Plasma calcitonin in chronic renal failure: relation to other factors of importance in bivalent ion metabolism

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Summary
1. Plasma concentrations of human calcitonin were measured in groups of patients with chronic renal failure, treated either conservatively or by haemodialysis, and compared with a normal group of persons.
2. Plasma calcitonin was statistically significantly elevated in both groups with renal failure.
3. When the data from the three groups were pooled, plasma calcitonin was found to be inversely correlated with total calcium and directly correlated with plasma creatinine.

Key words: calcitonin, calcium, creatinine, parathyroid hormone, renal failure, thyrocalcitonin, urea.

Introduction
The physiological relevance of human calcitonin is unclear, although there is evidence that its concentrations are related to serum calcium in patients with chronic renal failure or to the serum calcium/phosphate ratio and that plasma calcitonin is increased in renal failure (Heynen & Franchimont, 1974; Silva, Becker, Selaway, Snider, Moore, Bivins & Shalhoub, 1974). In this paper it is shown that concentrations of calcitonin correlated with various plasma biochemical measurements in normal persons and in patients with renal failure, including blood urea, creatinine, calcium, magnesium, serum immunoreactive parathyroid hormone and phosphate.

Material and methods
Subjects
Group 1. A control group of 20 normal volunteer nurses and soldiers, who gave informed consent, donated blood in the forenoon, in the non-fasting state without stasis.

Group 2. A group of 22 patients with chronic renal failure with a mean serum creatinine concentration of $0.67 \pm 0.42$ mmol/l gave blood samples as part of routine follow-up in the 'glomerular filtration rate under 10 ml/min' clinic at this hospital. They were maintained on an appropriate selected low-protein diet (Berlyne, Shaw & Nilwarangkur, 1965). Blood was taken in the forenoon in the non-fasting state without stasis. The chronic renal failure patients (not on dialysis) were nine females, mean age $51.5 \pm 8.1$ (SD) years and 13 males, mean age $50.2 \pm 15.0$ years.

Group 3. A group of 19 regular haemodialysis patients, receiving treatment of $18 \, m^2 \, h$ per week on $1 \, m^2$ area Cuprophane coil kidneys. Blood was drawn immediately before dialysis in the forenoon without stasis. The dialysis patients were nine females, mean age $31.6 \pm 8.2$ years, and ten males, mean age $31.0 \pm 7.9$ years.

Radioimmunoassay for human calcitonin

Material. Human synthetic calcitonin (Ciba) and rabbit antiserum to human synthetic calci-
Assay conditions. A non-equilibrium system was used in phosphate buffer (100 mmol/l, pH 7.5). Portions (100 and 200 µl) of serum were incubated with 100 µl of antibody solution in buffer containing human serum albumin (2.5 g/l). Samples without antibody were used for control systems. The reaction mixture was brought to a final volume of 0.6 ml with buffer. After 24 h of preincubation at 4°C, 100 µl of labelled calcitonin (approximately 2000 c.p.m., freshly labelled or kept frozen at -20°C for not more than 2 weeks after labelling, was added to the reaction mixture. Tubes were kept at 4°C for another 24 h. Separation of bound from free hormone was achieved with dextran-coated charcoal by the method of Herbert, Lau & Gotlieb (1962) with a modification in the amounts of charcoal and dextran to final concentrations: charcoal, 10 g/l, and dextran 70, 2.5 g/l in human serum albumin (3.5 g/l). After addition of the charcoal suspension, tubes were kept for 40 min to reach equilibrium and then were centrifuged. Both supernatant and precipitate radioactivity were measured in a Packard automated γ-counter.

A standard curve was obtained in the presence of hypocalcitonic serum from a patient who had undergone total thyroidectomy some years earlier. This was for the control of non-specific interaction in the range 5–250 pg/tube. Ten curves were obtained, each with freshly dissolved antigen, antibody and tracer. The coefficient of variation was 8.2%.

Serum samples were assayed at two or more dilutions, each in triplicate with appropriate blank serum in the absence of antibody as a control for serum interaction with tracer in reaction conditions.

