Mobilisation of Calcium and Inorganic Phosphate by Heparin in Metastatic Calcification during RDT

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In view of the longer expectation of life now obtainable by regular haemodialysis, special interest must be given to the complication of metastatic calcification which appears in about 12% of patients on RDT (Drukker et al, 1967). The pathogenesis at first sight appears related to the calcium-phosphate product, which is usually increased in renal insufficiency (Kuhlencordt et al, 1970). Hyperparathyroidism may appear secondarily and lead to further increase in the solubility product.

Soyannwo et al (1968) suggested that potentiation of the effect of parathormone by intermittent heparinization during haemodialysis might be a further factor. Osteoporosis and spontaneous fractures are well known complications of a long term heparin therapy (Griffiths et al, 1965) and an influence on the metabolism of skeletal calcium and phosphate seems possible.

To investigate this question the following parameters of calcium, phosphate and citric acid metabolism were determined in the serum of 8 patients undergoing regular haemodialysis, and in a control group of 8 subjects without renal disease before, and 30 minutes after the intravenous injection of 20,000 U of heparin. The total calcium (Ca) and ionized calcium (Ca\(^{++}\)) were measured according to the method of Lumb (1963), citric acid according to Antener et al (1966). Isocitrate dehydrogenase (ICDH), inorganic phosphate (PO\(_4\)) and alkaline phosphatase were also measured as well as the clearance of inorganic phosphate (C\(_{PO_4}\)). In addition, total calcium and inorganic phosphate were measured every half an hour during haemodialyses lasting 9 hours in 4 chronic uraemic patients. The doses of heparin (10,000 U at the beginning of dialysis, 5,000 U every further 2 hours) were injected immediately after the collection of blood.

RESULTS

The following results were obtained (Table I, Figures 1 and 2). The total calcium increased in patients without renal failure by 10%, in haemodialysis
Table I. Serum levels of calcium-, phosphate- and citric acid metabolism after intravenous injection of 20,000 U of heparin in subjects without renal disease and regular haemodialysis patients (mean level of 8 experiments; C = controls, H = 30 minutes after heparin injection)

<table>
<thead>
<tr>
<th></th>
<th>without renal disease</th>
<th></th>
<th>regular haemodialysis</th>
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<tr>
<td></td>
<td>C</td>
<td>H</td>
<td>C</td>
<td>H</td>
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<tr>
<td>Ca (mg/100 ml)</td>
<td>9.78 ± 0.48</td>
<td>10.76 ± 0.22</td>
<td>8.68 ± 1.08</td>
<td>9.14 ± 1.22</td>
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<td>Ca++ (mg/100 ml)</td>
<td>5.95 ± 0.50</td>
<td>6.24 ± 0.48</td>
<td>6.06 ± 0.24</td>
<td>6.14 ± 0.24</td>
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<td>Ca++ : Ca (%)</td>
<td>60.9 ± 6.77</td>
<td>58.3 ± 7.4</td>
<td>70.8 ± 9.15</td>
<td>68.2 ± 8.99</td>
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<tr>
<td>citric acid (mg/100 ml)</td>
<td>1.88 ± 1.05</td>
<td>2.39 ± 1.37</td>
<td>1.16 ± 0.46</td>
<td>1.44 ± 0.33</td>
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<tr>
<td>ICDH (mU/ml)</td>
<td>2.31 ± 0.88</td>
<td>1.04 ± 1.18</td>
<td>2.96 ± 2.17</td>
<td>1.64 ± 2.32</td>
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<tr>
<td>(\text{PO}_4) (mg/100 ml)</td>
<td>3.96 ± 0.46</td>
<td>4.29 ± 0.60</td>
<td>9.87 ± 2.71</td>
<td>13.7 ± 3.97</td>
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<td>Ca x (\text{PO}_4)</td>
<td>38.8 ± 5.30</td>
<td>46.1 ± 7.04</td>
<td>84.8 ± 20.5</td>
<td>124 ± 35.2</td>
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<tr>
<td>alkaline phosphatase (mU/ml)</td>
<td>35.9 ± 16.7</td>
<td>34.9 ± 15.9</td>
<td>125 ± 85.9</td>
<td>115 ± 78.9</td>
</tr>
<tr>
<td>(C_{\text{PO}_4}) (ml/min)</td>
<td>9.67 ± 2.92</td>
<td>8.07 ± 2.32</td>
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</table>
patients by 5.3% over the initial level, while the ionized fraction was only elevated by 5.1 and 1.3% respectively. In both cases, therefore, a decrease of 2.6% in the degree of ionization resulted. Thus the increase of total serum calcium is accompanied by a mobilization of non-ionized calcium. In both groups the serum citric acid increased by 27.1 and 24.1% respectively, while the activity of the isocitrate dehydrogenase decreased by 55% and 44.6%. All these alterations were significant.

