IONIZED, ULTRAFILTRABLE AND TOTAL CALCIUM IN SERUM IN HYPERPARATHYROIDISM

By
Ib Transbøl, Steffen Hahnemann and Ib Hornum

ABSTRACT

The present study has been performed in order to evaluate whether analysis of the ionized and ultrafiltrable fractions of calcium in the serum (Ca++ and UFCa) is superior or not to the analysis of total calcium (TOCa) in demonstrating the presence of hypercalcaemia. For this evaluation determinations have been carried out in 60 patients with hyperparathyroidism.

The material has been divided into one group of 47 patients with definite hypercalcaemia (average TOCa > mean control + 2.6 sd) and another of 14 patients with borderline hypercalcaemia (mean control + 2.6 sd ≥ TOCa ≥ mean control + sd; average Ca++ ≥ mean control + 2.6 sd). In the group with definite hypercalcaemia the average concentrations of TOCa, UFCa and Ca++ are all greater than the mean control + 2.6 sd, but the UFCa and Ca++ provide a greater distinction between the control group and the patients than that indicated by the concentration of TOCa. In the group with borderline hypercalcaemia large parathyroid glands were removed in all patients. Histological classification characterized 8 as adenomas and 6 as normal. Identity between these two subgroups as far as the preoperative level of TOCa, UFCa and Ca++ and the postoperative fall in this level is concerned makes it probable that the parathyroid tissue which was removed in both groups had the same secretory capacity irrespective of the histological classification. It is concluded that the UFCa and Ca++ which were clearly elevated in 9 and 14 patients, respectively, were both superior to the TOCa in demonstrating hypercalcaemia, not only in the 8 patients with definite histological evidence of hyperparathyroidism but also in the group as a whole.

When the total of 147 preoperative determinations of Ca++, UFCa and TOCa is estimated, it is found that 3%, 15% and 31%, respectively,
of the determinations fall within the mean control level + 2.6 sd and 1%, 9% and 25% within the mean control level ± 2 sd. Accordingly, it is suggested that UFCa and/or Ca++ should be determined in all patients with borderline hypercalcaemia.

Ultrafiltration of serum brings about separation of its content of total calcium (TOCa) into protein-bound and ultrafiltrable fractions (PBCa and UFCa) (Rona & Takahashi 1911). The latter mainly consists of free or ionized calcium (Ca++) but also comprises a modest complex-bound fraction (CBCa) (McLean & Hastings 1935). Ca++ is the physiologically active and the regulated fraction (McLean & Hastings 1935; Sherwood et al. 1966), and thus the fraction which is believed to be the most sensitive indicator of hypercalcaemia.

The first practically applicable method for chemical determination of Ca++ was introduced by Rose (1957). In more recent studies Rose and collaborators suggested that for diagnostic purposes the determination of Ca++ is superior to that of TOCa (Fanconi & Rose 1958; Lloyd & Rose 1958). The normal ranges for TOCa and Ca++ were, however, not sufficiently well established to warrant this conclusion. Other investigators who have argued against the conclusions of Rose and collaborators have either worked with Rose’s technique applied on very limited patient and control materials (Hyde et al. 1960; Fowler et al. 1961) or with Walser’s technique comprising very wide normal ranges for Ca++ and UFCa (Walser 1962).

The modifications of Rose’s method presented in this paper has made it possible to determine Ca++ and UFCa with the same accuracy as TOCa, and we have therefore used these in an investigation of 60 patients with hyperparathyroidism. The main purpose of the investigation was to elucidate whether determinations of Ca++ and UFCa represent an advantage in diagnosis.

MATERIAL

Determinations of TOCa, UFCa and Ca++ were carried out simultaneously in 60 patients, in whom symptoms consistent with the diagnosis of hyperparathyroidism made it necessary to determine the serum calcium, and in whom parathyroidectomy was subsequently performed (Table 1). Fifty-three of the patients were studied from Nov. 1964 to Nov. 1966 and from June 1967 to June 1968. This part of the material is not consecutive, the determinations of the calcium fractions being occasionally left out when the analytical capacity was exceeded.

