



## Detection of Primary Hyperparathyroidism, With Special Reference to its Occurrence in Hypercalciuric Females with "Normal" or Borderline Serum Calcium

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**T**HE diagnosis of primary hyperparathyroidism calls for a high index of suspicion in a variety of clinical situations. The possibility of this diagnosis should be entertained not only in patients with renal calculus and bone disease but also in those with peptic ulcer,<sup>1</sup> pancreatitis,<sup>2</sup> psychoneurosis or psychosis,<sup>3</sup> renal failure<sup>4</sup> and myopathy.<sup>5, 6</sup> It should also be kept in mind that the disorder may be familial<sup>7</sup> and that it may be associated with other endocrine adenomas, especially of the pituitary gland and pancreatic islets.<sup>8</sup> It has even been reported that the occurrence of neonatal tetany may be a clue to hyperparathyroidism in the mother.<sup>9</sup>

The incidence of the disorder appears to vary directly with the diligence with which it is sought. This has been the case at the Toronto General Hospital, where there have been 71 cases of proved primary hyperparathyroidism during the years 1939 to 1966 (Table I). However, during the first 15 years (1939 to 1954) the total number of cases was only 16, or an average of approximately one per year, whereas during the last two years of this study there was a total of 19 cases, or 9.5 per year. At the present time the great majority of cases are found in

TABLE I.—INCIDENCE OF PRIMARY HYPERPARATHYROIDISM AT THE TORONTO GENERAL HOSPITAL, 1939-1966

	Cases	Average per year
1939-1954.....	16	0.8
1955-1964.....	36	3.6
1965-1966.....	19	9.5
Total.....	71	

patients with renal calculi. The presenting problems in the last 55 cases of the study are tabulated in Table II, and it can be seen that approximately 80% of the patients sought medical advice for renal calculi.

TABLE II.—PRESENTING COMPLAINTS IN 55 PATIENTS WITH PRIMARY HYPERPARATHYROIDISM SEEN DURING THE YEARS 1955-1966

Renal calculi.....	42
Bone disease.....	8
Peptic ulcer.....	1
Headaches*.....	1
Mental depression.....	1
Pancreatitis.....	1
"Idiopathic" edema*.....	1

\*The two patients presenting with headaches and idiopathic edema also had kidney stones. There was no apparent relationship between the hyperparathyroidism and the edema which has persisted following the removal of the parathyroid adenoma.

To suspect primary hyperparathyroidism is one thing; to diagnose it is another. Only rarely is it possible to do so on the basis of history or physical examination alone, and skeletal changes that are diagnostic can be demonstrated radiologically in only a small proportion of cases. The

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clinician is therefore entirely dependent upon the biochemical laboratory for the final answer. On the basis of our experience, we suspect that the diagnosis of primary hyperparathyroidism is not being made in one-third of the patients who harbour parathyroid adenomas because the clinicians do not have access to the high-quality laboratory service which is required for diagnosis.

The clinician who does not have easy access to a laboratory that can provide a sufficiently accurate serum calcium determination is also faced with the confusion in the medical literature dealing with the diagnosis of the disorder. New tests, or new versions of old tests, which are supposed to facilitate diagnosis have been described, but these reports often confuse rather than clarify the situation.

It is the purpose of this paper to evaluate the discriminatory value of a number of these tests on the basis of experience gained in the investigation of 55 cases of proved primary hyperparathyroidism during the years 1955 to 1966 inclusive. Fifty-two of these patients had parathyroid adenomas, two had adenomatous or chief cell hyperplasia and one had primary water cell hyperplasia. All cases were investigated personally by one of the authors (E.R.Y.) and the same method for calcium determinations was used throughout the study. During this 11-year period there were only three unsuccessful neck explorations. Attention is also drawn to the high incidence of primary hyperparathyroidism in hypercalciuric females with kidney stones, and it is demonstrated that such patients may have parathyroid adenomas even though the serum calcium is considered to be "normal" or borderline.

