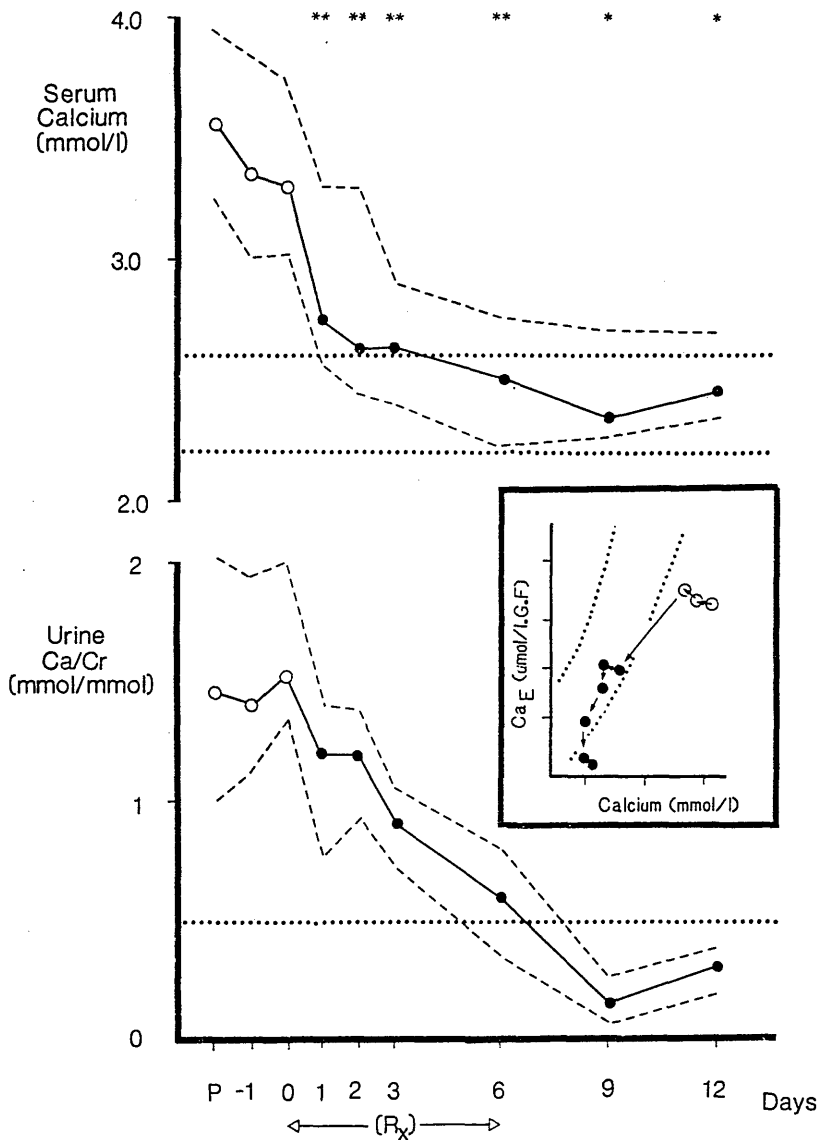
In the previous study (chapter 7.1), aminohydroxypropylidene diphosphonate (APD) was found to give better long-term control of hypercalcaemia in malignancy than either mithramycin or corticosteroids plus calcitonin. APD was found to be the least suitable agent for the acute treatment of hypercalcaemia however, because of its relatively slow onset of action. In this study the effects of combined treatment with APD and calcitonin were studied in patients with cancer-associated hypercalcaemia.

### 7.2.2 PATIENTS AND METHODS

Eight patients with hypercalcaemia due to various malignant tumours were studied. Four patients had squamous lung carcinoma and one patient each had carcinoma of breast, kidney, gallbladder and myeloma. All patients were initially rehydrated with 0.9% saline 3 litres daily for at least 48 hours, followed by 0.9% saline 2 litres daily for 12 hours prior to, and during treatment until the patient's clinical state of hydration was judged adequate.

Drug treatment was given as follows; APD as an intravenous infusion of 15mg daily in 250 mls 0.9% saline and salmon calcitonin 100 IU every eight hours by subcutaneous injection. Both agents were given for six consecutive days.

Biochemical analyses were made on true fasting urine samples and blood samples, obtained after an overnight fast.



**FIGURE 7.5**

Biochemical response to treatment with APD and calcitonin. Top panel shows serum calcium, bottom panel fasting urinary calcium/creatinine (Ca/Cr) and inset, serum calcium vs urinary  $Ca_E$ . Dotted lines indicate normal ranges, which in the case of the inset is that defined by Peacock (114). Points are medians, interrupted lines are interquartile ranges. \*= $p < 0.05$  and \*\*= $p < 0.02$  from day 0 for serum calcium and Ca/Cr. P = presentation.

The paired Wilcoxon test was used for statistical analysis.

### 7.2.3 RESULTS

Both serum calcium and urinary Ca/Cr values fell significantly and progressively between days 1-12 of the study (figure 7.5). Urinary OHP/Cr values also fell significantly from a mean (SEM) of 63(9)  $\mu\text{mol}/\text{mmol}$  before treatment to 38 (9) on day 1 and 42 (8) on day 12 ( $p < 0.02$ ,  $p < 0.05$ , respectively from pretreatment value). Serum creatinine fell from 152 (22.5)  $\mu\text{mol}/\text{l}$  at presentation to 129 (20.9) after rehydration ( $p < 0.05$ ). Creatinine fell further to 110 (19.6) on day 1 and 99 (20.5) on day 12 ( $p < 0.02$ ,  $p < 0.05$ , respectively from pretreatment value).

In terms of the "normal" relation between serum calcium and urinary calcium excreted ( $\text{Ca}_E$ ), the calcium lowering effect of drug treatment was due to a further fall in renal tubular calcium reabsorption from that achieved by rehydration and a reduction in filtered calcium load, as reflected by a downward and leftward shift of the points in figure 7.5 (inset). The fall in filtered calcium load was in turn, due mainly to a reduction in bone resorption, but also to an improvement in glomerular filtration rate. After treatment was stopped (days 9 and 12), the points shifted vertically downwards, indicating that renal tubular calcium reabsorption had risen, although bone resorption remained suppressed.

### 7.2.4 DISCUSSION

In this study, the rapid calcium lowering effect was largely due to an acute reduction in both bone resorption and renal tubular



calcium reabsorption. A similar response was noted in the previous study (7.1), using a combination of corticosteroids and calcitonin, and it was probably mediated by the renal and skeletal effects of calcitonin alone (236). In accordance with previous findings, the reduction in renal tubular calcium reabsorption was sustained for the duration of calcitonin treatment, but reversed thereafter. Indeed, longer term control of hypercalcaemia was achieved mainly by suppression of bone resorption, and this was probably due to the more sustained anti-osteoclast effect of APD (148,259). The small increase in OHP/Cr values which remained after treatment concurs with previous data which has indicated that, in this situation, a substantial proportion of urinary hydroxyproline may derive from tissues other than bone (236,259). In all cases, correction of hypercalcaemia was associated with an improvement in symptoms in the absence of any drug-related side effects.

