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A Simple Modification to the Radiochemical Assay of Cortisol which Avoids the Extraction from Serum *

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A modification to the radiochemical assay method to measure cortisol in serum, that renders unnecessary the extraction of cortisol from a serum sample is described. The serum is heated at 60° C for 3 hours, thereby transcortine is destroyed, then competition binding assay follows as usual.

There are three different methods for cortisol estimations in blood, colorimetric (7, 10, 12, 14), fluorimetric (13) and competition binding (5, 6). In all of them, cortisol has to be extracted from the serum before carrying out the analysis. In colorimetric and fluorimetric methods, cortisol extraction is neccessary to prevent interference from other steroids. In the competition binding methods, the extraction is needed to prevent the transcortine of the sample from interfering with the protein used in the development of the technique.

A modification to the usual competition binding technique, where the extraction of cortisol from the serum sample is unnecessary, is described in this paper.

Materials and Methods

Sample Collection. Samples were obtained by venous puncture. They were allowed to clot and then centrifuged. The serum was kept at —20° C.

Elimination of transcortine. 0.02 ml of serum was added to test tubes containing 0.8 ml of phosphate buffer (0.04 M, pH = 7.4). The tubes were stoppered, heated at 60° C for 3 hours and then allowed to cool.

Competition binding analysis. 0.1 ml of transcortine solution (0.012 g/100 ml

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of water) and 0.1 ml of H^3-labeled cortisol solution (30 μCi/100 ml of phosphate buffer) were added to each tube, they were shaken and incubated at 37° C for 5 minutes and then at 4° C for 30 minutes.

After incubation to each tube 0.5 ml of charcoal-dextran suspension (0.13 g of charcoal 2, 11, F.Q.P. and dextran T-70 in 60 ml of phosphate buffer) was added. The tubes were left at 0° C for 10 min and then centrifuged at 4° C for 10 min at 3,000 r.p.m. 0.1 ml of the supernatant was decanted into a counting vial, mixed with 10 ml of scintillating liquid (7 g of PPO, 10 g of naphtalene, 100 ml of toluol and 900 ml of dioxane) and counted in a liquid scintillation counter. The values of the unknown samples were calculated by interpolation on the standard curve. All measurements were done in triplicate.

Standard curve. Known quantities of a cortisol solution (16 μg/ml of ethanol) were added to test tubes containing phosphate buffer, to obtain concentrations of 5, 10, 20, 40 and 80 μg/100 ml, and submitted to the same procedure as the problem samples.

H^3-labeled-cortisol, transcortine, standard cortisol, charcoal-dextran and phosphate buffer were provided by Cea-Ire-Sorin.

Results

Sensitivity. To measure the sensitivity of this method, Ekins criterium was used. The projection of the zero point error on the standard curve is considered as the limit of sensitivity (2). At the 95 % confidence limit the sensitivity found was 0.3 ng.

Precision. The within and between assays variance was evaluated by triplicate measurement of identical samples in one single assay as well as in different assays (11). The coefficient of variation (CV) within one single assay was 3.5 % while the between assays variance was 4.2 %.

Specificity. To study specificity of this method, the recovery of a known quantity of cortisol (ranging from 1 to 8 ng %) added to normal serum was studied. Male and female sera were used to detect possible sex differences. In both cases the correlation coefficient between added and recovered cortisol was 0.99, however the male serum had a recovery of 90 ± 7.5 % and female serum of 62 ± 8.6 %.

Biological comprobation of the technique. The serum levels of cortisol were measured by this method on 34 normal subjects, 23 Addison's patients and 23 obese subjects (table I). Significant differences ($P < 0.01$) were observed between groups in respect to cortisol levels measured at 9 a.m. In the normal groups of subjects cortisol levels were also measured at 9 p.m. Our technique was able to detect the changes due to the circadian rhythm.

Results obtained at 2, 14 and 24 hours after the administration of 0.25 mg of a synthetic ACTH are shown in table II. In the normal and pituitary hypofunction groups, a significant increase of the levels of serum cortisol is observed 2 hours after ACTH administration. However in the suprarenal hyperplasia group, there are no significant differences between the basal values of cortisol and the values

Table I. *Serum levels of cortisol on different groups at different hours.*
In parenthesis number of experiments per group.

Experimental groups	Serum cortisol (μg/100 ml) 9 a.m.	9 p.m.
Normal subjects (34)	10.33 ± 1.4	2.17 ± 1.02
Addison's disease (23)	1.95 ± 1.37	—
Obese patiens (23)	18.30 ± 3.6	—

Table II. *Serum levels of cortisol after ACTH administration.* In parenthesis number of experiments per group.