Labelling of calcitonin was performed with 125I by the method of Hunter & Greenwood (1962) with the following modification. Oxidation with 2.0 µg of chloramine T was for 30 s and separation of 125I-labelled calcitonin from free 125I was performed by absorption on QUSO (Philadelphia Quartz Co., Philadelphia, Penn., U.S.A.) and washing twice with water to remove free 125I; the labelled hormone was extracted from QUSO with an acid solution containing acetone (2.5 g/l) in aqueous acetic acid (20%, v/v).

Specificity of the radioimmunoassay. Serum analysed in volumes of 50, 100 and 250 µl gave assay values of 260, 270 and 260 pg/ml respectively. These results indicate the identity of serum calcitonin with human synthetic calcitonin by this assay. Additional evidence for the identity of the assay with calcitonin is the response to an intravenous calcium infusion.

Calcium gluconate was infused in each of two volunteer subjects at a rate of 1 mmol h⁻¹ kg⁻¹ for 2.5 h until the plasma calcium concentration reached 2.8 mmol/l. Plasma samples were analysed for calcium every 15 min and calcitonin was measured before, in the middle and at the end of infusion. The response of serum calcitonin is as shown in Table I.

Radioimmunoassay for human parathyroid hormone

Highly purified bovine parathyroid hormone and guinea-pig antiserum to bovine parathyroid hormone were from Wilson Laboratory, Chicago, Ill., U.S.A.

Radioimmunoassay conditions. A non-equilibrium system was used in barbital/saline buffer (50 mmol/l, pH 7.4) containing aprotinin (Trasylol; 100 units/ml). Portions (100 µl and

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<thead>
<tr>
<th>Table 1. Calcium infusion test in normal man: effect on plasma calcium and calcitonin concentrations</th>
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<tr>
<td><strong>Time of testing</strong></td>
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<tr>
<td>---------------------</td>
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<tr>
<td>Subject 1</td>
</tr>
<tr>
<td>Before infusion</td>
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<td>Middle</td>
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<td>At end</td>
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<td>Subject 2</td>
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<tr>
<td>Before infusion</td>
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<tr>
<td>Middle</td>
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<td>At end</td>
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200 μl) of serum were incubated with 100 μl of antibody solution, the volume being brought to 0.6 ml with buffer. After 3 days of preincubation at 4°C, 100 μl of 125I-labelled parathyroid hormone (approximately 2000 c.p.m.) was added and the reaction mixture was kept at 4°C for a further 4 days. The reaction was stopped by adding dextran-coated charcoal as described above for calcitonin.

A standard curve was performed in the presence of 100 μl of hypoparathyroid serum taken from a patient after total thyroparathyroideectomy. Ten standard curves with freshly dissolved reagents were performed and coefficient of variation was 6.3%.

**Labelling of parathyroid hormone.** This was by the method of Hunter & Greenwood (1962) with modification. Oxidation with 60 μg of chloramine T was for 15 s and separation of 125I-labelled hormone from free 125I was done with QUSO as described for calcitonin. 125I-labelled parathyroid hormone was purified a day before use on a Bio-gel P100 column (Bio-Rad Laboratories, Calif., U.S.A.), HCI/saline, pH 3.2, being used in the presence of human serum albumin. The peak tubes of pure 125I-labelled hormone were used as tracer in the radioimmunoassay system.

**Other determinations.** Urea, creatinine, calcium, magnesium and inorganic phosphate were measured by standard laboratory techniques detailed elsewhere (Berlyne, Ben-Ari, Kushlevsky, Idelman, Galinsky, Hirsch, Shainkin, Yagil & Zlotnik, 1975).

Statistical analyses were performed on a CDC 3600 computer with UCL1 software for multiple regression analysis. Student's t-test was used for analysis of significance of difference of mean values.

**Results**

Plasma calcitonin results are given in Table 2 for each of the three groups, together with the pertinent biochemical findings.

