Urinary and nephrogenous cyclic AMP and renal phosphate handling in normal subjects and patients with parathyroid dysfunction

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Abstract. Studies were performed in 60 patients with proven primary hyperparathyroidism pre-operatively and in 54 of these patients post-operatively, 22 patients with permanent hypoparathyroidism and 34 normal subjects. Urinary and nephrogenous cyclic AMP excretion were increased in the hyperparathyroid patients with an overlap of values with the normal group of 10 and 9%, respectively. Values fell in all patients post-operatively, and were decreased in those with permanent hypoparathyroidism. TmPO4/GFR was decreased in the pre-operative hyperparathyroid patients and rose post-operatively while it was increased in the hypoparathyroid patients with an overlap of values with the normal group of 9%. Post-operative hypocalcaemia due to bone hunger was associated with continuing normo- or hypophosphataemia and urinary cyclic AMP that exceeded 4.5 nm/dl GF while those who developed permanent hyperparathyroidism had hyperphosphataemia, increased TmPO4/GFR and urinary cyclic AMP that was less than 3.5 nm/dl GF. Urinary and nephrogenous cyclic AMP were equally effective in characterizing patients with primary hyperparathyroidism and less effective in distinguishing patients with hypoparathyroidism from normal while TmPO4/GFR estimates were more effective in delineating the hypoparathyroid state.

Urinary cyclic AMP measurements have been used extensively in assessing patients suspected of parathyroid dysfunction but some have questioned their utility (Babka et al. 1976; Broadus 1979; Drezner et al. 1976; Dohan et al. 1972; Neelon et al. 1973; Pak 1980; Shaw et al. 1977; Walinder et al. 1978). Such disagreement may be related to the different modes of expression (Broadus 1979), non-parathyroid hormone factors, and possibly environmental and nutritional influences (Taylor et al. 1970; Hardman et al. 1969; Broadus et al. 1981). The more physiologically relevant expressions of urinary and nephrogenous cyclic AMP in nm/dl of glomerular filtrate (GF) enhance their sensitivity in characterizing parathyroid function and have almost completely replaced older, indirect tests (Bijvoet 1977). However, estimates of tubular maximum reabsorptive capacity for phosphate (TmPO4/GFR) provide an index of renal phosphate handling in relation to parathyroid hormone (PTH) activity which is less subject to other influences (Bijvoet 1977). Thus far, only limited data have been reported comparing its value with that of urinary cyclic AMP in assessing parathyroid function (Alston et al. 1980; Walker et al. 1977).

This report describes the results of studies conducted under conditions of a constant dietary calcium and phosphate intake in which urinary and nephrogenous cyclic AMP, serum PTH and TmPO4/GFR were measured in successive, unselected patients with surgically proven primary hyperparathyroidism pre-operatively and in most cases post-operatively, patients with permanent hypoparathyroidism, and control subjects. The data strongly suggest that urinary and/or nephrogenous cyclic AMP expressed in parametric terms and TmPO4/GFR are sensitive indices which facilitate the assessment of parathyroid function.
Materials and Methods

Sixty patients with surgically confirmed primary hyperparathyroidism and 22 patients with permanent hyperparathyroidism were studied after equilibration on a diet containing 150 mg of calcium and 500 mg of phosphorus per day. The hyperparathyroid patients included 44 females and 16 males (17 to 76 years). Fifty-four patients were studied post-operatively and data, collected between the 4th and 7th days after surgery, are reported. The hypoparathyroid group consisted of 5 with idiopathic and 17 with surgically related hypoparathyroidism and included 18 females and 4 males (21 to 82 years). Normal subjects including 19 females and 15 males (26 to 44 years) were asked to eliminate dairy product intake during and for the 2 days preceding their study.