In 47 patients the diagnosis of hypercalcaemia was established by a definite elevation of TOCa (mean above the 99 percentile limit of normal). The remaining 13 cases constituted a borderline group, in which hypercalcaemia was suspected by a high but normal concentration of TOCa (in the range of mean of control group + one standard deviation (sd) to mean + 2.6 sd) combined with a definite elevation of Ca++ (mean above the 99 percentile limit of normal). In one patient from the first group (no. 20)
Table 1.
Serum calcium fractions: the number of observations and their distribution among the various groups of patients. For further details, see the section on material.

<table>
<thead>
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<th>No. of observations</th>
<th>No. of patients</th>
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<td>Preoperative</td>
<td>147</td>
<td>60</td>
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<td>Postoperative</td>
<td>60</td>
<td>30</td>
<td>Appendix¹</td>
</tr>
<tr>
<td>Total</td>
<td>207</td>
<td>60</td>
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</table>

Preoperative
All fractions determined

- Definite hypercalcaemia 86 47
- Borderline hypercalcaemia 45 14
- Total 131 60
- No obvious hypoproteinaemia² 126 58
- Complete observations³ 96 50
- No obvious hypoproteinaemia³ 92 48

¹ Available from authors on request
² All patients except no. 9 and 27
³ Complete observations = all calcium fractions + serum proteins + plasma pH

TOCa decreased from 11.0 to 10.3 mg/100 ml after removal of one adenoma, but the Ca++ remained definitely elevated; accordingly she was also included in the borderline group and so made up the total 14 cases (Fig. 2). When possible, the diagnosis of hyperparathyroidism was made by the exclusion of other known cases of hypercalcaemia by the case history, the clinical examination and x-ray and laboratory investigations. In some cases, however, additional diagnostic measures such as the cortisol test (Dent 1956), the maximal tubular reabsorptive capacity for glucose (TmG/GFR) (Halver 1968) or the tubular reabsorption of calcium (TRCa°/o) (Transbol et al. 1968, 1970) had to be resorted to. In 51 cases the diagnosis was supported by histologically verified adenoma or hyperplasia, while in the remaining 9 patients it was confirmed by sustained normalization of Ca++ initiated by neck exploration (see Results and Discussion).

METHODS

In principle, the collection of blood, ultrafiltration and the determination of Ca++ was carried out according to the method of Rose (1957), but with several minor and a few major modifications as indicated in the following.

Collection of blood and ultrafiltration. Forty ml of venous blood, drawn from fasting subjects between 7 a.m. and 9 a.m., was collected directly into glass tubes containing 1 cm of paraffin oil. After spontaneous coagulation at room temperature and centrifugation (3000 g, 15 min) the serum (Hahnemann 1965) was transferred anaerobically to another glass tube containing paraffin oil. The »dead space« of the
syringe used for transfer was filled with the mixture of CO$_2$-O$_2$ to be used for equilibration during the ultrafiltration. It should be pointed out that the pCO$_2$ in the air mixture deviated by no more than ±5 mmHg from the pCO$_2$ determined in the capillary blood. In most cases this requirement was fulfilled by means of an air mixture containing 5% CO$_2$ (pCO$_2$ = 40 mmHg), but in a few cases the desired pCO$_2$ had to be provided by a microflowmeter-controlled mixing of two air mixtures of different CO$_2$ tension.

Reagents. Calcium solutions in concentrations from 10.0 to 16.0 mg/100 ml were prepared with intervals of 1 mg/100 ml. Calcium carbonate p.a. was dried at 105°C for two hours and weighed; 1.0 N HCl was then added in equimolar quantities and subsequently redistilled water to obtain the desired final concentration. Bicarbonate solutions were prepared, the concentration intervals for bicarbonate being 4 mEq./l (from 38 to 58 mEq./l), and the contents of sodium (296 mEq./l), potassium (10 mEq./l) and chloride (from 268 to 248 mEq/l), to make the ionic strength 0.306 μ in all solutions. Redistilled water and reagents of analytic grade were used. Murexide solution 1 (300 mg/l) and 2 (100 mg/l) were made from murexide (Siegfried) in redistilled water. Murexide was reprecipitated every third month by precipitation with ammonium chloride. Solution 1 should be used no later than 30 min after preparation, whereas solution 2, kept in the dark, should be used no later than 5 h following the preparation.