In this study, the combination of APD and calcitonin gave better long-term control of hypercalcaemia than the corticosteroid/calcitonin regime previously studied and was more rapidly acting than APD alone. No significant side effects were encountered as the result of treatment, making this regime superior to mithramycin and intravenous phosphate, which are rapidly acting but potentially toxic (218,190,191,192,193). Combined treatment with APD/calcitonin may therefore be particularly useful in the treatment of severe hypercalcaemia where a rapid but sustained effect is desired. As the main action of calcitonin was to "buy time" during the initial 48 hours while the APD took effect, it may be possible to limit the use of calcitonin to this initial period, with obvious advantages in terms of cost.

### 7.3 RELATIONSHIP BETWEEN MECHANISMS OF HYPERCALCAEMIA IN MALIGNANCY AND RESPONSE TO ANTIHYPERCALCAEMIC THERAPY

#### 7.3.1 INTRODUCTION

The calcium-lowering response to antihypercalcaemic therapy in hypercalcaemia associated with malignant disease is variable (303). Although the reasons for this are at present unclear, they may relate to differences in the pathophysiological mechanisms of hypercalcaemia in different patient-groups (5,93). In this study, the pathophysiological mechanisms of hypercalcaemia in 50 patients with hypercalcaemia of malignancy were related to the calcium-lowering response after antihypercalcaemic drug therapy.

#### 7.3.2 PATIENTS AND METHODS

Fifty patients who presented consecutively to Glasgow Royal Infirmary were studied, selected on the basis that the clinician in charge of the patient had considered that antihypercalcaemic therapy was an appropriate form of management. Prior to the study, all patients were sodium-repleted with a standard regime of intravenous saline 0.9%, 3 litres daily for a minimum of 48 hours, then 2 litres daily for at least 12 hours before administration of antihypercalcaemic therapy. Patients were treated with one of four antihypercalcaemic regimens; aminohydroxypropylidene diphosphonate (APD), (n=14), mithramycin (n=15), corticosteroid/calcitonin (n=13), APD/calcitonin (n=8). These patient-group are essentially as described in the previous sections (7.1 and 7.2), with the addition of a few extra patients who were treated with one of the above regimens, but were not included in either of the above studies. Drug therapy was

administered as described in sections 7.1 and 7.2.

Biochemical analyses were made on fasting blood samples and true fasting urine samples, usually obtained after an overnight fast. The relative contribution of impaired renal glomerular filtration, increased renal tubular calcium reabsorption and increased calcium flow from bone resorption to the pathogenesis of hypercalcaemia was assessed using the method described by Nordin (279)

The presence and extent of metastatic bone disease was assessed by bone scan examination as described in chapter 3.1. In three patients with myeloma and in others where abnormalities of doubtful significance were seen on the bone scan, account was taken of additional information gained by radiological skeletal survey and/or post-mortem examination.

In assessing the calcium-lowering response, the lowest post-treatment calcium values were used in statistical analysis. In two patients where serum calcium did not fall after antihypercalcaemic therapy was given, the last serum calcium value prior to death or administration of an alternative drug regime was used.

Biochemical analyses were made on true fasting blood and spot urine samples.

Methods used in statistical analysis were the paired and unpaired Wilcoxon's tests and Spearman's rank correlation coefficient.

### 7.3.3 RESULTS

In analysis of results, patients were divided into three groups on the basis of the extent of metastatic bone disease. In patients without bone metastases and a light skeletal tumour load (n=32), squamous carcinoma of lung was the commonest tumour accounting for 65% of cases, followed by hepatoma (12%), other squamous carcinoma (9%), adenocarcinoma, unknown primary (9%), renal carcinoma (3%) and anaplastic carcinoma (3%). In the heavy tumour load group, breast carcinoma was most common (55%), followed by myeloma (16%), squamous lung carcinoma (5%), oat cell lung carcinoma (5%), anaplastic lung carcinoma (5%), adenocarcinoma, unknown primary (5%) and renal carcinoma (5%).

Relevant biochemical variables after sodium repletion and before administration of antihypercalcaemic therapy in the three groups are shown in Table 7.1.  $Ca_E$  values were significantly higher in the heavy skeletal tumour load group when compared with both other groups. While serum creatinine, phosphate,  $TmPO_4$  and urine Ca/Cr values were significantly higher in the heavy skeletal tumour load group when compared with those with no metastases, there was no significant difference with respect to the light tumour load group. Plasma iPTH values (data not shown) were low or undetectable in all but two patients; one with squamous carcinoma of the gallbladder (PTH-1600ng/l) and one with squamous carcinoma of the lung (PTH-850ng/l).

In figure 7.6, the levels of serum calcium are shown in the three groups, after breakdown into separate components of altered glomerular filtration rate (GFR), renal tubular calcium reabsorption,

TABLE 7.1

BIOCHEMICAL VARIABLES BEFORE ANTIHYPERCALCAEMIC THERAPY

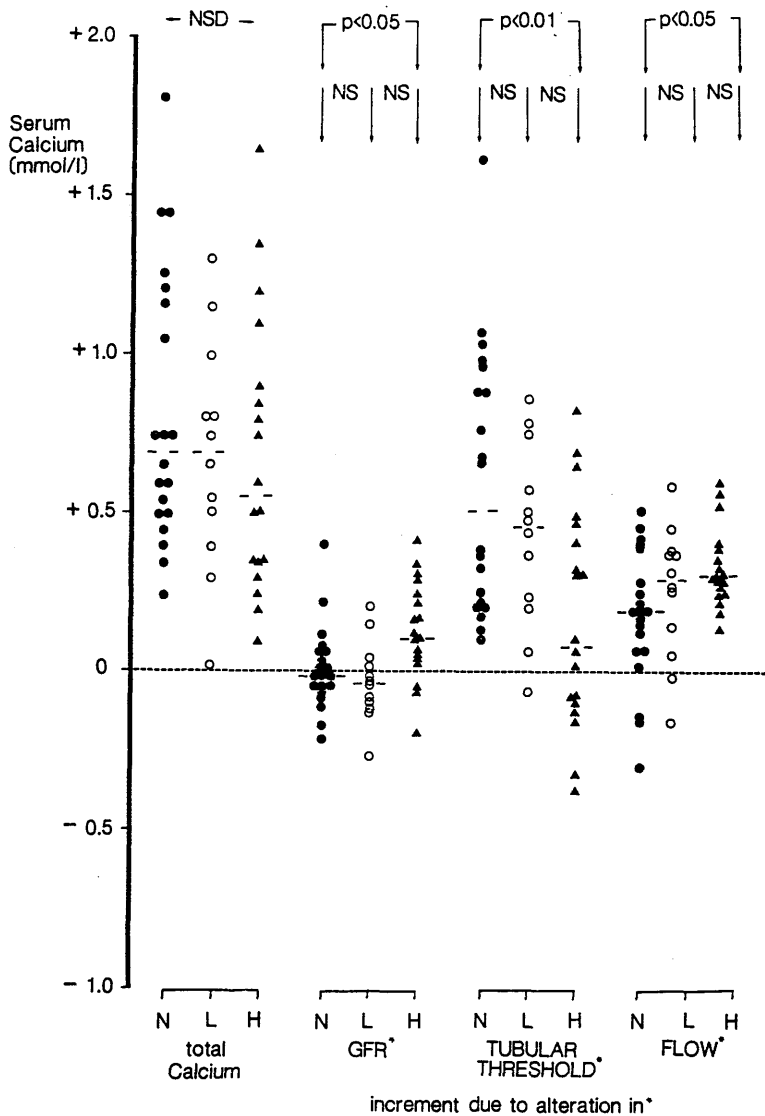
	<u>NO DETECTABLE METASTASES</u>	<u>LIGHT SKELETAL TUMOUR LOAD</u>	<u>HEAVY SKELETAL TUMOUR LOAD</u>
<u>NUMBER</u>	20	12	18
<u>SERUM CALCIUM (mmol/l)</u>	3.12 (2.75-4.30)	3.20 (2.50-3.80)	3.05 (2.60-4.15)
<u>SERUM PHOSPHATE (mmol/l)</u>	0.72 (0.40-1.55)	0.85 (0.55-1.25)	1.00 <sup>***</sup> (0.65-1.80)
<u>SERUM CREATININE (µmol/l)</u>	72 (45-240)	70 (45-190)	100 <sup>***</sup> (55-160)
<u>SERUM ALBUMIN (mmol/l)</u>	30 (22-41)	29 (23-35)	29 (24-41)
<u>URINE Ca/Cr (mmol/mmol)</u>	1.17 (0.22-2.40)	1.54 (0.12-2.50)	1.45 <sup>*</sup> (0.76-3.20)
<u>Ca<sub>E</sub> (µmol/IGF)</u>	89 (13-556)	110 (7-182)	151 <sup>***</sup> + (64-384)
<u>TmPO<sub>4</sub> (mmol/IGF)</u>	0.50 (0.30-0.90)	0.65 (0.30-1.50)	0.82 <sup>*</sup> (0.25-2.10)
<u>URINE CHP/Cr (µmol/mmol)</u>	56 (35-146)	49 (36-161)	69 (33-130)
<u>URINE Na<sub>E</sub> (mmol/IGF)</u>	2.83 (0.37-6.70)	3.15 (0.67-6.70)	2.74 (0.21-4.55)

