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Research Article

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Nephrogenous Cyclic Adenosine Monophosphate as a Parathyroid Function Test

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ABSTRACT Nephrogenous cyclic AMP (NcAMP), total cyclic AMP excretion (UcAMP), and plasma immunoreactive parathyroid hormone (iPTH), determined with a multivalent antiserum, were prospectively measured in 55 control subjects, 57 patients with primary hyperparathyroidism (1° HPT), and 10 patients with chronic hypoparathyroidism.

In the group with 1° HPT, NcAMP was elevated in 52 patients (91%), and similar elevations were noted in subgroups of 26 patients with mild (serum calcium ≤ 10.7 mg/dl) or intermittent hypercalcemia, 19 patients with mild renal insufficiency (mean glomerular filtration rate, 64 ml/min), and 10 patients with moderate renal insufficiency (mean glomerular filtration rate, 43 ml/min). Plasma iPTH was increased in 41 patients (73%).

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Results for NcAMP and UcAMP in nonazotemic and azotemic patients with hypoparathyroidism confirmed

the validity of the measurements and the expressions employed.

INTRODUCTION

The development of the immunoassay for parathyroid hormone (PTH)¹ in the mid-1960's (1) represented a distinct methodologic advance in the study of parathyroid physiology and pathophysiology. However, a number of technical and biologic difficulties, including the heterologous nature of the assays in current use (1, 2) and the heterogeneity of circulating forms of human PTH (3-6), have combined to restrict the availability and applicability of the technique. Although a partial resolution of these problems has been provided by the use of defined antisera, which recognize specific regions of the circulating peptides (2, 3, 5, 6), there remains a need for a simple and readily standardizable parathyroid function test.

In the late 1960's, the actions of PTH were shown to be mediated by cyclic AMP (cAMP), and the effects of the hormone on the renal cortex were found to be associated with dramatic increases in the excretion of the nucleotide (7-10). Based on these findings, a number of laboratories have attempted to use measurements of cAMP excretion (UcAMP) diagnostically in patients with parathyroid disease, with varying success (11-18). The fact that this analysis has not gained widespread acceptance as a valid index of parathyroid function can be attributed principally to three aspects of the data base currently available: (a) the lack of a demonstrably parametric expression for total cAMP excretion, (b) the description of several clinical and (or) experimental circumstances, including renal impairment (11, 12, 19) and high extracellular calcium concentrations (20),

¹Abbreviations used in this paper: cAMP, cyclic AMP; NcAMP, nephrogenous cyclic AMP; UcAMP, cyclic AMP excretion; GF, glomerular filtrate; GFR, glomerular filtration rate; 1° HPT, primary hyperparathyroidism; PTH, parathyroid hormone; iPTH, immunoreactive parathyroid hormone.

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which might interfere with the cAMP analyses or their interpretation, and (c) the alterations in plasma and (or) urinary cAMP which have been reported after pharmacologic doses of a number of agents (11, 21–24) and the apparent increases in UcAMP which have been noted in several disorders other than primary hyperparathyroidism (1° HPT) (13, 25–27). Thus, there has been reasonable doubt concerning the clinical specificity of *in vivo* measurements of cAMP.

That these potential problems in the performance and interpretation of clinical cAMP studies may be more apparent than real derives from the unique aspects of cAMP clearance and excretion in man (28). Plasma cAMP is excreted by a process of simple glomerular filtration, and to this filtered load of the nucleotide is added a quantity of cAMP formed *de novo* in the kidney (nephrogenous cAMP). Nephrogenous cAMP (NcAMP): (a) is the only “pool” of the nucleotide easily quantified *in vivo* (28), (b) reflects almost exclusively the effects of circulating PTH (9, 29), and (c) appears to be added directly to the tubular urine (9, 10), thus accounting for the magnitude, rapidity, and sensitivity of the PTH-induced changes in cAMP excretion. Although vasopressin also acts directly on a renal adenylate cyclase system (8, 30), at physiologic concentrations it has little, if any, effect on cAMP excretion (13, 29, 31, 32). Thus, the determination of NcAMP should provide a sensitive and specific assessment of parathyroid function, and several recent reports suggest that this measurement is extremely useful in the diagnosis of 1° HPT (33–35).

This report presents the results of cAMP measurements and plasma immunoreactive PTH (iPTH), employing a multivalent antiserum, in a prospective series of control subjects and patients with 1° HPT and chronic hypoparathyroidism. The general findings indicate that NcAMP validly reflects parathyroid function in a variety of clinical situations. In addition, expressed parametrically as a function of glomerular filtration rate (GFR), total cAMP excretion provides a readily interpretable index of parathyroid function, which should allow this simple determination to become a widely available, and functionally quite specific, diagnostic test for 1° HPT.

METHODS

Patients

Control group. The control group included 55 individuals with normal serum calcium and phosphorus and 24-h calcium excretion. The group contained 30 normal volunteers and 25 patients with a variety of disorders (6 patients with hypertension, 6 patients with osteoporosis, and 13 patients with miscellaneous diseases). The patients were taking a wide assortment of medications. The normal subjects and patients did not differ significantly from each other in any of the parameters measured and were considered together. The group

consisted of 32 women (age range 19–66 yr, mean 39 yr) and 23 men (age range 19–63 yr, mean 35 yr).

Patients with hypoparathyroidism. 10 patients with chronic hypoparathyroidism (2 idiopathic and 8 postoperative) were studied. Nine of the patients were receiving long-term vitamin D and calcium supplements and were deliberately being maintained with a relatively low serum calcium (mean \pm SD, 7.9 ± 0.8 mg/dl). There were four women and six men, and the mean age was 44 yr. 9 of the 10 patients had significant medical illness other than hypoparathyroidism for which they were taking a variety of medications.

Patients with hyperparathyroidism. 57 patients with 1° HPT were studied. There were 35 women (age range 9–78 yr, mean 51 yr) and 22 men (age range 16–74 yr, mean 46 yr). 23 were patients at the National Institutes of Health, and the remaining 34 patients were at the Massachusetts General Hospital.

For purposes of discussion, the patients have been divided into two groups. 40 patients were considered to have a “definite” diagnosis of 1° HPT based on one of the following criteria: (a) neck exploration with tissue confirmation (adenomas in 18 patients and hyperplasia in 11 patients), (b) familial hyperparathyroidism with tissue confirmation in at least one family member (7 patients), or (c) significant step-up in iPTH during neck vein catheterization (36) in a hypercalcemic patient (4 patients). An additional 17 patients were considered to have “clinical” diagnoses of 1° HPT based on documented hypercalcemia on a minimum of two determinations of serum calcium and exclusion of other causes of hypercalcemia by thorough clinical and biochemical evaluation. 14 of these 17 patients had clinical and (or) chemical histories suggestive of 1° HPT of greater than 1-yr duration. Approximately one-half of these 57 patients had significant additional medical illness for which they were receiving a variety of medications.

