falls within the range reported by Valentine et al., who used atomic absorption spectrophotometry (19). Others have reported values that differ somewhat from ours: 25 µg/L (9); 42 (SD 18) µg/L, range 0–69 (14); and 34 (SD 24) µg/L, range 0–150 (15). However, the interindividual variations are great, probably reflecting dietary intake of the element or individual environmental exposure.

Our simplified technique represents a valuable tool for measurement of selenium in body fluids with precision, accuracy, and reproducibility. Using this technique, we recently reported on the percutaneous absorption of selenium from a commercial shampoo that contains selenium sulfide (16).

References

Radioimmunoassay and Chemical Ionization/Mass Spectrometry Compared for Plasma Cortisol Determination
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We describe a method for determination of cortisol in plasma and urine, based on chemical ionization/mass spectrometry with deuterium-labeled cortisol as the internal standard. The within-run precision (CV) was 2.5–5.7%, the between-run precision 4.6%. Results by this method were compared with those by a radioimmuno- logical method (RIANEN Cortisol, New England Nuclear) for 395 plasma samples. The latter method gave significantly higher (approx. 25%) cortisol values.

Because of its speed and the sensitivity with which a large number of samples can be analyzed, radioimmunoassay (RIA) is commonly used in routine laboratory work for measuring cortisol in plasma. As compared with other methods, such as fluorometry, RIA also has a relatively high selectivity; however, there is some cross reaction with other steroids—e.g., cortisol metabolites and synthetic corticosteroids, which may become important in the diseased state and during drug treatment (1).3 Gas chromatography/mass spectrometry (GCMS) with selected-ion monitoring is recognized as one of the most selective analytical techniques available today and has been suggested as a "definitive" method in clinical chemistry (2). Björkhem et al. (3) described a GCMS method with electron-impact ionization for the determination of plasma cortisol. Cortisol labeled with 14C was originally used as internal standard, but has recently been replaced with a deuterium-labeled analog (4). We present here a modified method based on chemical-ionization (CI) GCMS, which we used to measure cortisol in plasma and urine during an investigation of the systemic effects of glucocorticoid ointments. We used RIA to rapidly check if plasma cortisol suppression was severe, in which case the steroid treatment was discontinued. For the final evaluation of cortisol concentrations, the same plasma samples were analyzed at a later date by the more laborious GCMS method. The data compared are those for 395 plasma samples from 16 healthy men.

Materials and Methods
 Subjects and blood collection. Sixteen healthy men gave

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3 Nonstandard abbreviations: RIA, radioimmunoassay; GC, gas chromatography; MS, mass spectrometry; CI, chemical ionization.
informed consent to take part in a cross-over study designed to compare the cortisol suppression after topical application of glucocorticoid ointments containing, per gram, 250 μg of butyrosone (18α,17α-butyldienodyoxy-11β,21-dihydroxy-pregn-1,4-diene-3,20-dione; Preferid®), 1 mg of cortisol-17-butyrate (Locoid®), or 0.5 mg of betamethasone-17,21-dipropionate (Diproderm®). Blood (10 mL) was drawn at 0800 hours and collected in heparinized Venoject tubes (Terumo Corp., Tokyo, Japan). After centrifugation, 2.00 mL of plasma was added to a glass tube and stored at −20 °C until analysis by GCMS. Another 0.5 mL was transferred to another tube for immediate analysis by RIA. Blood was collected on 15–30 occasions from each subject during a period of three months. Twenty-four-hour urine specimens were collected for the determination of free cortisol by GCMS. The study is described in detail elsewhere (5).

Reagents. [2H3]Cortisol ([9,12,12-2H3]11β,17α,21-trihydroxy-4-pregnene-3,20-dione) was obtained from KOR Isotopes, Cambridge, MA 02142; trimethylalilylimidazole from E. Merck, Darmstadt, F.R.G.; and methoxyamine hydrochloride from Supelco, Bellefonte, PA 16823. Pyridine, hexane, and dichloromethane were of analytical grade and used without further purification.

A kit for radioimmunoassay of plasma cortisol (RIANNEN Cortisol [125I]) was obtained from New England Nuclear, North Billerica, MA 01862.

Apparatus. The mass spectrometer (Model 4000; Finnigan Corp, Sunnyvale, CA 94086) was operated in the CI mode and interfaced to a Finnigan 6100 data system (revision 1 software). The GC column (40 cm × 2 mm i.d.) was packed with 1% OV-1 on Gas Chrom Q, 100–120 mesh, and connected to the mass spectrometer via the direct transfer line. Helium was used as carrier gas at a flow rate of 25 mL/min. The CI reagent gas (ammonia) was added as make-up gas to the GC effluent to give an indicated ion source pressure of 67 Pa (0.5 Torr) and a pressure of 7.3 × 10−3 Pa (5.5 × 10−6 Torr) in the analyzer region. The temperatures of the injection block, GC oven, transfer line, and ion source were 260, 240, 250, and 250 °C, respectively. The ion source was operated at an ionizing energy of 125 eV and an emission current of 0.25 mA. During selected-ion monitoring the instrument was set by the data system to measure m/z 637 and 640.