In patients without renal failure measurement of inorganic phosphate showed a non significant elevation of 8.3%. In the dialysis patients the already higher average level of 9.87 ± 2.71 mg/100 ml increased far more, by 38.8% to 13.7 ± 3.97 mg/100 ml. The extent of this increase correlates with the

![Graph showing calcium, calcium ionized, calcium ionized x 100, citric acid, and isocitrate dehydrogenase levels before and after heparin administration.]

Figure 1. Total and ionized calcium, degree of ionization, citric acid and isocitrate dehydrogenase before and 30 minutes after 20,000 U of heparin in subjects without renal disease (clear columns) and in regular haemodialysis patients (shaded columns)
level of phosphate before injection; the correlation-coefficient (Spearman) is 0.7719; that is, the higher the initial level, the greater the increase. In patients without renal disease the calcium-phosphate product increased by 18.8%; in the uraemic patients the considerably higher product (average level of $84.8 \pm 20.5$) increased by 46.2% to $124 \pm 35.2$. The activity of alkaline phosphatase decreased by 2.8 and 8% in control and uraemic groups respectively. The clearance of inorganic phosphate measured in the control group showed a significant decrease of 16.5% of the initial level. During 9 hours of haemodialysis only two of the patients (with a high level of phosphate before dialysis of 10.0 and 9.57 mg/100 ml) showed a distinct increase of the calcium-phosphate product after heparin injection (Figure 3). In spite of simultaneous phosphate and calcium elimination the solubility-product exceeded the initial
level even after the second dose of 5000 U of heparin, two hours after the start of haemodialysis. After the fourth dose of heparin no increase could be shown. In the two other patients with lower initial levels (7.45 and 9.43 mg/100 ml) the increase was too small to appear during the simultaneous elimination of both substances by haemodialysis.

DISCUSSION

The well known clinical observation of the development of osteoporosis after long term heparin therapy suggests the possibility that calcium was mobilised from the skeleton. The exchange of the calcium between blood and bone is probably effected through easily soluble calcium citrate complexes. The experimental finding of an increase of the citric acid level after injection of parathormone (Neumann et al., 1956) supports this hypothesis; about 12% of the total serum calcium is complex-bound, mainly as citrate complex.

Heparin forms macro-anionic complexes with proteins and can lead to inhibition of a number of enzyme-systems by shifting the isoelectric point (Gastpar, 1965). The decrease of the activity of the isocitrate dehydrogenase (which catalyses the first step of citrate degradation) explains the increase of serum citric acid, as being due to diminished degradation. The simultaneous elevation of non-ionized calcium seems to be the result of
mobilization of citrate-complexes. The increased phosphate level, the
decrease of the phosphate-clearance as well as the decrease of the activity of
alkaline phosphatase argue against enhanced secretion of parathormone as
suggested by Soyannwo et al (1968). On the contrary the increase of calcium
may induce a diminished secretion of the hormone.

The slight rise of phosphate in patients without renal failure can be re-
lated to the decreased phosphate-clearance and to the simultaneous mobiliza-
tion from bone with the calcium. In the uraemic patients this interpretation
cannot be considered for quantitative reasons. The considerably elevated
inorganic phosphate in renal insufficiency is not only the consequence of
diminished renal elimination but also of decreased incorporation of phosphate
in high energy organic phosphate-compounds by the disturbance of metabolism
by uraemia (Papenberg et al, 1962). This process is performed under the
influence of several enzymes. Possibly the enzyme-suppressing effect of the
heparin induces a further disturbance of the oxidative phosphorylation, and
therefore a shift in favour of the inorganic phosphate fraction. In this connec-
tion the correlation of the initial level with the dimension of the increase sug-
gests a heparin-induced potentiation of the disturbance of metabolism by
uraemia.

Thus, intermittent heparinization can lead to further increases of the
calcium-phosphate product at high initial levels and can provoke precipitation
of the two substances during haemodialysis. The therapeutic goal should be
to keep the calcium-phosphate product as low as possible. Sufficient dialysis,
and restriction of intestinal absorption of phosphate should be considered.

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OPEN DISCUSSION

G POSEN (Ottawa): The doses of heparin that you used in your study were considerably greater than a lot of us use routinely in our dialysis centres. Could you tell me if you have done any studies with smaller doses? In usual KII dialysis we use anywhere from 1200 units per hour to 2000 units per hour without an initial 2000 or 10000 units. Have you done any studies using these smaller doses?

KORZ: No, we haven't done any study to investigate the dose-dependence of this effect I related to you.

A C KENNEDY (Glasgow, Chairman): Could I ask you, Dr Korz, whether you have done any studies on phosphate elevation in other types of patient receiving heparin therapeutically?

KORZ: No, we didn't.

V PARSONS (London): How much phosphate is in your heparin? It contains quite a large amount.

KORZ: In heparin solution? I don't know, but I can tell you that we have studied the in vitro effect of heparin on serum and there was no change in the concentration of serum phosphate by adding a large dose of heparin to the serum.

PARSONS: My second question is: have you measured the change in serum proteins during the dialysis period? In other words, is the calcium rising because the protein is rising?

KORZ: No.

KENNEDY: Well, Dr Korz, there are at least three questions there that could lead to another paper for the future.