Five patients included in the “definite” subgroup of patients were members of two generations of a single kindred regarded as having “familial hypocalciuric hypercalcemia” (patients 36–40, Table II). This kindred had quite severe, but relatively asymptomatic, hypercalcemia, and a histologically hyperplastic gland had been removed from one of the patients. However, determinations of iPTH and NcAMP were repeatedly normal in these 5 patients, in contrast to the frank elevations in one or both of these analyses which were noted in each of the remaining 52 patients. This kindred was included in this series because of the prospective nature of the study, and its members are referred to as “biochemically normal” in the text below. Several patients from other kindreds with familial hyperparathyroidism were also studied (Table II) and demonstrated clearcut biochemical evidence of disease.

Study protocol

All patients and control subjects underwent an identical study protocol. Urine specimens were collected over a 2-, 3-, or 4-h period between 6 a.m. and 12 noon. Midpoint blood samples were drawn for the analysis of plasma cAMP and iPTH and serum calcium and creatinine. Approximately 1-ml aliquots of the urine specimens were taken, and the remaining urine was pooled into an ongoing 24-h collection.

During the morning collection periods, fluid intake was encouraged to ensure adequacy of the collections (urine flow averaged 1.5–2 ml/min), beverages containing methyl xanthines were excluded, and activity was limited to bed, chair, and bathroom privileges. Although the state of hydration (13, 29, 31, 32) or the consumption of methyl xanthines (29, 37) do not appear to influence cAMP excretion, physical activity has been reported to increase plasma cAMP (25) and to have

variable effects on UcAMP (29). We examined the effects of physical activity in six of the control subjects and found that mean plasma cAMP levels rose from 16.39 ± 0.85 nM (SEM) under basal conditions to 20.26 ± 1.19 (SEM) after 30 min of casual walking (a mean increase of 24%), with a coincident mean rise of 8% in cAMP excretion. In this series, all blood samples were drawn after the patients had been supine and relaxed for a minimum of 30 min. Activity, hydration, and the consumption of xanthine-containing beverages were not controlled during the 24-h collections.

Both plasma iPTH (3, 38, 39) and plasma and urinary cAMP (18, 29, 37) have been reported to exhibit a diurnal variation, although in each case there is disagreement as to the exact pattern and extent of variation. In 52 patients, plasma cAMP was determined between 8 and 9 a.m. and again between 11 a.m. and 12 noon. The results \pm SEM for these 104 plasma samples were 15.91 ± 0.51 and 15.55 ± 0.47 nM, respectively. Serial clearance determinations (two or three clearance periods) were performed in 15 control subjects and 8 patients with 1° HPT between 6 a.m. and 12 noon and the results were within the variability of the methodology. Finally, in a series of five normal subjects and two patients with 1° HPT in whom no attempt was made to control diet or activity, cAMP excretion, expressed as nanomoles per 100 glomerular filtrate (GF), varied by only 10–24% from mean values in 12 sequential 4-h collections.

The iPTH and cAMP data represent the mean of multiple measurements in almost every patient and control subject. The control group had an average of 2.2 determinations of iPTH and cAMP clearance per individual and 1.2 analyses of 24-h cAMP excretion per individual. The group with 1° HPT had an average of 2.3 measurements of iPTH and cAMP clearance and 1.8 analyses of 24-h cAMP excretion per patient. The group with hypoparathyroidism had an average of 3.0 determinations of iPTH and cAMP clearance and 2.4 samples for 24-h cAMP excretion per patient. Blood for iPTH was unavailable in one patient with 1° HPT, and 24-h collections were not obtained from five control subjects and five patients with 1° HPT.

Collection and preparation of samples

Urine. Urine samples were collected into 6 N HCl (1 ml/h of collection), aliquoted, and frozen at -20°C until analyzed. After the addition of about 1 pmol of [^3H]cAMP, aliquots of the urine samples (containing approximately 100 pmol cAMP) were applied to 5×0.5 -cm columns of AG1-X8 (formate form, 200–400 mesh) prepared in Pasteur pipettes, washed with 10 ml water, and eluted with 1.0 N formic acid. The cAMP eluates (2nd through 11th ml) were collected in glass scintillation vials, lyophilized, dissolved in 1 ml water, and assayed. Recovery averaged approximately 80% (range 65–91%). We have retrospectively reassayed 100 nonchromatographed samples from 70 patients after appropriate dilution and have found excellent general agreement (within 10–15%) with the values for cAMP content after chromatography.

Plasma. Blood samples were drawn into chilled heparinized tubes for iPTH and into EDTA tubes for cAMP (vacutainer tube 3,200 QS containing 15 mg of tripotassium EDTA in 0.1 ml solution). The samples were centrifuged at 1,600–2,000 *g* for 20 min at 4°C , and the plasma was aspirated, with particular care to leave the buffy coat undisturbed in the cAMP sample. Plasma was stored at -20°C until analysis. Approximately 1 pmol [^3H]cAMP was added to 3-ml samples of plasma (containing about 45 pmol cAMP), and the samples were deproteinized by the addition of 7 ml of 5.7% TCA. The extracts were neutralized with potassium hydroxide, with bromthymol blue as an internal indicator, and applied to

7.5×0.8 -cm columns of AG1-X8 (formate form, 200–400 mesh) prepared in disposable chromaflex columns. The columns were washed with 10 ml water and eluted with 1.0 N formic acid, cAMP appearing in the 5th through 10th ml (insoluble material appearing in the first 4 ml was avoided by discarding this fraction). The eluates were collected in glass scintillation vials, lyophilized, dissolved in 500 μl water, and aliquots were counted for recovery (which averaged about 60%, range 53–71%). Earlier attempts to use AG 50-X8 chromatography revealed that eluates from this resin gave a variable blank in our assay, which could contribute significant error in the assay of plasma samples.

Analyses

Plasma iPTH was measured in previously unfrozen samples at the Nichols Institute for Endocrinology, San Pedro, Calif. This method (40) employs a guinea-pig anti-bovine antiserum (GP-101), bovine ^{125}I -PTH, a standard pool of serum from patients with hyperparathyroidism (results expressed in equivalent units), and a second antibody for separating bound and free hormone. Antiserum GP-101 is multivalent and has approximately equal affinity for the amino-terminal and carboxy-terminal sites of the human hormone.² Samples were assayed in triplicate, and control tubes (lacking antiserum) were run in duplicate for each unknown for detection of non-specific binding. The limits of detection (approximately 25 $\mu\text{leq/ml}$) were reported as 10% displacement of iodinated ligand; the upper limits of normal (90 $\mu\text{leq/ml}$) corresponded to 25–30% displacement. With serum pools of 60, 103, and 743 $\mu\text{leq/ml}$, the interassay coefficients of variation were 19% ($n = 20$), 14% ($n = 11$), and 9% ($n = 15$), respectively.

cAMP was measured by a modification of the protein-binding assay of Gilman (41). The assay was designed to optimize cAMP measurement between 0.5 and 10 pmol (approximately 20 and 80% displacement, respectively); about 90% saturation was achieved by the addition of 1–1.5 pmol of [^3H]cAMP. Plasma and urine samples from a given patient were always assayed simultaneously. The interassay coefficient of variation for urine specimens is 10.2% (58 assays) and for plasma samples, 15.4% (24 assays).