To quantitate the bound tracer in the radioimmunoassay, we used an LKB-Wallac 80 000 gamma sample counter (LKB, 16126 Bromma, Sweden).

GCMS assay. The extraction and derivatization procedure of Björkhem et al. (3) for cortisol was adopted, with some modifications:

Add 1.00 mL of internal standard solution (400 nmol/L of [2H3]cortisol in water) to 2.00 mL of plasma or urine in a glass tube and shake slowly with 6.0 mL of dichloromethane for 20 min. Centrifuge at 1500 × g for 5 min and evaporate the organic phase, under a stream of nitrogen, at 70 °C. Incubate the residue with 25 μL of a 100 g/L solution of methoxyamine hydrochloride in pyridine at 70 °C for 15 min. Remove the pyridine under a stream of nitrogen, add 25 μL of trimethylalilylimidazole, and heat the mixture at 100 °C for 30 min. Allow the reaction vial to cool, then add 50 μL of hexane and shake the vial so that the two phases are thoroughly mixed. Use about 4 μL of the hexane phase for GCMS analysis. The retention time of the dimethoximetris(trimethylsilyl) derivative of cortisol is about 0.8 min.

Samples for the standard curve were prepared by adding known amounts of cortisol to 2.0 mL of water, corresponding to concentrations of 50–600 nmol/L. The samples were then taken through the complete analytical procedure. A complete standard curve was prepared every day.

Precision studies were performed by taking replicate samples, either from urine and plasma containing normal concentrations of cortisol or from an aqueous solution containing a known amount of cortisol, and processing them as described above.

Radioimmunoassay. In the RIANNEN Cortisol [125I] kit, 10 μL of plasma is used. Separation of the bound from the free antigen involves use of a prereacted first- and second-antibody complex. After a 30-min incubation followed by centrifugation, the supernate is discarded and the radioactivity of the pellet containing the bound tracer is counted with a gamma counter. The standard curve covers the range 55–1380 nmol/L, and values of the unknowns are obtained by interpolation.

Results and Discussion

The method described by Björkhem et al. (3) for plasma cortisol determination with electron-impact–GCMS shows selectivity and sufficient sensitivity. Our mass spectrometer, however, is operated most of the time in the CI mode, and we find that a few days of CI operation drastically reduces the sensitivity of the instrument in the electron-impact mode. Therefore, we needed a method based on CI-GCMS for the analysis of cortisol. Figure 1 shows the CI mass spectrum of the dimethoximetris(trimethylsilyl) derivative of cortisol when ammonia was used as the reagent gas. The protonated molecular ions (m/z 637 and 640) of cortisol and the deuterium-labeled internal standard, respectively, were used for selected-ion monitoring. Figure 2 shows a selected-ion current profile of a derivatized plasma extract containing 68 nmol of cortisol per liter.

The standard curve was constructed by plotting the peak-height ratio for m/z 637/640 vs known cortisol concentration (Figure 3). Because cortisol contributes to the recording at m/z 640, the relationship is not linear. Therefore, the measured peak-height ratio for each standard concentration was corrected with a factor equal to:

\[
\frac{C_{\text{cortisol}} (C_{\text{IA}} + 0.064 \cdot C_{\text{cortisol}})}{C_{\text{IA}} (C_{\text{cortisol}} + 0.002 \cdot C_{\text{IA}})}
\]

where \(C_{\text{cortisol}}\) is the concentration of cortisol, \(C_{\text{IA}}\) is the concentration of [2H3]cortisol, 0.064 is the peak-height ratio m/z 640/637 for cortisol, and 0.002 is the peak-height ratio m/z 637/640 for [2H3]cortisol. After this correction, a straight line could be fitted to the data by least-squares regression analysis (Figure 3). The cortisol concentration in unknown samples was calculated by iteration. The measured peak-height ratio was used to estimate a preliminary cortisol concentration,
which was used to calculate a correction factor, and in turn a corrected peak-height ratio. This sequence was repeated five times by use of a simple BASIC program, after which the sixth digit did not change, and the calculated cortisol concentration was taken as the analytical result.

The within-run precision of the GCMS method—investigated for water, urine, and plasma samples—ranged between 2.5 and 5.7% (Table 1). Björkhem et al. (3) reported a relative standard deviation (CV) of 3.5% for their method. For the estimation of day-to-day variation identical samples were taken from a plasma pool and analyzed with each new standard curve. The mean cortisol concentration of the pool was found to be 157.9 (SD 7.3) nmol/L (n = 29), CV 4.6%.