values are medians  
(range)

<sup>\*\*\*</sup> p<0.001 from no metastases group

<sup>\*</sup> p<0.05 from no metastases group

<sup>+</sup> p<0.05 from light tumour load group



**FIGURE 7.6**

Pathogenic mechanisms of hypercalcaemia before treatment in terms of renal and skeletal components on division by the extent of metastatic bone disease. Serum calcium values on the abscissa are referred to a zero value of 2.50mmol/l. GFR-increment of hypercalcaemia due to impaired glomerular filtration; TUBULAR THRESHOLD-increment due to alteration in renal tubular calcium reabsorption; FLOW-increment attributable to increased bone resorption.

- ▲ -Heavy skeletal tumour load
- -Light skeletal tumour load
- -No detectable bone metastases

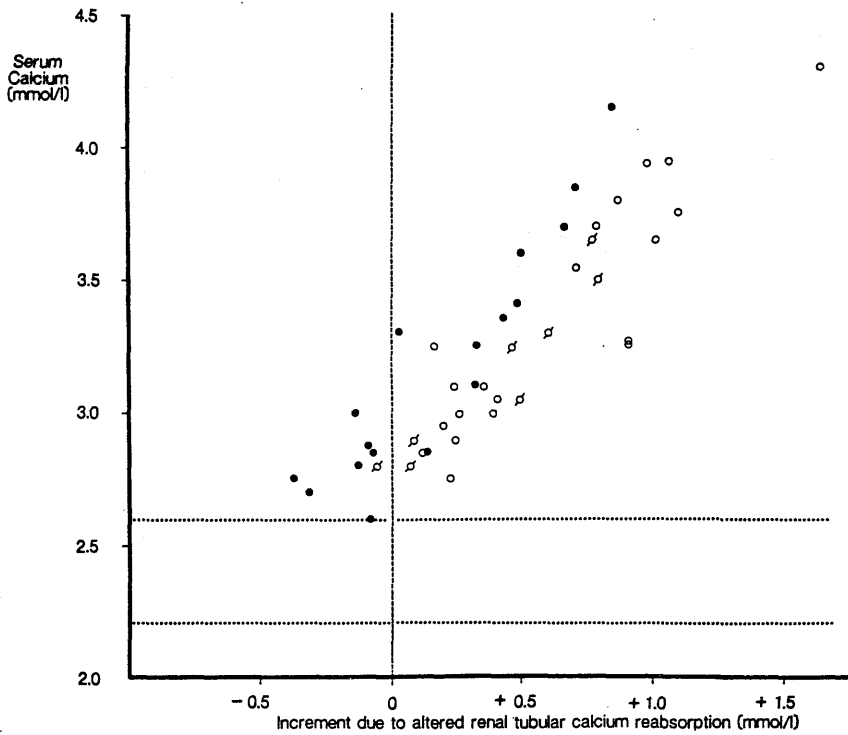
Bars are medians. NS/NSD - no significant difference

and calcium flow. Patients with a heavy skeletal tumour load had a significantly ( $p < 0.05$ ) greater impairment of GFR than those with no metastases. Conversely, the renal tubular component of hypercalcaemia was significantly ( $p < 0.01$ ) lower in the heavy skeletal tumour load group. The calcium flow component was significantly ( $p < 0.05$ ) higher in the heavy tumour load group when compared with the no metastases group.

A highly significant correlation was found between serum calcium values before treatment and the renal tubular component of hypercalcaemia ( $r = 0.83$ ,  $p < 0.001$ ). In absolute terms, the renal tubular component of hypercalcaemia was raised in most patients prior to treatment (figure 7.7). Patients in the heavy skeletal tumour load group tended to have lower renal tubular calcium reabsorption than those with no metastases and a light skeletal tumour load.

On statistical analysis, other significant correlations were observed between the following pre-treatment variables; serum calcium vs serum creatinine ( $r = 0.52$ ,  $p < 0.005$ ), serum calcium vs GFR component of hypercalcaemia ( $r = 0.53$ ,  $p < 0.005$ ) and serum creatinine vs serum phosphate ( $r = 0.36$ ,  $p < 0.05$ ).

The response to the individual antihypercalcaemic drug regimes in terms of the components of hypercalcaemia are shown in figures 7.8-7.10. The flow component was reduced in most cases, although the response was least consistent in the corticosteroid/calcitonin treated patients (figure 7.8). The renal tubular component was significantly reduced after treatment with corticosteroid/calcitonin, mithramycin and APD/calcitonin regimens. While APD alone did not significantly reduce the tubular component of hypercalcaemia overall, the tubular