Urinary creatinine was measured by the method of Bonses and Taussky (42). Other routine analyses were determined by standard techniques in the clinical chemistry laboratories of the Clinical Center and the Massachusetts General Hospital.

In the early phases of this study, it became clear that the serum creatinine was the least reliable of the various analyses required for the calculation of NcAMP (and cAMP excretion, expressed as a function of GFR). This potential problem can be minimized by the use of the mean of several measurements of serum creatinine in each study.

Expression and calculation of cAMP data

NcAMP was expressed: (a) as an absolute rate (nanomoles per minute), (b) in relative terms, provided by the clearance ratio (cAMP:creatinine), and (c) as a function of GFR, as given by the expression:

$$\frac{(UcA \cdot V) - (PcA \cdot Ccr)}{Ccr} \times 100,$$

where *C*, *P*, *U*, and *V* represent the conventional symbols for clearance, plasma and urinary concentration, and urine flow rate; *cA* is cyclic AMP and *cr* is creatinine. The units of this

² Nichols, A., and S. Krutzik. Unpublished observations.

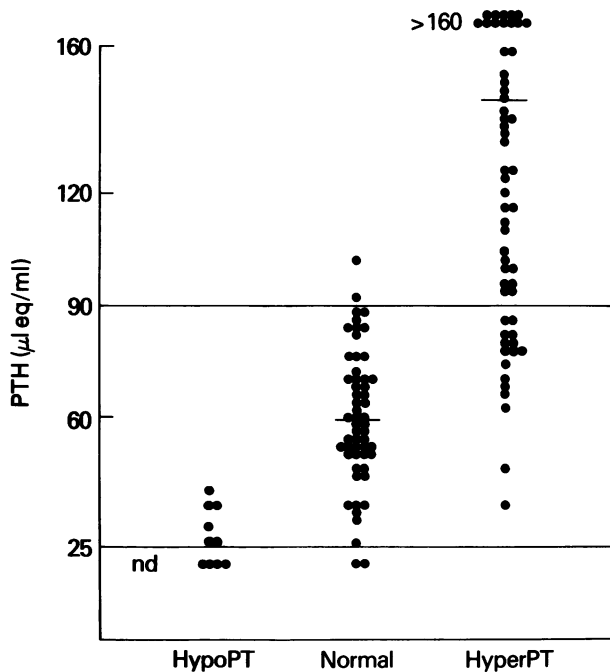


FIGURE 1 Plasma iPTH in the control subjects and in patients with hypoparathyroidism and 1° HPT. Most points represent the mean of more than one observation (see Methods), and the horizontal bars are the mean values for the control group and patients with 1° HPT. The limits shown for this assay (25–90 µeq/ml) were derived from the range of observed values in 150 normal subjects (40). 95% of the subjects in the control group ($n = 55$) of this study had levels of iPTH ≤ 90 µeq/ml (mean ± 1.645 SD), and two subjects had undetectable (nd) levels.

expression are nmol/min per 100 ml GFR, or more correctly, nmol/100 ml GF (where GF is glomerular filtrate).

UcAMP was expressed: (a) as a rate (nanomoles per minute, micromoles per 24 h), (b) as a function of urinary creatinine (nanomoles per milligram creatinine, micromoles per gram creatinine) and, (c) as a function of GFR, as given by:

$$\frac{(UcA \cdot V)}{C_{cr}} \times 100,$$

where the symbols and units correspond to those employed above.

UcAMP, expressed as a function of GFR (nanomoles per 100 ml GF), may be conveniently computed by the product of the serum creatinine and UcAMP (in nanomoles per milligram creatinine), and NcAMP (nanomoles per 100 ml GF) may be easily obtained by subtracting the plasma cAMP concentration (in nanomoles per deciliter) from this value; these simple calculations can be applied to spot sample collections.

Statistical analysis

The cAMP and iPTH analyses in the control group appeared to be normally distributed, and these data were treated with conventional statistics. In the patients with 1° HPT, however, both parameters were positively skewed; no attempt was made to normalize these data.

RESULTS

Plasma PTH. The results for plasma iPTH are shown in Fig. 1 and Tables I and II. In the group with 1° HPT, iPTH was increased (>90 µeq/ml) in 41 (73%) of the 56 patients in whom it was measured. Excluding the 5 patients who were “biochemically normal” (see Methods), iPTH was increased in 80% of these patients.

TABLE I
Summary of Analyses

| Group | iPTH | Creatinine clearance* | cAMP excretion | cAMP excretion | cAMP excretion | Plasma cAMP | Clearance cAMP |
|------------------------------|---------------|-----------------------|-----------------|--------------------|-----------------|------------------|----------------|
| | µeq/ml | ml/min | nmol/min | nmol/mg creatinine | nmol/100 ml GF | nM | ml/min |
| Entire group ($n = 55$) | 59 \pm 19 ‡ | 102 \pm 23 | 3.22 \pm 0.91 | 3.11 \pm 0.78 | 3.19 \pm 0.68 | 16.35 \pm 3.27 | 204 \pm 61 |
| Normal range § | 21–97 | — | 1.40–5.04 | 1.55–4.67 | 1.83–4.55 | — | — |
| 95% Limit [¶] | 90 | — | 4.72 | 4.39 | 4.31 | — | — |
| Females ($n = 32$) ¶ | 61 \pm 19 | 96 \pm 21 | 3.09 \pm 0.86 | 3.42 \pm 0.69** | 3.25 \pm 0.70 | 16.14 \pm 2.78 | 194 \pm 47 |
| Normal range § | — | — | — | 2.04–4.80 | — | — | — |
| 95% Limit [¶] | — | — | — | 4.56 | — | — | — |
| Males ($n = 23$) ¶ | 56 \pm 18 | 111 \pm 23 | 3.41 \pm 0.94 | 2.68 \pm 0.69** | 3.09 \pm 0.63 | 16.65 \pm 3.82 | 217 \pm 74 |
| Normal range § | — | — | — | 1.30–4.06 | — | — | — |
| 95% Limit [¶] | — | — | — | 3.82 | — | — | — |

* Creatinine clearance data are taken from the clearance periods.

‡ All data are shown as mean \pm SD.

§ Normal range represents 95% confidence limits (mean ± 2 SD).

As anticipated, 8 of the 11 patients with 1° HPT and greater than twofold increases in iPTH also had significant azotemia (mean GFR 48 ml/min), a circumstance in which inactive fragments of the hormone are known to accumulate in plasma (5, 43). Immunoreactive PTH was at or above the limit of detection (10% displacement) in six patients with hypoparathyroidism.

Nephrogenous cAMP. NcAMP values are shown in Figs. 2 and 3 and in Tables I and II, expressed: (a) in relative terms (as the clearance ratio, cAMP:creatinine), (b) as an absolute rate (nanomoles per minute), and (c) as a function of GFR (nanomoles per 100 ml GF).