In the radioimmunoassay the within-run precision, as determined by duplicate analysis, in the range 110–663 nmol/L was 4.5%. Day-to-day variation was assessed by determining two plasma pools for each new standard curve. The mean cortisol concentrations were found to be 110 (SD 5.0) nmol/L (n = 10) and 594 (SD 27.1) nmol/L (n = 10), with CVs of 4.5% and 4.6%, respectively.

Cortisol concentrations were measured by both GCMS and RIA in a total of 423 plasma samples. Because the precision of the GCMS method had been investigated only down to 40 nmol/L, 28 samples with an estimated cortisol concentration below this value were excluded from the comparison of methods. The remaining 395 samples ranged between 54 and 697 nmol/L as determined by GCMS or 86 and 1049 nmol/L as determined by RIA; the results are plotted in Figure 4. Linear regression analysis of RIA values (y) on GCMS values (x) produced a line with the equation y = 5.4 + 1.24x. The correlation coefficient was 0.929.

The equation y = −42 + 1.30x (with r = 0.99) was reported for RIANEN in a similar investigation, including only 12 plasma samples, where GCMS and four RIA kits were compared (6). That study, however, was performed with a kit from New England Nuclear, which differs considerably from the kit used in our investigation (O. Lantto, personal communication) and the results should not be compared. In the same paper (6), RIANEN was shown to give lower values than GCMS at cortisol concentrations below about 150 nmol/L and higher values than GCMS above that value. In the present investigation such a trend was not seen. For only 11 of 395 samples was a higher concentration found with GCMS than with RIA.

A t-test of log(RIA/GCMS) showed highly significant difference (p < 0.001) between the two methods. The geometric mean ratio RIA/GCMS was 1.25. This difference between the
two methods cannot be explained by cross reaction of the RIA method with synthetic glucocorticoids absorbed through the skin, because this possible interference was investigated and found to be insignificant for the low concentrations expected in plasma. The higher RIA values compared to GCMS probably reflect interferences with cortisol metabolites and other endogenous steroids (7). According to the instruction manual from New England Nucelar the cross reaction against corticosterone is 25% [normal value for male subjects 11.4 ± 6.6 nmol/L (8)] and 11-deoxycortisol 12% [normal range 1.4–34.7 nmol/L (9)].

Latto et al. (6) noted that when standards supplied with a RIA kit were used for calibration, falsely high cortisol values were found for authentic plasma samples. They ascribed this effect to the absence of an intact biological matrix in the standards. It is possible that a similar matrix effect has contributed to the high RIA values in this investigation.

We thank Dr. C. J. Lamm for valuable discussions concerning the statistical evaluation of the results.

References

Evaluation of Four Assay Methods for Determination of Tobramycin in Human Serum

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Four assay procedures for tobramycin in serum—enzyme immunooassay (I), substrate-labeled fluorescent immunooassay (II), radioimmunoassay (III), and bioassay (IV)—were compared and evaluated by replicate and analytical recovery studies. I and II were about 50% more precise than III and IV. II was substantially more nearly accurate than the other methods and also gave the best reproducibility (correlation coefficient 0.992 between-day). The least expensive method was IV. Ease of handling favored I and II. Overall, we find II to be the most acceptable procedure for use in the clinical laboratory.

Additional Keyphrases: drug assay · economics of laboratory operation

The therapeutic usefulness of tobramycin is tempered by its potential for ototoxicity (1, 2) and nephrotoxicity (3). Several factors influencing its toxicity and therapeutic response have been identified (4). Therapeutic concentrations in serum range from 4 to 10 mg/L, with toxic symptoms when peak serum concentrations exceed 10 mg/L and caution advised when trough values remain above 2 mg/L (5, 6). Because the therapeutic index of tobramycin is narrow and many factors may influence toxicity and therapeutic response, careful and frequent monitoring of the drug's concentrations in serum has been advocated, especially in the case of patients with renal insufficiency (3).

The many assay methods for measuring tobramycin in serum are discussed in recent reviews (3, 7) of serum aminoglycoside measurement techniques. BA (8), RIA (9), EIA (10–12), and FIA (7, 13) seem to have found the widest acceptance among clinical laboratories.2 Here, we compare these methods on the basis of precision, accuracy, reproducibility, cost, and ease of handling.

Materials and Methods

Tobramycin controls used with each assay technique included a pooled tobramycin serum control containing 6.0 (SD = 0.4) mg/L (Syva Co., Palo Alto, CA 94304), and tobramycin controls of 1, 4, and 14 mg/L that we prepared from tobramycin sulfate powder (USP, Nebcin; Eli Lilly & Co. Indianapolis, IN 46206) in a matrix of normal human serum.

The technique of the bioassay method is described else-

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