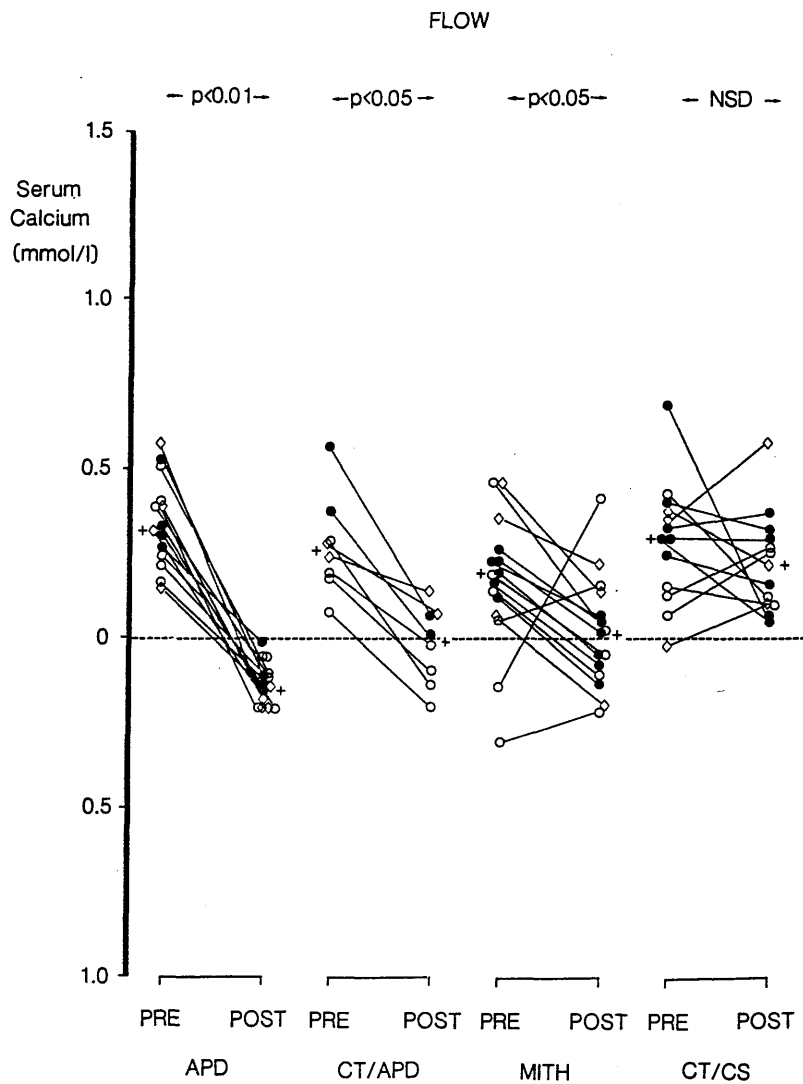
**FIGURE 7.7**

Relation between serum calcium values before antihypercalcaemic therapy and the renal tubular component of hypercalcaemia before therapy.

- - Heavy skeletal tumour load
- / - Light skeletal tumour load
- - No detectable bone metastases

The horizontal dotted lines indicate the reference range for serum calcium.





**FIGURE 7.8**

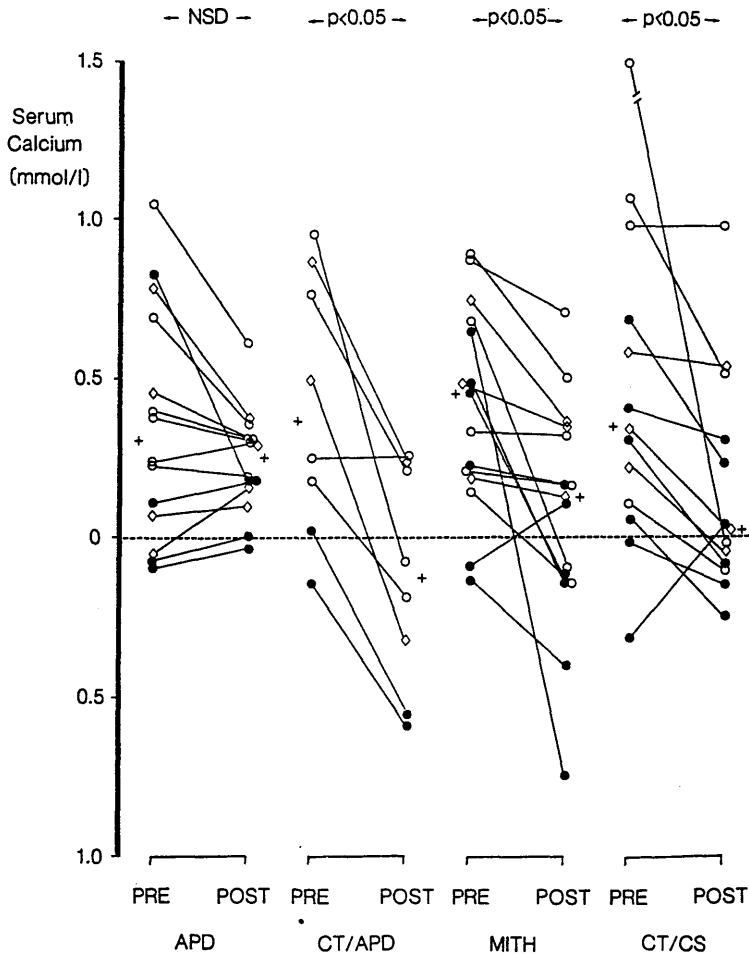
Response of calcium "flow" component to anti-hypercalcaemic therapy, on division of patients by type of treatment received. PRE -before treatment, POST -after treatment.

APD- aminohydroxypropylidene diphosphonate  
 CT/CS- calcitonin and corticosteroids  
 MITH- mithramycin  
 APD/CT- calcitonin and APD

● -Heavy tumour load  
 ◇ -Light tumour load  
 ○ -No detectable metastases

+median values

TUBULAR  
REABSORPTION



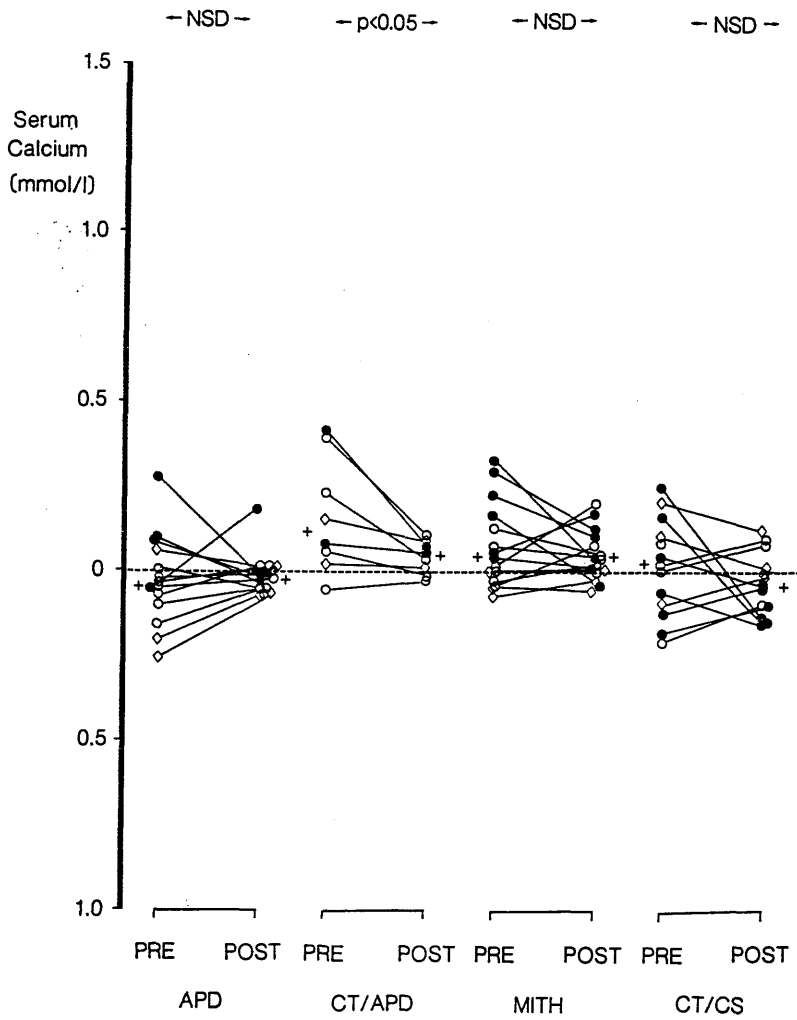
**FIGURE 7.9**

Response of the renal tubular component of hypercalcaemia to antihypercalcaemic therapy, on division of patients by the type of treatment received. PRE- before treatment, POST- after treatment.

