
James T. Wu
Terry Miya
Joseph A. Knight

Dept. of Pathol.
Univ. of Utah School of Med.
Salt Lake City, UT 84132

Determination of Calcium Concentration in Pancreatic Juice with the Corning 940 Calcium Titrator

To the Editor:

The Corning 940 Titrator (Corning Ltd., IMA Analysengeräte, Giessen, F.R.G.) which measures total calcium concentrations by titration with EGTA [ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid] has been shown to measure accurately calcium concentrations in blood serum and urine (1). We evaluated the instrument for calcium measurement in pancreatic juice. Comparative studies were carried out with an atomic absorption spectrophotometer (AAS, Model 400; Perkin-Elmer, Uberlingen, F.R.G.), with the method of Trudeau and Freier (2). Pure feline pancreatic juice samples of 1 mL were obtained as previously described (3) and collected during intravenous infusion of secretin (4 Crick units·kg·h⁻¹) with or without simultaneous intravenous infusion of cholecystokinin (16 Iry dog units·kg·h⁻¹; both secretagogues from Karolinska Institute, Stockholm, Sweden). After estimation of secretory rate and protein concentration (by absorbance at 280 nm), we acidified the samples to pH 1.0 with 100 µL of HCl (1 mol/L) to prevent calcium precipitation in the alkaline juice (3). The specimens were then analyzed for calcium.

An evaluation of the precision (4) was based on repeated analyses of 100-µL samples of pooled juice with either a low (1.28 g/L) or a high (6.71 g/L) protein content. The calcium concentrations of both samples were measured on four different days within one month, six times each within the same run. The high-protein juice was additionally analyzed with a sample volume of 50 µL. Within-run imprecision for low-protein juice (mmol/L; ± SD) was 0.1908 ± 0.0049, CV = 2.68%; for high-protein juice (100 µL), 0.036 ± 0.00075, CV = 2.25%; for 50 µL of high-protein juice, 0.332 ± 0.0099, CV = 2.99%. Between-run imprecision for low-protein juice was SD = 0.0065 mmol/L, CV = 3.41%; for high-protein juice (100 µL), SD = 0.0110, CV = 3.28%; for 50-µL samples, SD = 0.0117, CV = 3.51%.

Linearity and sensitivity were tested by serial stepwise dilution of a juice sample containing about 0.60 mmol of calcium per liter. Titers and concentrations were linearly related down to about 0.07 mmol/L. At lower concentrations, no precise results could be obtained.

For analytical recovery studies, eight different amounts of calcium (0.25–2.5 µmol) were added to samples of pooled pancreatic juice. The mean recovery was 97.3 (SD 3.1) %, as calculated from six analyses on each sample.

For correlation studies, 16 juice samples with calcium content ranging from about 0.17 to 1.40 mmol/L were analyzed in quadruplicate with both the Corning Titrator and the atomic absorption spectrometer (Figure 1). The results obtained with the titrator were systematically lower than with AAS (p < 0.001); the bias was 0.0268 mmol/L. The differences between results increased with increasing calcium concentrations, reflecting a proportional error.

In summary, the instrument was precise and provided results of close linearity between 0.07 and 0.80 mmol/L, thus covering sufficiently the range of physiological pancreatic juice concentrations (5). The observation that the calcium concentrations obtained with the Corning Titrator are lower than with AAS, the bias increasing at higher concentrations, is in accordance with earlier findings (1). However, at the low concentration normally found in pancreatic juice, the difference is negligible. Because increased concentrations of pancreatic juice calcium may be related to a disturbed diffusion barrier between the extracellular space and the pancreatic ductular system and thus may be of diagnostic and (or) pathogenic importance in pancreatic disease (3, 5–8), the titrator provides a useful aid for gastrointestinal clinical or research laboratories.

References

P. Layer J. Hotz D. Maruhn H. Goebell

Dept. Internal Med.
Div. of Gastroenterol.
Univ. of Essen
Hufeland str. 55
D-4300 Essen 1, F.R.G.