Thyrocalcitonin concentrations were significantly higher in both chronic renal failure and regular haemodialysis groups than in normal subjects (P < 0.01 and P < 0.01 respectively). There was no significant difference between the concentrations in the two renal failure groups (0.3 > P > 0.2).

In the normal group of patients there was a highly significant positive correlation between calcitonin and parathyroid hormone concentrations (r = 0.631, P < 0.01) (see Fig. 1). No correlations of statistical significance were found between calcitonin and the remaining biochemical measurements shown in Table 2 in the normal group.

In patients with chronic renal failure, maintained without dialysis, plasma concentrations of calcitonin were inversely correlated with filterable calcium (r = 0.488, P < 0.01) (see Fig. 2). No other significant correlations were found in this group (see Table 1).

In patients undergoing regular dialysis, no significant correlations were found between calcitonin concentrations and any other biochemical measurement shown in this group (Table 2).

When all data were pooled, plasma calcitonin was inversely correlated with total plasma calcium (r = 0.357, P < 0.01) (see Fig. 3) and directly correlated with plasma urea (r = 0.401, P < 0.01) (see Fig. 4).

There was also a direct correlation between calcitonin and plasma creatinine concentrations (r = 0.326, P < 0.05) (see Fig. 5).

**Discussion**

Other workers (Heynen & Franchimont, 1974) have noted an increase in concentration of calcitonin in chronic renal failure and dialysis patients, findings similar to those reported here. In acute renal failure, Ardaillou, Beaufils, Nivez, Isaac, Mayaud & Sraer (1975) have noted high concentrations of plasma calcitonin, but these diminished with time and the suggestion has been made that the raised plasma concentrations of renal failure, both acute and chronic, are caused by gastrin stimulation of secretion of calcitonin. Serum gastrin concentrations are high in renal failure (Henessey, Gray, Cooper & Ontjes, 1974; Silva et al., 1974). Glucagon has also been implicated (Melvin, Voelkel & Tashjian, 1970). However, we have not measured gastrin or glucagon concentrations.

The inverse relation between plasma calcitonin in chronic renal failure and serum calcium demonstrated in this paper has been found by others in both acute and chronic renal failure (Heynen & Franchimont, 1974; Silva et al., 1974). In the present work, however,
<table>
<thead>
<tr>
<th>Groups</th>
<th>No.</th>
<th>Thyrocalcitonin (pg/ml)</th>
<th>Parathyroid hormone (ng/ml)</th>
<th>Calcium (mmol/l)</th>
<th>Magnesium (mmol/l)</th>
<th>Inorganic phosphate (mmol/l)</th>
<th>Plasma alkaline phosphatase (K.A. units/100ml)</th>
<th>Urea (mmol/l)</th>
<th>Serum creatinine (mmol/l)</th>
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<tr>
<td>Normal</td>
<td>20</td>
<td>288 ± 209</td>
<td>3.21 ± 0.68</td>
<td>2.59 ± 0.125</td>
<td>1.14 ± 0.27</td>
<td>0.79 ± 0.18</td>
<td>0.43 ± 0.09</td>
<td>1.02 ± 0.09</td>
<td>9.44 ± 0.75</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>21</td>
<td>585 ± 485</td>
<td>7.70 ± 0.92</td>
<td>2.05 ± 0.21</td>
<td>1.62 ± 0.10</td>
<td>0.98 ± 0.22</td>
<td>0.61 ± 0.16</td>
<td>1.65 ± 0.10</td>
<td>12.78 ± 5.40</td>
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<tr>
<td>Dialysis patients</td>
<td>19</td>
<td>582 ± 475</td>
<td>5.59 ± 0.39</td>
<td>2.09 ± 0.28</td>
<td>1.00 ± 0.34</td>
<td>1.46 ± 0.38</td>
<td>0.82 ± 0.26</td>
<td>2.27 ± 0.34</td>
<td>32.33 ± 13.73</td>
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Fig. 1. Relation between plasma concentrations of calcitonin and parathyroid hormone in normal subjects. Plasma parathyroid hormone (PTH) refers to a bovine standard supplied by Wilson Laboratories Incorp. (Chicago, Ill., U.S.A.). $r = 0.631$, $P < 0.01$.