Standards and blind for determination of Ca$^{++}$. Six standards were made up of 1000 μl each of calcium and bicarbonate solutions. The former was so selected that the concentration of Ca$^{++}$ in the standards were equally distributed to both sites of the expected concentration of Ca$^{++}$ in the ultrafiltrate. The bicarbonate solution, to be used for all six standards, was so selected that the bicarbonate concentration deviated at most 1 mEq/l from that of the ultrafiltrate. Finally, the ionic strength of standards was kept close to that of most normal and pathological ultrafiltrates. The blind solution was made by adding 1000 μl redistilled water to 1000 μl of the bicarbonate solution.

Analytical methods. Simultaneously with the drawing of venous blood, capillary blood was drawn from the ear lobe for determinations of pH, pCO$_2$, base excess and standard bicarbonate (Siggaard-Andersen et al. 1960). Estimation of the pH of ultrafiltrates and of known bicarbonate solutions diluted 1:1 with redistilled water were carried out in duplicate after equilibration of all specimens with the 5% CO$_2$ air mixture for 10 min at 38°C (pH meter model 27 and micro electrode unit (E 5021), accuracy: ± 0.001 pH unit (Radiometer)). From these determinations the bicarbonate content of the ultrafiltrate could be calculated. For the determination of Ca$^{++}$ 100 μl of murexide solution 1 was added to 2000 μl of ultrafiltrates, standards and blind, which then were bubbled through simultaneously with the 5% CO$_2$ air mixture for at least 3½ minutes. Finally, double determinations of the extinction were made in matched quartz cuvettes (width 10 mm) at 470 nm, slit width 1.0, by the Beckmann spectrophotometer, model DU. TOCa and UFCa were determined titrimetrically (EEL-titrator with microburette and Unigalvo halation galvanometer (Evans Ltd.) by the use of murexide solution 2, with added 1.0 N KOH up to pH > 12, as indicator and Na$_2$EDTA as titrant (Wilkinson 1957). PBCa and CBCa were determined indirectly, by subtraction: PBCa = TOCa – UFCa, and CBCa = UFCa – Ca$^{++}$. The reproducibility of the analyses on sera was found to be about 1.5% for UFCa and Ca$^{++}$ as has been found for TOCa.

Over a period of 18 months 83 control subjects were examined. These included healthy adults (4 males and 28 females, aged 21–32 years) and patients with minor
illnesses unrelated to the calcium or acid/base metabolism (23 males and 28 females, evenly distributed between 22 and 75 years of age). The following ranges were established, $\text{TOCa}$: 9.2–10.6, $\text{PBCa}$: 1.93–3.57, $\text{UFCa}$: 6.55–7.65, $\text{CBCa}$: 0.83–1.29 and $\text{Ca}^{++}$: 6.00–6.60 mg/100 ml (mean ± 2 sd). All values are expressed per 100 ml serum without correction for serum solids or Donnan effect. In the control group and in some of the patient material, the determination of serum proteins was carried out by refractometry (Schmidt 1959) while in the remaining patients the biuret method was used (Aronsson et al. 1966). The control ranges for these methods are almost identical: 7.2 ± 0.9 g/100 ml (Table 3, group 1) and 7.4 ± 0.8 g/100 ml, respectively.

Statistical methods. Comparisons were made between the control material and various patient groups, and within the patient groups, by Wilcoxon's rank test for two samples (Diem 1962), while the effect of parathyroidectomy (Table 3, group 6a and b) was evaluated by means of Wilcoxon's rank test for pair differences (Diem 1962). The group results are expressed as mean values ± one sd (Table 3).

RESULTS

Correlation of serum calcium fractions to the outcome of parathyroid surgery

Confirmation of the preoperative diagnosis in the group of definite hypercalcaemia ($n = 47$)

According to the method of selection all patients in this group had definite elevations of the average concentrations of TOCa; the same was also found to be the case regarding the average concentrations of UFCa and $\text{Ca}^{++}$ (Fig. 1). In all cases but three, histologically verified adenomas or hyperplasia were found. In the negative cases TOCa measured 15.7, 11.8 and 11.1 mg/100 ml (no. 4, 12 and 19), and the neck exploration was followed by normalization of TOCa as well as $\text{Ca}^{++}$ in all cases.