In azotemic and nonazotemic patients with chronic hypoparathyroidism, values for the clearance ratio clustered around unity, with a mean of 1.23 ± 0.09 (\pm SD) and a range of observed values of 1.08–1.36. In the control group, the mean clearance ratio was 2.01 ± 0.43 , with 95% of values falling below 2.72. The clearance ratio was greater than 2.72 in 52 of the patients with 1° HPT (91%).

19 patients with 1° HPT had coincident mild renal insufficiency (mean GFR 64 ml/min, range 52–80 ml/min), and an additional 10 patients had moderate renal insufficiency (mean GFR 43 ml/min, range 30–50 ml/min). Although the absolute rate of NcAMP production (nanomoles per minute) correlated poorly with the hyperparathyroid state in these patients (Fig. 2 and Table II), the clearance ratio was greater than 2.72 in 28 of these 29 patients. Thus, there was a clear parallel between the decreases in cAMP clearance and creatinine clearance in the patients with 1° HPT and renal insufficiency (Table II) and, therefore, an almost linear

relationship between GFR and the ability to generate NcAMP in these patients (Fig. 2).

These findings indicated that NcAMP might parametrically be expressed as a function of GFR, and in Fig. 3, NcAMP is represented as nanomoles per 100 ml GF. NcAMP was low in the patients with chronic hypoparathyroidism (0.40 ± 0.20 nmol/100 ml GF, mean \pm SD). In the control group, NcAMP was 1.55 ± 0.63 nmol/100 ml GF (mean \pm SD), with 95% of values below 2.59 nmol/100 ml GF. In the group with 1° HPT, NcAMP was greater than 2.59 nmol/100 ml GF in 52 patients (91%), and all 29 patients with 1° HPT and renal impairment (identified in Fig. 3) displayed abnormally high values. Only the five familial patients classified as “biochemically normal” (patients 36–40, Table II) displayed normal NcAMP. Excluding patients with moderate azotemia (GFR \leq 50 ml/min), there was a positive correlation ($r = +0.40$) between NcAMP and iPTH in the group with 1° HPT. Eight control subjects with renal impairment (mean GFR 65 ml/min, range 42–80 ml/min) had slightly higher mean values for iPTH (65 μ leq/ml) and NcAMP (1.90 nmol/100 ml GF) than the general control population.

In 26 of the 57 patients with 1° HPT, the hypercalcemia was mild and (or) intermittent, yet the disease in these patients was not “mild” in terms of biochemical documentation; both iPTH (above 90 μ leq/ml in 84%) and NcAMP (abnormal in 100%) were elevated in the great majority of these patients, and the biochemical abnormalities in some patients were striking (Table II). There was a positive correlation between the level of serum calcium and both iPTH ($r = +0.49$) and

in the Control Group

| Clearance ratio | Filtered cAMP | Nephrogenous cAMP | Nephrogenous cAMP | Creatinine excretion | cAMP excretion | cAMP excretion |
|------------------------|-----------------|-------------------|-----------------------|----------------------|---------------------------------|---|
| <i>cAMP:creatinine</i> | <i>nmol/min</i> | <i>nmol/min</i> | <i>nmol/100 ml GF</i> | <i>mg/24 h</i> | <i>μmol/24 h</i> | <i>μmol/g creatinine</i> |
| 2.01 ± 0.43 | 1.66 ± 0.48 | 1.56 ± 0.68 | 1.55 ± 0.63 | $1,494 \pm 398$ | 4.42 ± 1.11 | 3.09 ± 0.85 |
| 1.15–2.87 | — | 0.20–2.92 | 0.29–2.81 | — | 2.20–6.64 | 1.39–4.79 |
| 2.72 | — | 2.68 | 2.59 | — | 6.24 | 4.49 |
| 2.06 ± 0.42 | 1.56 ± 0.49 | 1.53 ± 0.59 | 1.64 ± 0.62 | $1,272 \pm 212^{**}$ | 4.27 ± 0.94 | $3.39 \pm 0.74^{**}$ |
| — | — | — | — | — | — | 1.91–4.87 |
| — | — | — | — | — | — | 4.61 |
| 1.94 ± 0.44 | 1.81 ± 0.43 | 1.59 ± 0.79 | 1.42 ± 0.62 | $1,766 \pm 404^{**}$ | 4.61 ± 1.27 | $2.70 \pm 0.82^{**}$ |
| — | — | — | — | — | — | 1.06–4.34 |
| — | — | — | — | — | — | 4.05 |

[‡] 95% limit indicates that 95% of observations are less than this value (mean \pm 1.645 SD).

[¶] 24-h collections were obtained from 28 females and 22 males.

