Southwestern Medical School

Medical Grand Rounds

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HYPERCALCEMIA OF MALIGNANCY

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The most frequent consultation request the Mineral Metabolism Section receives is to assist in the diagnosis and management of hypercalcemia. Primary hyperparathyroidism and neoplasm constitute the most frequent causes for hypercalcemia, with an estimated incidence of 50 and 25 percent, respectively.[1] Last Grand Rounds I discussed the differential diagnosis of primary hyperparathyroidism. Today, I would like to focus on the pathogenesis, clinical presentation, diagnosis and therapy of hypercalcemia of malignancy.

Hypercalcemia is a common complication of malignancy, with an overall incidence of 10-20 percent.[2] Breast carcinoma is the most common malignancy associated with hypercalcemia in women and carcinoma of the lung is most common in men. Multiple myeloma, head and neck tumors, esophageal tumors and hypernephromas were other malignant lesions most often found to cause hypercalcemia in a prospective ongoing study at Los Angeles County, University of Southern California Medical Center.[2]

Hypercalcemia of malignancy traditionally has been divided into two groups on the basis of whether it is associated with invasion of the skeleton. In most patients (80-85 percent), skeletal metastases can be demonstrated.[2] Since malignant lesions are often osteolytic, the hypercalcemia has been explained on the basis of localized bone destruction by neoplasm. In some hypercalcemic patients (15-20 percent), however, no evidence for tumor can be found in bone. Hypercalcemia in this group is believed to result from the elaboration from the extraskeletal neoplasm of humoral substances that cause osteoclastic resorption.[3] It is apparent that this division is somewhat arbitrary since methods of detection of bone metastases in living subjects are not sufficiently sensitive to detect all instances of skeletal invasion, tumor present in bone may still produce hypercalcemic humoral factors, and local bone destruction by metastases may involve stimulation of osteoclastic resorption by humoral factors.

In this presentation, oncogenic hypercalcemia will be categorized according to the different forms of hypercalcemic factors elaborated. Such factors may be either humoral (extraskeletal) or local (skeletal) in origin. Evidence for direct osteolysis by malignancy will also be discussed.

Production of PTH-Like Substance by Neoplasm

In the search for humoral factors that cause hypercalcemia, initial emphasis was placed on measurements of the known calcium-regulating hormones. Although production of parathyroid hormone by non-parathyroid tumors is now believed to be uncommon,[4] it has been well documented in the literature in studies of tumor extracts (by immunofluorescent and immunoradiographic techniques as well as by radioimmunoassay),[5] arteriovenous gradients across the tumor bed,[6] and biosynthesis of hormone by tumor tissue in vitro.[7] Representative measurements from these types of studies are shown below in Fig. 1 and Table 1 from reference 6. In a patient with primary hepatoma, the circulating concentration of im-

munoassayable parathyroid hormone was elevated before and fell to normal after transplantation (Fig. 1). Immunologically reactive parathyroid hormone was demonstrated in extracts of the resected tumor. At operation, arteriovenous and venovenous gradients across the liver suggested that the hepatic tumor was actively secreting a parathyroid hormone-like polypeptide into the circulation (Table 1).

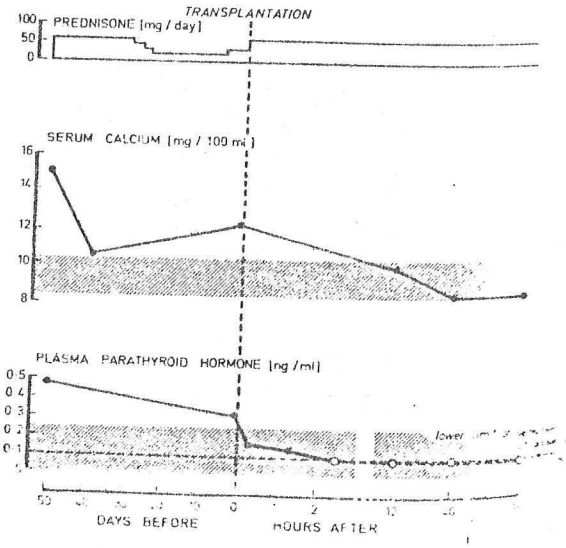


FIGURE 1 *Plasma Parathyroid-Hormone Levels before and after Hepatic Transplantation.*

TABLE 1 *Comparison of the Concentration of Parathyroid Hormone in the Arterial and Venous Supply to the Liver and the Venous Drainage from the Liver.**

CONCENTRATION (NG/ML)†			HEPATIC-VEIN-HEPATIC-ARTERY GRADIENT‡	HEPATIC-VEIN-PORTAL-VEIN GRADIENT‡
IN HEPATIC ARTERY	IN PORTAL VEIN	IN HEPATIC VEIN		
0.39 ± 0.015	0.20 ± 0.015	0.54 ± 0.018	0.15	0.34

*Parathyroid hormone expressed in terms of its bovine equivalent.

†Mean ± 1 SE of 3 dilutions in 3 separate assays.

‡All gradients significant — $p < 0.0001$ (Student's t test).

Since the initial demonstration of PTH in tumor extracts by Tashjian et al.,[8] the hormone has been reported to be present in extracts of at least 23 additional tumors as measured by radioimmunoassay. The production of PTH is more commonly encountered in "solid" tumors than in malignancies of the hemopoietic system. Malignancies of the kidney and lung are most commonly involved. Though initially considered rare, breast carcinoma has

also been implicated. Other associated solid neoplasms include carcinomas of the pancreas, colon, parotid gland, gallbladder, esophagus, bladder, penis, hepatoma and melanoma. Recently, the production of PTH has been described in soft tumors, including acute lymphocytic leukemia, acute lymphoblastic leukemia, undifferentiated lymphoma, acute myeloblastic leukemia, and acute myelofibrosis. The content of hormone in the neoplasms is generally less than 2 μg/g[2], whereas that in parathyroid adenoma is in the rage of 60[9] to 280 μg/g.[5] This perhaps explains why patients who present with hypercalcemia of malignancy, frequently have tumors large enough to be easily located.

The chemical nature of PTH elaborated by nonparathyroid neoplasm is not known. On the basis of available data, it is not possible to determine whether the substance of neoplastic origin is different from the native hormone or whether it undergoes different metabolic transformation or degradation. It is apparent, however, that the serum from patients with neoplasm contains different immunoreactive components (to PTH antiserum) than does serum from patients with primary hyperparathyroidism. The Mayo Clinic group compared the immunoreactive forms of parathyroid hormone in the plasma of 6 patients with primary adenomatous hyperparathyroidism and 6 patients with ectopic hyperparathyroidism due to non-parathyroid cancer by using gel filtration on columns of Bio-gel P-150.[10] They found much less carboxy-terminal fragments of immunoreactive PTH in plasma samples from patients with hypercalcemia of malignancy, although the amount of intact PTH 1-84 was the same in both groups. This finding was used to explain an earlier report that for any given value of serum Ca, serum immunoreactive PTH tended to be lower in the patients with oncogenic hypercalcemia than in patients with primary hyperparathyroidism.[11] Nevertheless, centers such as the Mayo Clinic[11] and Slatopolsky's St. Louis group[12] which utilize PTH antisera that are very sensitive to intact PTH and C-terminal fragments continue to report abnormally elevated serum iPTH (for the concomitant serum Ca level) in the majority of patients with hypercalcemia of malignancy. In Fig. 2 below from the Maylo Clinic,[11] serum immunoreactive PTH was increased in 95 percent of 108 unselected hypercalcemic patients with malignant disease.

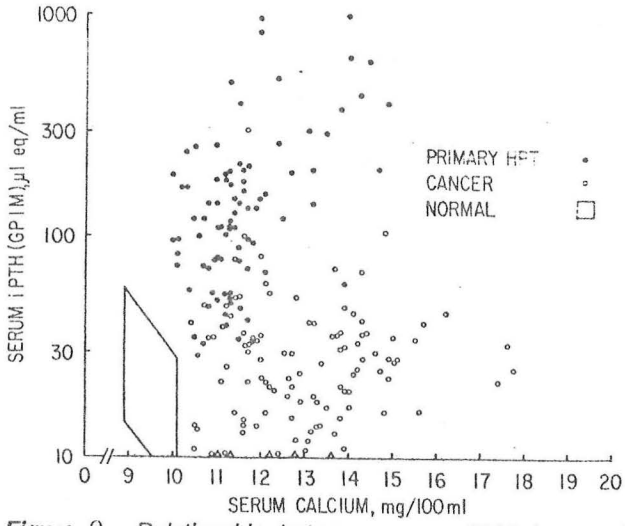


Figure 2 Relationship between serum iPTH (assayed using GP 1M) and serum calcium in primary hyperparathyroid (●) and in hypercalcemic patients with cancer (○). Serum iPTH is given in terms of equivalents of a standard hyperparathyroid plasma. For a given serum calcium value, serum iPTH was lower in patients with ectopic hyperparathyroidism and was undetectable (△) in 5 of the 108 patients with cancer. Shaded region indicates area in which normal values would fall.

Our own radioimmunoassay for PTH (Fig. 3) is a C-terminal assay and provides results very comparable to those of the Mayo Clinic. Normal individuals are depicted with open circles, patients with primary hyperparathyroidism are shown as closed circles, and the patients with hypercalcemia of malignancy are shown by asterisks. These types of assay results may be depicted schematically as in Fig. 4.[13] Although there is obviously a great deal of overlap, most primary hyperparathyroidism patients with serum Ca levels above 12, have significantly elevated values for iPTH, whereas the values for malignancy patients tend to remain in the high normal range. Since malignancy patients frequently present with serum Ca greater than 13 mg/dl, the finding of a PTH value in the normal range or only slightly above on the assay utilized here would be a helpful clue.