#### METHODS

Calcium in serum, urine and plasma ultrafiltrate was measured by the method of Campbell<sup>10</sup> which involves titration with EDTA using an ammonium purpurate indicator with end-point determination by photometric means. This method is rapid and capable of giving highly reproducible results. With it we were able to establish a narrow normal range of serum calcium of 8.9 to 10.3 mg. per 100 ml. Plasma ultrafiltrates were obtained using a modified Toribara technique.<sup>11</sup> Our methods for measuring phosphorus clearance (Cp), % tubular reabsorbed phosphate (% TRP) and the phosphorus excretion index (PEI) have been described in a previous publication.<sup>12</sup> Inorganic phosphorus in serum and urine was measured by the Gomori method.<sup>13</sup> The procedure followed for calcium infusion is a modification<sup>14</sup>

TABLE III.—NORMAL RANGES: SERUM CHEMISTRIES MEASURED IN THE EARLY MORNING FASTING STATE; URINE CALCIUM MEASURED DURING A NORMAL DIETARY CALCIUM INTAKE OF APPROXIMATELY 800–1000 MG. PER DAY; PHOSPHORUS CLEARANCE (Cp), % TUBULAR REABSORBED PHOSPHATE (TRP) AND PHOSPHORUS EXCRETION INDEX (PEI) MEASURED DURING NORMAL DIETARY PHOSPHATE INTAKE

Serum calcium	8.9–10.3 mg. per 100 ml.
Serum plasma	2.3–4.5 mg. per 100 ml.
Urine calcium	male < 300 mg. per day female < 250 mg. per day
Cp	6–16 ml. per min.
% TRP	82–97
PEI	–0.09 to +0.09

of that originally described by Howard, Hopkins and Connor.<sup>15</sup> Normal ranges are given in Table III. Unless otherwise stated, all blood samples were taken in the early morning fasting state, and all determinations were performed in a research laboratory.

#### RESULTS AND DISCUSSION

Our experience with serum calcium, serum plasma, urine calcium, Cp, % TRP, PEI and the calcium infusion is summarized in Table IV. In the case of serum calcium and phosphorous and urine calcium, the table refers to the mean of many determinations. In many instances, Cp, % TRP and PEI were repeated on one or more occasions, and again the table refers to mean values. At first glance it might seem that the response to calcium infusion is of great diagnostic value since only one false negative test was encountered. However, false positive tests are so common in euparathyroid patients with renal calculi that the procedure is of little value in discriminating between the hyperparathyroid and euparathyroid state.

TABLE IV.—TEST RESULTS IN 55 PATIENTS WITH PRIMARY HYPERPARATHYROIDISM, 1955–66

	Patients tested	Number with mean determination in normal range
Serum calcium	55	5
Serum plasma	55	10
Urine calcium	54	15
PEI	49	11
% TRP	49	12
Cp	44	23
Calcium infusion	41	1

The test procedure and normal results for the calcium infusion test have been reported elsewhere.<sup>14</sup>

Similarly, tests designed to demonstrate a renal phosphate leak—Cp, % TRP and the PEI are of limited value, because of the high incidence of false negative tests. However, false positive results for % TRP and PEI, although they do occur, are much less frequent than false

positive results with the I.V. calcium test, and in our experience occur in only about 15% of euparathyroid patients with renal calculi. Consequently, one should not dismiss lightly the possibility of hyperparathyroidism, if these tests are repeatedly abnormal in patients whose glomerular filtration rate is not significantly impaired.

That the urinary calcium excretion is frequently normal in hyperparathyroidism is also demonstrated in Table IV. The commonest cause of hypercalciuria in patients with calcium stone is the condition called idiopathic hypercalciuria, which in our experience is at least five times as common as primary hyperparathyroidism. However, there is again an important qualification, for in our experience the disorder is almost entirely confined to males. The finding of unexplained hypercalciuria in the female therefore should suggest a strong possibility of hyperparathyroidism. In the investigation of approximately 600 patients with renal calculi, we have found that although the hypercalciuric male has only about a 12% chance of having primary hyperparathyroidism, the hypercalciuric female has a 65% chance of having this disorder.<sup>16</sup>

The serum phosphorus, too, is of limited value in diagnosis. The mean serum phosphorus level was normal (above 2.8 mg. per 100 ml.) in 20% of our cases. Furthermore, serum phosphorus levels are not infrequently below normal in idiopathic hypercalciuria. However, a mean serum phosphorus level above 3.5 mg. per 100 ml. may be taken as strong evidence against primary hyperparathyroidism in the absence of some other explanation for this finding. In only two of our patients was the mean serum phosphorus above 3.5 mg. per 100 ml., and one of these had acromegaly and the other chronic renal insufficiency.