** Significantly different ($P < 0.001$).

TABLE II
Summary of Analyses in Patients with

| Patient | Age/sex | Serum calcium‡ | iPTH‡ | Creatinine clearance§ | cAMP excretion | cAMP excretion | cAMP excretion |
|-------------------------|---------|----------------|---------|-----------------------|----------------|--------------------|----------------|
| | yr | mg/dl | µeq/ml | ml/min | nmol/min | nmol/mg creatinine | nmol/100 ml GF |
| 1 | 43 F | 10.6 | 80 | 52 | 3.24 | 7.28 | 6.23 |
| 2 | 72 F | 12.1 | 422 | 33 | 1.72 | 3.85 | 5.21 |
| 3 | 30 F | 12.4 | 104 | 126 | 10.55 | 10.51 | 8.37 |
| 4 | 43 F | 10.6 | 93 | 113 | 5.73 | 7.24 | 5.07 |
| 5 | 41 F | 10.7 | 94 | 72(104) | 5.11 | 8.44 | 7.10 |
| 6 | 45 M | 11.5 | 123 | 111 | 7.22 | 5.90 | 6.50 |
| 7 | 61 F | 10.3 | 85 | 54 | 3.52 | 6.82 | 6.52 |
| 8 | 51 M | 11.5 | 188 | 110 | 7.63 | 8.10 | 6.94 |
| 9 | 52 F | 10.9 | 152 | 83 | 4.52 | 8.39 | 5.44 |
| 10 | 78 F | 12.1 | 375 | 44 | 2.75 | 6.86 | 6.25 |
| 11 | 41 F | 11.8 | 270 | 98 | 4.69 | 7.36 | 4.79 |
| 12 | 26 M | 11.8 | 61 | 119 | 5.45 | 4.59 | 4.58 |
| 13 | 70 M | 13.7 | 646 | 50 | 6.01 | 6.18 | 11.78 |
| 14 | 65 M | 11.5 | 139 | 66 | 5.60 | 8.48 | 8.48 |
| 15 | 22 F | 10.8 | 112 | 70 | 5.53 | 9.49 | 7.90 |
| 16 | 55 F | 11.0 | 147 | 80 | 4.29 | 7.33 | 5.36 |
| 17 | 68 M | 10.4 | 101 | 64 | 3.01 | 5.08 | 4.70 |
| 18 | 57 M | 10.3 | 205 | 79 | 4.22 | 6.31 | 5.34 |
| 19 | 54 M | 14.0 | 204 | 41 | 3.07 | 2.76 | 7.49 |
| 20 | 65 F | 12.1 | 69 | 57 | 4.43 | 5.42 | 7.77 |
| 21 | 56 F | 10.8 | 116 | 113 | 5.86 | 5.97 | 5.19 |
| 22 | 26 M | 11.4 | 67 | 120 | 5.34 | 3.71 | 4.45 |
| 23 | 54 M | 10.8 | 81 | 127 | 5.29 | 3.98 | 4.17 |
| 24 | 31 F | 11.2 | 115 | 105 | 6.96 | 6.55 | 6.63 |
| 25 | 44 M | 10.7 | — | 57 | 2.96 | 3.47 | 5.19 |
| 26 | 55 F | 10.0 | 125 | 45 | 2.24 | 3.76 | 4.98 |
| 27 | 52 F | 10.1 | 158 | 63 | 3.98 | 5.60 | 6.32 |
| 28 | 45 F | 12.7 | 218 | 86 | 11.32 | 13.12 | 13.16 |
| 29 | 74 M | 10.1 | 275 | 42 | 3.85 | 6.32 | 9.16 |
| 30 | 58 M | 12.8 | 232 | 68 | 3.91 | 6.73 | 5.75 |
| 31 | 72 F | 10.7 | 280 | 30 | 2.26 | 6.36 | 7.53 |
| 32 | 42 M | 11.4 | 158 | 73 | 5.98 | 4.90 | 8.19 |
| 33 | 34 F | 10.2 | 150 | 90 | 8.40 | 10.42 | 9.33 |
| 34 | 9 F | 11.0 | 80 | 47 | 3.31 | 7.86 | 7.04 |
| 35 | 11 F | 10.0 | 139 | 106 | 9.42 | 9.85 | 8.89 |
| 36 | 49 M | 11.4 | 27 | 118 | 3.19 | 2.17 | 2.70 |
| 37 | 43 M | 11.0 | 66 | 120 | 4.45 | 3.04 | 3.71 |
| 38 | 17 F | 12.1 | 86 | 104 | 2.73 | 2.64 | 2.63 |
| 39 | 16 M | 12.5 | 79 | 107 | 3.21 | 2.30 | 3.00 |
| 40 | 16 M | 12.8 | 74 | 108 | 3.44 | 2.27 | 3.19 |
| Mean±SD | | 11.3±1.0 | 159±116 | 82±29 | 4.91±2.19 | 6.19±2.52 | 6.33±2.25 |
| “Clinical” group¶ | | 10.5±0.5 | 109±29 | 75±21 | 4.48±1.48 | 6.59±1.53 | 6.33±1.57 |
| Entire group** (n = 57) | | 11.1±1.0 | 145±100 | 80±27 | 4.78±2.01 | 6.31±2.28 | 6.33±2.07 |
| Females** (n = 35) | | 10.9±0.8 | 142±79 | 74±26 | 4.86±2.33 | 7.21±2.09 | 6.69±1.92 |
| Males** (n = 22) | | 11.5±1.1 | 146±130 | 90±27 | 4.66±1.35 | 4.86±1.76 | 5.75±2.16 |

* See Methods for definition of “definite” subgroup. Patients 1–18 had adenomas, patients 19–29 had hyperplasia, patients 30–33 had significant localization by iPTH at neck catheterization, and patients 30–40 had familial hyperparathyroidism.

‡ Serum calcium and iPTH values drawn during the clearance periods; other values were excluded.

§ Creatinine clearance data were taken from the clearance periods. Data from the 24-h collections agreed within 15% in all but 4 of the 57 patients, for whom the 24-h values are shown in parentheses. These patients and patient 34 were specifically excluded from the azotemic groups (see text).

¶ The absolute 24-h excretion of cAMP is not shown but may be easily calculated from the data given. The values for 24-h cAMP excretion, expressed as a function of GFR, may also be obtained by multiplying the data given (µmol/g creatinine)

“Definite” Hyperparathyroidism*

| Plasma cAMP | Clearance ratio | Nephrogenous cAMP | Nephrogenous cAMP | - Creatinine excretion | cAMP excretion [¶] |
|----------------|---------------------|----------------------|----------------------|------------------------------|--------------------------------|
| nM | cAMP: creatinine | nmol/min | nmol/100 ml GF | mg/24 h | μmol/g creatinine |
| 18.47 | 3.38 | 2.28 | 4.38 | 600 | 6.51 |
| 15.20 | 3.37 | 1.22 | 3.70 | 700 | 3.17 |
| 17.00 | 5.08 | 8.41 | 6.67 | 1440 | 8.76 |
| 13.35 | 3.80 | 4.22 | 3.73 | 1100 | 6.22 |
| 14.70 | 4.82 | 4.05 | 5.63 | 1270 | 5.75 |
| 18.00 | 3.61 | 5.22 | 4.70 | 1750 | 5.37 |
| 17.57 | 3.70 | 2.57 | 4.76 | 880 | 6.07 |
| 19.20 | 3.62 | 5.52 | 5.02 | 1500 | 7.77 |
| 14.80 | 3.68 | 3.29 | 3.97 | 760 | 7.15 |
| 16.98 | 3.64 | 2.01 | 4.57 | 640 | 6.66 |
| 11.36 | 4.21 | 3.58 | 3.65 | 1030 | 6.81 |
| 13.78 | 3.33 | 3.81 | 3.20 | 1930 | 4.59 |
| 24.31 | 4.93 | 4.77 | 9.35 | 900 | 8.34 |
| 27.28 | 3.11 | 3.80 | 5.76 | 950 | 8.48 |
| 18.71 | 4.23 | 4.22 | 6.03 | 930 | 8.55 |
| 15.65 | 3.41 | 3.04 | 3.86 | 710 | 7.75 |
| 17.45 | 2.69 | 1.89 | 2.96 | 980 | 4.33 |
| 17.07 | 3.13 | 2.87 | 3.63 | 890 | 5.78 |
| 23.27 | 3.22 | 2.12 | 5.17 | 1980 | 2.86 |
| 23.04 | 3.45 | 3.12 | 5.47 | 940 | 6.48 |
| 12.21 | 4.27 | 4.48 | 3.96 | 1260 | 6.40 |
| 14.60 | 3.05 | 3.59 | 3.00 | 1710 | 3.63 |
| 15.00 | 2.78 | 3.39 | 2.67 | 1630 | 3.71 |
| 16.51 | 3.94 | 5.23 | 4.98 | 1560 | 7.43 |
| 18.24 | 2.85 | 1.92 | 3.37 | — | — |
| 16.22 | 3.09 | 1.51 | 3.36 | 960 | 4.59 |
| 16.61 | 3.80 | 2.93 | 4.66 | 1120 | 5.71 |
| 19.27 | 6.81 | 9.66 | 11.23 | 1570 | 10.63 |
| 27.89 | 3.43 | 2.68 | 6.38 | 960 | 6.18 |
| 15.00 | 3.97 | 2.89 | 4.25 | 730 | 6.88 |
| 23.80 | 3.23 | 1.55 | 5.17 | 580 | 5.66 |
| 19.96 | 4.13 | 4.52 | 6.19 | 1840 | 4.84 |
| 17.81 | 5.27 | 6.80 | 7.56 | 1160 | 10.62 |
| 19.82 | 3.58 | 2.38 | 5.06 | 410 | 9.17 |
| 19.85 | 4.47 | 7.31 | 6.90 | 920 | 12.20 |
| 10.04 | 2.70 | 2.01 | 1.70 | 1970 | 2.17 |
| 12.59 | 2.96 | 2.93 | 2.44 | 1960 | 3.29 |
| 12.90 | 2.04 | 1.30 | 1.25 | — | — |
| 16.18 | 1.85 | 1.46 | 1.36 | — | — |
| 14.51 | 2.19 | 1.87 | 1.73 | — | — |
| 17.38±4.09 | 3.62±0.91 | 3.56±1.90 | 4.58±1.99 | 1173±448 | 6.40±2.25 |
| 17.85±4.37 | 3.63±0.81 | 3.22±1.14 | 4.54±1.33 | 1006±330 | 6.23±1.25 |
| 17.52±4.14 | 3.62±0.88 | 3.46±1.72 | 4.57±1.82 | 1122±422 | 6.34±2.03 |
| 17.62±3.81 | 3.87±0.91 | 3.62±1.98 | 4.94±1.73 | 1030±639 | 6.98±1.85 |
| 17.86±4.58 | 3.23±0.67 | 3.20±1.44 | 3.98±1.80 | 1456±426 | 5.25±1.76 |