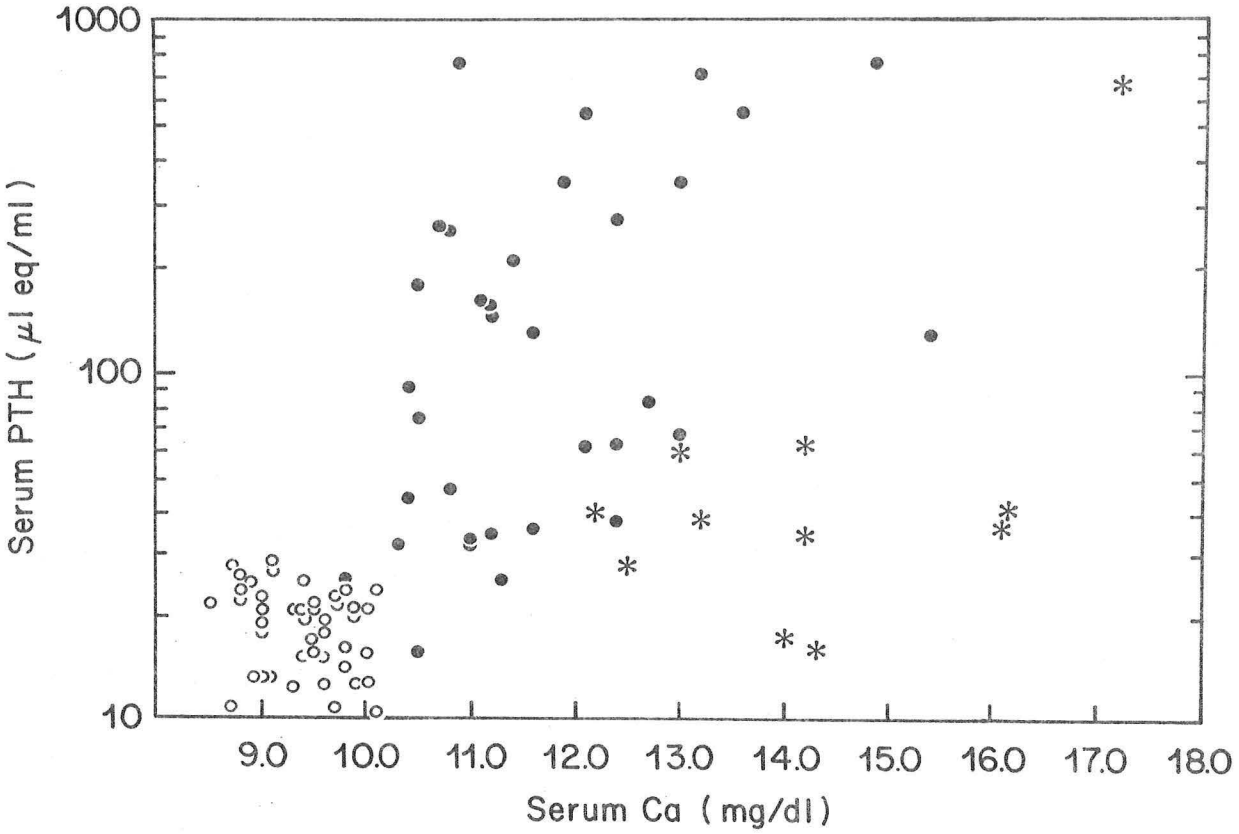

1000
100
10
Serum PTH (μl eq/ml)
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
17.0
18.0
Serum Ca (mg/dl)

Fig. 3.

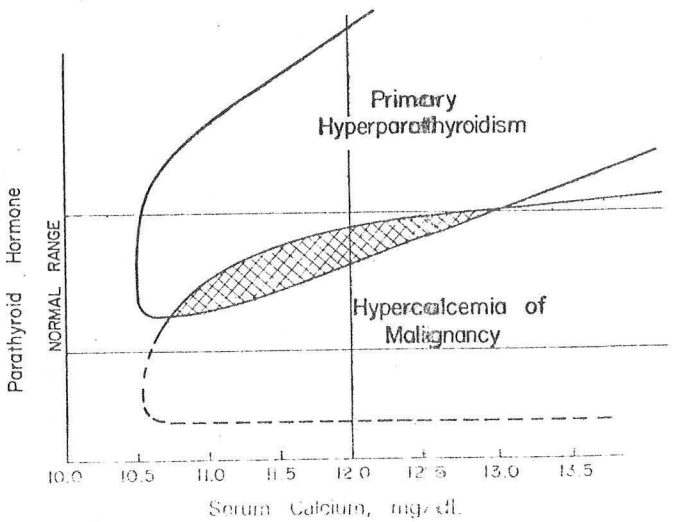


Figure 4. Schematic diagram of generalized pattern of immunoreactive parathyroid hormone and serum calcium values in patients with either primary hyperparathyroidism or hypercalcemia of malignancy. Most primary hyperparathyroidism patients with serum calcium levels above 12 mg/dL have elevated values for immunoreactive parathyroid hormone. Dashed line indicates the region of the assays where sensitivity and precision are poor.

Different assay systems for PTH may provide different results because their antiserum may have different antigenic recognition sites. Thus, the groups in Boston[14] and Los Angeles[15] generally find undetectable iPTH levels in their patients with hypercalcemia of malignancy (Fig. 5). Raisz et al.[16] recently compared several commercially available parathyroid hormone immunoassays on the differential diagnosis of hypercalcemia due to primary hyperparathyroidism or malignancy (Fig. 6.).

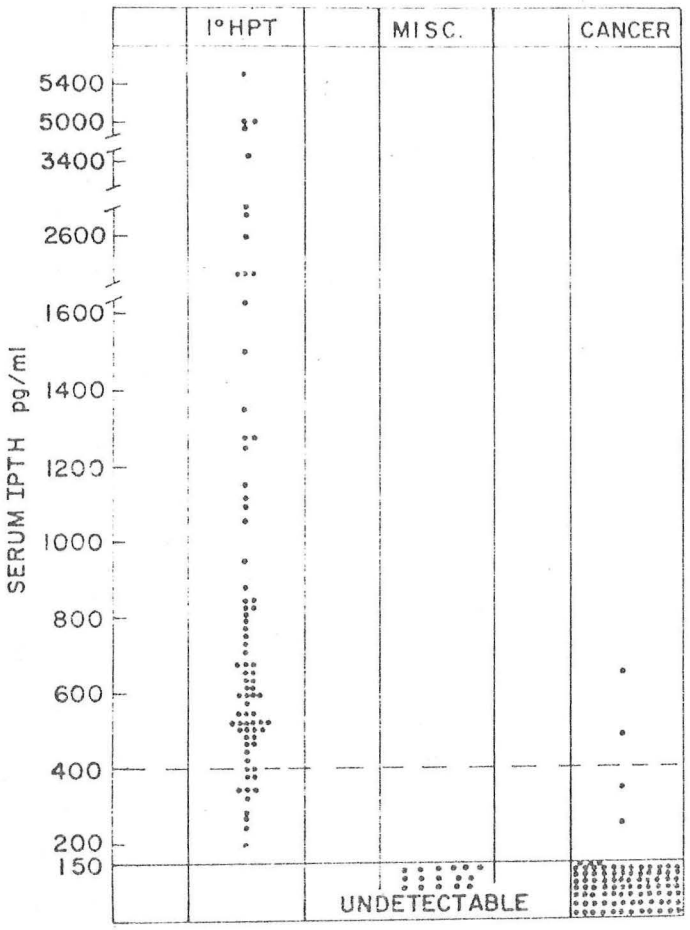


FIG. 5 Serum IPTH concentrations in patients with primary hyperparathyroidism, hypercalcemia and malignancy (CANCER column), and other hypercalcemic states (MISC. column), *i.e.* thyrotoxicosis (n = 8), sarcoidosis (n = 3), immobilization (n = 4), and the milk alkali syndrome (n = 4). ——, The level of detectability (150 pg/ml). – – –, The upper limit of normal (400 pg/ml).

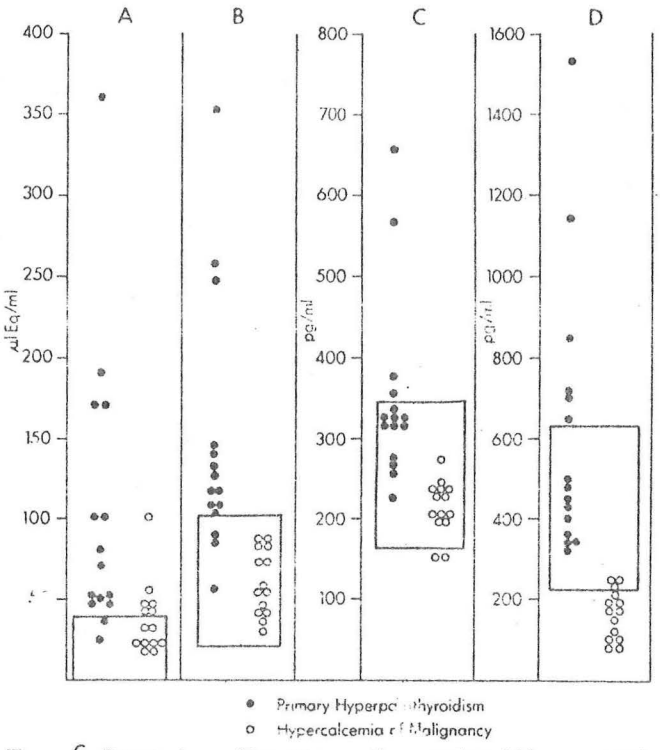


Figure 6 Comparison of immunoreactive parathyroid hormone values determined by four different laboratories in patients with primary hyperparathyroidism and hypercalcemia of malignancy. Outlined rectangles indicate the reference ranges for each assay.

A represents Mayo Medical Laboratories, Rochester Mn; B is Nichols Institute, San Pedro, Ca; C is Upjohn Company, Kalamazoo, Mi, and D is Bioscience Laboratory, Van Nuys, Ca. In assays A, B and C which measured circulating carboxy-terminal fragments and intact PTH (the most sensitive for diagnosing primary hyperparathyroidism) there was considerable overlap. In assay D which reacted largely with the amino terminal portion of the PTH molecule (least sensitive for diagnosing hyperparathyroidism), there was the least overlap and most oncogenic hypercalcemia patients had undetectable PTH. For any particular PTH assay system employed, its value in the diagnosis of neoplastic production of PTH requires comparison with the control group and with primary hyperparathyroidism at the same level of circulating Ca concentration.

The meaning of the circulating iPTH that is detected by certain assays (such as our own) in patients with hypercalcemia of malignancy is not entirely clear. Some patients may have coexistent primary hyperparathyroidism. The coincidence of malignant disease with primary hyperparathyroidism is higher than would be expected by the chance occurrence of these two diseases together: 10% to 30% of patients with primary hyperparathyroidism have had or will develop neoplastic disease of organs other than the parathyroids.[17] Any patient with hypercalcemia, neoplasia and significantly elevated iPTH, who is viable, certainly deserves to have a parathyroid venous drainage study of the neck. Second, animal studies indicate that the parathyroid glands continue to secrete small amounts of immunoreactive hormone even after sustained hypercalcemia for up to 2 days.[18] Whether secretion of iPTH would persist after long periods of hypercalcemic suppression is uncertain, but it might account for its detection in some patients with hypercalcemia of malignancy. In our preliminary screening of patients with hypercalcemia of malignancy, it is apparent that one patient with poorly differentiated carcinoma of the cervix had a serum iPTH far in excess of what we usually see (Table 2). Unfortunately, the patient was near-terminal, and PTH venous drainage study was not performed. A biopsy from the cervix did not grow in tissue culture. However, Dr. Zerwekh of our group is searching for other patients of this type in order to characterize the biochemistry and bioactivity of the "PTH-like substance" in vitro.