Confirmation of the preoperative diagnosis in the group of borderline hypercalcaemia ($n = 14$)

This group attracts the greatest interest by offering an excellent opportunity for evaluating whether determinations of UFCa and $\text{Ca}^{++}$ are really advantageous in the diagnosis of hypercalcaemia. The average preoperative concentrations of TOCa were all at or below the 99 percentile limit of the control group, while the average concentrations of UFCa and $\text{Ca}^{++}$ were above this limit in 9 and 14 cases, respectively (Fig. 2).

In 8 out of 14 patients the preoperative diagnosis was confirmed by the generally accepted criterion: the histological demonstration of adenomas or hyperplasia. Five and 8 of these had mean concentrations of UFCa and $\text{Ca}^{++}$ above the respective 99 percentile limits of the control group.

The remaining 6 patients, who had average concentrations of TOCa, UFCa and $\text{Ca}^{++}$ comparable to those of the former group (Fig. 2), had suspiciously
The average preoperative levels of serum total (TOCa), ultrafiltrable (UFCa) and ionized (Ca++) calcium in 60 patients with hyperparathyroidism. The order of the patients is determined by their average level of TOCa. The upper limits of the normal are demarcated by double lines representing the 95 and 99 percentile limits. The discontinuity of the slope of the average values of TOCa, which is also reflected in the more irregular slopes of UFCa and Ca++, remains unexplained.

large but histologically normal parathyroid glands removed. Nevertheless, the parathyroidectomy initiated a reduction in the average concentrations of TOCa, UFCa and Ca++ identical to that observed in the first group, as well as a lasting normalization of Ca++ in all cases. In this group 4 and 6 patients had mean concentrations of UFCa and Ca++ above the respective 99 percentile limits.

The response to parathyroidectomy evaluated by postoperative determination of Ca++

The establishment of Ca++ as the most sensitive indicator of hypercalcaemia makes it particularly useful in controlling the efficiency of surgical therapy.
PARATHYROID ADENOMAS
OR HYPERPLASIA
(n = 8)

TOCa
PTX

11.0
10.0
9.0

UFCa
PTX

8.50
7.50
6.50

Ca++
PTX

8.00
7.00
6.00

mg/100 ml

Fig. 2.

Average pre- and postoperative (PTX) determinations of serum total (TOCa), ultrafiltrable (UFCa) and ionized (Ca+++) calcium in 14 patients with hyperparathyroidism and borderline hypercalcaemia. All postoperative determinations were carried out more than 3 weeks after parathyroidectomy. The upper limits of the normal are demarcated by double lines representing the 95 and 99 percentile limits. Note that, despite histological disparity, there is a striking identity among the sub-groups regarding preoperative levels and response to parathyroidectomy (see text). The fact that only the 5 patients with the slightest degree of hypercalcaemia in the adenoma-group had postoperative determinations makes this identity complete.

Such evaluation was carried out in 30 patients. Two patients with proven adenoma or hyperplasia remained hypercalcaemic, two became permanently hypoparathyroid, and a fifth was afflicted with prolonged hypocalcaemia due to healing of severe ostitis fibrosa. Among the remaining 25 patients the Ca++ was normal in 24 and doubtfully increased in one: 6.65 mg/100 ml. Only 21 out of the 30 patients had complete series of serum calcium fractions + serum proteins + pH determinations carried out both pre- and postoperatively; these
patients were selected for the evaluation of changes in PBCa and CBCa in response to parathyroidectomy (Table 3, group 6a and b).

**Detailed evaluation of the preoperative determinations of serum calcium fractions**

**Preoperative determinations of Ca\(^{++}\), UFCa and TOCa**

These determinations are evaluated in two ways, namely 1. on the basis of the number of individual observations within the 95% and 99% limits of the control ranges (Table 2, Fig. 3), and 2. on the basis of the number of patients in whom a. one or more observations (Table 2) or b. the average of the observations fall within the above mentioned limits (Fig. 1, Table 2). The results of the evaluation clearly appear in the tables and figures.