Experimental groups	Serum cortisol (μg/100 ml)			
	Basal	Hours after ACTH administration		
	9 a.m.	2	14	24
Normal (20)	10.6±1.9	31.2±4.6	1.5±0.8	10.6±1.97
Pituitary hypofunction (5)	3.2±1.3	26.6±4.5	0.5±0.4	3.1±1.6
Suprarenal hyperplasia (5)	21.0±4.7	25.9±8.0	19.5±3.3	20.1±6.0

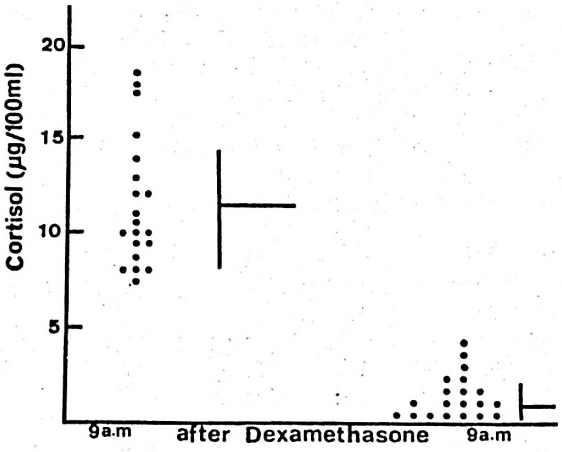

Fig. 1. *Inhibition on cortisol secretion after dexamethasone administration.*

obtained 2, 14 and 24 hours after ACTH administration.

To test dexamethasone inhibition activity on cortisol secretion, serum levels of cortisol were measured at 9 a.m. on 20 normal and obese subjects. One mg of dexamethasone was administrated at 11 p.m. of the same day. Serum levels of cortisol were measured again at 9 a.m. the following morning (fig. 1). The values obtained after dexamethasone are significantly low ($P < 0.001$) when compared with basal level.

Discussion

All methods to measure cortisol in blood have, as a common handicap, the need for previous cortisol extraction (7, 10, 12-14). Lack of precision, unspecificity and being time-consuming are some of the drawbacks inherent in all the methods requiring such a previous step, which make them unfeasible in the daily routine.

It is a well established fact that cortisol circulates in plasma bound to transcortin in a reversible manner (5) and that cortisol must be extracted prior to the analysis to avoid the transcortine interference in the competition union with the exogenous binding protein used in the competition assay.

Heating the serum sample for 3 hours at 60° C, transcortine is denaturalized losing its binding power and its ability to interfere, therefore making the cortisol extraction unnecessary.

The technique studied is more sensitive than the one used by LECLERQ *et al.* (4) and FIORELLI *et al.* (3) although less sensitive than that used by ABRAHAM *et al.* (1).

The proposed technique has a coefficient of variation within and between assays equal or less than those used by other authours (1, 4).

The difference between male and female recovery could be due to the interferences of estrogens present in the female serum. This interference could only be avoided by improving the specificity of antiserum used for the competition binding analysis.

The values of cortisol in sera obtained in normal subjects and patients with suprarenal disease are similar to those found

by other authors (8). At 9 p.m. the levels of cortisol on normal subjects are 9 % to 32 % of values at 9 a.m.

The present technique is able to detect the variations in cortisol levels after synthethic ACTH or dexamethasone administration.

In conclusion the technique described in this paper is useful for the determination of cortisol in clinical medicine due to the great simplification that it presents when compared with usual procedures.

Resumen

Se describe una modificación al análisis radioquímico para medir cortisol en suero, que hace innecesaria la extracción de cortisol del suero problema. El suero se calienta a 60° C durante 3 horas para destruir la transcortina y el análisis se continúa como es usual en los métodos de competición en la unión a proteínas.

References

1. ABRAHAN, G. E., BUSTER, J. E. and TELLER, R. C.: *Anallyt. Letters*, **5**, 757-765, 1972.
2. EKINS, R. P.: *Clin. Chim. Acta*, **5**, 453-458, 1960.
3. FIORELLI, G., PIOLANTI, P., FORTI, G. and SERIO, M.: *Clin. Chim. Acta*, **37**, 179-182, 1972.
4. LECLERQ, R., COPINSCHI, C. and FRANCKSON, J. R. M.: *Rev. Franç. Etud. Clin. Biol.*, **14**, 815-819, 1969.
5. MURPHY, B. E. P.: *Nature* (London), **201**, 679-682, 1964.
6. MURPHY, B. E. P., ENGELBERG, W. and PATTE, C. S. J.: *J. Clin. Endocr.*, **23**, 293-298, 1963.
7. NELSON, D. H. and SAMUELS, L. T.: *J. Clin. Endocr.*, **12**, 519-522, 1952.
8. NENITA, F., ITURZAETA, D., HILLMAN, A. and COLLE, E.: *J. Clin. Endocr.*, **30**, 185-189, 1970.
9. OSORIO, C. and SCHATS, D. L.: *J. Clin. Endocr.*, **24**, 1067-1070, 1964.
10. PORTER, C. C. and SILBER, R. H.: *J. Biol. Chem.*, **185**, 201-206, 1950.
11. QUESADA, T., RUIZ, E., MARTÍN, A. and OSORIO, C.: *Rev. Clin. Esp.*, **13**, 211-218, 1974.
12. RECKNAGEL, R. O. and LITTERIA, M.: *J. Clin. Endocr.*, **17**, 889-894, 1957.
13. SWEAT, M. L.: *Analyt. Chem.*, **26**, 773-779, 1954.
14. WICHSELBAUM, T. E. and MARGRAF, H. W.: *J. Clin. Endocr.*, **17**, 959-962, 1957.