THE DIAGNOSTIC VALUE
OF CALCIURIA DURING HORMONAL TREATMENT
OF METASTASIZED MAMMARY CARCINOMA

By

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INTRODUCTION

One of the great drawbacks in hormonal treatment of metastasized mammary carcinoma is the lack of sufficient objective standards. The importance of diagnostic criteria is not only related with the choice of the most efficient therapy, but also with the sometimes tumour-stimulating effect of hormonal substances. In patients with skeletal metastases the calcium excretion in the urine may be one of these objective standards, as has been shown already by Laszlo et al. (1952) and Pearson et al. (1952). The reliability of this standard is emphasized by the following 4 facts:

a. The bones contain about 99 per cent of the total amount of calcium in the body. That means, the calcium metabolism reflects to a great extent the metabolism of the skeleton.

b. The total amount of calcium amounts to about 2 per cent of the body weight.

c. One gram of bone contains about 150 mg. of calcium. That means a negative balance of 150 mg. of calcium per day constitutes a loss of 1 gram of bone per day.

d. With a low-calcium diet (± 200 mg. Ca per day) the normal urinary calcium excretion ranges from 50 to 150 mg./day. In these circumstances usually a negative calcium balance exists because of the calcium excretion in the faeces.

To evaluate the diagnostic use of the urinary calcium excretion in patients with metastasized mammary carcinoma we investigated the following problems:

1. The calcium excretion in the daily urine of patients with metastasized mammary carcinoma with and without demonstrable skeletal lesions. In the latter case we differentiated between osteolytic and osteoplastic metastases.
2. The relationship between the calcium content of the serum and the calcium excretion in the daily urine of these patients and the influence of some factors on this relationship.

3. The relationship between the course of the calciuria and the clinical results during hormonal treatment.

METHODS

The calcium content in serum and urine has been determined by a direct microtitration with Ethylene-Diamine-Tetra-Acetate using murexide as indicator (N. J. Poulie, 1954, L. A. de Vries, 1955).

All patients have been on a low calcium diet with distilled water, without milk and milk-products and without some calcium rich vegetables. This constitutes a relatively normal diet with ± 175 mg. of calcium per 24 hours (Gerbrandy & Ebeling, 1956). In other cities and countries with a lower calcium content of the drinking water patients may have tap water; in Amsterdam, however, this contains about 10 mg. % calcium.

The urinary calcium excretion is related to calcium intake and to bodyweight (Knapp, 1947). The differences of calcium excretion caused by differences in bodyweight, however, are not large enough to apply a correction factor.

RESULTS

1. Urinary calcium excretion in patients with metastasized mammary carcinoma

In 114 patients with metastasized mammary carcinoma who latterly received no surgical, radiological or hormonal treatment, the daily calcium excretion on a low calcium diet has been determined. In the same period the skeleton was radiologically examined (X-rays of skull, cervical, thoracic and lumbar vertebrae and of the pelvis). In Table 1 the results are shown. It appears that 53 per cent of the 72 patients with and only 7 per cent of the 42 patients without radiological evidence of skeletal metastases had a calciuria of more than 200 mg. per day. A calciuria of more than 300 mg. per day was only found in patients with visible skeletal lesions: in 20 (= 28 per cent) of the 72 patients.

The 72 patients with demonstrable skeletal lesions have been subdivided into 57 patients with predominantly or entirely osteolytic metastases, 10 patients with mixed osteolytic-osteoplastic metastases and 5 patients with generalized osteoplastic metastases. Table 2 shows the rate of calcium excretion in these 3 groups. It appears, that a calciuria of more than 300 mg. per day is only seen in patients with osteolytic metastases and that a generalized osteoplastic
Table 1.

<table>
<thead>
<tr>
<th>X-ray findings in 114 patients with metastases mammary carc.</th>
<th>Number of patients</th>
<th>Calciuria mg./24 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-95</td>
</tr>
<tr>
<td>Skeletal metastases visible</td>
<td>72</td>
<td>18</td>
</tr>
<tr>
<td>( = 25% )</td>
<td></td>
<td>( = 22% )</td>
</tr>
<tr>
<td>No skeletal metastases visible</td>
<td>42</td>
<td>20</td>
</tr>
<tr>
<td>( = 48% )</td>
<td></td>
<td>( = 45% )</td>
</tr>
<tr>
<td>Total</td>
<td>114</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 2.