**Table 2.**

One hundred and thirty-one observations of serum total, ultrafiltrable and ionized calcium in 60 patients with hyperparathyroidism. Table 2 indicates the number of observations, which fell within the 95 and 99 percentile limits of normal, as well as the number of patients in whom one or more of the observations and the mean of the observations fell within these percentile limits.

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<th>Observations within the upper limits of normal</th>
<th>Patients with:</th>
<th>Mean of observations within the upper limits of normal</th>
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<td>Percent</td>
<td>Number</td>
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<td>0.8</td>
<td>1</td>
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<td>Ultrafiltrable calcium</td>
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<tr>
<td>Ultrafiltrable calcium</td>
<td>7.65(^1)</td>
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<td>9.2</td>
<td>11</td>
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<tr>
<td>Ultrafiltrable calcium</td>
<td>7.80(^2)</td>
<td>20</td>
<td>15.3</td>
<td>13</td>
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<tr>
<td>Total calcium</td>
<td>10.6(^1)</td>
<td>33</td>
<td>25.2</td>
<td>14</td>
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<tr>
<td>Total calcium</td>
<td>10.8(^2)</td>
<td>41</td>
<td>31.3</td>
<td>17</td>
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\(^1\) 95 percentile limit (= mean + 2 sp)

\(^2\) 99 percentile limit (= mean + 2.6 sp)
Eighty-three observations of serum calcium fractions in 83 control subjects (hatched) and 126 observations in 58 patients with hyperparathyroidism (blank). Five observations in two patients with obvious hypoproteinaemia were omitted.

*Preoperative determinations of PBCa*

This fraction was only found to be increased in about 7% of the determinations (Fig. 3). Since PBCa is dependent on the concentrations of Ca++ and serum proteins and also on the pH, we have not only expressed PBCa in mg/100 ml serum, but also in percentage of Ca++, PBCa %, as the mean values for PBCa, PBCa %, serum proteins and pH are compared group by group (Table 3). We have preferred this simple method rather than calculating the dissociation constant of the calcium proteinate since such calculations are based on a number of conditions, several of which cannot be considered as being fulfilled. Nevertheless, for the information of those who may find it of interest, we have calculated the dissociation constant in some of the groups.

With *slight* hypercalcaemia (mean Ca++ ≤ 8.00 mg/100 ml, n = 27) PBCa does not increase significantly, though the PBCa % falls from 43.6 to 38.0 % (Table 3, group 2, *P < 0.01*). In *severe* hypercalcaemia (mean Ca++ > 8.00 mg/100 ml, n = 21) an increase in PBCa is seen, but the relative value shows a further fall to 33.7 % (Table 3, group 3, *P < 0.01*). The mean values for
Table 3.
Serum calcium fractions, serum proteins and plasma pH in controls (group 1) and in hyperparathyroidism (group 4). The latter group is divided into slight and severe hypercalcaemia (group 2 and 3). Groups of patients with high concentrations of serum proteins (group 5) and with complete pre- and postoperative observations (group 6a and 6b) are also selected from group 4. All values are expressed as the mean ± one standard deviation.