by the ratio of the clearance period values (nmol/100 ml GF: nmol/mg creatinine, which is equivalent to the serum creatinine in the patients).

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** The data for the “clinical” subgroup is included.

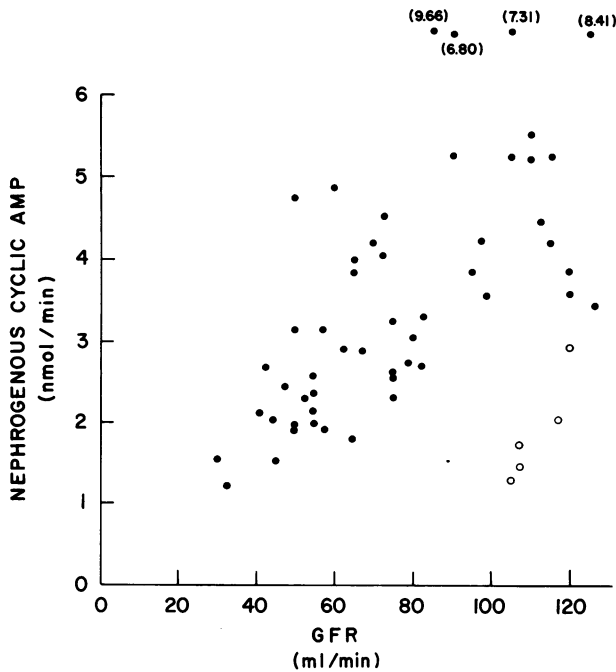


FIGURE 2 The relationship between NcAMP (nanomoles per minute) and glomerular filtration rate in 57 patients with 1° HPT. The values for NcAMP in four patients with severe biochemical disease are shown in parentheses, and the values for five patients from a kindred found to be "biochemically normal" are depicted by open circles. There was a clear parallel between cAMP clearance and creatinine clearance in the patients with 1° HPT and mild or moderate renal impairment (mean respective cAMP and creatinine clearances in the patients with mild azotemia were 232 and 64 ml/min and, in the patients with moderate azotemia, 153 and 43 ml/min). Excluding the patients with severe biochemical disease and those who were "biochemically normal," there was a linear correlation between NcAMP (nanomoles per minute) and GFR ($r = +0.47$).

NcAMP ($r = +0.33$) in the patients with 1° HPT (excluding patients 36–40 in Table II).

Total cAMP excretion. UcAMP during the clearance periods, expressed as: (a) a rate (nanomoles per minute), (b) a function of creatinine excretion (nanomoles per milligram creatinine), and (c) a function of GFR (nanomoles per 100 ml GF), is shown in Fig. 4 and in Tables I and II.

As expected, when expressed in absolute terms (nanomoles per minute), UcAMP correlated poorly with the hyperparathyroid state: 25 of the 29 patients with 1° HPT and mild or moderate renal insufficiency displayed "normal" values, and several additional patients (identified in Table II) had clearly inadequate urine collections during the clearance periods.

On initial inspection, the expression of UcAMP as a ratio to urinary creatinine (nanomoles per milligram creatinine, Tables I and II) appeared to provide a much improved diagnostic yield (approximately 81%)

in the patients with 1° HPT, thus confirming, in a phenomenologic sense, many previous reports (11–18). However, a detailed examination of the data revealed a number of examples of substantive and (or) interpretive difficulties which derived from the non-parametric nature of this expression: (a) Expressed as nanomoles per milligram creatinine, values for UcAMP were significantly different for the male and female control subjects ($P < 0.001$), and these differences were entirely attributable to the significantly ($P < 0.001$) lower creatinine excretion by female individuals (Table I). Results for the male and female patients with 1° HPT were also significantly ($P < 0.001$) different and for largely the same reason (Table II). (b) There was an obvious fortuitous aspect in the apparent improvement in diagnostic potential provided by expressing results as a function of urinary creatinine, in that the patients with 1° HPT (male and female) demonstrated greater than a fourfold range in creatinine excretion, with a mean value which was 25% less than that of the control group (Tables I and II). Thus, the "elevated" UcAMP in a number of patients with 1° HPT was as much apparent as real, particularly in the 27 patients with relatively low (<1,000 mg/24 h) creatinine excretion (Table II). Conversely, several heavily muscled patients with 1° HPT displayed "normal" UcAMP as a result of high levels of creatinine excretion (e.g., patient 22, Table

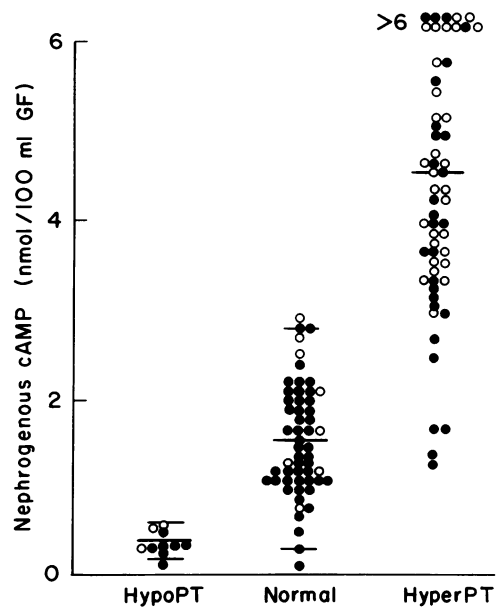


FIGURE 3 NcAMP, expressed as a function of GFR, in the control subjects and patients with 1° HPT. The open circles depict the subjects and patients with renal impairment (mean creatinine clearance ≤ 80 ml/min). The horizontal bars represent the mean values, and the control group is shown as mean ± 2 SD.