APD- aminohydroxypropylidene diphosphonate  
 CT/CS- calcitonin and corticosteroids  
 MITH- mithramycin  
 CT/APD- calcitonin and APD

● -Heavy tumour load  
 ◇ -Light tumour load  
 ○ -No detectable metastases

+Median values



**FIGURE 7.10**

Response of renal glomerular component of hypercalcaemia to antihypercalcaemic therapy on division of patients by type of treatment received. PRE- before treatment, POST- after treatment.

APD- aminohydroxypropylidene diphosphonate  
 CT/CS- calcitonin and corticosteroids  
 MITH- mithramycin  
 CT/APD- calcitonin and APD

● -Heavy skeletal tumour load  
 ◇ -Light skeletal tumour load  
 ○ -No detectable metastases

+median value

component did fall in patients with the most severe hypercalcaemia before treatment (fig 7.9). Thus, initial serum calcium values were (median (range)); (3.37 (3.00-4.50) mmol/l) in 8 patients where the renal tubular component fell, compared with (2.90 (2.80-2.92) mmol/l) in 6 where the renal tubular component rose or remained static ( $p < 0.01$ ). The increment of hypercalcaemia attributable to impaired GFR was relatively minor in most cases (figure 7.10), but tended to fall after treatment. The fall in the GFR component was significant only in the APD/calcitonin group. From figures 7.8 - 7.10, it can be seen that, there was no quantitative difference in the response to antihypercalcaemic therapy in patients with different degrees of metastatic bone disease.

Relevant biochemical variables after antihypercalcaemic therapy are shown in table 7.2, on division of patients by the extent of metastatic bone disease. Although four distinct drug regimens were used, the relative proportion of patients treated with any one regimen was similar in each "metastatic" group (figures 7.8 - 7.10).

In each of the three groups, serum calcium, urinary Ca/Cr, OHP/Cr and  $Ca_E$  values fell significantly from the pre-treatment level. In the light tumour load group, serum phosphate fell and in the no metastases group,  $Na_E$  values fell.

In figure 7.11, serum calcium values and the components of hypercalcaemia are shown after antihypercalcaemic therapy, on division into three groups by the extent of metastatic bone disease. The only significant differences between the groups after antihypercalcaemic treatment were in terms of serum calcium values and the renal tubular component of hypercalcaemia, which were significantly lower in

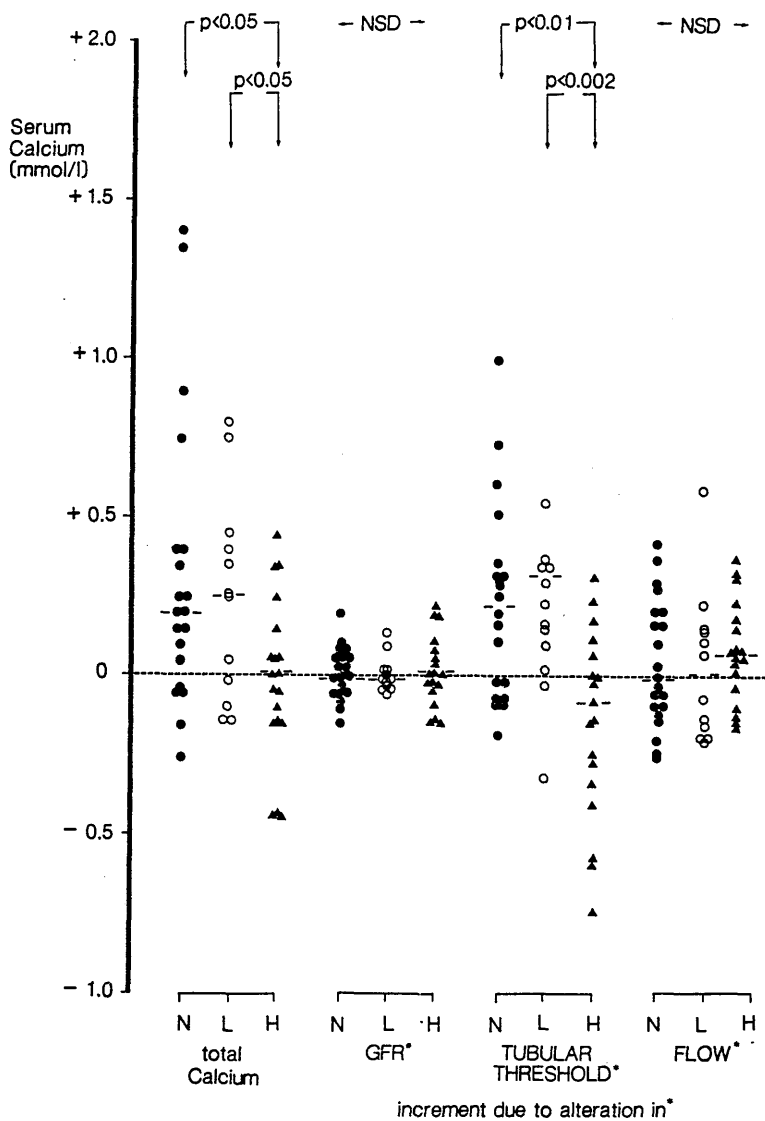
**TABLE 7.2**

**BIOCHEMICAL VARIABLES AFTER ANTIHYPERCALCAEMIC THERAPY**

	<u>NO DETECTABLE METASTASES</u>	<u>LIGHT SKELETAL TUMOUR LOAD</u>	<u>HEAVY SKELETAL TUMOUR LOAD</u>
<u>NUMBER</u>	20	12	18
<u>SERUM CALCIUM (mmol/l)</u>	2.70 <sup>+++</sup> (2.25-3.90)	2.75 <sup>+++</sup> (2.35-3.30)	2.50 <sup>+++ *</sup> (2.05-2.95)
<u>SERUM PHOSPHATE (mmol/l)</u>	0.70 (0.50-1.40)	0.77 <sup>+</sup> (0.45-1.05)	0.82 (0.55-1.95)
<u>SERUM CREATININE (µmol/l)</u>	77 (50-210)	72 (45-120)	72 (40-180)
<u>SERUM ALBUMIN (g/l)</u>	30 (21-40)	28 (24-35)	30 (24-41)
<u>URINE Ca/Cr (mmol/mmol)</u>	0.36 <sup>++</sup> (0.06-2.40)	0.45 <sup>++</sup> (0.07-2.80)	0.52 <sup>+++</sup> (0.13-1.53)
<u>Ca<sub>E</sub> (µmol/IGF)</u>	34 <sup>++</sup> (5-264)	33 <sup>++</sup> (3-196)	39 <sup>+++</sup> (8-116)
<u>TmPO<sub>4</sub> (mmol/IGF)</u>	0.55 (0.10-1.30)	0.70 (0.30-0.80)	0.50 (0.20-1.65)
<u>URINE OHP/Cr (µmol/mmol)</u>	39 <sup>++</sup> (15-147)	35 <sup>+</sup> (18-120)	41 <sup>++</sup> (14-112)
<u>Na<sub>E</sub> (mmol/IGF)</u>	2.07 <sup>++</sup> (0.24-6.24)	2.60 (0.10-5.84)	2.27 (0.10-3.85)

values are medians  
(range)