<table>
<thead>
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<th>No.</th>
<th>Group</th>
<th>No. of subjects</th>
<th>TOCa</th>
<th>PBCa</th>
<th>UFCa</th>
<th>CBCa</th>
<th>Ca++</th>
<th>PBCa % of Ca++</th>
<th>Serum proteins g/100 ml</th>
<th>Plasma pH</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>mg/100 ml</td>
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<td>1</td>
<td>Control</td>
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<td>9.85</td>
<td>2.74</td>
<td>7.10</td>
<td>0.81</td>
<td>6.29</td>
<td>43.6</td>
<td>7.16 ± 0.45</td>
<td>7.39 ± 0.02</td>
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<td></td>
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<td></td>
<td>± 0.35 ± 0.40</td>
<td>± 0.28 ± 0.24</td>
<td>± 0.16</td>
<td>± 0.16</td>
<td>± 6.7</td>
<td>± 0.45</td>
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<td>11.06</td>
<td>2.80</td>
<td>8.26</td>
<td>0.90</td>
<td>7.37</td>
<td>38.0</td>
<td>7.20 ± 0.52</td>
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<td>± 0.53 ± 0.28</td>
<td>± 0.44 ± 0.20</td>
<td>± 0.39</td>
<td>± 0.39</td>
<td>± 4.0</td>
<td>± 0.52</td>
<td>± 0.02</td>
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<td>Patients Ca++ &gt; 8.00 mg/100 ml</td>
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<td>14.06</td>
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<td>10.81</td>
<td>1.15</td>
<td>9.68</td>
<td>33.7</td>
<td>7.14 ± 0.47</td>
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<td>± 1.67 ± 0.50</td>
<td>± 1.37 ± 0.36</td>
<td>± 1.12</td>
<td>± 1.12</td>
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<td>12.38</td>
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<td>± 1.90 ± 0.45</td>
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<td>5</td>
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<td>12.34</td>
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<td>± 2.00 ± 0.48</td>
<td>± 1.68 ± 0.27</td>
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<td>Patients Group 6b preoperative</td>
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<td>± 1.55 ± 0.40</td>
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<td>± 3.4</td>
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<td>Patients Group 6a postoperative</td>
<td>21</td>
<td>9.66</td>
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<td>± 0.65 ± 0.38</td>
<td>± 0.44 ± 0.20</td>
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<td>± 5.6</td>
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Significance of differences (P):

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<td>N. S.(^3) &amp; &lt; 0.05</td>
<td>N. S.(^3)</td>
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<td>2 : 3</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01 &amp; &lt; 0.01</td>
<td>&lt; 0.02</td>
<td>&lt; 0.01</td>
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<td>N. S.(^3)</td>
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<td>6a : 6b</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01 &amp; &lt; 0.01</td>
<td>N. S.(^3)</td>
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<td>N. S.(^3)</td>
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\(^1\) Determinations of serum proteins were carried out in only 63 of 83 control subjects. Identity with regard to the mean values of calcium fractions in these subjects and the control group as a whole permits the assumption that the concentration of serum proteins is representative also of the latter group.

\(^2\) Acid-base status of capillary blood was not determined in the control group. The mean value originates from another control group examined by the same method.

\(^3\) N. S. = non significant (\(P > 0.05\)).
serum proteins and pH are identical for these groups. If, from among the patient material, we pick out all the patients with high mean concentrations of serum proteins ($\geq 7.4$ g/100 ml, n = 17) we find that these patients who are in every other respect comparable to group 4, offer a similar reduction in PBC $\%$, 36.2 $\%$ (Table 3, group 5). Thus, the relative fall in PBCa can not be explained by hypoproteinaemia or by a fall in plasma pH; neither could the modest increase in CBrca be the explanation (see below). Parathyroidectomy (n = 21) causes a definite fall in PBCa, but only an insignificant increase in PBCa $\%$ (Table 3, group 6b), see Discussion.

For the groups no. 1–4 of Table 3 the dissociation constant of calcium proteinate is calculated according to the directions given by McLean & Hastings (1935). The dissociation constants are $10^{-1.71}$, $10^{-1.65}$, $10^{-1.66}$ and $10^{-1.62}$, respectively, and reflect the changes in PBCa $\%$ mentioned above.

In some investigations PBCa has been evaluated by expressing this value or UFCa in percentage of TOCa. In the control and patient material the respective values of UFCa constitute 72.1 ± 3.5 $\%$ and 75.8 ± 2.2 $\%$ of TOCa ($P < 0.01$).

Preoperative determinations of CBCa

Only about 13 $\%$ of the determinations are found to be increased (Fig. 3). The mean values for the control material, for the groups of slight and severe hypercalcaemia and for the total patient material are 0.81, 0.90, 1.15 and 1.01 mg/100 ml (Table 3, group 1–4). Only the means of the last mentioned two groups are significantly above that of the control group ($P < 0.01$).

**DISCUSSION**

This paper is concerned with 60 patients with symptoms consistent with the diagnosis of hyperparathyroidism and in whom parathyroidectomy was carried out. According to the average level of TOCa the patients were divided into groups of definite and borderline hypercalcaemia comprising 47 and 14 cases, respectively.