Table 2. Serum iPTH In Hypercalcemia of Malignancy (N=11) Compared to Primary Hyperparathyroidism (N=7)

Diagnosis	Number	Ca_S	Cr_S	iPTH
Primary Hyperparathyroidism	7	11.5±0.3	0.9±0.1	80±40
Histiocytic Lymphoma	2	15.0±1.0	5.2±1.4	29±11
Multiple Myeloma	2	13.2±1.1	2.8±1.4	46±20
Sq cell Ca Lung	6	13.9±0.6	1.2±0.2	41±7
Poorly Differentiated Carcinoma of Cervix	1	17.2	2.0	700

Diagnostically, the presentation of neoplastic hypercalcemia due to PTH secretion resembles primary hyperparathyroidism in that patients may present with a high renal phosphate clearance, hypophosphatemia and enhanced urinary cyclic AMP. This provides indirect evidence that the PTH of neoplastic origin can also cause phosphaturia and stimulate renal adenylate cyclase. Urinary calcium is generally elevated in the presence of normal renal function. Bone histology generally reveals increased resorption.[2] The presentation differs from primary hyperparathyroidism in the following general respects: (1) the onset of hypercalcemia is more sudden and more

rapidly progressive; (2) the degree of hypercalcemia and hypercalciuria is more marked, often exceeding 14 mg/dl and 600 mg per day, respectively; (3) symptoms of peptic ulceration, skeletal fractures and renal stones are usually absent; (4) serum alkaline phosphatase is more frequently elevated; and (5) although rarely measured, intestinal Ca absorption has been low or normal.[19] Serum 1,25-$(OH)_2D$ has not been systematically measured in cancer patients with high iPTH.

Finally, successful treatment of the neoplasm either by surgical or by medical means, has been found to restore normal serum calcium concentration and to reverse other biochemical abnormalities enumerated above. The fact that biochemical restoration may follow surgical resection of an extra-skeletal neoplasm provides the best evidence that the disorder is the consequence of ectopically produced PTH.

Neoplastic Production of "Non-PTH Renotropic Factor"

Recently, Broadus' Group has accumulated data that elevated nephrogeneous cyclic AMP may be a useful marker of humorally mediated cancer-associated hypercalcemia, that this type of hypercalcemia is common, and that the humoral factor responsible for this syndrome is not native 1-84 parathyroid hormone.[20] Nephrogenous cyclic AMP (NcAMP) was determined from fasting blood and urine samples in 50 patients with hypercalcemia of malignancy. There was a bimodal distribution of NcAMP excretion among these patients: 41 high and 9 low, with no overlap (Fig. 7). The high NcAMP group tended to include "solid" tumors such as squamous carcinomas of head, neck and lung; renal and bladder tumors. The low NcAMP group were mainly breast, lymphoma and myeloma--tumors generally associated with local bone resorption.

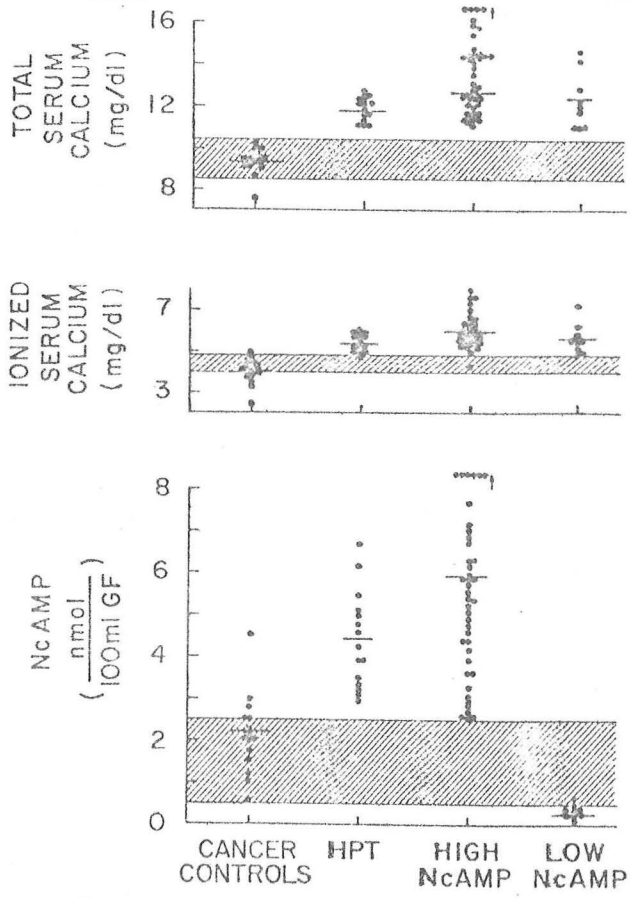


Figure 7 Total Serum Calcium, Ionized Serum Calcium, and Nephrogenous Cyclic AMP (NcAMP) in the Four Patient

Among the patients in the high NcAMP group, 39 of 41 had depressed renal P thresholds, whereas 6 of 9 patients in the low NcAMP group had normal thresholds (Fig. 8). Patients with both types of cancer-associated hypercalcemia had higher values for fasting calcium excretion than either normocalcemic controls with cancer or patients with primary hyperparathyroidism (Fig. 9). Mean values for 1,25-$(OH)_2D$ were elevated in patients with primary hyperparathyroidism, but were markedly depressed in both groups with hypercalcemia and cancer (Fig. 10).

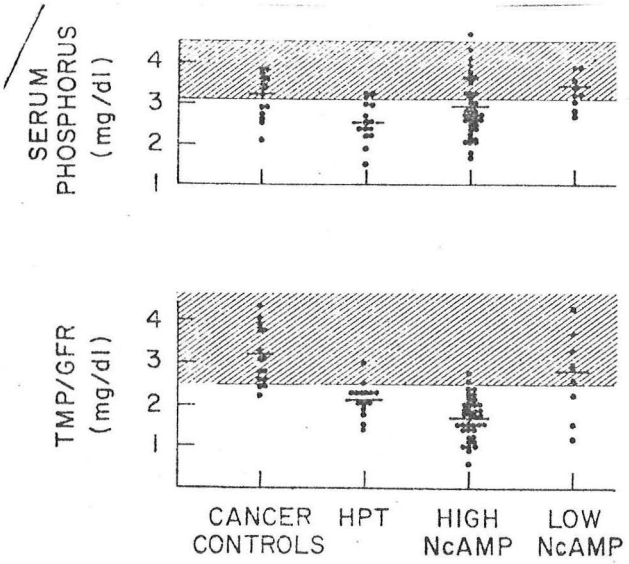


Figure 8 Serum Phosphorus and Renal Phosphorus Threshold in the Four Groups of Patients.

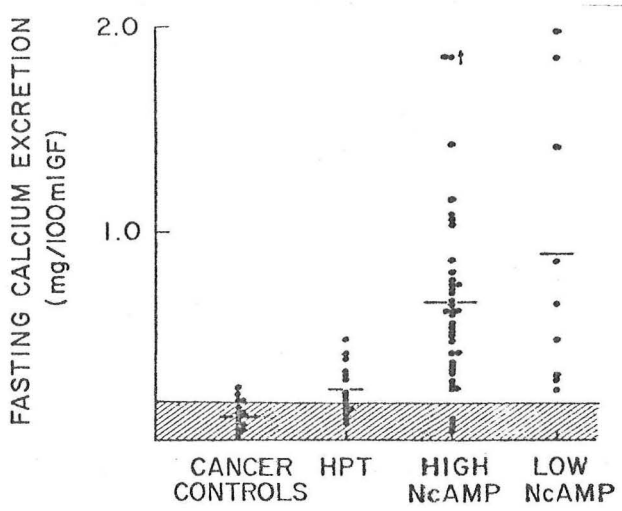


Figure 9 Fasting Calcium Excretion in the Four Patient Groups.

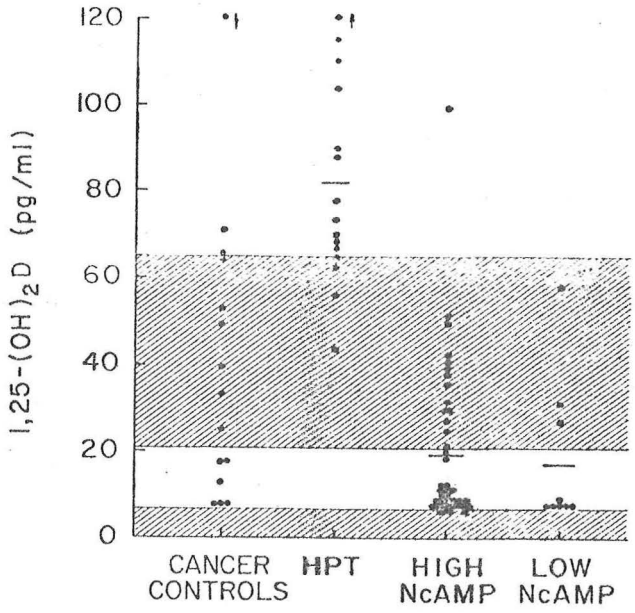


Figure 10 Levels of 1,25-dihydroxyvitamin D in the Four Patient Groups.

Fig. 11 shows the results of four region-specific parathyroid hormone radioimmunoassays, with value expressed as a percentage of the upper limits of normal for comparison. Patients with primary hyperparathyroidism generally had frankly elevated values, whereas both groups of patients with cancer-associated hypercalcemia had low to undetectable values (except for the multivalent assay GP 101). Bone scans were generally more positive in the low NcAMP cancer group than in the patients with high NcAMP.