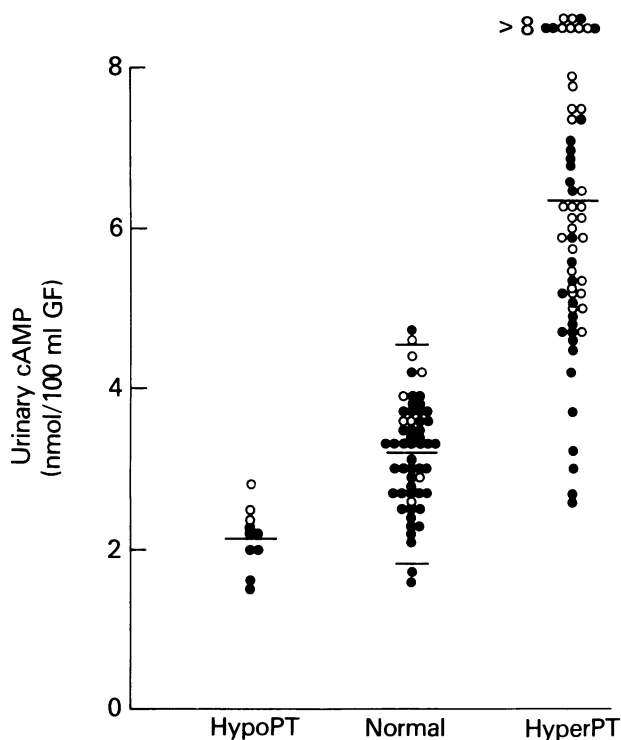


FIGURE 4 UcAMP, expressed in nmol/100 GF, in the control subjects and in patients with hypoparathyroidism and 1° HPT. The open circles depict the subjects and patients with renal impairment (mean GFR \leq 80 ml/min). The horizontal bars represent the mean values for the patients with hypoparathyroidism and 1° HPT, and the control group is shown as mean \pm 2 SD.

II). (c) The expression did not provide a reliable index of the hyperparathyroid state in a number of patients with 1° HPT and moderate renal impairment (patients 2, 19, 25, 26, and 32, Table II). (d) Although the mean UcAMP was low in the patients with chronic hypoparathyroidism (2.08 ± 0.72 nmol/mg creatinine, mean \pm SD), results varied over greater than a fivefold range (from 0.72 nmol/mg creatinine in an azotemic patient to 3.61 nmol/mg creatinine in a cachectic patient with low creatinine excretion).

A partial, but artificial, solution to the interpretive problems inherent in the expression may be provided by employing different male and female control ranges (Table I).

Based on the unique features of cAMP clearance in man (28), both the filtered and nephrogenous components of cAMP excretion would be predicted to be functions (direct or indirect) of the GFR, and the data in this series readily confirmed this prediction. The data relating NcAMP to GFR is presented in Fig. 2. Fig. 5 depicts the relationship between GFR and the filtered load of cAMP in 79 patients and control subjects with normal renal function and in 46 patients with

mild to moderate renal impairment. There was a good correlation between filtered cAMP and GFR, so that, when expressed as a function of GFR, there was a relatively constant quantity of the nucleotide filtered by azotemic and nonazotemic individuals (see legend of Fig. 5). Thus, if the total excretion of cAMP were to be expressed as a function of GFR, there would be a relatively stable "background" of filtered nucleotide upon which the variable nephrogenous component would be superimposed.

UcAMP in the control and patient groups, expressed as a function of GFR (nanomoles per 100 ml GF), is depicted in Fig. 4 (see also Tables I and II). The parametric nature and advantageous features of this expression are immediately apparent, based on the following considerations: (a) The control subjects demonstrated a relatively narrow range of values for UcAMP (1.83–4.55 nmol/100 ml GF, with 95% of subjects excreting less than 4.31 nmol/100 ml GF), and there were no differences between male and female control subjects (Table I). (b) 51 (89%) of the patients with 1° HPT excreted more than 4.31 nmol/100 ml GF of the nucleotide, and the increased UcAMP in these patients was real rather than apparent (Fig. 4 and Table II). All patients with "normal" UcAMP also had "normal" iPTH and were in the "definite" subgroup of patients

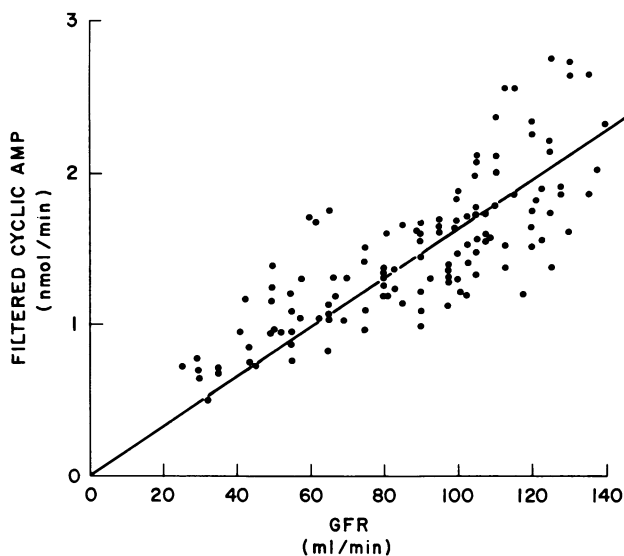


FIGURE 5 The relationship between GFR and the filtered load of cAMP in patients and control subjects with and without renal impairment. The figure depicts 79 patients and control subjects with normal renal function (GFR 108 ± 16 ml/min, mean \pm SD) and a mean filtered load of cAMP of 1.72 ± 0.41 nmol/min (\pm SD) and 46 patients with mild to moderate azotemia (GFR 56 ± 15 ml/min, mean \pm SD) and a mean filtered load of the nucleotide of 1.08 ± 0.29 nmol/min (\pm SD). Expressed as a function of GFR, the mean (\pm SD) filtered loads in the azotemic and nonazotemic patients were 1.92 ± 0.45 and 1.59 ± 0.30 nmol/100 ml GF, respectively.

with 1° HPT (Table II). (c) UcAMP was appropriately elevated in all patients with 1° HPT and mild or moderate renal impairment (identified in Fig. 4). (d) The patients with hypoparathyroidism excreted 2.16 ± 0.38 nmol/100 ml GF of cAMP (mean \pm SD), with a relatively narrow range of values (1.52–2.83 nmol/100 ml GF).

The data from the 24-h samples provided findings essentially identical to those noted above (Tables I and II, the values expressed as a function of GFR are not shown). The 24-h analyses did not identify abnormalities in patients with 1° HPT who demonstrated normal UcAMP during the clearance periods.

DISCUSSION

Immunoassay data. Our iPTH data are consonant with those of other laboratories employing multivalent antisera (2, 43) but do not provide the 90% or greater increases in iPTH in patients with 1° HPT noted by some investigators with antisera reported to be primarily carboxyl specific (3, 5, 6, 38). However, the present method remains very useful clinically, providing accurate diagnostic information in the great majority of patients with 1° HPT when results are discriminated as a function of serum calcium in the patients. The detectable iPTH in a number of the patients with hypoparathyroidism may have derived from some residual parathyroid function in patients whose serum calcium was deliberately being kept low (7.9 ± 0.8 mg/dl, mean \pm SD).

