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Note

Reliability of the estimation of serum cortisol by high-performance liquid chromatography

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Because of its non-radioactive feature, the determination of serum cortisol by high-performance liquid chromatography (HPLC) is promoted as a useful alternative to radioimmunoassay (RIA) techniques in routine laboratory analyses [1—11]. In almost all of the HPLC methods hitherto published, the UV absorbance of cortisol is used for quantitation [1—10]. The interest of the present study focused on the question, as to whether UV detection is indeed sufficiently selective for cortisol assessment in serum. Therefore, we estimated cortisol by HPLC in a series of 195 serum samples arising in our routine laboratory work. The HPLC results were correlated with the values obtained by the specific RIA method.

EXPERIMENTAL

Materials

Solvents, reagents, extraction devices, non-labelled steroids and labelled cortisol were as previously described [12]. The solid phase ^{125}I-RIA kit was obtained from Clinical Assays (Cambridge, MA, U.S.A.). The liquid phase ^{3}H-RIA and the characteristics of the corresponding antiserum used have also been described previously [12].

Instrumentation

The high-performance liquid chromatograph equipped with a UV detector at a fixed wavelength of 254 nm, an automatic sampler and fraction collector was from Hewlett-Packard (Model 1084B). For HPLC, a polar coated silica, Diol® (particle size 5 μm; Knauer, Berlin, G.F.R.), as stationary phase, *n*-hexane and isopropanol as eluent and gradient mode were used. Details of the HPLC system applied have been reported recently [12].

Serum samples

Parameters measured in the batch of serum samples studied included cortisol base levels and levels before and after adrenal stimulation (ACTH, insulin) or suppression (dexamethasone). Serum was stored at —20°C until analysis.

Analytical procedures

HPLC (method I). Serum (1 ml) traced with 100 ng of prednisone, was extracted with diethyl ether using a solid phase extraction technique [13]. The dried residue was dissolved in the eluent and automatically chromatographed. The ratios of peak heights of cortisol and prednisone were evaluated by a standard curve established from cortisol standards ranging from 27.6—1380 nmol/l. The standard curve itself was linear up to 540 nmol/l.

Solid phase RIA (method II). The protocol followed the instructions of the manufacturer using aliquots of 10 μl of serum.

HPLC—RIA (method III). The cortisol-containing fractions eluted by HPLC (see method I) were automatically collected, evaporated, redissolved in 5 ml of water, and finally quantitated by liquid phase ^{3}H-RIA [12]. In this technique, [^{3}H]cortisol added to the serum sample prior to the assay, was used for the recovery measurement.

RESULTS AND DISCUSSION

Fig. 1a demonstrates the UV chromatogram of steroidal drugs commonly used in steroid therapy. Fig. 1b shows the corresponding chromatogram of

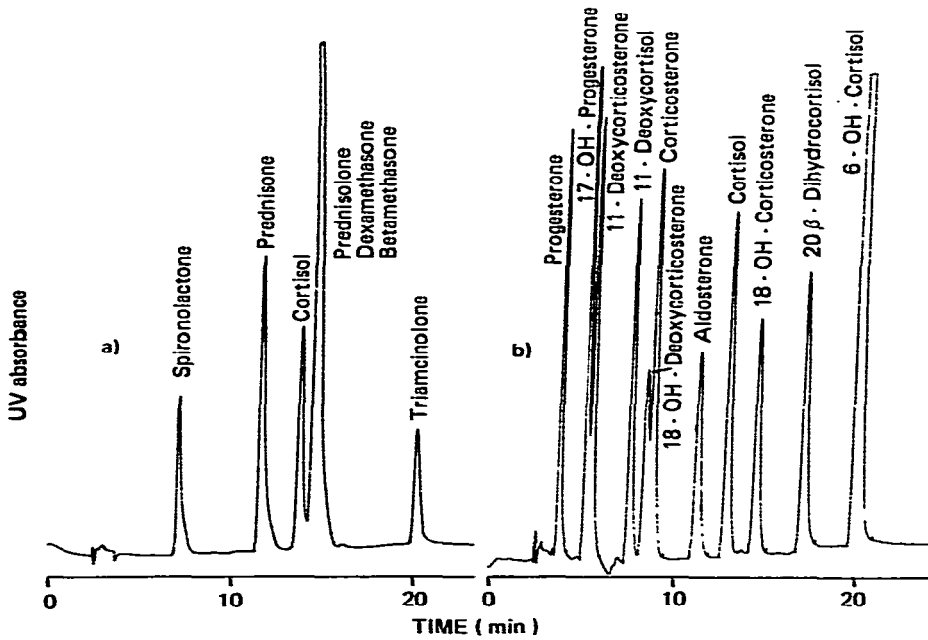

Fig. 1. UV chromatograms of steroidal drugs (a) and of biogenic steroids (b). HPLC system used: column, Diol, 250 x 4.5 mm I.D.; solvent A: *n*-hexane; solvent B: *n*-hexane—isopropanol (75:25); flow-rate: 1.3 ml/min; sample volume injected: 150 μl; temperature of column, 30°C; detection at 254 nm.

the adrenal steroids naturally prevailing in human serum. A distinct separation of cortisol from both drugs and biogenic steroids is apparent. The degree of resolution is equivalent to or better than that of the HPLC systems described hitherto [1—10].