<table>
<thead>
<tr>
<th>Radiological character of skeletal metastases in 72 patients with mammary carc.</th>
<th>Number of patients</th>
<th>Calciuria mg./24 hr.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0-95</td>
</tr>
<tr>
<td>Osteolytic metastases</td>
<td>57</td>
<td>10</td>
</tr>
<tr>
<td>Mixed osteolytic-osteoplastic metastases</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Generalized osteoplastic metastases</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>72</td>
<td>18</td>
</tr>
</tbody>
</table>

The way of metastasizing is always accompanied with a calciuria of less than 100 mg. per day.

The 57 patients with osteolytic metastases have been subdivided again according the radiological extent of the osseous lesions, using a rough estimation of course (Table 3). Three plusses means: generalized osteolytic lesions; two plusses: several osteolytic lesions; one plus: a few or one osteolytic lesion(s). This table shows a positive correlation between urinary calcium excretion and radiological extent of osteolytic metastases. The differences between the calciu-
Radiological evidence of osteolytic metastases in 99 patients with mammary carcinoma

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Mean calciuria mg./24 hr.</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ + +</td>
<td>19</td>
<td>312 ± 33</td>
</tr>
<tr>
<td>+ +</td>
<td>19</td>
<td>240 ± 21</td>
</tr>
<tr>
<td>+</td>
<td>19</td>
<td>157 ± 20</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>106 ± 8</td>
</tr>
</tbody>
</table>

Conclusions: a) For practical purposes we may consider the level of 200 mg. urinary calcium per day with a low-calcium diet as the upper limit of normal, and 300 mg. calcium per day as a definitely abnormal level.

b) In 114 patients with metastasized mammary carcinoma 41 (= 36 per cent) had a calciuria of more than 200 mg. per day and 20 (= 18 per cent) of more than 300 mg. per day. Practically only patients with radiologically visible skeletal metastases had a raised calcium excretion in the daily urine.

c) Predominantly osteoplastic metastases were accompanied with a low calcium excretion; a highly raised calcium excretion was only seen in patients with predominantly osteolytic metastases. A rough correlation appeared to exist between the radiological extent of the osteolytic lesions and the calcium excretion in the daily urine.

d) These rules can be explained if one assumes that the calcium that is freed by malignant skeletal destruction, is excreted in the daily urine.

2. Relationship between serum-calcium and urinary-calcium excretion

From the practical point of view repeated determinations of serum-calcium are easier than a long term investigation of the urinary-calcium excretion. Therefore we compared serum-calcium and urinary-calcium in 103 patients with metastasized mammary carcinoma and in 30 patients we compared the changes in serum-calcium with the changes in urinary-calcium during hormonal treatment.

In 103 patients with mammary carcinoma serum-calcium and daily urinary-calcium excretion during a period of 3–4 days were determined simultaneously. All these patients have been on a low-calcium diet and no one had been treated...
CORRELATION BETWEEN SERUM CALCIUM AND URINARY CALCIUM IN 103 PATIENTS WITH METASTASIZED MAMMARY CARCINOMA.

\[ r = 0.50 \]
\[ p \ll 0.001 \]

Fig. 1.

of late either surgically, radiologically or hormonally. In Fig. 1 every experiment is represented by a dot on the graph. A line is drawn through all these points according to the formula of Dent (1935) and compared with that of Housner & Brennan (1948). A rough correlation appeared to exist with \( r = 0.50 \) and with \( p \ll 0.001 \). The slope of this line represents quantitatively the relationship between serum-calcium and urinary-calcium excretion: a difference of 1 mg. % serum-calcium corresponded with a difference of 108 mg. of calcium in the daily urine. This line cuts the ordinate at 8.7 mg. %.

During commencing or stopping hormonal treatment in 30 patients with metastasized mammary carcinoma similar observations have been made. The hormonal treatment consisted of administration of androgens (40 ×) and of cortisone (5 ×) and withdrawal of androgens (13 ×) and of cortisone (2 ×). Together 60 experiments have been carried out on 30 patients. In these experiments the changes of serum-calcium were compared with the changes of urinary-calcium excretion. In Fig. 2 every experiment is represented by a dot on the graph. A highly statistically significant correlation was observed with \( r = 0.85 \) and \( p \ll 0.001 \). The slope of the line, drawn through all the points, gives a remarkably similar relationship between serum-calcium and urinary-calcium as found in the experiments of Fig. 1: a change of 1 mg. % of serum-calcium corresponded with a change of 103 mg. of urinary-calcium excretion.