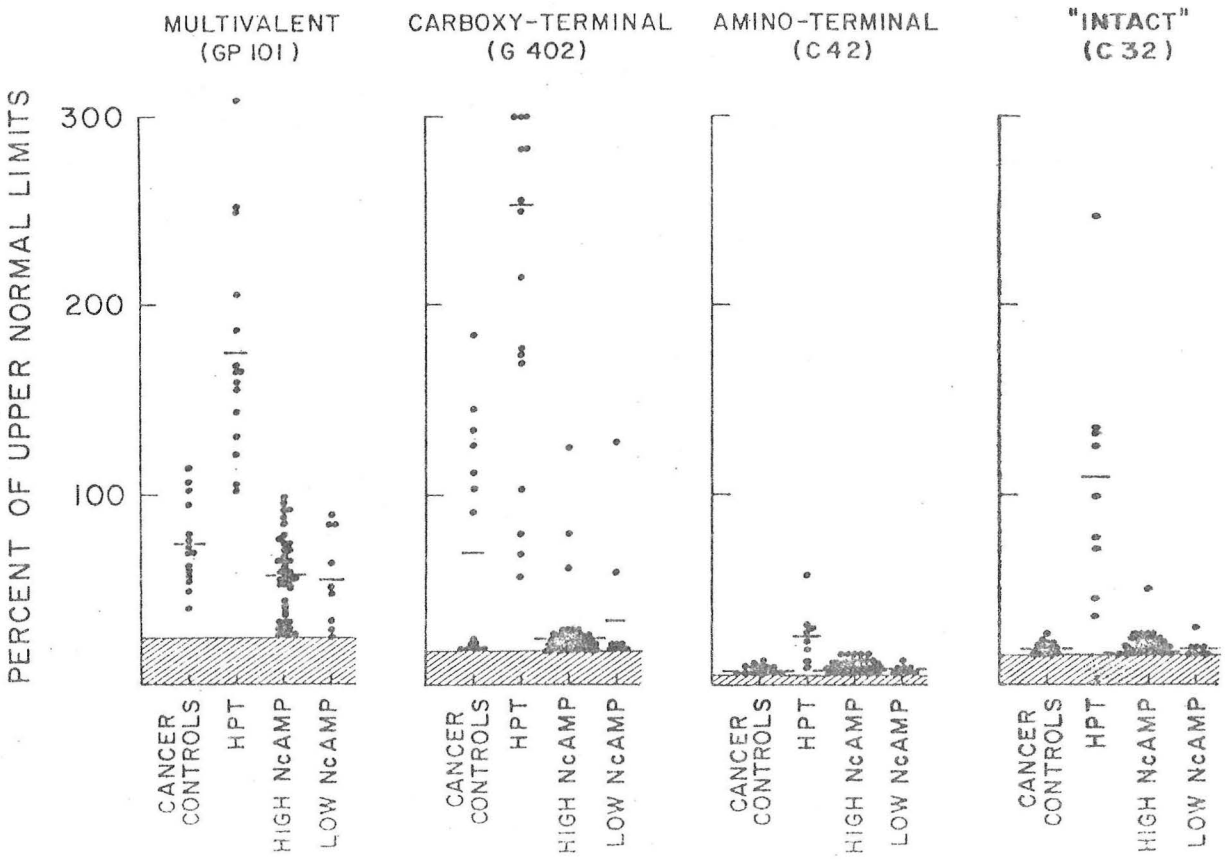


Figure 11 Results of Four Parathyroid-Hormone Radioimmunoassays in the Four Patient Groups.

Finally, separation into the groups described has recently been independently corroborated by a cytochemical bioassay for parathyroid-hormone-like humoral activity.[21] The index of bioassay response, glucose-6-phosphate dehydrogenase (G6PD) activation in renal tubular cells, was shown to be stimulated by cAMP. In this assay, mean cytochemical activity was 10 times higher in plasma from patients in the high NcAMP group than in plasma from patients in the low NcAMP group (Fig. 12).

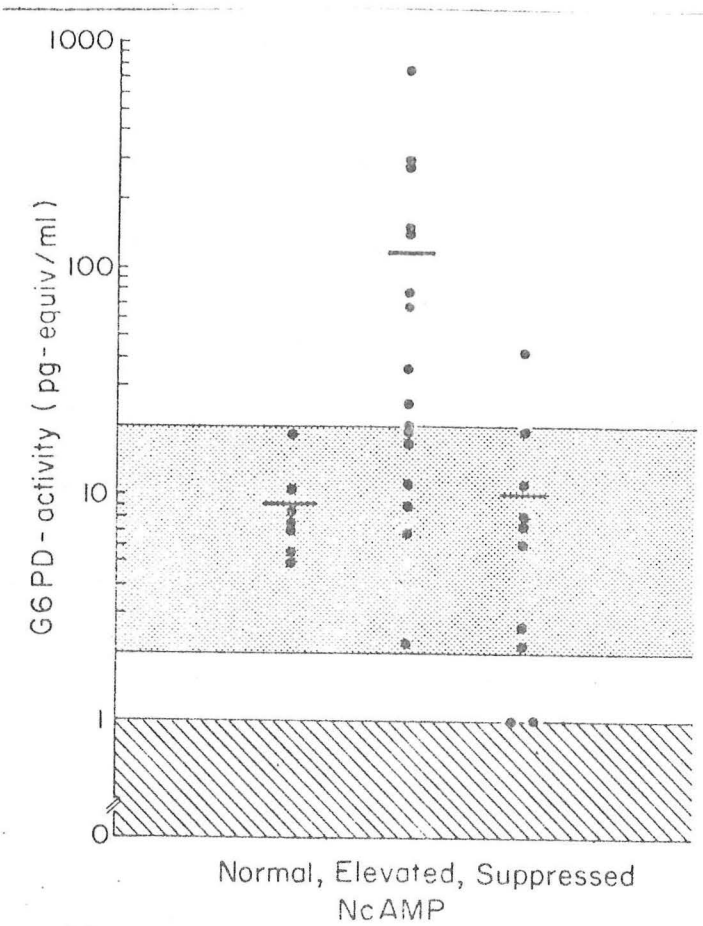


FIG. 12 Levels of G6PD activity in the plasma of patients with malignancies and normal, elevated, or suppressed excretion of NcAMP. Patients with normal excretion of NcAMP were normocalcemic, whereas the other 2 groups were hypercalcemic. G6PD levels were determined by cytochemical bioassay, as described in *Materials and Methods,* and were expressed as picogram equivalents per ml relative to a PTH standard. Each *point* represents the mean of 20 measurements from duplicate sections. The *horizontal line* indicates the mean level in each group, the *shaded region* indicates the normal range (11), and the *hatched area* represents levels below the detection limit of the assay.

The data presented suggest that patients in the high NcAMP group have a circulating renotropic factor that resembles native parathyroid hormone in its ability to stimulate proximal tubular adenylate cyclase and G6PD activity and to inhibit proximal tubular phosphate reabsorption; however, the factor differs from native parathyroid hormone in its relative inability to stimulate distal tubular calcium reabsorption, its inability to stimulate 25(OH) vitamin D 1-α-hydroxylase, and its diminished or absent reactivity with a variety of region-specific parathyroid-hormone anti-erums. The marked reduction of $1,25\text{-}(OH)_2D$ in the humoral hypercalcemia of malignancy patients was particularly striking, since this metabolite is increased in the vast majority of patients with primary hyperparathyroidism. This finding supports the data of Coombes et al.[19] on fractional Ca absorption and suggests that calcium absorption via the gastro-intestinal tract is not a prominent source of calcium entry into the extracellular pool in patients with cancer-associated hypercalcemia (in contrast to the findings in patients with primary hyperparathyroidism). The data also sug- gest that the final common pathway of hypercalcemia in both groups with hypercalcemia and cancer appears to be bone resorption, in that intestinal absorption and re-

nal Ca reabsorption appear to be depressed. In a preliminary study comparing bone histomorphometry in humoral hypercalcemia of malignancy (N=5) vs primary hyperparathyroidism (N=7) there were striking differences.[22] As compared to patients wtih hyperparathyroidism, those with humoral hypercalcemia of malignancy had 3-fold greater bone resorption, marked reduction in bone formation, lower bone volume, and complete uncoupling of osteoclast/osteoblast activity. Urinary hydroxyproline was also markedly elevated compared to nomocalcemic cancer patients.

Additional support for the non-PTH renotropic factor has been obtained in a patient with ovarian cancer and hyercalcemia. This patient had undetectable iPTH by a multivalent radioimmunoassay, elevated NcAMP excretion (5.41 nmol/100 ml GF, normal <2.5) and elevated plasma bioactivity by the cytochemical bioassay (740 pg eq/ml, normal <25). Surgery resulted in a clinical and biochemical cure, with post-operative nomocalcemia, normal NcAMP excretion (1.68 nmol/100 ml GF) and normal plasma bioactivity (25 pg eq/ml). This patients ovarian carcinoma was grown in tissue culture. Cytochemical bioactivity (132 pg eq/ml) was demonstrable in the culture medium harvested and pooled after 10 days of growth. This material also contained marked activity in a bone-resorbing assay. PTH immunoreactivity was undetectable in this medium. No PTH immunoreactivity or cytochemical bioactivity was detectable in control culture medium.

Additional proof that the humoral hypercalcemia factor which raises NcAMP is not native PTH (1-84) derives from gel filtration analysis and immunoabsorption studies. Gel chromatographic analysis revealed that a major component of plasma bioactivity eluted before rather than with PTH (1-84) in patients with malignancy in contrast with that in patients wtih primary hyperparathyroidism (Fig. 13).[21] Of 3 plasma samples tested in which activity eluted before bPTH (1-84), prior incubation with PTH antiserum only partly reduced the activity in each (Table 3), whereas the plasma activity of the patient with primary hyperparathyroidism was almost completely inactivated by similar incubation with antiserum.

TABLE 3 Effect of preincubation with anti-PTH serum on cytochemical bioactivity in patients with hyperparathyroidism or malignancy with hypercalcemia and increased NcAMP

Patient no.	Activity	
	pgeq/ml	% Remaining
1. Hyperparathyroid[a]	430	
+ antiserum[b]	4	<1
2. Malignancy	150	
+ antiserum	102	68
3. Malignancy	35	
+ antiserum	24	69
4. Malignancy	860	
+ antiserum	456	53

Samples were assayed after preincubation in the absence ([a]) or presence ([b]) of AS211/32, as described in *Materials and Methods*.

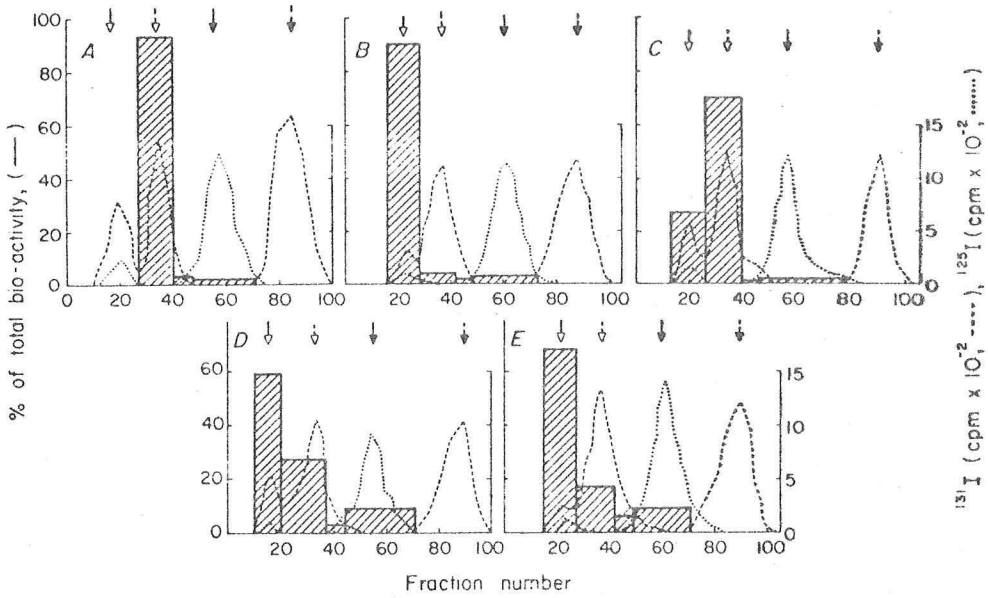


FIG. 13 Gel filtration profiles of G6PD activity (bioactivity) after chromatography on Bio-Gel P-100 of plasma from one patient with primary hyperparathyroidism (A) and four patients with hypercalcemia, malignancy, and elevated NcAMP excretion (B–E). ^{131}I and ^{125}I profiles were obtained by chromatographing the 3-ml plasma samples with labeled intact PTH, [^{131}I]bPTH-(1-84), labeled active PTH fragment, [^{125}I]hPTH-(1-34), and salt ([^{131}I]Na). In each panel, *vertical arrows from left to right* denote, respectively, the elution position of the void volume (↓), labeled intact PTH (↓), labeled active fragment (↓), and salt (↓). Details of chromatography and cytochemical G6PD bioassay are provided in *Materials and Methods*. The *height of the hatched bars* indicates the percentage of total bioactivity represented by pooled eluted fractions, and the *width of the bars* indicates the size of the pools assayed.