The UV chromatograms of normal serum samples are shown in Fig. 2. While the chromatogram in Fig. 2a was well suited for peak height evaluation, there was a strong UV-absorbing background in the area of the prednisone reference peak (Fig. 2b), which made a reliable assessment of serum cortisol exclusively through UV detection impossible. In a series of 195 routine samples, there were 145, which exhibited a chromatographic profile similar to that in Fig. 2a. The analytical variables under these conditions were as follows:

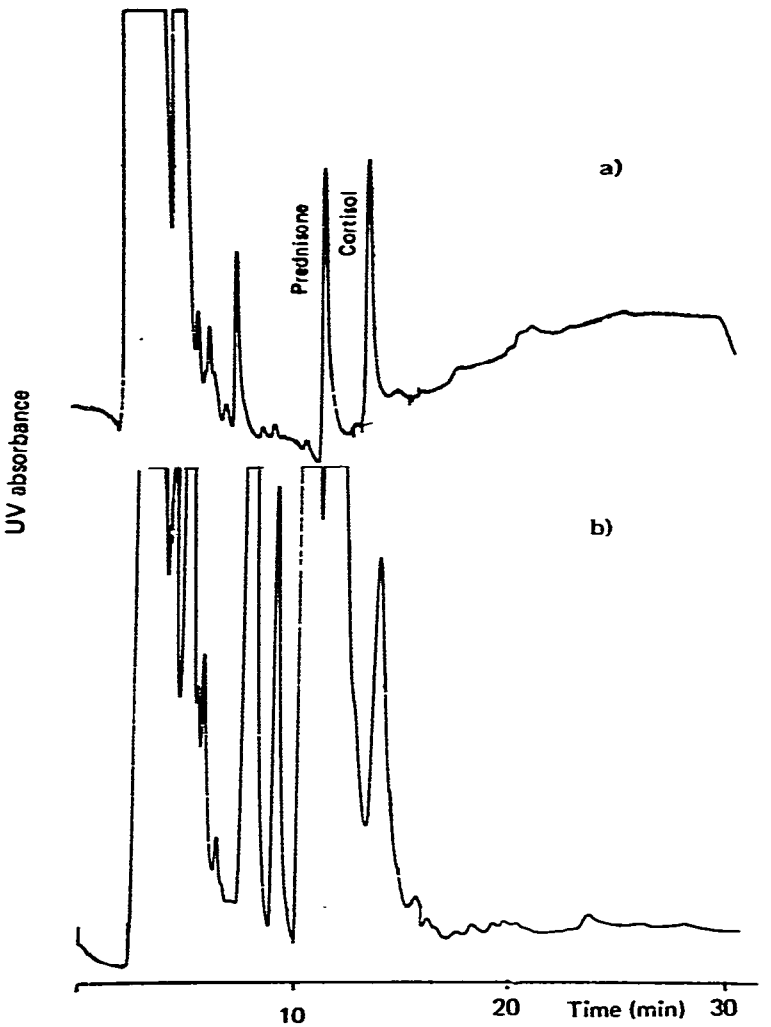

Fig. 2. Representative UV chromatograms from normal serum samples. Ether extracts of 1 ml of serum were applied. Chromatographic conditions as in Fig. 1. The chromatogram in Fig. 2a was well suited for peak height evaluation. In Fig. 2b, the reference peak of prednisone was completely covered by unspecific UV-absorbing compounds of the serum sample.

detection limit, 20 nmol/l; intra- and interassay variability, 7.05% and 10.5% (coefficient of variation), respectively; equation of correlation, $cortisol_{found} = -6.7$ (nmol/l) + 1.01 · $cortisol_{added}$ ($r = 0.99$). These data are comparable to those obtained by the RIA technique [14].

However, 21% of all samples studied, exhibited a UV chromatogram similar to that in Fig. 2b. In these samples, absolute serum cortisol concentrations were not at all, or only speculatively assessable because of considerable absorptions of the serum background, thus overlapping the peaks of prednisone, of cortisol itself, or of both steroids.

Due to the constant absorption background of the serum samples of an individual subject, the problem of unspecific interferences was reduced in samples of function tests. In these cases, the relative changes of the cortisol peak are often sufficient for a chemical diagnosis.

When correlating the results obtained from the 145 samples assessable by UV absorption (method I) with those obtained by HPLC—RIA (method III) as reference method, the following equation was found: $cortisol_{method\ I} = -9.6$ (nmol/l) + 1.25 · $cortisol_{method\ III}$ ($r = 0.965$). The corresponding correlation between method II and III was: $cortisol_{method\ II} = 60.0$ (nmol/l) + 1.41· $cortisol_{method\ III}$ ($r = 0.971$).

In conclusion, the present data emphasize the findings of other workers [8,9] that HPLC assessment of serum cortisol is feasible in principle and that the analytical quality of HPLC may achieve a level comparable to the current RIA techniques. If, however, UV absorbance is used for quantitation, a reliable estimation of all samples arising in routine laboratory analysis is not guaranteed. The fluorescence method recently published for cortisol quantitation after HPLC [11] may be a potential solution to this problem.

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