Serum calcium determinations are by far the most useful means of detecting hyperparathyroidism despite the fact that the mean serum calcium was "normal" in five of our patients. Most authorities have stressed that the demonstration of hypercalcemia by reliable determinations of the serum calcium should be the *sine qua non* for diagnosis. Dent<sup>17</sup> has gone so far as to state that "a single normal value if obtained with impeccable techniques, makes a diagnosis of primary hyperparathyroidism unlikely".

There have been occasional reports of patients with primary hyperparathyroidism and normal serum calcium levels, but in the majority of these it is apparent, either on the basis of the method used or from the excessively wide normal ranges reported, that the laboratory was unable to detect mild degrees of hypercalcemia. In his excellent report of the vast experience with

hyperparathyroidism at the Mayo Clinic, Keating<sup>18</sup> has demonstrated that even with a relatively difficult method such as the Clarke-Collip method it is possible to establish a normal range for serum calcium which is narrower than that generally reported. The 95% range with this method was 9.2 to 10.4 mg. per 100 ml. Keating also stressed that the degree of hypercalcemia may be extremely slight. In 12 of his patients the mean serum calcium level fell within the 99% limits for normals (at or below 10.6 mg. per 100 ml. and in one patient the mean serum calcium level fell within the 95% limit for normal (at or below 10.3 mg. per 100 ml.).

Our experience has also demonstrated that the degree of hypercalcemia in hyperparathyroidism is often extremely slight. In more than one-third of our patients (20 of our last 55 cases), the initial serum calcium determination has been 11.0 mg. per 100 ml. or less. We suggest that these are the cases which frequently escape detection. It does not seem to be good enough for a laboratory merely to set up a method which is capable of extreme accuracy. When differences of 0.1 mg. per 100 ml. (or 1%) may mean the difference between euparathyroidism and hyperparathyroidism, any method needs constant supervision and the estimations must be carried out by experienced, reliable personnel who are familiar with the method and aware of the possible significance of slight lapses in technique. Not only must meticulous care be given to methodology, but care must also be taken in obtaining the specimens. The application of a tight tourniquet for more than a few seconds will cause spurious elevations of serum calcium.<sup>17</sup> In addition, the blood should be taken under constant conditions, preferably in the early morning fasting state, and test tubes should not be stoppered with cork which contains sufficient calcium to cause a false elevation of serum levels.<sup>19</sup>

Does the observation that the mean serum calcium levels in five of our patients were less than 10.3 mg. per 100 ml. indicate that excessive secretion of parathyroid hormone may be unaccompanied by elevated blood calcium levels? An initial inspection of Fig. 1 would indicate that this indeed appears to be the case. Of a total of 46 calcium determinations in these five patients, only seven were above the normal range. Normocalcemia is known to occur when serum protein levels are diminished or when phosphate levels are raised as in chronic renal insufficiency. None of these five patients, however, had hypoproteinemia or renal insufficiency. An alternative explanation is that levels of ionized calcium in the plasma may be elevated

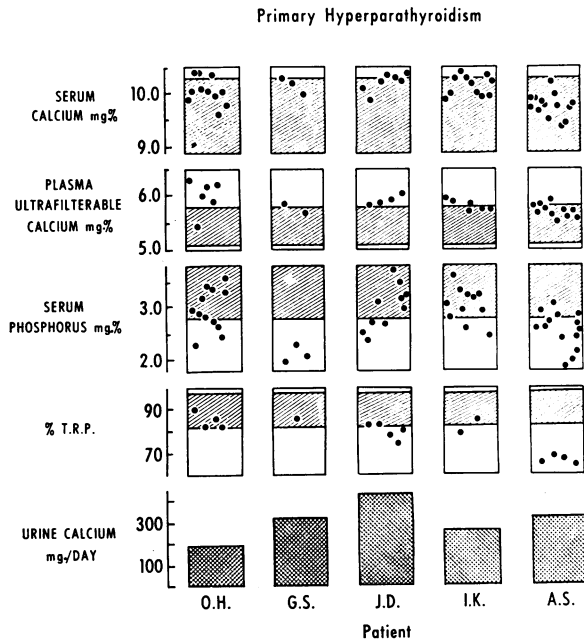


Fig. 1.—Laboratory values in five female patients with parathyroid adenomas. All had recurrent renal calculi, with the exception of A.S., who presented with osteoporosis. The shaded areas represent normal ranges. The normal range depicted for serum calcium is 8.9 to 10.3 mg. per 100 ml. and is that of a mixed male and female population. The normal range for plasma ultrafilterable calcium is 5.0 to 5.8% and is also that of a mixed male and female population. Each dot represents a single determination.

