“Improved” Sample Extraction before Liquid Chromatography of Prednisone and Prednisolone in Serum

To the Editor:

A recent Letter (1) reported on improvements in our extraction procedure for prednisone and prednisolone (2) by use of a commercially supplied column-type extraction tube (“Clin-Elut,” cat. no. 1003; Analyticem International, Harbor City, CA 90710).

The sample extraction procedure proposed (2) was repeated by our group using two different batches of Clin-Elut tubes. We do agree that the extraction procedure was shortened, but we do not agree that the method was improved because, as demonstrated in Figure 1 (left), the chromatogram shows additional unidentified peaks and a delay in reaching the baseline after injection of the sample. A few injections of “Clin-Elut” extracts onto the column containing a 2-μm frit let the pressure rise from 1000 psi to an unacceptable pressure of 3000 psi. This plugging of the column was probably due to the few fine particles eluted from the extraction tube. Thus we found it necessary to open the column in order before liquid chromatography of prednisone and prednisolone in human serum. Clin Chem 28, 2326–2327 (1982).

Fig. 1. Dual-pen recordings of chromatograms (Right) For human plasma supplemented with prednisone and prednisolone, with use of the extraction procedure as described by Frey et al. (2). PO, prednisone; HC, cortisol; D, dexamethasone (int. std.); P, prednisolone, and C, corticosterone. The attenuation of the upper pen is 10 times that for the lower pen recording. (Left) Same, but with use of the extraction procedure as described by Stewart et al. (1) to replace the frit after every 3rd to 6th injection.

The standard curves for prednisone and prednisolone were linear, as reported by Stewart et al. (1).

The method developed by our group (2) allowed one to analyze simultaneously and specifically for prednisone, prednisolone, and the endogenous steroids cortisol and hydrocortisone (2). Stewart et al. (1), however, changed our conditions in such a way that for measuring prednisone and prednisolone “the interference from endogenous serum components such as cortisone and hydrocortisone was negligible” (1). However, as demonstrated for hydrocortisone on our chromatogram (Figure 1, right), endogenous cortisone and hydrocortisone are extracted by the method proposed by Stewart et al (1). These endogenous glucocorticoid concentrations may indeed not be negligible, as was recently shown in patients being treated chronically with prednisone (3).

We agree with the intention of Stewart et al. (1) that a simplified extraction procedure should be developed, but the modification of our method as proposed is inadequate because it shortens the extraction procedure at the expense of the quality of the chromatogram.

This work was supported by Grant 3.914-0-82 of the Swiss National Foundation for Scientific Research.

References
1. Stewart JT, Honigberg IL, Turner BM, Davenport DA. Improved sample extraction

Somatostatin in Diabetes

To the Editor:

Somatostatin, besides being of importance in the control of pituitary hormone release, may also have an important physiological role in the regulation of endocrine pancreas. The development of an immunoassay for somatostatin can contribute to an understanding of the pathogenesis of different types of diabetes mellitus. Only a few groups have reported values for man, and changes in somatostatin after some stimuli are not yet quite clear.

We have measured somatostatin in plasma of normal persons, in persons with impaired glucose tolerance (IGT), and in diabetic type II subjects after an oral glucose tolerance test (OGTT), according to the National Diabetes Data Group criteria (1), always with glucose basal concentrations <7.8 mmol/L.

Samples of whole blood were collected into pre-chilled tubes containing EDTA and aprotinin (Trasyrol, Bayer), and the plasma was immediately separated by centrifugation (2000 × g, 10 min, 4 °C). The plasma was stored at −80 °C until use, then thawed in an ice bath.

We used an RIA kit to measure somatostatin (Immunonuclear Corp., Stillwater, MN 55082), involving an extraction procedure (acetone) and washing with organic solvent. The RIA was performed as described in the manufacturer’s literature.

For a group of 119 subjects, the mean basal reference value was 21.5 (SD 7.8) ng/L. The OGTT was performed in a group of 38 subjects (18 control, 10 IGT, and 10 diabetes type II). In the three groups, the maximum increase among the basal values and those obtained during the OGTT were
measured. The absolute values after stimulation were also studied in the same groups.

Table 1 summarizes our data. After OGTT, the somatostatin concentration in plasma increased in all subjects in the three groups, usually at 60 and 90 min. The results, expressed as the maximum increase, showed that the control group was significantly lower ($p < 0.01$) than the IGT and diabetic groups; likewise, the IGT group was significantly lower ($p < 0.05$) than the diabetic one. On the other hand, the results expressed as absolute values showed that the control group was lower ($p < 0.05$) than the diabetic one. There was no correlation between values for insulin, glucose, and somatostatin in plasma.

Unfortunately we have only limited information at this time to use in determining the clinical significance of this finding, and although it is difficult to speculate about it, data on somatostatin may be important in explaining the insulin disregulation found in diabetes.