Fig. 2. Relation between plasma calcitonin and plasma filterable calcium in the chronic renal failure group. $r = 0.488$, $P < 0.01$.

Fig. 3. Relation between plasma calcitonin and total plasma calcium (pooled results from all three groups). $r = 0.357$, $P < 0.01$.

Fig. 4. Relation between plasma calcitonin and plasma urea concentrations (pooled results from all three groups). $r = 0.401$, $P < 0.01$.

A negative correlation was found between plasma calcitonin and both serum calcium and ionized calcium concentrations in chronic renal failure patients and in pooled data. In contrast to these findings, Silva et al. (1974) reported an inverse correlation between plasma calcitonin and calcium in patients on dialysis but a direct correlation in non-dialysis patients between plasma calcitonin and calcium. We are unable to explain this discrepancy.

In normal subjects there is a direct correlation between plasma parathyroid hormone and calcitonin. This is surprising in view of the elevation of plasma calcitonin caused by intravenous infusion of calcium and the depression of plasma parathyroid hormone by the same
procedure. There is a highly significant direct correlation in the total pooled results from renal failure patients and normal subjects between plasma calcitonin and urea \( (P < 0.001) \) and between plasma calcitonin and creatinine \( (P < 0.05) \). This relation has not hitherto been noted in chronic renal failure. However, it is known that parathyroid hormone and urea concentrations are directly correlated in chronic renal failure (Reiss, Canterbury & Kanter, 1969) and it is possible that the stimuli for secretion of both parathyroid hormone and calcitonin are related directly or indirectly to another factor such as inorganic phosphate, as found by Silva et al. (1974). Alternatively, urea concentrations may act as indirect indices of plasma gastrin levels in renal failure, and the apparent direct correlation between calcitonin and urea may be fortuitous. However, the statistical significance of the correlation coefficient between urea and calcitonin is \( P < 0.001 \), higher than that of other factors which correlate significantly with calcitonin. In the present study there was no significant correlation in any group between plasma calcitonin and inorganic phosphate concentrations, but it has been shown elsewhere that, owing to the low dietary phosphorus intake in Israel, plasma inorganic phosphate concentrations are lower in renal failure than in Western Europe and U.S.A. and clinical hyperparathyroidism is rare in undialysed chronic renal failure in Israel (Berlyne, Ben-Ari, Epstein, Booth & Yagil, 1973).

It remains to be elucidated whether calcitonin has an actual physiological role in chronic renal failure or whether the increase in immunoreactive calcitonin demonstrated in the present work and that of others (Heynen & Franchimont, 1974; Silva et al., 1974) is due to the accumulation of metabolites of calcitonin which otherwise would have undergone renal excretion in healthy persons. The histological picture of osteitis fibrosa or of osteomalacia commonly seen in renal failure would not indicate hypercalcitonemia, but in the occasional case where osteopetrosis is marked it is possibly the result of hypersecretion of calcitonin. There is no direct evidence as yet to corroborate this and we did not perform any histological studies on bone in these patients to assess the type of bone disease present.

A crucial question in all calcitonin studies by radioimmunoassay is whether the assay procedure is measuring calcitonin or not. In this study we have shown that serum behaves like a standard solution of synthetic human calc-
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It was found that calcitonin increased pari passu with increase in plasma calcium concentration during an intravenous calcium infusion in two subjects. This is adequate proof that the hormone measured is calcitonin.

Certainly at present plasma calcitonin concentrations are of clinical value only in the diagnosis of medullary carcinoma of the thyroid and possibly of bony metastases from other neoplasms, the elevated values in chronic renal failure being of little diagnostic use, although perhaps contributing to the documentation, if not understanding, of the complex of factors responsible for skeletal and bivalent ion metabolism in renal disease.

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References