Two or more blood specimens and 24 h urines were obtained in all subjects for measurement of creatinine, calcium, phosphorus, and cyclic AMP. Blood specimens were obtained at 08.00 h while patients remained supine and in normal subjects after 20 min of rest. Blood for cyclic AMP was collected in tubes containing EDTA, centrifuged at 4°C, and plasma was stored in the frozen state. Blood was also obtained for routine chemistries, PTH and ionized calcium. Urines were collected under ice and in containers to which 15 ml of 6 N HCl was added and aliquots were frozen at -20°C until assayed for cyclic AMP. Urine cyclic AMP was measured using a competitive protein binding assay and plasma cyclic AMP by radioimmunoassay. Serum PTH was determined by the Nichols Institute, San Pedro, California using a carboxyterminal radioimmunoassay. Urinary and nephrogenous cyclic AMP were expressed in nM/dl GF. TmPO4/GFR was calculated using the nomogram of Walton & Bijvoet (1975). Student's t-test (two-tailed) was used to compare group data and differences were considered to be significant for P values of < 0.05.

Table 1.
Incidence (%) of clinical manifestations in 60 patients with primary hyperparathyroidism.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental disturbances/personality changes</td>
<td>47</td>
</tr>
<tr>
<td>Arthralgias</td>
<td>44</td>
</tr>
<tr>
<td>Hypertension</td>
<td>41</td>
</tr>
<tr>
<td>Renal colic/uro lithiasis</td>
<td>40</td>
</tr>
<tr>
<td>Weakness</td>
<td>31</td>
</tr>
<tr>
<td>Constipation</td>
<td>29</td>
</tr>
<tr>
<td>Polyuria/polydipsia</td>
<td>29</td>
</tr>
<tr>
<td>Fatigability</td>
<td>26</td>
</tr>
<tr>
<td>'Serendipity'</td>
<td>25</td>
</tr>
<tr>
<td>Weight loss</td>
<td>14</td>
</tr>
<tr>
<td>Documented peptic ulcer</td>
<td>12</td>
</tr>
<tr>
<td>Muscle pain/cramps</td>
<td>12</td>
</tr>
<tr>
<td>Bone pain</td>
<td>6</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>

Clinical presentation and outcome of the hyperparathyroid group

The incidence of symptoms and clinical manifestations in the pre-operative hyperparathyroid group are listed in Table 1. Two patients 17 and 20 years of age were brothers. Five years after removal of
a single large parathyroid adenoma the older brother had a recurrence of hypercalcaemia and a second parathyroid adenoma was removed. Three patients had parathyroid hyperplasia. One patient had parathyroid cancer and despite surgery, ultimately died of the consequences of her cancer. Two patients had two adenomata each. All other patients in this series had a solitary benign adenoma. As a result of surgery, 5 of 54 patients developed permanent hypoparathyroidism and required chronic vitamin D and calcium supplementation.

**Chemical data**

Data are expressed as mean ± SD. Serum calcium was greater than normal in the patients with primary hyperparathyroidism (11.70 ± 1.09 vs 9.24 ± 0.36, \( P < 0.001 \)) and less than normal in those with hypoparathyroidism (6.69 ± 1.38, \( P < 0.001 \)). Pre-operatively, total serum calcium levels were consistently increased in all but 8 patients with primary hyperparathyroidism while serum ionized calcium was consistently increased whenever measured. Surgery was followed by a fall in serum calcium to or below normal and mean value for the group was less than in the normal subjects (8.46 ± 0.76, \( P < 0.001 \)). Forty-three of 54 patients studied post-operatively became hypocalcaemic. Thirty patients had a transient hypocalcaemia with levels returning to normal within a week of surgery, 5 exhibited 'bone hunger', 5 developed permanent hypoparathyroidism, and 3 were lost to follow-up but are presumed to have had transient hypoparathyroidism.