despite normal levels for total blood calcium as in the two cases reported in 1958 by Lloyd and Rose.<sup>20</sup> We have had difficulty measuring ionized calcium in our laboratory. However, using a modified Toribara method for obtaining plasma ultrafiltrates, Dr. Barbara Hunt, while working in our laboratory, was able to establish a narrow normal range for plasma ultrafilterable calcium of 4.98 to 5.80 mg. per 100 ml. We believe that this range can be narrowed further since in retrospect a few of the earlier determinations in our normal subjects seem unduly high.\* In these five patients many of the determinations for plasma ultrafilterable calcium were elevated. We suspect, therefore, that elevated levels of calcium ion in the blood remain a *sine qua non* for the diagnosis of primary hyperparathyroidism.

To explain the discrepancy between ionized or ultrafilterable and total blood calcium levels, which is especially striking in patient O.H., Lloyd and Rose<sup>20</sup> presented data which suggested to them that parathyroid hormone in some way interferes with the binding of calcium by plasma proteins. Our data also indicate that in the hyperparathyroid patient approximately

\*In 40 normal middle-aged females studied since the presentation of this paper the normal level for calcium in the plasma ultrafiltrate was  $5.11 \pm 0.20$  (mean  $\pm$  S.D.) with a 95% range of 4.71-5.51 mg. per 100 ml.

TABLE V.—ULTRAFILTERABLE CALCIUM EXPRESSED AS A PER CENT OF THE TOTAL PLASMA CALCIUM IN 60 NORMAL SUBJECTS AND 35 PATIENTS WITH PRIMARY HYPERPARATHYROIDISM. A SLIGHTLY GREATER (2.5) PER CENT OF TOTAL PLASMA CALCIUM IS ULTRAFILTERABLE IN PRIMARY HYPERPARATHYROIDISM

	Mean	Standard error
Normal subjects..... (60)	57.3	0.29
Hyperparathyroid patients.... (35)	59.8	0.51
	p < .001	

2% more of the total plasma calcium is ultrafilterable than in normal subjects (Table V).

This difference is small but statistically significant. Unlike Lloyd and Rose, however, we have not been able to demonstrate a significant change in the ultrafilterability of calcium after the removal of parathyroid adenomata; and if this is so, a direct effect of parathyroid hormone on the propensity of plasma protein to bind calcium seems unlikely. Further work on this aspect of the problem is obviously required.

Another possible explanation for the finding of normal total serum calcium levels in these five patients was suggested by the fact that they were all women. Might the normal range for total serum calcium be lower for women than for men? Over the past 10 years we had gained the impression that values over 10.0 mg. per 100 ml. were extremely infrequent in women but we had never subjected this to a statistical analysis. Recently, however, we have done this and the data are presented in Table VI. Our normal subjects were chiefly medical students, interns, nurses and laboratory technicians and the data are rather heavily weighted for the third and fourth decades. Unfortunately, the sex of some of our early normal subjects was not recorded. However, the mean serum calcium level was 0.2 mg. per 100 ml. lower for women than for men—and this difference is of statistical significance (p < 0.01). If the data on these five patients are replotted on the basis that the upper limit for total serum calcium levels in women is 10.05 mg. per 100 ml., the problem assumes somewhat different dimensions—for now the mean serum calcium level is elevated in all patients with the exception of A.S. (Fig. 2).

TABLE VI.—SERUM CALCIUM LEVELS IN 113 NORMAL STUDENTS, INTERNS, NURSES AND LABORATORY TECHNICIANS

	Number of subjects	Mean	S.D.	95% range
Total.....	113	9.62	0.30	9.02-10.22
Males.....	50	9.68	0.31	9.04-10.30
Females.....	32	9.49	0.28	8.93-10.05
Sex unrecorded..	31			