Reference


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**Table 1. Somatostatin Concentrations in Plasma: Responses to Oral Glucose Tolerance Test**

<table>
<thead>
<tr>
<th></th>
<th>Maximum increase</th>
<th>Absolute values</th>
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<tr>
<td>Controls</td>
<td>9.39 ± 5.7*</td>
<td>29.2 ± 10.1</td>
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<tr>
<td>(n = 18)</td>
<td></td>
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<tr>
<td>IGT (n = 10)</td>
<td>16.9 ± 4.0</td>
<td>35.4 ± 10.1</td>
</tr>
<tr>
<td>Diabetics</td>
<td>32.9 ± 18</td>
<td>56.2 ± 22.8</td>
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<tr>
<td>Type II</td>
<td>(n = 10)</td>
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<td></td>
<td>*SD</td>
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Paracetamol and Blood-Glucose Analysis with the YSI Analyzer

To the Editor:

Two years have passed since the first report of a high serum paracetamol concentration interfering with blood glucose determination by the Yellow Springs Glucose Analyzer. In this case (1) the value for blood glucose was spuriously increased and inappropriate insulin therapy was commenced.

Other authors (2, 3) have reported similar interference, and recently the manufacturers (Yellow Springs Instrument Co., Yellow Springs, OH) have introduced a modified membrane to overcome the problem.

I report a series of nine patients with paracetamol self-poisoning in whom plasma glucose was determined with two YSI instruments, one fitted with the unmodified membrane, the other with the new paracetamol-resistant membrane. These values were compared with a COBAS BIO (Roche Ltd.) plasma glucose determination (hexokinase technique).

The results show a highly significant ($r = 0.98, p < 0.01$) correlation between the increase of plasma glucose concentration and the serum paracetamol concentration when the unmodified membrane was used (regression line: $y = 3.3x + 0.11$) and no significant correlation between serum paracetamol concentration and plasma glucose concentration with the modified membrane ($r = 0.01$; regression line: $y = 0.01x + 0.16$).

Thus the manufacturer’s claim of decreased paracetamol sensitivity is upheld by these findings, and blood glucose in patients who have taken a paracetamol overdose may be reliably determined by this method.

I thank Mr. D. Ferguson, F.R.C.S., of the Royal Hallamshire Hospital, for the cooperation of his department in this study.

References


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**"Tandem" Urine Pregnancy Test Evaluated**

To the Editor:

The Tandem Visual HCG pregnancy test for urine, an immunoenzymometric assay produced by Hybritech Inc., La Jolla, CA 92037, involves use of a solid-phase monoclonal antibody directed toward a site of the human chorionic gonadotropin (hCG) molecule on the alpha subunit. After the first antibody binds with the specimen, a monoclonal antibody labeled with an enzyme (alkaline phosphatase) is added. This second antibody is directed toward a separate antigenic site, located on the beta subunit. After the solid-phase beads are washed, enzyme substrate is added and the beads are incubated. The presence of a distinct blue color of greater intensity than in the positive reference is reported as a positive result. The sensitivity of the method is 50 int. units/L and total assay time is about 45 min. The dual-antibody system is specific for the intact hCG molecule (alpha plus beta subunits).

We compared the Tandem Visual pregnancy test with Wampole’s Beta Stat urine pregnancy test by hemagglutination inhibition (Wampole Laboratories, Cranbury, NJ 08512) for 85 selected patients’ specimens. Serum from some of these patients was also submitted for hCG determinations by radioimmunoassay, with Hybritech’s Tandem HCG RIA method. All tests were performed according to the manufacturers’ instructions. Approximately half of the patients studied were randomly selected; the rest were undergoing workups for possible ectopic pregnancy, early pregnancy, or problem pregnancies. Some of the latter group of patients’ specimens had previously resulted in inconclusive results by our currently used urine test (Beta Stat).

Results by the two methods agreed for urine samples in only 72% (61/85) of the patients. There were outright disagreements in another 7% (6/85) of the patients. The remaining 21% (18/85) had inconclusive results by the Beta Stat method; i.e., the results were neither clearly positive nor negative. Of these, 61% (11/18) gave negative results and 39% (7/18) gave positive results by the Tandem urine method.

The two methods were found to give significantly different results at the $p = 0.05$ level (chi square test).

When we compared the Beta Stat with the Tandem RIA method, these results also were statistically different at the $p = 0.05$ level, agreement being obtained in only 64% (31/48) of the cases and outright disagreement in 19% (9/48) of the cases. The latter included six false negatives and three false positives by the Beta Stat procedure. Beta Stat results were inconclusive in 17% (8/48) of the cases. Of these cases, 62% (5/8) were negative by RIA and 38% (3/8) were positive.

In comparing the results of the urine Tandem method with the serum RIA method we found no statistically sig-