**Conclusions:** a) The calcium excretion in the urine is a more sensitive standard than the level of the serum-calcium. For instance, if the serum-calcium rises from 10.0 to 11.0 mg. %, a rise of 10 per cent is seen. In the meantime the calcium-excretion in the urine rises from 150 to 250 mg. per day; that means a rise of 80 per cent. However, repeated determinations of the serum
CORRELATION BETWEEN CHANGES OF SERUM CALCIUM AND URINARY CALCIUM DURING 60 EXPERIMENTS IN 30 PATIENTS WITH METASTASIZED MAMMARY CARCINOMA (45 x ADMINISTRATION OF HORMONES, 15 x WITHDRAWAL OF HORMONES)

![Graph showing correlation between serum calcium and urinary calcium changes](image)

\[ r = 0.85 \]
\[ p << 0.001 \]

Calcium content remain useful in the hormonal treatment of metastasized mammary carcinoma.

b) In this sort of experiment the »point of attack« of the androgens presumably lies in the bones; these hormones probably do not alter primarily the kidney function. This in contrary, for instance, to the presumed influence of parathyroid hormones on phosphate-metabolism.

However, significant these relationships between serum-calcium and urinary-calcium may be, a large scattering of points is seen in the graphs of Fig. 1 and Fig. 2. This might be caused by the intrinsic inaccuracy of our methods or by biological variations. Two factors, till now discarded, influence the relationship between serum-calcium and urinary-calcium excretion: a. the calcium-protein binding in the plasma and b. the »clearance« by the kidneys. Other factors of importance are diet (acids or bases), diuresis and chemotherapy.

Calcium-protein binding in the plasma. In the relationship between serum-calcium and urinary-calcium excretion probably only the ionized calcium-fraction in the plasma matters. If one assumes that 1 gram % of normal plasma protein binds about 0.8 mg. % of calcium (McLean & Hastings, 1935; Gutman & Gutman, 1937; Miller, 1937) two patients with the same serum-calcium of 10 mg. % but with a plasma protein content of 5.0 and 8.0 gram % resp. will have an ionized serum-calcium of 6.0 and 3.6 mg. % resp. In fact every serum-calcium determination should be corrected in this way.
Changes of kidney function. The relationship between calcaemia and calciuria as we calculated in our patients, actually represents the calculation of the «clearance». A large tubular resorption reduces the calcium «clearance» to about 2 ml. per minute. However, a sudden change in the renal clearance causes an equally sudden change in the relationship between serum-calcium and urinary-calcium excretion as we observed during the occurrence of a hypercalcaemia in the following 2 patients:

Miss A., 44 years, with mammary carcinoma, osteolytic metastases, a hypercalciuria of ± 350 mg. per day and a normal serum-calcium of 10 mg. % was treated with daily injections of 100 mg. testosterone-propionate during 11 days (Fig. 3). During this shortlasting period a hypercalcaemia of 17.5 mg. % developed presumably by stimulation of the tumour growth. The creatinine-clearance, before the administration of the hormone of 100 ml. per minute, fell during the occurrence of the hypercalcaemia to 23 ml. per minute, together with a fall of the calcium excretion. When the hormone

![Graph representing changes in various parameters such as serum calcium, urinary calcium, urinary phosphate, urinary creatinine, creatinine clearance, and diuresis over time.](image-url)
was withdrawn the serum-calcium fell rapidly to a normal level again and the normal relationship between serum-calcium and urinary-calcium excretion was restored. (After ovariectomy no hypercalcaemia occurred when testosterone-propionate was given again. This remarkable fact possesses all sorts of theoretical possibilities.)

Mrs. B., 40 years, with mammary carcinoma and extensive osteolytic metastases, had been treated a year ago with ovariectomy and adrenalectomy. A clinical remission of 3/4 of a year was observed; afterwards an exacerbation of the malignant process took place. On 3rd September she was readmitted in the hospital in a poor condition. The next few days she deteriorated rapidly and a spontaneous hypercalcaemia till a maximum value of 17.1 mg. % occurred, presumably by a progressive destruction of the skeleton (Fig. 4). After this period a spontaneous clinical improvement was seen with a fall of serum-calcium to a normal level. During this observation period, the creatinine-clearance fell from 18 ml. per minute (for this small woman of about 45 kg. ± 30 % of the normal value) to 6.8 ml./min. During the following 10 days the creatinine clearance gradually rose to the original value. During the occurrence of the hypercalcaemia the blood urea rose from 61.8 mg. % to 121.5 mg. %, afterwards fell to normal again. At the height of the hypercalcaemia the calcium excretion fell temporarily to a lower level.