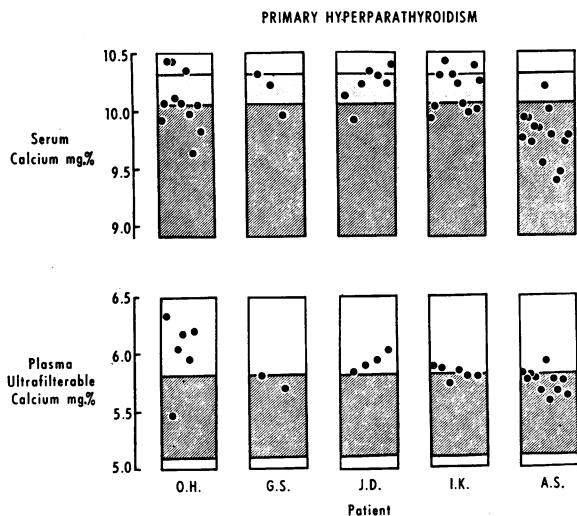


Fig. 2.—Serum calcium and plasma ultrafilterable calcium values in the same five patients depicted in Fig. 1 have been replotted. The shaded areas are normal ranges. Two upper limits of normal are now given for serum calcium levels. The line above the stippled area is 10.3 mg. per 100 ml., which is the upper limit of normal for a population of mixed sex originally established in our laboratory. The line above the cross-hatched area is at 10.05 mg. per 100 ml., the upper limit of normal for females recently established in our laboratory. If 10.05 mg. per 100 ml. is taken as the upper limit of normal in these patients, all but one (A.S.) had many elevated values.

Note: Since the presentation of this paper, the normal 95% range for plasma ultrafilterable calcium in a group of 40 middle-aged women was found to be 4.71 to 5.51 mg. per 100 ml. On this basis, all values for plasma ultrafilterable calcium are elevated with the exception of one determination in patient O.H.

A few words are in order concerning the clinical problem presented by these five patients. It has been suggested that the urgency for correct diagnosis may perhaps not be great in patients in whom the evidence of parathyroid overactivity is so minimal. Unfortunately, the experience with four of these five patients (O.H., G.S., J.D. and I.K.) does not bear this out. These four patients had recurrent renal calculi and each of them had had so much trouble resulting from the calculi that the possibility of a negative parathyroid exploration was thought to present much less of a hazard than the risk of further stone formation. Among the four there had been a total of 10 major operations including a nephrectomy in one patient and a partial nephrectomy in another. In addition, recurrent urinary tract infection was troublesome in all four, and it was tempting to ascribe their tendency to stone formation to underlying urinary tract infection. However, in each instance we were alerted by the finding that the urinary calcium was either in the high normal range or distinctly elevated. It is worth while repeating that, although idiopathic hypercalciuria is extremely common in male stone formers, it is much less common in females. Since the removal of their parathyroid adeno-

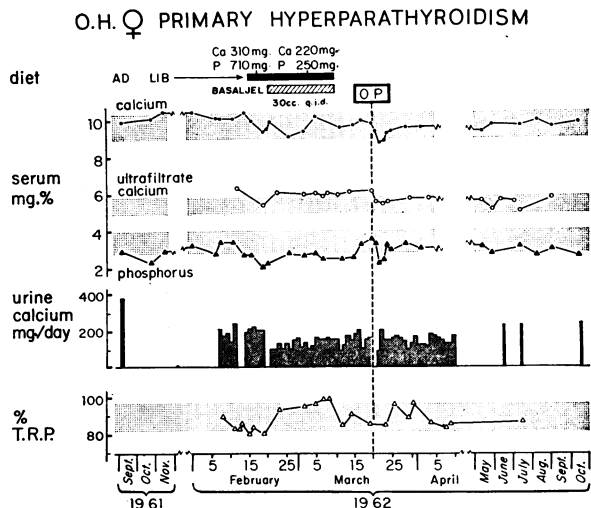


Fig. 3.—Metabolic studies in patient O.H.

mas none of these patients has had further difficulty with either stones or urinary tract infection.

In this paper little has been said concerning a number of other procedures which have been reported to be useful in the diagnosis of hyperparathyroidism. It has been our experience that usually they are unreliable when assistance is most urgently needed. For example, in one of the five patients previously discussed (O.H.), phosphorus deprivation neither aggravated the hypercalcemia nor increased urinary calcium excretion (Fig. 3). This was our first "normocalcemic" patient with primary hyperparathyroidism, and illustrates the most marked discrepancy between calcium levels in serum and plasma ultrafiltrate. The latter were unquestionably elevated even on the basis of our initial normal range of 5.0 to 5.8 mg. per 100 ml., whereas serum levels were often well within the normal range. This study also demonstrates that a phosphate deprivation regimen (low phosphorus diet and Basaljel) did not accentuate the hypercalcemia or increase the urinary excretion of calcium.