Expression of cAMP data. The clearance ratio (cAMP:creatinine) is the simplest expression of NcAMP and provides accurate quantitative information under most circumstances. However, as a relative expression, this ratio is subject to quantitative variation resulting from differences in plasma levels of cAMP among individuals. The biological variables which alter plasma cAMP and which must be recognized as potential influences on the clearance ratio are physical activity (25), severe renal failure (9, 44), and adrenergic discharge (21, 25). In this series, there were several examples of patients with moderate renal failure (patients 13, 14, 19, 20, 29, and 31, Table II) or patients who were considered to be "stressed" in whom plasma cAMP was moderately elevated with a coincident "apparent" lowering of NcAMP as expressed by the clearance ratio. The expression nanomoles per 100 ml GF provides an accurate quantitation of NcAMP under all circumstances, and we favor it for routine use.

Our data regarding the inadequacies of expressing UcAMP as an absolute rate or as a function of creatinine excretion require little amplification. The latter expression (nanomoles per milligram creatinine) is not based on physiologic rationale and came into use because of the empirical observations that results ex-

pressed on this basis gave a somewhat narrower normal range and a better apparent segregation of patients with 1° HPT from normal (9, 11–18, 33–35, 37). It is of interest that in most of these studies, as in this series, expressing UcAMP as a function of creatinine excretion led to a mean decrease in values from control subjects and a mean increase in values from patients with 1° HPT, findings which could only have resulted from differences in creatinine excretion between the control and diseased populations. An additional, and extremely important, example of the potential hazard of expressing results on this basis is the inherent nonspecificity of this expression. That is, it is self-evident that a reduction in creatinine excretion accompanies any disease process with a significant wasting and (or) neuromuscular component and that this occurs independent of the variables which control cAMP excretion. In our experience, this nonparametric feature accounts entirely for the "apparent" increases in UcAMP which have been reported in patients with thyrotoxicosis (26, 27, 45), the syndrome of inappropriate antidiuretic hormone secretion (13), and the carcinoid syndrome (13). Expressed parametrically (nanomoles per 100 ml GF), UcAMP is perfectly normal in these patients (29).

The expression of UcAMP as a function of GFR is eminently rational, based on the physiology of the excretion of the nucleotide (28). This expression (nanomoles per 100 ml GF) avoids entirely the inadequacies of alternate expressions and provides a parametric index of UcAMP in a variety of clinical situations, including mild to moderate renal impairment. A key feature of this expression is its functional specificity, for, in our experience, the only condition (other than hyperparathyroidism) associated with a "true" increase in UcAMP is pheochromocytoma (29). This specificity is not unexpected, in that: (a) the urinary excretion of cAMP is an insensitive index of altered rates of cAMP production in the extracellular pool, due to the wide distribution and rapid turnover of the nucleotide in this pool (28, 29), (b) in contrast, the excretion of cAMP provides a rapid and sensitive index of altered rates of production of the nucleotide in the nephrogenous pool (9, 10), and (c) the relationship between NcAMP and circulating PTH in vivo appears to be specific (29). Thus, with added clinical experience, we have begun to rely increasingly on the simple measurement of UcAMP (expressed as nanomoles per 100 ml GF) and have come to view the routine determination of NcAMP as unnecessary in most hypercalcemic patients.

Although several laboratories have empirically expressed UcAMP as a function of GFR in evaluating patients with varying degrees of azotemia and secondary hyperparathyroidism (46, 47), at present, this expression should not be employed in evaluating pa-

tients with a GFR less than approximately 20 ml/min. Plasma cAMP is increased in moderate to severe renal failure (9, 44), so that, expressed as a function of GFR, the filtered load of the nucleotide is relatively increased in this setting. The influence of this phenomenon on results of total cAMP excretion, expressed on a GFR basis, was quantitatively rather insignificant over the range of renal function encountered at this series (i.e., a disproportionate increase approximating 10% in the total excretion of the nucleotide was noted in the azotemic patients in this study, see Table II and Fig. 5). However, this potential problem clearly becomes significant at levels of GFR in the range of 10 ml/min, and this important area requires more detailed investigation.

For the sake of brevity and because the collections were less well controlled, the results of the 24-h cAMP analyses were not emphasized in this series. However, these results were virtually superimposable with those obtained during the clearance periods (see Tables I and II), and the determination of the 24-h excretion of cAMP may be preferable for some laboratories and/or under certain clinical or experimental conditions. In addition, although modest physical activity has a slight but significant influence on plasma cAMP concentrations, such activity has a minimal influence in the results of total UcAMP and does not appear to require control for routine clinical interpretation of these results.

Clinical spectrum of parathyroid disease. The large prospective experience with 1° HPT provided by this series encompassed the entire clinical spectrum of the disease and was essential in evaluating the significance of several potential problems which might interfere with the cAMP analyses on their interpretation. First, with regard to the conflicting reports concerning possible direct inhibitory effects of high extracellular calcium concentrations on the activity of the PTH-sensitive renal adenylate cyclase system (20, 48, 49), it was of interest that there was a positive, rather than a negative, correlation between serum calcium and NcAMP in the patients with 1° HPT. Second, our data confirmed the previously reported low UcAMP and reduced cAMP response capacity (to endogenous PTH) in patients with renal impairment (11, 12, 19); yet the parametric expression of both NcAMP and UcAMP as a function of GFR provided an excellent index of parathyroid status over the range of GFR encountered in this series. Finally, other clinical variables (e.g., diurnal changes, medications, etc.) did not appear to exert a discernible influence on the cAMP results.

The inclusion in this series of the kindred which was "biochemically normal" could be questioned, and the results in these five patients clearly influenced the apparent diagnostic potential of the various parameters which were compared. However, these patients ful-

filled one set of criteria in a prospective study and could not, therefore, be excluded. This kindred has been reported separately as an example of "familial hypocalciuric hypercalcemia" (50) and represents an as yet undefined variant of hypercalcemia and/or hyperparathyroidism similar to that of another kindred reported in the literature (51). The other patients in this series with familial hyperparathyroidism or multiple endocrine neoplasia (types I and II) were clearly biochemically abnormal (patients 25, 28, 34, and 35, Table II).

In addition to the hyperparathyroid patients included in this series, we have studied several patients suspected of having hypoparathyroidism on the basis of modest depressions in serum calcium and elevations in serum phosphorous. As might be anticipated, measurements of NcAMP in these patients overlapped with the low-normal range (i.e., values up to 1 nmol/100 ml GF were seen).

In five patients with nonparathyroid hypercalcemia, NcAMP averaged 0.26 nmol/100 ml GF (range: nondetectable to 0.40 nmol/100 ml GF), and plasma iPTH averaged 31 μ leq/ml (range: nondetectable to 56 μ leq/ml). In three patients with apparent "ectopic hyperparathyroidism", NcAMP averaged 4.57 nmol/100 ml GF (range: 3.97 to 5.32 nmol/100 ml GF), and plasma iPTH averaged 51 μ leq/ml (range: 49 to 52 μ leq/ml).

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