The nature of the material responsible for the NcAMP and cytochemical bioactivity is unclear. The bioactivity is unlikely to be related to prostaglandins in that prostaglandins E_1 and E_2 are not believed to raise urinary cyclic AMP excretion in man and are not detected by the cytochemical bioassay. Some might argue that the "non-PTH renotropic" factor is actually a species of PTH. After all, in some radio-immunoassays (Fig. 11) serum immunoreactive PTH is detectable in these patients, although lower than in primary hyperparathyroidism. This situation resembles that found in ectopic PTH production in which the "hormone" produced may not be immunologically as reactive as the native hormone by the particular assay system used. The humoral substance was 30-40% inactivated by preincubation with PTH antiserum. As indicated, the material shares many characteristics of PTH action, including osteoclastic bone resorption, reduction of renal tubular P threshold, stimulation of NcAMP and reactivity in the cytochemical bioassay. However, attempts at preliminary characterization by gel chromatography provided evidence that the material was not analogous to the characteristic form of PTH circulating in hyperparathyroidism, and was inactivated only partially with PTH antiserum. In

addition, heterogeneous patterns were observed, as has been reported to occur in other ectopic humoral syndromes.[23] It is certainly possible that the humoral mediator is an altered form of PTH with selective target tissue effects, lacking the ability to stimulate 25-hydroxy-D_3 1α-hydroxylase activity and to enhance Ca reabsorption in the distal tubule. There is a beginning awareness in endocrinology that the endocrine system may be more complex than specific glands producing specific hormones. Rather, a multitude of tissues may be capable of producing a variety of polypeptides, each with specific and selective activities. The chemical nature of the material reactive in the bioassay now requires further elucidation. Dr. Broadus informs me that he is planning to take a sabbatical and work with Henry Kronenberg at the Massachusetts General to define the chemistry of this "humoral hypercalcemic factor".

Neoplastic Production of Vitamin D-like Sterols

Breast cancer patients may have hypercalcemia with normal or high levels of serum P in the absence of osseous metastases. The hypercalcemia generally responds well to adrenal cortocosteroid therapy. These findings led to the suggestion that breast cancers may produce vitamin D-like osteolytic sterols. An initial report that lipid extracts of breast cancer tissue contained an osteolytic sterol similar to 7-dehydrocholesterol created a great deal of excitement.[24] Subsequently, the osteolytic sterols were identified as phytosterols and phytosteryl esters, and were extracted from the plasma of patients with breast carcinoma. However, equivalent circulating concentrations of such sterols were found in normal women and patients with breast cancer, and in hypercalcemic patients and normocalcemic patients with breast cancer.[25] Extripation of the breast cancer tissue in one patient resulted in no change in circulating phytosterol levels.[25] It is now believed that these plant-derived vitamin-D like sterols are simply consumed in the diet and stored in fat tissues. Breast cancer, normal breast and non-mammary adipose tissues were found to contain comparable amounts of phytosterols (Table 4). Such sterols are unlikely to play an important role in the pathogenesis of hypercalcemia in breast cancer.

CIRCULATING PHYTOSTEROLS

TABLE 4. Phytosterol concentrations in human tissues (ng/g tissue)

Patient	Tissue	Campesterol	Stigmasterol	β-Sitosterol
LS	Breast carcinoma (surgery)	2,280	437	4,836
KW	Breast carcinoma (surgery)	991	1,207	7,451
BG	Breast carcinoma (surgery)	3,400	1,787	7,684
HF	Breast carcinoma (autopsy)	1,015	236	2,040
HA	Buttock fat (surgery)	2,620	561	2,247
MT	Abdominal wall fat (autopsy)	2,025	1,116	5,260
MT	Normal breast (autopsy)	3,674	2,088	4,843
SN	Normal breast (autopsy)	1,667	844	2,304
SN	Normal bile (autopsy)	18,686	3,993	20,255

Nevertheless, the possibility that vitamin D-like sterols may be involved in certain types of oncogenic hypercalcemia has not been adequately explored. The current availability of sensitive radio-receptor assays for the various circulating vitamin D metabolites should permit large scale screening of patients with hypercalcemia of malignancy. The preliminary results of such a study initiated at this institution are shown in Table 5.

Table 5. Serum 1,25-$(OH)_2D$ In Hypercalcemia of Malignancy (N=9) Compared to Primary Hyperparathryoidism (N=7)

Diagnosis	Ca_S	Cr_S	iPTH	1,25-$(OH)_2D$
Primary Hyperparathyroidism	11.5±0.3	0.9±0.1	80±40	73±9
Histiocytic Lymphoma	15.0±1.0	5.2±1.4	29±11	72±0
Multiple Myeloma	13.2±1.1	2.8±1.4	46±20	10
Sq cell Ca Lung	13.9±0.6	1.2±0.2	41±7	18±6
Poorly Differentiated Carcinoma of Cervix	17.2	2.0	700	37

Several features of this table are of interest. The normal range of serum 1,25-$(OH)_2D$ is 20-50 pg/ml. We generally find high levels in patients with primary hyperparathyroidism. In 7 patients with assorted types of hypercalcemia of malignancy, exclusive of the 2 patients with histiocytic lymphoma, the serum 1,25-$(OH)_2D$ level was 19±5 pg/ml (mean ± SE). This low level of circulating 1,25-$(OH)_2D$ in patients with hypercalcemia of malignancy, is almost identical to the low level reported by Broadus' group in such patients (20 pg/ml).[20] Studies of serum vitamin D metabolites in hypercalcemic cancer patients with high iPTH levels have not yet been reported, so it is of interest that the patient with carcinoma of the cervix and a very high immunoreactive PTH level had only normal serum 1,25-$(OH)_2D$. Of greatest interest was the finding of high serum 1,25-$(OH)_2D$ levels in 2 patients with histiocytic lymphoma, raising the possibility of production of this vitamin D metabolite by the tumor. An abstract summarizing our findings in these 2 patients has been submitted for the spring research meetings and is appended here:[26]

HYPERCALCEMIA CAUSED BY HIGH SERUM 1,25-DIHYDROXYVITAMIN D IN TWO PATIENTS WITH HISTIOCYTIC LYMPHOMA. N Breslau,* J McGuire,* J Zerwekh,* E Frenkel,** and C Y C Pak**, Department of Medicine, University of Texas Southwestern Medical School, Dallas, Texas.

The cause of hypercalcemia in 2 patients with histiocytic lymphoma (A and B) was explored:

	Ca mg/dl	C_{Cr} ml/min	PTH (C-terminal assay) µl-eq/ml	1,25-$(OH)_2$D pg/ml	Frac. Intes. ^{47}Ca Abs
Normal	8.5-10.5	80-120	10-30	20-50	.39-.60
A	14.0	18	18	77	.94
B	16.0	8	39	72	-

Despite chronic renal failure and relatively suppressed serum parathyroid hormone (PTH) levels, both patients had increased serum 1,25-$(OH)_2$D. The high serum 1,25-$(OH)_2$D, determined by radioreceptor assay, differed from other patients with hypercalcemia of malignancy (19±5 pg/ml, mean±SE, N=7). Fractional intestinal ^{47}Ca absorption (α), measured in patient A, was increased. Neither patient responded to indomethacin. In response to prednisolone 50 mg daily for 7 days, patient A decreased serum Ca to 11.4, serum 1,25-$(OH)_2$D to 34 and α to .82. As the bone marrow became hypocellular after chemotherapy, serum Ca decreased to 10.5, serum 1,25-$(OH)_2$D to 28 and α to .28. When tumor recurred, serum Ca rose to 12.4, 1,25-$(OH)_2$D to 68 and α to .73. In patient B, prednisolone reduced an inguinal mass, and decreased serum Ca to 9.5 and 1,25-$(OH)_2$D to 43. Post-mortem exam revealed normal parathyroid glands and no evidence of sarcoidosis or tuberculosis. In summary, 2 patients with histiocytic lymphoma had high serum 1,25-$(OH)_2$D and hypercalcemia despite renal failure. The correction of these abnormalities by steroids suggests that an extrarenally produced 1,25-$(OH)_2$D may be responsible for hypercalcemia.

It should be emphasized that the hypercalcemia, increased serum 1,25-$(OH)_2$D, and increased intestinal Ca absorption of primary hyperparathyroidism generally does not respond to glucocorticoids.[27] The pattern seen in the 2 patients with histiocytic lymphoma more closely resembles that found in sarcoidosis,[28] a condition for which extra-renal generation of 1,25-dihydroxyvitamin D has been demonstrated.[29] Other extra-renal tissues that were recently proved capable of extra-reanl 1-hydroxylation of 25-(OH)D include bone cells[30] and placenta.[31] In vitro confirmation of this capability has been obtained only for the latter two tissues.

Neoplastic Production of Prostaglandins

The potentially important role of prostaglandin in the pathogenesis of oncogenic hypercalcemia was first suggested by studies in vitro and in experimental animals. Prostaglandins of the E series (PGE) stimulate skeletal adenylate cyclase[32] and augment bone resorption in tissue culture.[33] Certain malignancies of experimental animals that cause hypercalcemia (mouse fibrosarcoma[34] and rabbit carcinoma[35]) also produce PGE_2. Inhibition of PGE_2 synthesis in these animals by indomethacin restores normal serum Ca, commensurate with a reduction in serum PGE_2.

There is now substantive evidence that prostaglandins may exert a similar role in the development of hypercalcemia in certain human neoplasms.[2] The most extensive studies of the role of prostaglandins in the hypercalcemia of malignancy have been carried out by Seyberth et al.[36,37] Figs 14-17 derive from this work:

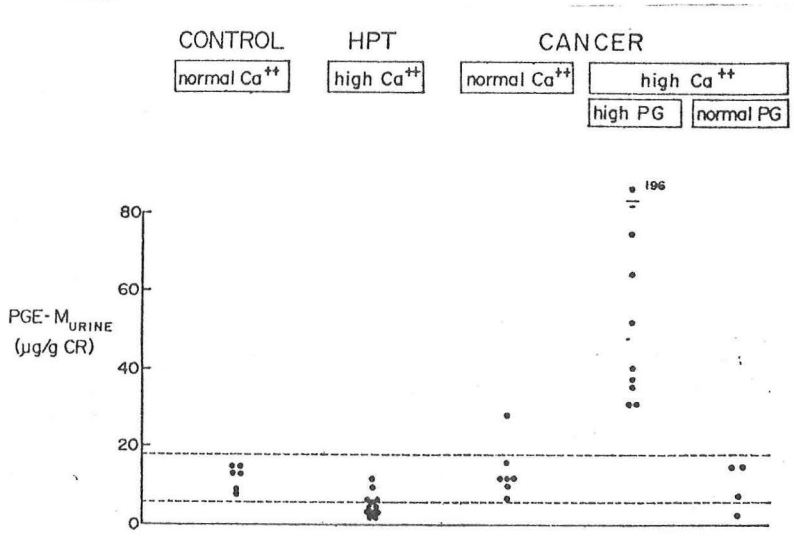


Fig. 14.