Serum phosphate was decreased in the patients with primary hyperparathyroidism (2.56 ± 0.49 vs 3.42 ± 0.43, \( P < 0.001 \)) and rose to normal post-operatively (3.54 ± 0.68, NS). Pre-operatively, 50% of the patients were hypophosphataemic. In the hypoparathyroid patients, levels were increased above normal (5.28 ± 1.19, \( P < 0.001 \)). Chloride/phosphate ratios in the pre-operative primary hyperparathyroid group were above 33 in all but 3 patients. Urinary calcium excretion was increased in the hyperparathyroid patients pre-operatively (0.25 ± 0.15 mg/dl GF vs 0.08 ± 0.04, \( P < 0.001 \)) with values post-operatively falling to 0.08 ± 0.06 (NS). In the hypoparathyroid patients urinary calcium excretion was comparable to normal (0.05 ± 0.06, NS). The pre-operative hyperparathyroid group exhibited the greatest range of values (0.05 to 0.75 mg/dl GF) while all values in the other groups were less than 0.20 mg/dl GF. Mean serum creatinine levels were comparable in all subject groups while endogenous creatinine clearances were lower than normal (109.3 ± 23.2) in the pre-operative hyperparathyroid (86.5 ± 32.0, \( P < 0.001 \)), post-operative hyperparathyroid (92.2 ± 34.6, \( P < 0.02 \)) and hypoparathyroid groups (85.5 ± 27.2, \( P < 0.005 \)). Pre-operatively, serum PTH values ranged from 69 to 595 µEq/ml with a mean value of 152 ± 93.2 (normal range, 40 to 90

<table>
<thead>
<tr>
<th>TmPO4/GFR and urinary and nephrogenous cyclic AMP data (mean ± 1 SD) and per cent overlap of values between normal subjects and patients with primary hyperparathyroidism pre- and post-operatively, and patients with permanent hypoparathyroidism.</th>
<th>TmPO4/GFR</th>
<th>Total urinary cyclic AMP nm/dl GF</th>
<th>Nephrogenous cyclic AMP nm/dl GF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>3.08 ± 0.47</td>
<td>3.65 ± 0.60</td>
<td>2.45 ± 0.72</td>
</tr>
<tr>
<td>Hyperparathyroid</td>
<td>1.95 ± 0.43</td>
<td>7.32 ± 2.43</td>
<td>6.26 ± 2.35</td>
</tr>
<tr>
<td>Pre-operatively % overlap</td>
<td>35%</td>
<td>10%</td>
<td>9%</td>
</tr>
<tr>
<td>Hyperparathyroid</td>
<td>3.80 ± 1.03</td>
<td>3.34 ± 1.11</td>
<td>1.58 ± 1.21</td>
</tr>
<tr>
<td>Post-operatively % overlap</td>
<td>55%</td>
<td>80%</td>
<td>70%</td>
</tr>
<tr>
<td>Hypoparathyroid</td>
<td>6.33 ± 1.84</td>
<td>2.31 ± 0.73</td>
<td>0.46 ± 0.62</td>
</tr>
<tr>
<td>% overlap</td>
<td>9%</td>
<td>59%</td>
<td>22%</td>
</tr>
</tbody>
</table>
µEq/ml). Eighty per cent of these patients had increased serum PTH levels. Serum PTH fell post-operatively (71.5 ± 30.3 µEq/ml, P < 0.001) with levels ranging from 38 to 166 µEq/ml. In the hypoparathyroid group mean serum PTH was 56.8 ± 19.1 with values ranging from 20 to 93 µEq/ml.

TmPO₄/GFR values were less than normal in the patients with primary hyperparathyroidism (1.94 ± 0.43 vs 3.10 ± 0.47, P < 0.001) and no patient had a value that exceeded 3.0 mg/dl (Fig. 1, Table 2). Post-operatively, values rose and were intermediate to those found in the normal and hypoparathyroid groups (3.80 ± 1.03, P < 0.001). In the hypoparathyroid group, mean TmPO₄/GFR value was elevated above normal (6.33 ± 1.84, P < 0.001) and all but 2 patients had values in excess of 4.70 mg/dl GF. There was an overlap of only 9% between the hypoparathyroid and normal groups.

In the hyperparathyroid group urinary and nephrogenous cyclic AMP were increased above normal (P < 0.001) (Table 2, Figs. 2 and 3). with an overlap of 10 and 9%, respectively. Post-operatively, urinary and nephrogenous cyclic AMP fell in all patients (Fig. 4). In the hypoparathyroid group, urinary and nephrogenous cyclic AMP were less than normal (P < 0.001). Urinary cyclic AMP, however, was not an effective discriminant with an overlap of 59% while there was an overlap of only 22% in nephrogenous cyclic AMP.