+++ p<0.001 from pretreatment values  
 ++ p<0.02 from pretreatment values  
 + p<0.05 from pretreatment values  
 \* p<0.05 from no metastases group

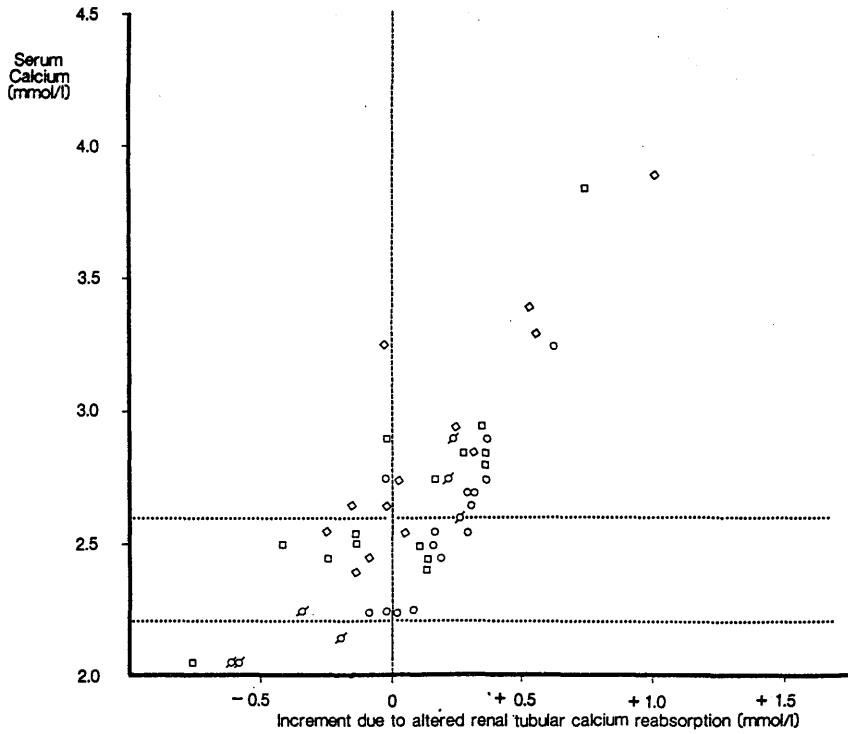


**FIGURE 7.11**

Pathogenic mechanisms of hypercalcaemia after administration of antihypercalcaemic therapy, on division by the extent of metastatic bone disease. Serum calcium values on abscissa are referred to a zero value of 2.50mmol/l. GFR- increment of hypercalcaemia due to impaired glomerular filtration rate; TUBULAR THRESHOLD- increment attributable to alteration in renal tubular calcium reabsorption; FLOW- increment attributable to increased calcium "flow" from bone resorption.

- ▲ -Heavy skeletal tumour load
- -Light skeletal tumour load
- -No detectable bone metastases

bars are medians. NS/NSD -no significant difference.



**FIGURE 7.12**

Relation between post-treatment renal tubular component of hypercalcaemia and serum calcium values after antihypercalcaemic treatment.

- - APD treated patients
- ⊙ - calcitonin/APD treated patients
- - mithramycin treated patients
- ◇ - corticosteroid/calcitonin treated patients

Horizontal dotted lines indicate reference range for serum calcium.

patients with a heavy skeletal tumour load ( $p < 0.05$ ).

On statistical analysis, only three pre-treatment biochemical variables correlated significantly with post-treatment serum calcium values. These were; serum calcium pre-treatment ( $r = 0.37$ ,  $p < 0.05$ ), serum phosphate pre-treatment ( $r = -0.39$ ,  $p < 0.02$ ), and the renal tubular component of hypercalcaemia pre-treatment ( $r = 0.61$ ,  $p < 0.001$ ). When patients were considered separately on the basis of the drug treatment given, there was a significant correlation between the renal tubular component of hypercalcaemia pre-treatment in the APD-treated patients ( $n = 14$ ,  $r = 0.65$ ,  $p < 0.01$ ), the APD/calcitonin-treated patients ( $n = 8$ ,  $r = 0.95$ ,  $p < 0.001$ ) and the corticosteroid/calcitonin treated patients ( $n = 13$ ,  $r = 0.65$ ,  $p < 0.01$ ). In mithramycin-treated patients, there was also a positive correlation, but this failed to reach statistical significance ( $n = 15$ ,  $r = 0.41$ ,  $p > 0.05$ ).

In figure 7.12, the relationship between post-treatment serum calcium values and the renal tubular component of hypercalcaemia post-treatment is shown. There was a highly significant correlation between these variables in the study group as a whole ( $r = 0.71$ ,  $p < 0.001$ ). Post-treatment serum calcium values and the post-treatment renal tubular component of hypercalcaemia were also significantly correlated when patients were divided by the type of treatment received; APD ( $r = 0.65$ ,  $p < 0.01$ ); APD/calcitonin ( $r = 0.78$ ,  $p < 0.05$ ); mithramycin ( $r = 0.82$ ,  $p < 0.005$ ), corticosteroid/calcitonin ( $r = 0.92$ ,  $p < 0.002$ ).

Other components of hypercalcaemia post-treatment which correlated with post-treatment serum calcium values were the post-treatment flow component of hypercalcaemia ( $r = 0.37$ ,  $p < 0.05$ ) in the study group as a



whole, and in the subgroup treated with APD alone ( $r=0.82$ ,  $p<0.005$ ). There was no significant correlation between the GFR component of hypercalcaemia and serum calcium values at any stage.

#### 7.3.4 DISCUSSION

In this study, patients without detectable bone metastases and those with a light skeletal tumour load had similar mechanisms of hypercalcaemia; renal tubular reabsorption of calcium and calcium "flow" from bone resorption were both increased, probably as the result of the renal tubular (chapter 3) and osteoclast-stimulating effects (5,93,139,140,141,142) of humoral mediators released by tumour tissue. In patients with a heavy skeletal tumour load, bone resorption was raised to a significantly higher level, possibly as the result of metastatic bone destruction (2,3,5,25). Serum creatinine values were also significantly higher in patients with a heavy skeletal tumour load, leading to a much higher filtered load of calcium per unit of glomerular filtrate when compared with those who had few or no metastases. This was offset by the generally lower levels of renal tubular calcium reabsorption, resulting in similar total serum calcium values in the three groups of patients.

The status of renal calcium handling in the hypercalcaemia of malignancy has been the subject of much debate in recent years. In early studies, renal tubular calcium reabsorption was found to be depressed (147,148), leading to the belief that hypercalcaemia of malignancy was mediated by a purely "bone-resorptive" mechanism, either as the result of metastatic bone destruction (2,3,25), or in association with humoral substances which stimulate osteoclastic bone resorption (64,77,78,93,105). The work previously presented in this

thesis suggests that renal tubular calcium reabsorption is frequently elevated in patients with cancer-associated hypercalcaemia, particularly in solid tumours such as squamous lung carcinoma. These observations indicate that hypercalcaemia in these tumour may be due, in part, to a PTH-like effect on the renal tubular calcium reabsorption. Recently, studies have suggested that humorally-mediated increases in renal tubular calcium reabsorption appear to be a common feature of malignancy-associated hypercalcaemia, irrespective of tumour type and extent of metastatic bone disease (304,305).