In the former group verified adenomas or hyperplasia were found in 44 of 47 cases. Normalization of TOCa and Ca++ was initiated by the neck exploration in the remaining cases two of which had marked hypercalcaemia preoperatively. These cases, at least, must be assumed to have had ordinary adenomas, which underwent necrosis *in situ* during or following the operation. The only advantage gained by the use of UFCa and Ca++ in this group is a more obvious indication of the hypercalcaemia as measured by the number of standard deviations by which the observations deviate from the mean of the control group (Fig. 1).
The group of borderline hypercalcaemia is interesting from several angles. In these 14 patients the average concentration of TOCa was in the same region as in 15.4% of the normal population, viz. the area between mean ± sd and mean ± 2.6 sd, but they were separated from the normals by having a clearly increased average concentration of Ca++. All the patients had those parathyroid glands removed which were considered to be enlarged by an experienced surgeon, but which according to histological criteria were classified differently. Eight were described as adenomas or hyperplasia, while six were considered to be normal. Everyone would agree that the first eight patients were suffering from hyperparathyroidism, and among these, five and eight of whom being clearly hypercalcaemic as judged from the average concentrations of UFCa and Ca++, respectively, we can thus maintain with certainty that the present methods of analysis were superior to the TOCa analysis. The six patients, who were classified as normal from a histological point of view, give rise to more problems: 1. Are they patients, who are extreme variants of the normal with regard to Ca++? 2. Are they patients with hypercalcaemia for »another cause«, a cause, which it has simply been impossible to discover? or 3. Are they patients suffering from a kind, or an early stage, of hyperparathyroidism, which manifest itself functionally, but does not fulfil the common histological criteria? The first possibility may be rejected immediately, since the average concentration of Ca++ deviates from 4.5 to 11 sp’s from the average level of the control group (Fig. 2). Hypercalcaemia due to »another cause« is also a rather hypothetical possibility, but it is difficult to exclude it definitively. We know, however, that non-parathyroid hypercalcaemia suppresses the secretion of parathyroid hormone (Sherwood et al. 1966), and even though animal experiments have given grounds for the assertion that a certain basal secretion cannot be suppressed (Gittes & Radde 1966), it would be most peculiar if the removal of these not very active glands would occasion a normalization of Ca++ in all six cases. If we compare the six histological normal with the eight histologically abnormal cases, we find identity as regards the preoperative average values for TOCa, UFCa and Ca++ and identity as to the reduction of these values following parathyroidectomy (Fig. 2). These functional criteria make it probable that the removed glands had identical secretory capacity, irrespective of the histological findings. At any rate, this conclusion, which also gains some support from the literature (Boyce & Bradshaw 1960; Hodgkinson & Edwards 1963; Davies et al. 1968), points to the fact that the determination of Ca++ is of essential diagnostic importance – not only for the first eight patients, but also for the group as a whole. The same is true for the determination of UFCa, but to a slightly lesser extent (Fig. 2).