A transient and functional hypoparathyroidism with serum calcium returning to normal within 7 days of surgery was noted in 30 of 54 patients. Five patients who developed permanent hypoparathyroidism had continuing hypocalcaemia, hyper-phosphataemia, impressive increases in TmPO₄/GFR, urinary cyclic AMP that was low or low normal and in all instances < 3.5 nM/dl GF, and nephrogenous cyclic AMP that was subnormal in 3 (< 0.48) and low normal in a fourth subject (1.10) in whom it was measured. Five patients with bone hunger had continuing hypocalcaemia, hypophosphataemia and urinary cyclic AMP excretion that exceeded 4.5 nM/dl GF.

Within the pre-operative hyperparathyroid group, patients with stones (n = 17) and patients without stones (n = 43) were not significantly different in respect to serum calcium, phosphate or creatinine, urinary calcium, creatinine clearances, TmPO₄/GFR or urinary cAMP.

**Fig. 2.**

Individual urinary cyclic AMP excretion data expressed in nM/dl GF in the normal subjects, patients with primary hyperparathyroidism pre- and post-operatively, and patients with permanent hypoparathyroidism. Horizontal bars represent mean values ± 1 SD.

Discussion

Serum calcium was consistently elevated in 52 of 60 patients with primary hyperparathyroidism. Eight were intermittently normocalcaemic particularly while on a calcium restricted diet supporting the concept of calcium intolerance in primary hyperparathyroidism (Broadus et al. 1981). In these patients though serum albumin levels were normal, when measured, serum ionized calcium levels were consistently increased. The lower mean post-operative serum calcium was attributable to a transient
hypoparathyroidism in 30 of 54 patients, permanent hypoparathyroidism in 5, and bone hunger in 5 others. Post-operative hypocalcaemia together with hypophosphataemia and high normal or increased urinary cyclic AMP excretion was characteristic of bone hunger (Albright & Reifenstein 1948). Patients who developed permanent hypoparathyroidism were characterized by hypophosphataemia, impressive increases in $T_mP_{O_4}/GFR$ values, and low or low normal excretion of cyclic AMP. These results parallel those reported by Spiegel et al. (1981).

Citing the concomitant tendency to hyperchloremia and hypophosphataemia in primary hyperparathyroidism, Palmer et al. (1974) suggested that a chloride/phosphate ratio might be an effective discriminant in evaluating patients with hypercalcaemia. More than 95% of their hyperparathyroid patients had a ratio greater than 33 while more than 90% with hypercalcaemia from other causes had ratios less than 30. Our data are in keeping with those observations. We would agree with Broadus (1981) that this ratio is a useful and simple test that can be utilized in the differential diagnosis of hypercalcaemia.

Serum PTH levels were increased in 80% of our patients with primary hyperparathyroidism and were subnormal in 13% of those with permanent hyperparathyroidism.
hypoparathyroidism. Some investigators have reported increases in circulating PTH in 90% or more of hyperparathyroid patients (Shaw et al. 1977; Silverman & Yalow 1973; Reiss & Canterbury 1974; Arnaud et al. 1974). Broadus et al. (1977), however, reported increases in plasma PTH in 41 of 56 (73%) patients with primary hyperparathyroidism and normal levels in 6 of 10 patients with hypoparathyroidism. Others have found elevated PTH values in 50 to 70% of patients with primary hyperparathyroidism (Alston et al. 1980; Drezner et al. 1976). Shaw et al. (1977) reported complete separation of normal and hyperparathyroid groups when serum PTH was analyzed as a function of serum calcium. Notwithstanding the problems with PTH assays, such measurements are helpful in delineating parathyroid function (Shaw et al. 1977; Broadus et al. 1977) and in delineating the hypercalcaemia of malignancy (Stewart et al. 1980; Rude et al. 1981).