In the present study, the renal tubular component of hypercalcaemia was found to progressively increase as serum calcium values rose; all patients with serum calcium values of more than 3.00mmol/l had elevated renal tubular calcium reabsorption in absolute terms, using the method of calculation described by Nordin (279). Although this may have been due to a PTH-like effect on the renal tubule, it is perhaps more likely to have been due to an artefact introduced by the method of calculation. In clinical practice assessment of renal tubular calcium reabsorption is problematic; it depends on comparing data from normal subjects who have been rendered acutely hypercalcaemic by calcium infusion, with those in chronically hypercalcaemic patients who often have far higher serum calcium values (268). Current methods of assessment fail to take account of possible differences in urinary sodium excretion between acutely hypercalcaemic control subjects and chronically hypercalcaemic patients, even though alterations in urinary sodium excretion may profoundly affect urinary calcium excretion in clinical practice (176,266,268,283,298,305). Since tubular reabsorption of sodium and calcium are closely linked in the proximal renal tubule (267,268), it may be anticipated that, in

any given patient, filtered sodium load would have to rise in proportion with filtered calcium load to maintain renal tubular calcium reabsorption at a constant level, with increasing degrees of hypercalcaemia.

In this study, a standard protocol of sodium repletion was used, with the result that urinary  $\text{Na}_E$  values were similar in most patients, notwithstanding the severity of hypercalcaemia. Accordingly the progressive rise in renal tubular reabsorption of calcium which was observed with increasing hypercalcaemia may have been due, in part, to relative sodium deficiency, rather than a humorally-mediated increase in renal tubular calcium reabsorption. The apparent reduction in renal tubular calcium reabsorption which occurred in APD-treated patients with more severe hypercalcaemia lends support to this hypothesis, since APD therapy does not alter renal tubular calcium reabsorption in Paget's disease or osteoporosis (148). From the above considerations, it would appear that the algorithm used for calculation of renal tubular reabsorption of calcium is not accurate in patients with severe hypercalcaemia, using the current methods of sodium repletion.

Notwithstanding these doubts about the accuracy, in absolute terms, of the renal tubular calcium reabsorption data, information can be gained by comparisons of renal tubular calcium reabsorption between different patient-groups. Thus, the significantly greater fall in renal tubular calcium reabsorption which was observed in calcitonin and mithramycin-treated patients suggests that these agents did indeed possess a true inhibitory effect on renal tubular calcium reabsorption (235, 236, chapter 7.1). Moreover, the levels of renal

tubular calcium reabsorption were significantly different in different groups of patients with cancer-associated hypercalcaemia; in patients with no metastases and a light skeletal tumour load levels were higher than those in patients with a heavy skeletal tumour load. This suggests that in the latter, a humorally mediated increase in renal tubular calcium reabsorption was less likely to have contributed to the pathogenesis of hypercalcaemia. The variable response to antihypercalcaemic therapy also lends support to the hypothesis that the mechanisms of hypercalcaemia were different in different patient-groups. Serum calcium values after treatment were strongly related to the level of renal tubular calcium reabsorption both pre- and post-treatment, irrespective of the type of treatment given. This was also reflected by the fact that patients with a heavy skeletal tumour load (who had generally lower levels of renal tubular calcium reabsorption), also had a significantly better response to antihypercalcaemic therapy. It should also be noted however, that the calcium-lowering effect of decreased bone resorption was also enhanced in the heavy tumour load group by the greater degree of renal glomerular failure before treatment.

It has previously been recognised that the calcium-lowering response to diphosphonate therapy is incomplete in patients with humorally-mediated elevations in renal tubular calcium reabsorption (148,253). The current data suggest that, an incomplete response is also observed in this situation, when other antihypercalcaemic agents such as mithramycin and corticosteroids/calcitonin are used.

The inverse relationship which was observed between the extent of metastatic bone disease, impairment of glomerular filtration rate and

levels of renal tubular calcium reabsorption in this study is also of interest. It suggests that the hypercalcaemia in patients with a heavy skeletal tumour load may principally have been of the disequilibrium type (4), where increased bone resorption leads to severe hypercalcaemia because of intravascular volume depletion, with a resultant impairment of GFR and hyperphosphataemia (306). In patients with few or no metastases however, the hypercalcaemia more closely resembled that of primary hyperparathyroidism and was due to humorally-mediated increases in renal tubular calcium reabsorption (chapter 3), and bone resorption (5), but with preservation of a relatively normal GFR. These differences in the mechanisms of hypercalcaemia can partly explain the variable response to antihypercalcaemic therapy in malignant disease; elevated levels of renal tubular calcium reabsorption as the result of humoral aetiology appear to be associated with a particularly poor response.

CHAPTER 8

NEW THOUGHTS ON THE PATHOGENESIS  
OF MALIGNANCY ASSOCIATED HYPERCALCAEMIA:  
AN APPRAISAL OF THE CURRENT WORK IN RELATION  
TO EXISTING KNOWLEDGE

8.1 NEW THOUGHTS ON THE PATHOGENESIS AND MANAGEMENT  
OF MALIGNANCY-ASSOCIATED HYPERCALCAEMIA

The hypercalcaemia associated with malignancy has classically been attributed to the excessive release of skeletal calcium by accelerated osteoclastic bone resorption, either on a multifocal basis, due to metastatic bone disease, or on a systemic basis due to the osteoclast activating effects of circulating humoral mediators which are released by tumour tissue (5,50). A variety of substances have been invoked in the pathogenesis of cancer-associated hypercalcaemia including; circulating prostaglandins (64), factors which stimulate bone resorption by enhancing local production of skeletal prostaglandins (77,78), transforming growth factors (90,97,104,105), platelet-derived growth factors (90), epidermal growth factor (103), tumour necrosis factors (51), interleukin 1 (47), parathyroid hormone (PTH) (124,125,126,127,128) and  $1,25(\text{OH})_2 \text{D}_3$  (87,88,89). These factors share in common, the ability to stimulate bone resorption in tissue culture. However, with the exception of PTH and  $1,25(\text{OH})_2 \text{D}_3$ , none have been shown to be capable of causing hypercalcaemia when administered systemically, either because of their rapid metabolism or because of the efficient renal homeostatic mechanisms which tend to preserve normocalcaemia at the expense of hypercalciuria (4,5,90). Recent evidence has indicated that ectopic PTH production is an extremely rare cause of cancer-associated hypercalcaemia (93,130,131, 137,138). Similarly, excessive production of  $1,25(\text{OH})_2 \text{D}_3$  by tumour tissue is observed only in a small group of patients with hypercalcaemia due to certain lymphomas (87,88,89).