Urinary PGE metabolites (PGE-M) were measured in the urine of cancer patients by mass spectroscopy. Hypercalcemic cancer patients were divided into two subsets: those with normal PGE-M and those who had an increased excretion of this metabolite. All the hypercalcemic cancer patients had undetectable iPTH (by the multivalent Boston radioimmunoassay GPY). Those with high urinary PGE-M had normal or reduced urinary cyclic AMP. Those with normal PGE-M had elevated urinary cyclic AMP, thus resembling patients with "non-PTH renotropic" factor.

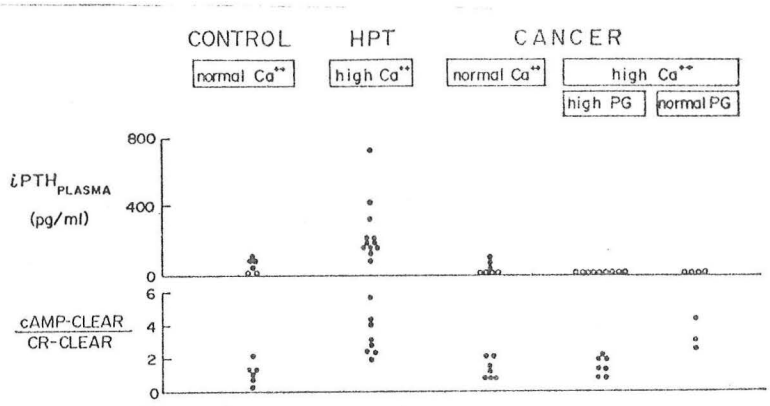


Fig. 15.

Hypercalcemic cancer patients with high urinary PGE-M excretion, who did not have bone metastases, responded very nicely to prostaglandin synthesis inhibitors. Patients with high urinary PGE-M excretion, who did have bone metastases (not shown) only had a partial response and were unable to decrease serum Ca to normal. Perhaps the local production of PGE by bone metastases is too high for the effects to be completely overcome by aspirin or indomethacin, or perhaps these drugs do not concentrate well at the site of bone metastases. Alternatively, direct bone metastases may invoke a process that is not PGE-mediated.

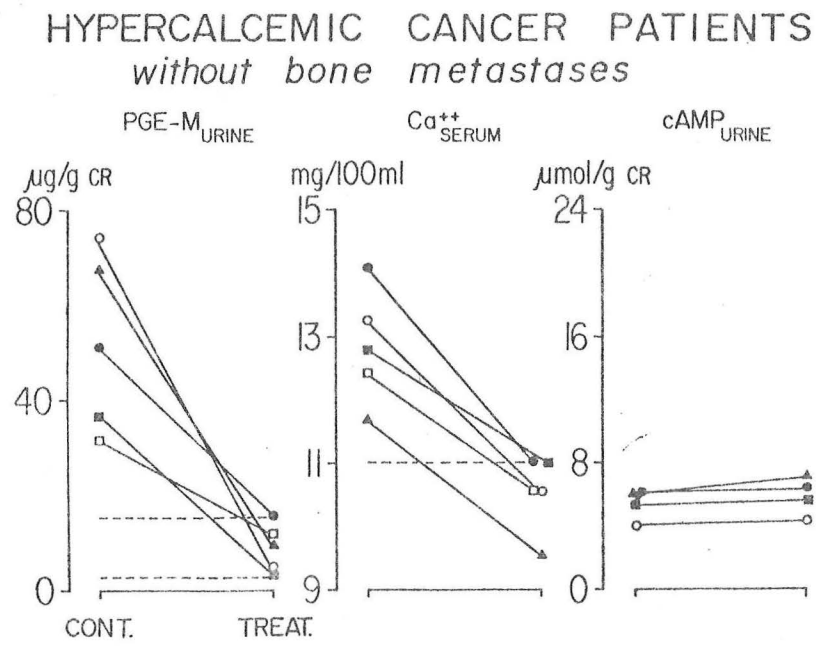


FIG. 16 Effect of indomethacin (open symbols) and aspirin (solid symbols) after three to five days of treatment on the urinary excretion of PGE-M, serum calcium concentration and urinary cyclic AMP excretion in five hypercalcemic cancer patients without bone metastases: Squamous-cell carcinoma of the lung, cervix, and pancreas, undifferentiated cell carcinoma of the lung, and metastatic adenocarcinoma of unknown origin. The areas between or under the broken lines represent the normal range.

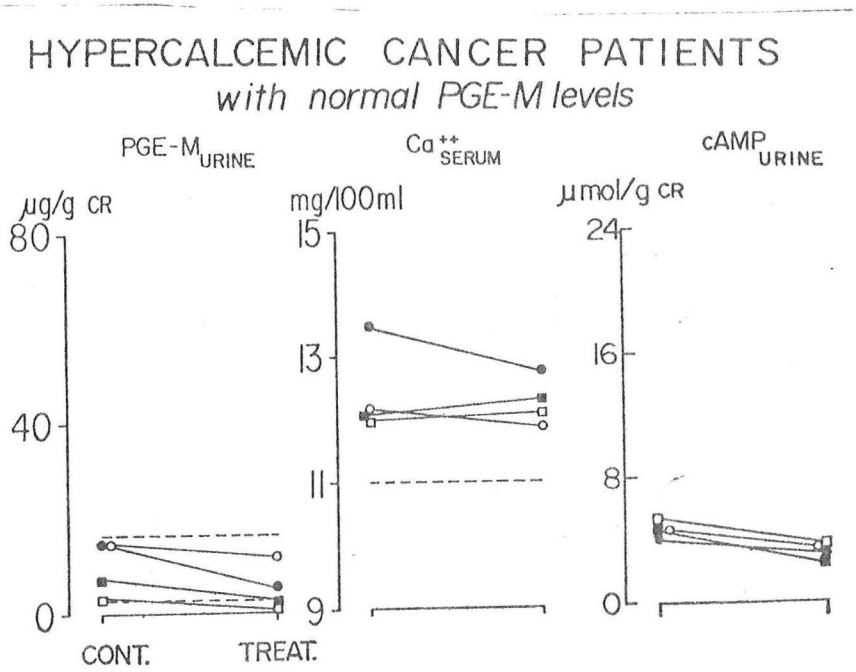


FIG. 17 Effect of indomethacin and aspirin in four hypercalcemic cancer patients, two with and two without bone metastases: Two times squamous-cell carcinoma of the lung, undifferentiated-cell carcinoma of the lung, and metastatic undifferentiated-cell carcinoma of unknown origin. (For further details see legend to Fig. 3.)

Hypercalcemic cancer patients with normal urinary PGE-M excretion did not respond to prostaglandin synthesis inhibitors. These observations taken together strongly support the concept of prostaglandin-mediated hypercalcemia in some patients with humoral hypercalcemia of malignancy.

There is still some question as to whether ectopically produced prostaglandins can be secreted in sufficient quantity to induce hypercalcemia. Even if sufficient amounts of PGE were produced by an extraskeletal neoplasm, the rise in the circulating concentration of PGE may be limited because of the high rate of its clearance and metabolism, particularly by the lung.[1] Even when an elevation in the circulating concentration of PGE occurs, the amount may be insufficient to influence bone resorption significantly and cause hypercalcemia. It is theoretically possible that hypercalcemia results from the action of a metabolite of PGE, which attains a higher concentration in circulation and shares similar bone resorptive capacity with PGE. However, such a metabolite has not been found. Nevertheless, the response to treatment with indomethacin or aspirin in the absence of a malignant invasion of bone suggests a probable etiologic role of ectopically produced PGE. The evidence that hypercalcemia may result from local production by bone metastases of PGE, either directly or by stimulating bone tissue to synthesize prostaglandins[38] is particularly secure.

Although serum 1,25-$(OH)_2D$ has not been systematically measured in patients with increased urinary PGE-M excretion, it is conceivable that this metabolite may contribute to the hypercalcemia. PGE_2 has recently

been demonstrated to stimulate 1-hydroxylation of 25-(OH)D in chick kidney cell culture.[39]

The neoplasms associated with increased PGE production are "solid" tumors such as carcinoma of lung, kdney, breast, esophagus, parotid, ovary, penis and tonsil; adenocarcinoma of pancreas; hepatoma and melanoma. The hematologic malignancies have not been associated with increased urinary PGE-M excretion.[36]

PGE production should be suspected in the presence of a "solid" tumor in which hypercalcemia is accompanied by suppressed iPTH and urinary cyclic AMP values. Serum P may be normal or reduced. Under these circumstances, a trial of aspirin (650 mg qid) or indomethacin (25-50 mg tid) would be warranted. Hypercalcemia should respond within 3 days if PGE is playing an etiologic role.

Not all patients with neoplastic production of PGE have amelioration of hypercalcemia after treatment with indomethacin or aspirin therapy despite an inhibition of PGE synthesis. This may result because of the presence of bone metastases, or the presence of other hypercalcemic humoral factors. Since there is an overlap in the types of neoplasms associated with the production of PTH and PGE, certain tumors may elaborate both humoral factors. Seyberth et al. have estimated that in an "unselected" series of patients with hypercalcemia and cancer, less than 10% will respond to indomethacin.[37]

Neoplastic Production of Osteoclast-Activating Factor (OAF)

In 1971, Raisz became intrigued with the problem of localized resorption of bone associated with chronic inflammation (such as periodontal disease). He then initiated his classic studies concerned with the delineation of osteoclast-activating factor (OAF). It was found that lymphocytes, upon stimulation by antigens to which the donor has developed cellular immunity or by nonspecific mitogens (solubilized dental plaque or phytohemagglutinen), produce a soluble substance that stimulates bone resorption in organ culture.[40] The substance was called "osteoclast-activating factor" because the treated bone showed numerous active osteoclasts. The OAF could be distinguished from PTH, PGE_2 and $1,25\text{-}(OH)_2D$ from dose-response curves (in terms of ^{47}Ca release from bone).[41] Moreover, OAF-stimulated bone resorption was inhibited more effectively by cortisol than was PTH stimulation.