MAMMARY CARCINOMA AND OSTEOLYTIC METASTASES

\[\text{Calcium} \quad \text{serum} \quad \text{mg/\%} \quad 18 \quad 16 \quad 14 \quad 12 \quad 10 \quad 8 \quad 6 \quad 4 \quad 2 \quad 0 \]
\[\text{Urinary} \quad \text{Calcium} \quad \text{mg/24 hrs.} \quad 300 \quad 200 \quad 100 \quad 0 \]
\[\text{Creatinine} \quad \text{serum} \quad \text{mg} \quad 3 \quad 2 \quad 1 \quad 0 \]
\[\text{Creatinine} \quad \text{clearance} \quad \text{ml/min} \quad 10 \quad 0 \]
\[\text{Diuresis} \quad \text{ml/24 hrs.} \quad 1000 \quad 100 \quad 0 \]

Fig. 4.
Injections of a mercurial diuretic, that in normal circumstances cause a raised calcium excretion in the urine, were not accompanied by a higher calcium excretion.

From the occurrence of a marked hypercalcaemia a vicious circle seems to arise: a further rise of the serum-calcium damages the kidney function and the impaired calcium excretion leads to a further rise of the serum-calcium. Maybe, during such a hypercalcaemia calciumphosphate-crystals are formed, which obstruct the tubules. The outcome of this process depends on the fact that the calcium delivery by the skeleton diminishes. In these two cases the kidney function was gradually restored to the original value when the serum-calcium content returned to a normal level.

3. Relationship between calciuria and clinical symptoms during hormonal therapy

To evaluate the diagnostic aid of calciuria during hormonal treatment of metastasized mammary carcinoma we compared the clinical results and the course of calciuria levels. The following clinical criteria were used during the hormonal treatment:

a) Pain. This is one of the most prominent features in evaluating hormonal therapy especially in osseous metastases. Disappearance of pain is often the first sign of clinical improvement and appearance of pain the first sign of relapse.

b) Neurological disorders, especially changes in reflexes and muscular strength of the limbs.

c) X-rays of the bones. Changes in the X-rays are, however, a late phenomenon. In all our patients we took biopsies from the iliac crest; in many patients we found tumour tissue in the bone marrow without any radiological abnormality at that site and in some cases without any radiological abnormality in the skeleton at all (Van Slooten & Hampe, 1956).

d) Pulmonary function (in patients with carcinomatous lymphangitis) and eventual effusions.

e) Haematological disorders (appearance or disappearance of normoblasts and immature leucocytes in the peripheral blood: anaemia, and rarely thrombocytopenia).

f) Measurable changes in palpable and visible lesions.

g) The general condition.

In 30 patients with mammary carcinoma and skeletal metastases, who during the course of their illness at some time suffered from a hypercalciuria, a hormonal therapy was started. In most of these patients several hormonal treatments were started at different times, together 70 different treatments in 30 patients. The hormonal treatments consisted of: $7 \times$ ovariectomy, $3 \times$ adrenalectomy, $4 \times$ ovariectomy + adrenalectomy, $31 \times$ testosterone-propionate, $5 \times$ methyl-androstenediole sublingually, $3 \times$ methyl-androstenediole intramuscularly, $7 \times$ nor-testosterone-phenyl-propionate and $10 \times$ cortisone.

In every experiment the change of calciuria was compared with the clinical
Relationship between changes of calciura and clinical results in 70 experiments with hormonal treatment of 30 patients with metastasized mammary carcinoma

![Graph showing relationship between calciura and clinical results]  

Fig. 5.

course. In Fig. 5 the results are represented. Every line represents one experiment; the left end of the line gives the mean calcium excretion for a few days before the administration of the hormone, the right end shows the stabilized calcium excretion during administration of the hormonal substance. The first column represents the clinical remissions (21 experiments); the second column shows the experiments without distinct clinical change (13 experiments) and the third column represents the cases with clinical deterioration (36 experiments). The cases with clinical remission all show a fall of calciuria to a low level; the cases, which remained clinically unchanged, generally showed a less fall of calciuria, the cases with clinical deterioration either showed a fall, remained equal or showed a rise of the calciuria. (The one case with a marked fall of calcium excretion from 600 to 25 mg. per 24 hours concerns a woman, who died of cervical cord lesion and respiratory paralysis).

In the 3 following cases examples are given of the diagnostic importance of the calcium determination. The first patient showed a clinical remission for 2 months, in the second patient the clinical course was not greatly influenced by hormonal treatment and the third patient shows (the same as in Fig. 3) the dangerous potentialities of androgens in patients with metastasized mammary carcinoma.
Mrs. C., 58 years, with mammary carcinoma and extensive osteolytic metastases was comatose because of cerebral metastases (ascertained later at the post-mortem); she also had a hypercalciuria (Fig. 6).

