IMMUNOASSAY OF HUMAN PITUITARY LUTEINIZING HORMONE (LH) IN URINE. A COMPARISON BETWEEN A HAEMAGGLUTINATION AND RADIOIMMUNOSORBENT METHOD

By

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ABSTRACT

A modified haemagglutination inhibition test (Luteonosticon) was used to estimate human pituitary LH in urines from 66 women and 2 men. The results were compared with those obtained by a radioimmunosorbent method on the same urine samples. The correlation between the results of the two methods was highly significant (P < 0.001).

Preliminary results with a modified haemagglutination inhibition reaction (HIR) were presented at a recent symposium devoted to the determination of human pituitary luteinizing hormone (LH). Emphasis was given to comparisons between this immunological method and biological assays. The purpose of this study was to compare the values of LH in urine as estimated by the HIR described by Schuurs (1969a) and Schuurs & van Wijngaarden (1970) with those obtained with the radioimmunosorbent technique (RIST) of Wide & Porath (1966).

MATERIALS AND METHODS

Twenty-four hour collections of urine were obtained from 66 women for whom gonadotrophin assays were required to assist in the investigation and diagnosis of a variety of gynaecological disorders. Urines from 2 hypogonadal men with Klinefelter's syndrome were also assayed.

All urines were assayed by radioimmunoassay using immunosorbents and by a haemagglutination inhibition assay.

ACTA ENDOCRINOLOGICA
67 (1971) 491-494

The Department of Obstetrics and Gynaecology, Hormone Laboratory, S. M. M. P., Royal Infirmary, Edinburgh, Scotland
of Human Menopausal Gonadotrophin (2nd IRP-HMG) was the standard used in both systems. The luteinizing hormone concentrations were expressed in International Units of LH by immunoassay per 24 hours (IU.LH.I.). In the radioimmunosorbent method each urine sample was assayed in duplicate (Wide 1969). A dose response curve (DRC) was obtained by plotting semi-logarithmically the counts per minute of the standard against the hormone concentration. The values for the unknowns were obtained by reading from the DRC.

The reagents used in the haemagglutination inhibition assays were available in test kit form (Luteonosticon, Organon). Only one batch of material was used and the sensitivity of the reagents was checked before and during this investigation against the IRP standard. Each urine was serially diluted with a buffer solution so that the interval between dilutions was equal to log 0.1761. A control tube containing 6.0 ml of urine, 0.5 ml of buffer solution instead of 0.5 ml of antiserum, and 0.5 ml of an erythrocyte suspension was set up for each sample tested. The end point of the reaction was taken as the highest dilution of urine which completely inhibited haemagglutination. This end point was matched with a similar one for the standard and from this the excretion of LH per 24 hours was calculated.

RESULTS

The relationship between the results of the 2 assay methods is shown (Fig. 1) and the coefficient of linear correlation \( r = + 0.83 \) was highly significant \( P < 0.001 \). Although few gross differences were found between the results of the 2 methods, a value of »less than« was obtained in 6 of the HIR assays. The concentration of LH in these urines was either close to or well below the limit of sensitivity of the method. In 3 instances this may have been due to the abnormally high diuresis by women whose 24 hour excretion exceeded 2.3 litres.

DISCUSSION

Haemagglutination inhibition assays have been used sucessfully to measure human chorionic gonadotrophin (HCG) and pituitary LH in urine (Wide 1967). Immunoassays for the estimation of HCG do not need to be very sensitive because this gonadotrophin is usually excreted in relatively large amounts. The measurement of LH in the urine of children or during the menstrual cycle, before and after the ovulatory rise in gonadotrophin, requires a more sensitive test system than that used to measure HCG. In the present investigation a modified haemagglutination inhibition assay was used which was sensitive enough to measure LH at concentrations between 20 and 25 IU per litre of urine. This method was compared with the RIST which was 10 times more sensitive than the HIR. It was found that, within the limits of sensitivity for the haemagglutination inhibition method, an estimate of urinary LH can be made which is similar to that obtained by the radioimmunosorbent method. When a small amount of LH was excreted a not very meaningful »less than« result was
obtained. When low levels of gonadotrophins were assayed by biological methods it was often necessary to first extract and concentrate the hormone. Immunological assays being more sensitive can usually be used with untreated urine. There were some urines in the present study whose LH concentration was below the sensitivity of the HIR and in these circumstances it would seem appropriate to concentrate the gonadotrophin before assaying.

However, extracting the urine may well concentrate non-specific substances which could influence the result of the haemagglutination assay (Schuurs 1969b). It has been reported that immunoassays of pituitary gonadotrophin with untreated urine give values which are much higher than those found when the same urines are extracted (Kalin et al. 1968; Stevens 1969; Schmidt-Elmendorff & Kaiser 1970). It may be significant that a kaolin acetone method was used by these authors to extract the pituitary gonadotrophin from the urine. Hobson & Wide (1964) showed that when HCG in urine was adsorbed on and eluted from kaolin 50 per cent of the immunological activity was lost and that unabsorbed immunologically active material could be detected in the supernatant. These observations suggest that the methods used to concentrate gonadotrophins for

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**Fig. 1.**
Scatter diagram showing the relationship between 68 assay results obtained with RIST and HIR (log dilution interval 0.1761) methods. Correlation between these two methods $r = +0.83$. 
use in bioassays may not be suitable when immunoassays are used. Like the majority of immuno-and bioassays, the haemagglutination inhibition method used in this investigation does not distinguish between LH and HCG. It reacts with luteinizing gonadotrophins but is not apparently affected by the presence of human pituitary follicle stimulating hormone (Schuurs & van Wijngaarden 1970). It is concluded that the haemagglutination test Luteonosticon is simple, easy to perform and sensitive enough to allow the direct estimation of LH in most unconcentrated urines.

ACKNOWLEDGMENTS

This work was done in the Department of Clinical Chemistry, University Hospital, Uppsala, Sweden. I should like to thank Dr. Leif Wide for providing laboratory facilities and for the urine samples used in this investigation. Thanks are also due to Organon Laboratories Ltd. for a generous supply of Luteonosticon.

REFERENCES


Received on January 1st, 1971.