Although the molecular structure of OAF has not been elucidated, the biological activity is probably due to a peptide component, since a loss of activity ensues upon treatment with trypsin or pronase.[42] It occurs in two forms, "big OAF" (12,500 to 25,000 daltons) and "small OAF" (1330 to 3500 daltons).[43] Both forms produce bone resorbing activity. A preliminary report indicated that prostaglandins may regulate OAF production.[44]

Although a specific assay for OAF has yet to be developed, the use of a bone culture bioassay has enabled studies to be carried out on cells obtained from patients with a variety of hematologic neoplasms. The data obtained suggest that OAF may be an important factor in mediating osteolysis in several disorders. Initially, OAF was detected in the supernatant fluid of cultured lymphoid cell lines obtained from patients with multiple myeloma, Burkitt's lymphoma and malignant lymphoma.[41] The characteristics of this bone-resorbing factor were indistinguishable from that obtained from stimulated normal leukocytes. Since the in vitro demonstration of OAF production by these cultured cell lines did not provide definitive evidence that the parent cell did this in vivo, the investigators carried out short-term cultures of myeloma cells obtained by marrow aspiration.[45] In 6 of 7 short-term cultures, OAF was detectable in the supernatant fluid. High concentrations of OAF also have been demonstrated in cell cultures of circulating lymphocytes obtained from a patient with lymphosarcoma cell leukemia who had hypercalcemia.[46] The fact that mononuclear cell lines from peripheral blood synthesize OAF indicates that OAF may be produced extraskeletally. Moreover, OAF may be produced by neoplasm indirectly by means of immunologic or inflammatory stimulation of monocytes and macrophages.

The major drawback to the study of OAF in oncogenic hypercalcemia has been inadequate structural identification and the lack of a reliable sensitive radioimmunoassay. Thus, it has not been possible to demonstrate the presence of OAF in the circulation or in the venous drainage of a tumor. Definitive proof of the role of OAF in the pathogenesis of hypercalcemia of malignancy must await the demonstration of OAf in circulation, in venous effluent of tumor, or in the extract of non-cultured tumor. It would also be necessary to demonstrate that successful treatment of the hypercalcemia with anti-tumor therapy coincides with a decline in OAF production.

OAF-mediated hypercalcemia has been described only in neoplasms of lymphoid origin. The most common hematologic cause of hypercalcemia is multiple myeloma.[2] Two clinical points with regard to the diagnosis of multiple myeloma are: (1) Roentgenographic skeletal surveys indicate bone involvement with greater sensitivity than technesium diphosphonate bone scan in patients with multiple myeloma.[47] (This contrasts with metastases to bone from lung, prostate or breast cancer which are more apt to be detected by bone scan). Technesium diphosphonate creates "hot spots" on bone scan when it adheres to hydroxyapatite crystals actively being formed and the degree of binding relates to the rate of blood flow (which is incresed in newly forming bone). In multiple myeloma, where there are mainly osteolytic lesions, bone scan is not too helpful. Similarly, alkaline phosphatase, which is released from osteoblasts which are actively forming new bone, is usually not elevated in multiple myeloma. (2) The second clinical point is that black women (who have 10% greater bone density than white women) rarely develop osteoporosis. In my recent ward rotation on medicine, two black women presented with "osteoporosis" and vertebral crush fractures. Both were eventually diagnosed to have multiple myeloma.

As in the malignant production of PGE (which has not been reported in hematologic malignancies), serum PTH and urinary cyclic AMP are normal or low. However, unlike the situation in neoplastic production of PTH or PGE, a favorable response to steroids (e.g. 30 to 60 mg prednisone per day) may be obtained. Hypercalcemia should respond within a few days of treatment.

Direct Osteolysis

Despite the fact that osteolytic bone metastases occur frequently in patients with advanced malignancy (80-85 percent),[2] the pathologic basis for the resorption of bone by skeletal metastases has not been extensively studied. All of the humoral hypercalcemic factors previously discussed (PTH, non-PTH renotropic factor, 1,25-$(OH)_2D$, PGE and OAF) may be produced locally by metastases in bone, resulting in osteoclast-mediated bone resorption. Furthermore, it is possible that osteoclasts could be stimulated at metastatic sites by factors released by cells other than cancer cells. Local bone-resorbing factors such as prostaglandins or OAF could be released by monocytes, activated lymphocytes or fibroblasts as part of a cell-mediated immune response to the tumor. There has been a recent report of humoral hypercalcemic factor[38] stimulating prostaglandin production in bone tissue itself, which would then presumably stimulate osteoclastic resorption.

To further complicate the picture, it is now apparent that in some situations of malignant invasion of bone there may be excessive bone destruction without osteoclastic stimulation. Both Faccini,[48] and Galasko[49] have found that transplanted tumors in rabbits appear to induce osteoclastic bone resorption in the adjacent bone at an early stage of tumor growth. In later stages as the tumor invades the bone, osteoclasts become difficult to detect although bone resorption continues. Galasko has noted similar histologic findings in patients with bone metastases due to a variety of tumors.[49]

Tumor-mediated bone resorption independent of participation of osteoclasts could ensue through one or two mechanisms:

1. Direct Resorption of Bone by Monocytes and Macrophages: Malignant tissue in bone may stimulate local concentration of monocytes and dmacrophages. Mundy has demonstrated that monocytes are capable of stimulating the release of bone mineral and matrix from "killed" bones devoid of live cellular components.[50] Bone marrow macrophages were also capable of resorbing the dead bone.

2. Direct Resorption of Bone by Tumor Cells: Confirmatory data which supports this concept has been reported by Mundy and Eilon.[51] They found that the supernatant fluid of one human breast cancer cell line could release ^{45}Ca from fetal rat bones in organ culture. Histologic examination of the bone revealed no increase in osteoclast number or activity. Addition of cortisol or phosphate in high concentrations (which inhibit osteoclastic bone resorption in organ culture) failed to inhibit the supernatant-induced bone resorption. The final evidence that the bone re-

sorption was not mediated by bone cells was provided by the observation that fetal rat bones "killed" by freeze-thawing and ultraviolet light could release ^{45}Ca when incubated with the breast cancer cell supernatant.

Direct osteolysis should be suspected in the presence of osteolytic metastases, when the production of the known hypercalcemic humoral factors can be excluded. The hypercalcemia of osteolytic metastases is more responsive to treatment with adrenal corticosteroids (with a reported 30 percent responsiveness) than is the oncogenic hypercalcemia of PTH or PGE production without obvious malignant invasion of bone.[1] This finding may reflect the inhibitory effect of steroids on the local inflammation and on the consequent induction of direct osteolysis by inflammatory cells.

Treatment

Emergent hypercalcemia is most commonly encountered in patients with malignancy, and rapid treatment may be life-saving.

General Measures:

Dehydration is common in severe hypercalcemia because of the impairment in renal concentrating ability and the frequency of associated nausea and vomiting. In a severely ill patient, rehydration alone may reduce the serum Ca by 2 mg/dl or more. In the absence of renal or heart disease, the initial rate of saline infusion is usually in the range of 200-300 ml/h. Typically 1 to 2 liters of physiologic saline are given to overcome dehydration, prior to starting furosemide. Furosemide is then given in a dosage of 80 to 100 mg every 2 hours, with appropriate replacement of losses of water, sodium and potassium. The basis for the "hypocalcemic" action of furosemide-induced natriuresis is the promotion of the renal loss of calcium. Ordinarily, calcium and sodium clearances are closely linked, because the two cations have the same reabsorptive site in the renal tubule. Furosemide and saline cause a sustained increase in sodium excretion and thus exaggerate the calcium loss. A significant fall in the serum concentration of calcium typically ensues from this therapy within 4 h. The renal loss of calcium often exceeds 1 g per day. These front-line measures usually lead to a reduction in serum Ca of approximately 3 mg/dl within 24 h. Both hemodialysis and peritoneal dialysis against calcium-free solutions have been successfully employed in patients with emergent hypercalcemia and severe renal impairment.[52]

Measures Which Inhibit Bone Resorption:

Calcitonin is a potent inhibitor of osteoclastic bone resorption,[52] and may be employed early in the treatment of emergent hypercalcemia. The usual dosage of standard salmon calcitonin (Armour) is 4 to 8 MRC units per Kg of body weight given SQ or IM every 12 hours. The hypocalcemic effect of a single IM injection is detectable by 2 hours, and a maximal reduction of approximately 2 mg/dl is observed by 6 to 10 hours. The acute hypocalcemic resonse to calcitonin is usually accompanied by hypophosphatemia. Experience with calcitonin has been somewhat disappointing because of its

transient effectiveness[53] and the unpredictable loss of a sustained response to the hormone in some patients (Fig. 18A), a finding due to the well known "escape" phenomenon. Raisz et al initially described the escape phenomenon in which calcitonin initially inhibited bone resorption in PTH- or vitamin D-stimulated bone, but with time, the inhibitory influence of calcitonin was progressively lost despite continued presence of biologically active hormone.[54] Recent studies by Tashjian et al. have shown this escape phenomenon to be the result of "down regulation", or a reversible loss of specific calcitonin-binding sites in bone.[55] In the case of calcitonin, down regulation appears to be due in large part to continuous receptor occupancy by tightly bound, poorly dissociable hormone. The calcitonin escape phenomenon can be inhibited in vitro by cortisol, even when bone resorption is stimulated by parathyroid hormone.[56] There is recent data that the escape phenomenon can be prevented at least for a number of days in most patietns with hypercalcemia of malignancy by simultaneous treatment with glucocorticoids (Fig. 18B,C).[53] Calcitonin is a rapid form of therpay, generally without complications.

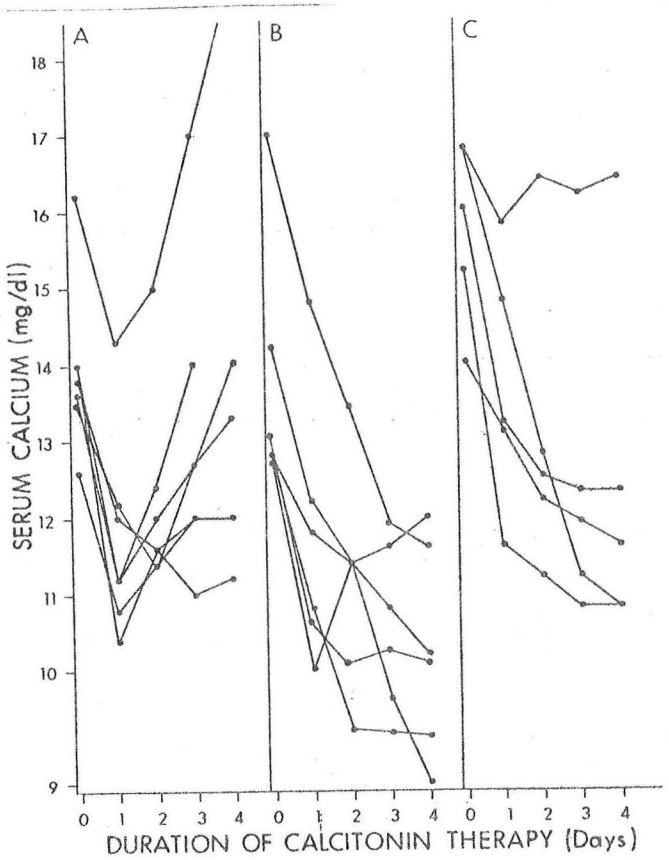


Figure 18 Responses of total serum calcium in individual patients with malignant hypercalcemia to initial treatment with calcitonin. **A.** Responses in the seven episodes treated with calcitonin alone. **B.** Initial effects of the combination of calcitonin and glucocorticoids in each of the six patients with lymphoproliferative malignancy and hypercalcemia treated with the combination. **C.** Initial effects in each of the five patients with solid tumors and hypercalcemia treated with the combination of calcitonin and glucocorticoids.