Recent studies by Bijvoet (1977) have served to further characterize renotubular handling of phosphate and facilitate determinations of $T_{m}P_{04}/GFR$. In keeping with data reported in 13 hyperparathyroid patients by Walker et al. (1977) and 12 of 14 patients reported by Alston et al. (1980), $T_{m}P_{04}/GFR$ values in our patients with primary hyperparathyroidism were significantly depressed. The higher mean $T_{m}P_{04}/GFR$ value after successful surgery in our patients resulted from normalization of parathyroid function in most patients and development of hypoparathyroidism in a few patients. Our hypoparathyroid group was characterized by increases in $T_{m}P_{04}/GFR$ with an overlap of values with the normal group of only 9% strongly suggesting that it may be an effective discriminant of the hypoparathyroid state.

Pak (1980) recently reviewed the general experience with urinary cyclic AMP measurements in normal subjects and patients with primary hyperparathyroidism. The overlap of values was greatest when cyclic AMP excretion was expressed in $\mu M$/day, less so when expressed in $\mu M$/g creatinine and least when excretion was expressed in nm/dl GF. In 35 patients with primary hyperparathyroidism, Broadus et al. (1977) reported an overlap of 51% when urinary cyclic AMP was expressed in $\mu M$/day, 20% when expressed in $\mu M$/g creatinine and only 6% when cyclic AMP was expressed in nm/dl GF. Similar or better separation was noted with nephrogenous cyclic AMP measurements (Pak 1980). Our urinary and nephrogenous cyclic AMP excretion results are in agreement with these reports with increased values in our pre-operative hyperparathyroid group with an overlap with the normal of 10 and 9%, respectively. As pointed out by others (Walinder et al. 1978; Broadus et al. 1977) such results are better than the general experience with PTH radioimmunoassays.

In keeping with the experience reported by Spiegel et al. (1981), in the present study, with corrective surgery, urinary and nephrogenous cyclic AMP levels fell abruptly and uniformly in all patients. In 5 instances, persistent post-operative increases in the face of hypocalcaemia and normo- or hypocophosphataemia were consistent with bone

**Fig. 4.**

Urinary cyclic AMP excretion expressed in nm/dl GF in patients with primary hyperparathyroidism before and after parathyroidectomy. The shaded area represents the range of values in the normal subjects.
hunger. In 8 other patients, subnormal post-operative values were in keeping with a hypoparathyroid state. In 4 of the 5 patients who developed permanent hypoparathyroidism in whom nephrogenous cyclic AMP was measured, excretion was subnormal in 3 and low normal in the 4th. These results indicate that urinary or nephrogenous cyclic AMP measurements can be of considerable value in the diagnosis of primary hyperparathyroidism, the assessment of the response to surgical therapy, and in the management of post-operative hypocalcemia.

Urinary and nephrogenous cyclic AMP excretion have been reported to be low in hypoparathyroidism but because of considerable overlap with the normal range, such measurements have been of limited clinical value (Pak 1980). In 10 patients with hypoparathyroidism, Broadus et al. (1977) reported urinary and nephrogenous cyclic AMP values that almost completely overlapped with those in their normal group. In the present series, 9 of 22 patients (41%) with hypoparathyroidism had subnormal urinary cyclic AMP excretion and 14 of 18 had subnormal nephrogenous cyclic AMP excretion.

Our results strongly suggest that urinary and nephrogenous cyclic AMP measurements are equally effective in characterizing patients with primary hyperparathyroidism and are less sensitive in distinguishing patients with hypoparathyroidism from normal. On the other hand, estimates of \( T_m \text{PO}_4 / \text{GFR} \) appear to be very effective in delineating the hypoparathyroid state. In view of the general availability of phosphorus and creatinine measurements, an estimate of \( T_m \text{PO}_4 / \text{GFR} \) appears to be a simple and practical measure of parathyroid function. Such estimates together with measurement of urinary cyclic AMP are especially helpful in the post-operative management of the hyperparathyroid patient.

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References


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