During methyl-androstenediol therapy a striking clinical improvement occurred together with a fall of the calcium excretion to a low level. After diminishing the doses of methyl-androstenediol patient relapsed 2 months later; in the same time the calcium excretion rose to an elevated level again. She became resistant to other hormones and died after a few weeks.

Mrs. D., 38 years, with mammary carcinoma and osteolytic metastases underwent successively all sorts of hormonal therapy: ovarectomy, testosterone-propionate, cortisone and adrenalectomy, again testosterone-propionate (Fig. 7). However, patient gradually deteriorated and died after a few months. The urinary-calcium excretion never fell to a normal or subnormal level.

Mrs. E., 58 years, with mammary carcinoma and osteolytic metastases received testosterone-propionate when she left the hospital (Fig. 8). This has been a wrong
decision, firstly because she did not show at that time a marked progression of her disease, and secondly because the first period of treatment with a hormonal substance has to be observed closely. After 2 months she was readmitted in the hospital in a bad condition with progressive skeletal destruction. The testosterone-propionate had been
stopped a day before she re-entered the hospital. This has been wrong again, because one has to observe as far as possible the effect of all these changes during the stay in the hospital. Withdrawal of the drug resulted in a fall of calcium excretion. Restarting administration of the drug resulted in a rise of calciuria and in a hypercalcaemia, which disappeared again after stopping the drug. Four months later, when patient was still in a clinical remission, we tried for a short period the new compound nor-androstenolone-phenyl-propionate (Durabolin).*) No hypercalciuria resulted from this. This fact has some interesting theoretical implications.

**Conclusions:** a) In cases with hypercalciuria a hormonal treatment with favourable clinical results is always accompanied with a fall of calcium excretion to a normal or subnormal level.

b) A fall of calcium excretion during hormonal treatment is *not* always accompanied with clinical improvement. Especially in the cases treated with androgens we have to make allowance for the unspecific anabolic calcium-retaining effect (F. Albright & E. C. Reifenstein, 1947, 1948; P. J. Dragsted & N. Hjorth, 1953; F. T. G. Pruntly et al., 1953; P. G. Ackerman et al., 1954; F. Fisher & B. Hastrup, 1954; A. Lichtwitz et al., 1955). This is in agreement with our observations in 5 patients (4 with bronchial carcinoma and 1 with renal carcinoma) who all had osteolytic metastases and a hypercalciuria. Administration of androgens resulted in all patients in a fall of urinary-calcium excretion of about 150 mg. per 24 hours but without clinical improvement.

c) A rise of calciuria is invariably accompanied with a clinical deterioration. In cases of a tumour-stimulating effect of androgens, oestrogens and other hormonal substances this phenomenon is of the most practical and theoretical importance.

d) The determination of the calcium excretion in the urine is an indispensable method to evaluate a hormonal treatment in patients with mammary carcinoma and osteolytic metastases.

**SUMMARY**

1. In 114 patients with metastasized mammary carcinoma a calciuria of more than 200 mg. per 24 hours was seen in 38 (= 53 per cent) of 72 patients *with* and in 3 (= 7 per cent) of 42 patients *without* radiological evidence of skeletal destruction. The highest calciuria appeared in patients with osteolytic, the lowest in patients with osteoplastic metastases. A rough correlation existed between the extent of skeletal destruction and the rate of urinary-calcium excretion.

2. In 103 patients with metastasized mammary carcinoma a positive correlation existed between serum-calcium and calciuria. A difference of 1 mg. /°

*) Kindly supplied by Organon Company, Oss, Netherlands.

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serum-calcium corresponded with a difference of 108 mg. urinary-calcium per 24 hours. In 60 experiments with administration of hormonal substances to 30 patients a positive correlation appeared to exist between the change in the serum-calcium and the change in the calciuria. In these experiments a change of 1 mg. per cent serum-calcium corresponded with a change of 103 mg. urinary-calcium per 24 hours. No correction was made for differences of plasma protein or renal function. In 2 cases a temporary hypercalciuria was accompanied with a rapid fall of creatinine clearance. In these cases the ordinary relationship between serum-calcium and calciuria was disturbed.

3. In 30 patients with skeletal metastases and hypercalciuria a hormonal therapy was carried out 70 times. Comparison between clinical course and changes of calcium excretion leads to the following conclusions:

a) A favourable result is invariably accompanied with a fall of the calcium excretion to a level of about 100 mg. per 24 hours or lower.

b) A fall of the calcium excretion is not always accompanied with a clinical remission.

c) A rise of calciuria is invariably accompanied with a clinical deterioration.

REFERENCES

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