Oral Phosphate Therapy: Phosphate therapy is effective in reducing serum Ca levels in all hypercalcemic conditions.[57] Phosphate both

inhibits bone resorption and promotes bone mineral accretion, and the anti-hypercalcemic effect persists several days after discontinuation of therapy. The principal concern associated with the use of phosphates is the potential complication of extraskeletal calcification. This complication is likely to occur whenever the normal Ca X P mineral ion product is exceeded. Intravenous P therapy should be avoided. Oral P therapy also should never be used in patients with severe hypercalcemia, azotemia or pre-treatmeant levels of serum P above 3 mg/dl. However, in non-azotemic, hypophosphatemic patients who have been initially stabilized at moderate levels of hypercalcemia (serum Ca less than 12 mg/dl) by saline diuresis, modest doses of oral P (1000 to 1500 mg elemental P daily in four divided doses) may be very useful in maintaining the serum Ca at acceptable levels. The mineral ion product should not exceed 35 to 40.

Trial of Specific Therapy: The preceeding discussion has emphasized that hypercalcemia of malignancy is primarily a bone-resorptive form of hypercalcemia. Once the initial steps have been undertaken and the hypercalcemia is no longer emergent, a trial of specific therapy for the presumed underlying disorder is warranted. Steroids are effective in multiple myeloma and other hematologic malignancies; in many patients with breast carcinoma; and are variably effective in patients with osteolytic metastases. Prednisolone 30-60 mg daily in three divided doses should be tried. A clear-cut response is usually apparent within 48 to 72h. Likewise a trial of indomethacin 50 mg thrice daily or aspirin 600 mg every 4h-6h may be attempted, particularly in solid tumors without obvious bone metastases. Again, response should be seen within 48 to 72 h. If a specific therapy is effective, it should be continued.

Mithramycin: When all else fails, or if the hypercalcemia is acutely life threatening (seum Ca greater than 18 mg/dl), mithramycin may be employed. This cytotoxic antibiotic inhibits RNA synthesis and is a useful chemotherapeutic agent in testicular tumors. The agent effectively inhibits osteoclastic bone resorption in doses of 25 µg/kg given intravenously over 3-8 h (approximately one tenth of the chemotherapeutic dosage). The hypocalcemic effect is usually noted within 24 h after a single intravenous dose and persists for approximately 1 week, although the duration of response is variable. Thrombocytopenia, nehprotoxicity and hepatotoxicity may occur. Fortunately, these complications are not too common at the dosage used to treat hypercalcemia of malignancy.

Diphosphonates: Encouraging hypocalcemic responses to diphosphonates have recently been reported in patients with multiple myeloma[58] and other malignancies.[59] These agents which are analogues of pyrophosphate are potent inhibitors of hydroxyapatite crystal formation, and were initially developed as water softeners to stop calcium deposition in plumbing. More to the point in their use to treat hypercalcemia of malignancy, the diphosphonates were found to be potent inhibitors of osteoclastic bone resorption,[60] particularly dichloromethylene diphosphonate. A summary of the effects of this diphosphonate in 12 patients with hypercalcemia of malignancy is shown below (Fig. 19).[59] Unfortunately, in April 1981, dichloromethylene diphosphonate had to be temporarily taken off the market because of a possible association with acute myelogenous leukemia.

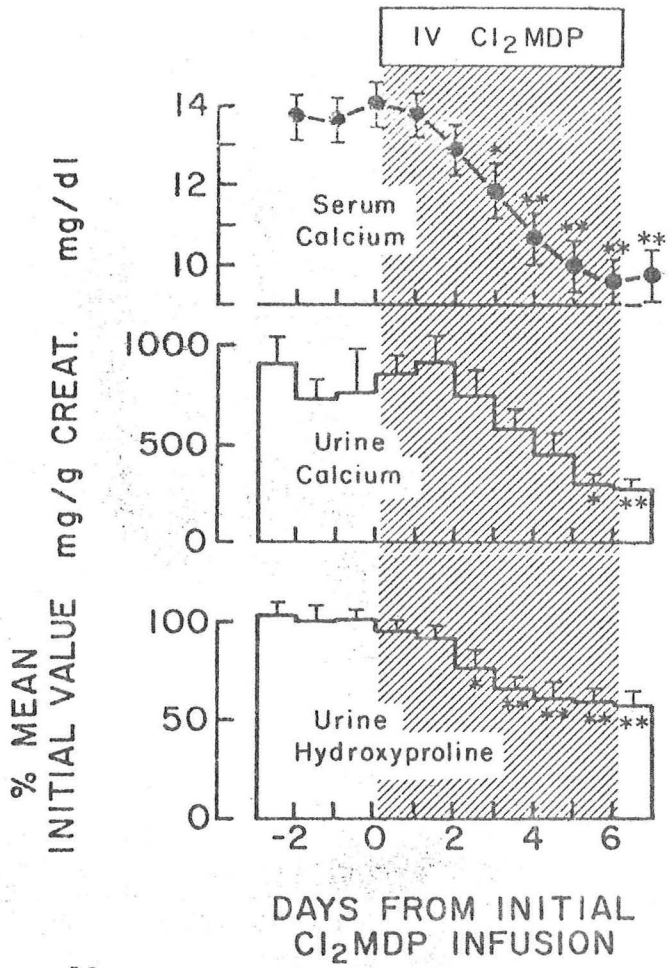
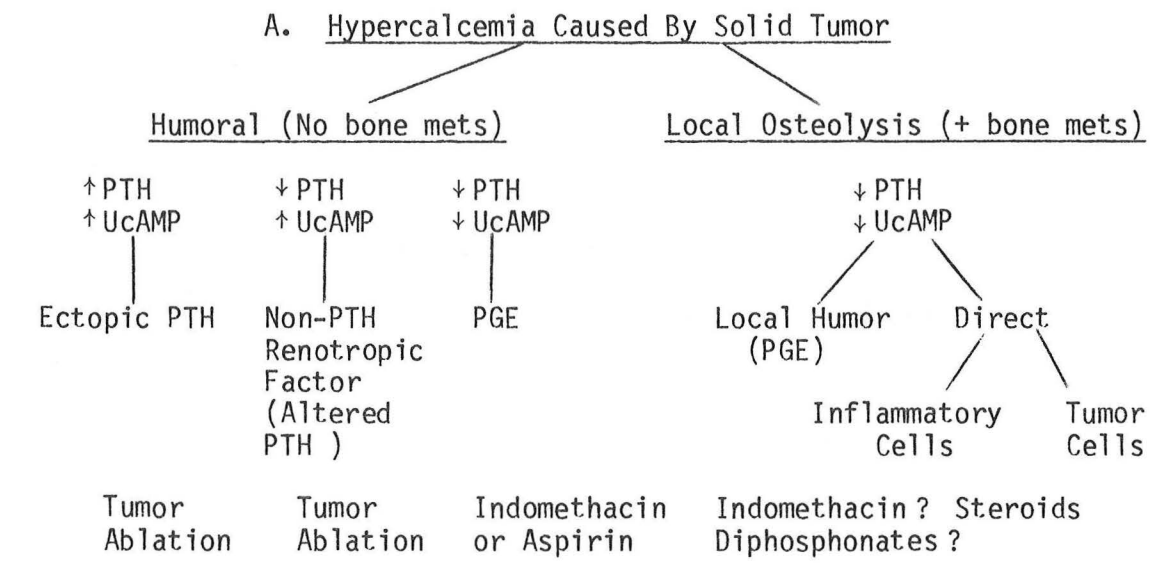
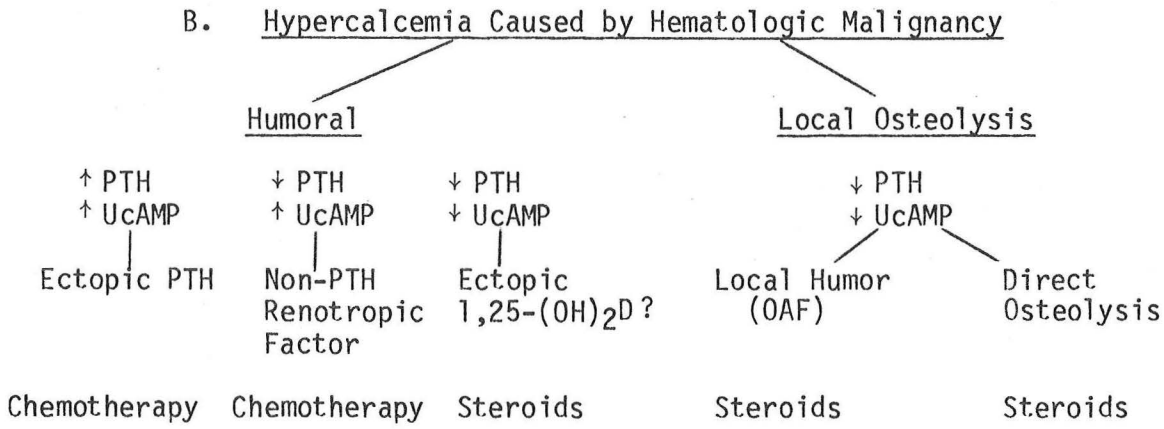


Figure 19 Summary of chemical responses to intravenous dichloromethylene diphosphonate (*IV Cl,MDP*). Responses of serum calcium and urine calcium concentrations and hydroxyproline excretion are shown in 12 patients given intravenous Cl,MDP for up to 7 days as treatment for malignant hypercalcemia. Mean values are expressed as ± SEM. Significant differences between each point and mean pretreatment values are denoted by a single ($p < 0.005$) or a double ($p < 0.005$) asterisk.

Based on the previous information, the causes and specific treatment of hypercalcemia of malignancy may be schematically depicted as follows:





The precise frequency of the various causes of hypercalcemia of malignancy are not yet established. The data of Broadus would suggest that in a series of cancer patients with hypercalcemia (composed mainly of solid tumors), a circulating humoral substance seemed to be the most common cause (80%). However, this series included very few patients with breast cancer. For the hematologic malignancies, local osteolysis mediated by OAF appears to be the most common cause for hypercalcemia.

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