In recent years, much interest has focussed on the release of

"PTH-like " factors by tumours associated with the hypercalcaemia of malignancy (93,143,144,145,146). Although these factors are structurally and immunologically distinct from PTH (130,137), they appear to interact with adenylyl-cyclase linked PTH receptors in vitro (143,144,145,146). It has been proposed that tumours which release these factors may be clinically identified by raised urinary excretion of nephrogenous cyclic AMP (NcAMP) and reduced renal tubular threshold for phosphate excretion ( $\text{TmPO}_4$ ) - end organ effects which are similar to those encountered in patients with primary hyperparathyroidism (93). The relationship between release of these factors and the pathogenesis of hypercalcaemia has hitherto been unclear however, since the PTH-like factors are relatively weak stimulators of bone resorption in tissue culture (90,150). Moreover, they have thought to differ from PTH in failing to stimulate  $1,25(\text{OH})_2 \text{D}_3$  synthesis and have not been considered to cause an elevation in renal tubular calcium reabsorption (93). In other words, they have been thought to lack the very properties which account for hypercalcaemia in primary hyperparathyroidism (113,114,116).

The data presented in this thesis suggests however, that humorally-mediated elevations in renal tubular calcium reabsorption play a significant role in the pathogenesis of malignant hypercalcaemia (chapter 4.1). Such elevations in renal tubular calcium reabsorption were not due to coexistent primary hyperparathyroidism or ectopic production of PTH, since iPTH levels were generally suppressed. Dehydration and sodium depletion were similarly excluded as possible causes, firstly since careful efforts were made to ensure that patients were normally hydrated on clinical basis, and secondly, since the renal handling of calcium in relation to sodium was



indistinguishable from that in primary hyperparathyroidism (chapter 4.3, reference 305). Nor did the raised levels of renal tubular calcium reabsorption appear to be a non-specific effect of chronic hypercalcaemia per se, since renal calcium handling was generally normal in patients with benign non parathyroid hypercalcaemia (chapter 4.3) and in some patients with malignant hypercalcaemia due to breast carcinoma and myeloma (chapters 4.3, 5.2, 5.3, 7.3).

In marked contrast to the accepted dogma that accelerated bone resorption plays the principal role in cancer-associated hypercalcaemia, raised levels of renal tubular calcium reabsorption were the predominant cause of hypercalcaemia in patients with early tumours, where little evidence of raised bone resorption was found on a histological basis (chapters 6.1, 6.2). These data corroborate recent findings in the rat Leydig cell tumour model of humoral hypercalcaemia, which indicate that an elevation in renal tubular calcium reabsorption is the initial event in the pathogenesis of hypercalcaemia, occurring before bone resorption becomes elevated (302).

In agreement with the data of Stewart, who identified patients with humoral hypercalcaemia on the basis of their raised NcAMP excretion (93), PTH-like effects on renal calcium handling were most frequently noted in patients with squamous lung cancer and genitourinary cancers and were less apparent in patients with extensive metastatic disease due to breast carcinoma and myeloma (chapters 7.3, 4.1, 5.2, 5.3). Indeed, patients falling into the latter category tended to exhibit fewer "PTH-like" features (raised urinary cyclic AMP excretion, detectable  $1,25(\text{OH})_2 \text{D}_3$  levels reduced

TmPO<sub>4</sub> levels), when compared with those who had other solid tumours (chapter 5.2, 5,3).

Although 1,25(OH)<sub>2</sub> D levels were elevated to within the hyperparathyroid range in some patients with solid tumour hypercalcaemia (chapters 5.1, 5.2), intestinal calcium absorption was generally suppressed, suggesting that there was end organ resistance to the effects of the active vitamin D metabolite in this situation (chapter 5.2). The finding of detectable 1,25(OH)<sub>2</sub> D levels suggests however that, as in the Leydig cell tumour model of humoral hypercalcaemia (149), the putative PTH-like humoral factor in human humoral hypercalcaemia of malignancy may frequently possess a stimulatory effect on renal 1- $\alpha$ -hydroxylase activity.

In some patients with extensive metastatic bone disease due to breast carcinoma and myeloma, elevations in renal tubular calcium reabsorption were noted, as were other PTH-like features such as raised urinary cAMP excretion, and depressed TmPO<sub>4</sub> levels. It is probable that in some of these patients, the hypercalcaemia was due, in part to the renal tubular effects of PTH-like humoral mediators. However, since the elevations in renal tubular calcium reabsorption were generally less marked in patients with these tumours when compared with those in patients with squamous carcinoma and other solid tumours (chapters 4.2, 5.2, 5.3), they may have been partly due to inaccuracies introduced by the methods of calculating the renal tubular component of hypercalcaemia (chapter 7.3).

However, it is apparent from these and other studies (93), that hypercalcaemic cancer patients may no longer simply be classified on the basis of metastatic bone involvement since evidence for a humoral

aetiology can be found in the majority of cases, irrespective of the presence of metastatic bone disease. From the current studies, possible exceptions are patients with extensive metastatic bone disease due to myeloma and breast carcinoma, where hypercalcaemia may occur as the result of multifocal osteolysis, in combination with renal glomerular failure (chapter 7.3). In breast carcinoma, the latter occurs as the inevitable result of a disequilibrium hypercalcaemia (4) and in myeloma, there is an additional nephrotoxic effect of the immunoglobulin fragments (28).

One of the most interesting findings to emerge from these studies was the difference in the mechanism of hypercalcaemia between patients with early and advanced malignant tumours (chapter 6.2). It has now become clear that most cancers associated with hypercalcaemia produce factors which are capable of resorbing bone (90). The PTH-like factors however, while released by about 80% of tumours associated with hypercalcaemia, do not possess strong bone resorbing properties in vitro (150). The progressive increase in bone resorption which was noted with advancing disease in chapter 6.2 lends support to Mundy's suggestion that the hypercalcaemia of malignancy may be due to the interaction of a number of tumour-produced factors (90). It may be that the PTH-like factors are exhibited at an early stage and mainly determine the renal tubular abnormalities associated with the humoral hypercalcaemia syndrome. Subsequently other factors, possibly the transforming growth factors, may be released in increasing amounts, explaining the accelerated bone resorption. A further possibility which has not been considered previously and which was raised by the current studies, is that immobilisation may have acted in combination with the PTH-like factors to synergistically increase

bone resorption in patients with advanced tumours. The relevance of elevated levels of NcAMP excretion in normocalcaemic cancer patients is difficult to explain at present (152), but may be due to the release of other factors by the tumour, ectopic antidiuretic hormone for example, which enhance NcAMP production by binding to non PTH adenylyl-cyclase linked receptors in the kidney (277).

From the point of view of patient management, the present studies indicated that control of hypercalcaemia is worthwhile in patients with advanced malignant disease to improve symptoms although antihypercalcaemic therapy appeared to have little effect on patient survival (chapter 7.1). Indeed, the only long term survivors were those in whom the tumour was a stage which was amenable to surgical resection.

Since hypercalcaemia in patients with advanced disease was due, in part, to the calcium-elevating effects of accelerated bone resorption, osteoclast inhibitory drugs continue to play an important role in the management of hypercalcaemia in this situation (303). Although the second generation diphosphonates such as APD and  $\text{Cl}_2$  MDP (253) appear to be the most effective agents currently available in this respect, the calcium lowering response is limited in patients with humorally mediated hypercalcaemia due to the elevations in renal tubular calcium reabsorption. In view of this, future approaches to the management of cancer associated hypercalcaemia may well involve the combined use of inhibitors of bone resorption and other agents which inhibit the renal tubular component of hypercalcaemia such as calcitonin (chapter 6.2), mithramycin (Chapter 6.1) or the new cytoprotective agent WR 2721 (265).

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