If we compare the outcome of the single determinations of Ca++, UFCa and TOCa for the material as a whole, we found that 3%, 15%, and 31%,
respectively, of the analyses come within the 99 percentile limits of the control group (Table 2, Fig. 3), and if we choose the 95 percentile limits as a criterion, the corresponding percentages turn out to be 1 %, 9 % and 25 %. These results which conform with our observations in other states of hypercalcaemia (Hahmemann et al. 1967; Hornum et al. 1968; Transbol et al. 1968, 1970) come up to the original expectations of Rose and collaborators regarding the chemical determination of the calcium fractions (Rose 1957; Fanconi & Rose 1958; Lloyd & Rose 1958). The investigations further available are, with a single exception (Yendt & Gagné 1968), either inconclusive or negative. The reasons seem to be due to small control and patient materials (Fanconi & Rose 1958; Lloyd & Rose 1958; Hyde et al. 1960; Fowler et al. 1961; Hodgkinson & Edwards 1963), non-comparable control ranges for the individual analyses (Fanconi & Rose 1958; Lloyd & Rose 1958) or analytical difficulties (Hyde et al. 1960; Fowler et al. 1961; Walser 1962). The last mentioned factor may in part be due to the use of varying quantities of heparin as anticoagulant (Hahmemann 1965). Walser’s surprising result from 17 determinations of Ca++, UFCa and TOCa in 14 patients with hyperparathyroidism was that 10, 3 and 1 of the observations fell within the respective 95 % limits (Walser 1962). The discrepancies between Walser’s and our own observations are due to differences in the width of the control ranges. In Walser’s study these ranges for Ca++, UFCa and TOCa are 1.68, 1.36 and 1.04 mg/100 ml, respectively, as compared with ours of 0.60, 1.10 and 1.40 mg/100 ml. This clearly implies that Walser’s method for determination of Ca++ and UFCa (Walser 1962) is not suitable for the diagnosis of slight cases of hypercalcaemia. Yendt & Gagné (1968), on the other hand, found that UFCa (modified Toribara technique) was superior to TOCa for diagnostic purposes, and our observations fully agree with this. However, the determinations of Ca++ and UFCa does not supersede the TOCa analysis, as the latter should be used to decide whether it is necessary to determine Ca++ and UFCa. The TOCa analysis, therefore, should be carried out «with extraordinary regard for technical precision and accuracy», as emphasized by Keating (1961). Assuming this is complied with, suitable methods will give normal ranges with a scale of about 1–1.6 mg/100 ml (mean ± 2 sn) (Keating 1961; Walser 1962; Hodgkinson & Edwards 1963; Dale & Kellerman 1967; Yendt & Gagné 1968; the present paper). In our experience, the determinations of Ca++ or UFCa are only required for the diagnosis of hypercalcaemia when the mean concentration of TOCa is within the range of mean ± sd to mean ± 3 sd (Figs. 1 and 2), but in the case of a less accurate TOCa analysis, this range should be widened.

The narrow range for Ca++ in the control group, 0.60 mg/100 ml, is supposed to reflect only the normal variation in the interactions between the hormones of the calcium homoeostasis and their target organs: tubules, intestine and bones. As regards UFCa and TOCa, the range are broader: 1.10
and 1.40 mg/100 ml, due to their incorporation of the normal variability in the concentrations of calcium-binding substances and in the factors influencing the calcium-binding capacity of these substances.

Assuming, in a hypothetical case, that Ca++ is increased by 0.48 mg/100 ml (= 3 sd) from the mean value, and that the other values are also increased proportionately, we find that UFCa and TOCa increase by 0.54 and 0.75 mg/100 ml, respectively, or only by approximately 2 sd for both. Now it is not a foregone conclusion that a proportional increase in the other fractions is to be expected, but even if it does occur, we understand that a net-addition of calcium ions to the blood will manifest itself primarily by exceeding the upper limit for Ca++. In the said example UFCa and TOCa will both increase by approximately 2 sd. The fact that we still find UFCa to be a more sensitive indicator of hypercalcaemia than TOCa must be due to PBCa not increasing proportionally to Ca++ (Fig. 3, Table 3). As regards this observation, i.e. a relative fall in PBCa with increasing hypercalcaemia, we are in agreement with most of the previous investigators (Fanconi & Rose 1958; Lloyd & Rose 1958; Breen & Freeman 1961; Lloyd et al. 1962; Hodgkinson & Edwards 1963; Yendt & Gagné 1968), but not with all of them (Gordan et al. 1962; Walser 1962; Leighton et al. 1964; Dale & Kellerman 1967). Contrary to us, Rose and collaborators observed a relative increase in PBCa following parathyroidectomy. However, our materials are not comparable; Rose's patients were examined immediately after the operation, when most of them were hypocalcaemic (and hypoparathyroid?), while our patients were examined weeks or months after the operation, when almost all cases were normocalcaemic. Secondary hyperparathyroidism in consequence of «bone hunger» may have occurred in some of our patients at the time of the follow-up. Thus, our data cannot exclude the validity of the suggestion by Lloyd et al. (1962) that the relative reduction in PBCa in hyperparathyroidism is due to the increased secretion of parathyroid hormone per se.

REFERENCES

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