The first urine collection and the last three were obtained on an outpatient basis on a diet *ad libitum*, whereas the remainder were obtained during an admission to the Clinical Investigation Unit. Dietary calcium was not restricted in the postoperative period, which may explain the apparent lack of a postoperative reduction in urinary calcium excretion. The last determination of plasma ultrafilterable calcium in this patient on May 12, 1967, was 5.08 mg. per 100 ml.

Similarly in these borderline cases we have had misleading results with the rapid calcium

infusion test,<sup>21</sup> and with the modification of % TRP by parathyroid hormone.<sup>22</sup> We have not attempted to increase the specificity of % TRP by giving supplemental phosphate as advocated by Gordan *et al.*<sup>23</sup> The practical role of external scanning of parathyroid adenomas after the administration of methionine tagged with <sup>75</sup>Se remains to be defined.<sup>24</sup>

Although new methods for the immunoassay of parathyroid hormone have recently been described, attempts to apply this technique to human subjects and to clinical medicine have met with considerable difficulty. In the most extensive experience with this method reported to date, the measured levels of circulating parathyroid hormone were within the normal range in 15 of 29 patients with parathyroid adenoma.<sup>25</sup> Nonetheless, it is hoped that the sensitivity of such direct measurements of parathyroid activity in human subjects may be improved to a degree that will permit this technique to aid or supplant the indirect measurements of parathyroid function presently in use.

**Summary** The diagnostic value of a number of indirect parameters of parathyroid function was evaluated in 55 consecutive patients with proved primary hyperparathyroidism. The phosphorus excretion index (PEI) and the % tubular reabsorbed phosphorus (% TRP) were normal in approximately one-quarter of the patients tested. Mean serum phosphorus levels were below 3.5 mg. per 100 ml. in all but two patients.

An accurate serum calcium determination was the most useful diagnostic test, although the mean serum calcium level was below 10.3 mg. per 100 ml. in five patients. These five patients were all females and all had hypercalciuria. It was subsequently demonstrated that the upper normal limit of serum calcium was lower in females (10.05 mg. per 100 ml.) than in males (10.30 mg. per 100 ml.), and on this basis the mean serum calcium was elevated in all but one patient. Calcium levels in the plasma ultrafiltrate were elevated in all five of these patients. Attention is drawn to the high incidence (65%) of primary hyperparathyroidism in hypercalciuric females with kidney stones as opposed to the relatively low incidence (12%) of this disorder in hypercalciuric males with kidney stones.

**Résumé** Les auteurs ont évalué la valeur diagnostique de quelques paramètres indirects de la fonction parathyroïdienne chez 55 malades pris consécutivement et souffrant d'hyperparathyroïdisme primaire notoire. L'indice d'excrétion du phosphore et le pourcentage de réabsorption tubulaire du phosphore étaient dans les limites normales chez un quart environ des malades. La phosphorémie moyenne était inférieure à 3.5 mg par 100 ml chez tous les malades sauf deux.

Une détermination précise de la calcémie a été l'épreuve diagnostique la plus utile, même si la calcémie moyenne était inférieure à 10.3 mg par 100 ml chez cinq malades. Ces cinq malades étaient toutes du sexe féminin et toutes présentaient de l'hypercalciurie. On a par la suite démontré que la limite supérieure normale était plus basse chez les femmes (10.05 mg par 100 ml) que chez les hommes (10.30 mg par 100 ml) et, tenant compte de ce fait, la calcémie moyenne était élevée chez toutes les malades sauf une. La concentration de calcium dans l'ultrafiltrat plasmatique était élevée chez ces cinq malades. Les auteurs attirent l'attention sur la grande fréquence de l'hyperparathyroïdisme primaire chez les femmes hypercalciuriques (65%) alors que ce trouble est relativement plus rare chez les hommes hypercalciuriques (12%).

We are grateful to our many urological confrères for referring cases for study and to Dr. John Palmer of the Department of Surgery, Toronto General Hospital, who has so ably carried out the surgical management of most of these patients. We are also indebted to our laboratory technicians, Mrs. E. Bennett, Miss N. Huber, Miss B. Munro, Miss H. Koop and Miss P. Spivak, whose care and accuracy have been so essential to this study; to the many research fellows, nurses and dietitians who have assisted with the care of these patients on the Farquharson Investigation Unit; and to Mr. F. Lammerich of the Department of Art as Applied to Medicine for the preparation of the charts.

#### ADDENDUM

Since the presentation of this paper we have studied two additional patients with parathyroid adenoma who fall into the category of "hypercalciuric females" with "normal" or borderline serum calciums.

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