DETERMINATION OF CORTISOL IN CAPILLARY BLOOD FROM MAN

By

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ABSTRACT

A spectrophotofluorometric micromethod for the determination of cortisol in plasma is shown to give satisfactory results when used on plasma from ear lobe blood as compared with determinations on simultaneously drawn vein puncture blood. 200 µl heparinized plasma is sufficient for a double determination. The method allows frequent multi-analysis of plasma cortisol, only small amounts of blood are drawn and there is little inconvenience for the patient.

In a previous communication we described a spectrophotofluorometric micromethod for the determination of cortisol in plasma and tissue obtained by needle biopsies (Jansen et al. 1967). This method which is based on the separation procedure described by van der Vies (1961) and the spectrofluorometric measurement of Glick et al. (1964) allows the determination of cortisol in plasma samples of 100 µl obtained from blood samples drawn from the ear lobe. Such a technique could provide the basis for multi-analysis on the same patient during a long period.

In view of this practical aspect we have compared the results of cortisol determination in 28 pairs of plasma samples from 10 human volunteers obtained from blood sampled simultaneously by vein puncture and by ear lobe puncture.

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MATERIALS AND METHODS

10 volunteers of either sex and various ages participated. Blood was sampled in heparinized polyethylene tubes at various times of the day in order to give a wider spectrum of results. Two ml were drawn by ven puncture from the cubital vein immediately before or after the sampling of approximately 400 µl ear lobe blood. There was an interval of one or two minutes between the two sampling procedures.

Separation of the plasma and the analytical procedure were initiated immediately after sampling. From both plasma samples two times 100 µl of plasma was processed for double determinations. The analytical procedure was identical with the one described previously (Jansen et al. 1967) and was used for all determinations in this investigation. Briefly, this involves two shakings with petroleum ether, shaking with dichloromethane, addition of 50 µl 0.1 N-NaOH and addition of MgSO4. An aliquot part of the dichloromethane residue is evaporated to dryness, water is added, and washed twice with tetrachloromethane. Cortisol is now transferred from water to dichloromethane and the amount of cortisol is determined by spectrophotofluorometry after addition of a fluorescence reagent (7 parts sulphuric acid and 3 parts of ethanol).

RESULTS AND DISCUSSION

In Fig. 1 the results are shown of cortisol determinations on paired samples of ear lobe blood and vein blood. In all cases the concentration presented is the mean of a double determination. There appears to be a high degree of

![Fig. 1.](chart.png)

The correlation between 28 pairs of cortisol determinations performed on plasma separated from vein blood (abscissa) and ear lobe blood (ordinate). The correlation coefficient is very high \((r = 0.961)\). The small positive abscissa intercept (8.9 ng/ml) does not differ significantly from zero at the 5% level, neither does the regression coefficient differ significantly from one at the 5% level.
correlation between the values obtained from ear lobe blood and vein puncture blood \((r = 0.961)\).

The deviation of the single points from a straight line is caused by a comparable accuracy in the analytical procedure on the two samples, as the standard error of the means are approximately the same for ear lobe and vein blood determinations \((123.8 \, \text{ng/ml} \pm 13.2 \text{ and } 122.4 \, \text{ng/ml} \pm 13.9, \text{respectively})\).

For both ear lobe and vein blood determinations the percentage deviation of the two double determinations from their mean was calculated. The results show that the precision of the analysis on ear lobe blood \((\text{mean deviation } 6.1 \% , \, \text{sd } = 5.0 \%)\) does not differ from the precision obtained with vein puncture blood \((\text{mean deviation } 6.2 \% , \, \text{sd } = 6.4 \%)\). These results are comparable to the values obtained in a previous investigation \((\text{Jansen et al. 1967})\). This precision seems acceptable for a micromethod.

On the basis of the present investigation it is concluded that this micromethod for plasma cortisol analysis can be applied to blood samples drawn from ear lobe punctures, although the error of the single result may be about 10\%. This procedure opens the possibility of performing frequent multi-analyses of plasma cortisol on the same patient with only minor inconveniences.

REFERENCES

\textit{Glick D., Redlich D. von & Levine S.}: Endocrinology 74 (1964) 653.

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