A Case of Cholesterol Gravel in the Urinary Tract

To the Editor:

The nephrological and urological department of our hospital usually sends urinary concrements, calculi or gravel, physiologically expelled or surgically extracted, to the laboratory for physico-chemical and chemical analysis. The analysis protocols we use are those described in well-known clinical chemistry books (1, 2), exploited also in several commercial kits for calculi analysis.

Recently we received for analysis a gravel expelled by a female patient. Its color was brownish-yellow, with a soft and waxy consistency. Because our sample did not dissolve in concentrated sulfuric acid, but instead became a sticky, deep brown mass, we tried a procedure that no commercial kit suggests for urinary concrement analysis, but which is reported for that of uro stealth calculi (2). We treated a por-
tion of our sample with chloroform; there was complete dissolution. The dried residue treated with Liebermann–Burchard reagent showed a positive reaction for steroids. Moreover, a small portion of the gravel, placed in a micro platinum crucible, burned completely, leaving no residue. The only textbook that reports a similar behavior, attributing it also to cholesterol, is the Merck "Clinical Laboratory" booklet (3).

The melting point of the material was 146 ± 1°C. Run in parallel with different steroids, in thin-layer chromatography (SG 81 impregnated paper sheets, Whatman Ltd, England; chloroform as a developer), our sample showed the same Rf as pure unesterified cholesterol, when colored with a cholesterol Trinder reagent or rendered fluorescent with dichlorofluorescein.

Pure cholesterol, the native concrement, and the recrystallized (from isopropanol) concrement were analyzed by the Debye–Scherrer powder technique (d), in a Philips X-ray Diffractometer (radiation Cu-Kα). All the three photographs evidenced the same structure (Figure 1). Table 1 the 2θ values of the observed reflections with the estimated intensities and the corresponding d values are reported for the three samples, and compared with the theoretical values calculated on the basis of the unit cell parameters and space group proposed by Shiieh et al. (5) for anhydrous cholesterol.

Evidently, the concrement we analyzed was practically pure anhydrous unesterified cholesterol. Now we are investigating how this substance could be passed in urinary ultrafiltrate. This very rare event has been reported exclusively in women (6).

No hypothesis must therefore be discarded a priori when one is analyzing human specimens, as "...a piece of work is a man... infinite in faculties. ..." (Shakespeare).

Table 1. Experimental and Theoretical Values (Scattering Angles: 2θ, Corresponding Interplanar Spacings: d) for the Same Samples As in Fig. 1

<table>
<thead>
<tr>
<th>Pure cholesterol, native concrement, recrystallized concrement</th>
<th>Theoret. values, anhydrous cholesterol (5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>d (nm)</td>
<td>2θ (°) (nm)</td>
</tr>
<tr>
<td>12.75</td>
<td>0.694</td>
</tr>
<tr>
<td>14.00</td>
<td>0.632</td>
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<tr>
<td>15.25</td>
<td>0.581</td>
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<tr>
<td>16.90</td>
<td>0.525</td>
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<tr>
<td>18.10</td>
<td>0.490</td>
</tr>
<tr>
<td>19.30</td>
<td>0.460</td>
</tr>
<tr>
<td>21.25</td>
<td>0.418</td>
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</tbody>
</table>

* Estimated intensities.

A reviewer comments:

Cholesterol is insoluble in water, so that if cholesterol occurs in urine, its origin must be cellular, i.e., from sloughed blood cells or sloughed epithelial cells, either normal or neoplastic. It is also possible that cholesterol could arise from passage of a gallstone into the urinary tract. Identification is simple if one has the idea that the stone may contain cholesterol. As noted by Zoppi et al., the stone may be dissolved in chloroform–methanol (2:1 by vol) and spotted on a TLC plate with a standard of cholesterol. The plate should be sprayed with concentrated sulfuric acid to generate a sort of Liebermann–Burchard reaction. For confirmation of composition, it is convenient to carry out an enzymatic analysis of cholesterol. The cholesterol is likely to be present as the monohydrate, whose behavior with temperature has recently been reported (1).

Obviously, structural proof could be obtained by gas chromatography/mass spectrometry, or by x-ray diffraction as reported by Zoppi et al.

Recently, our group reported a patient with gallstones composed of predominantly calcium oxalate (2). Thus, urinary concretions may have a composition one expects to find in gallstones, and vice versa.

References
