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Determination of Pregnanediol in Urine for Diagnostic Purposes

By DORA HUBER, *Laboratory of the University Gynaecological and Obstetrical Clinic, Zurich, Switzerland*

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Pregnanediol, excreted in urine in the form of sodium pregnanediol glucuronidate, is the chief metabolite of the corpus luteum hormone, progesterone, and the relation between pregnanediol excretion and corpus luteum activity makes it important to have a reliable and convenient method for determination of pregnanediol. It is physiologically inactive, in spite of its close relation to the hormone, and biological tests cannot, therefore, be used. Pregnancy may be diagnosed at an early stage from the pregnanediol excretion, which is, during the first 2 months' gestation, about equal to the maximum in the latter half of the menstrual cycle, rises progressively to a maximum and then falls precipitously

after delivery. Pregnanediol excretion drops to a low level when miscarriage is imminent (cf. Guterman, 1946). Also, the level of pregnanediol excretion may be considerably increased in adrenal cortical carcinoma or adrenal hyperplasia (Venning, Weil & Browne, 1939).

Various methods for the determination of pregnanediol in urine have been proposed, but they are either too complicated or too inaccurate for routine clinical use. The method of Venning & Browne (Venning, 1937, 1938; Venning & Browne, 1940) and the modification of Westphal (1944) do not, in the author's experience, allow the determination of less than 5 mg. of sodium pregnanediol glucuronidate,

because this substance is not quantitatively precipitated by acetone-water. The method of Allen & Viergiver (1941), depending on the titration of glucuronic acid liberated from the glucuronide, yields high results, because of the precipitation of other glucuronides at the same time. The method of Astwood & Jones (1941) is simple and reproducible, but only 70–80% of the pregnanediol present is recovered. Guterman (1944) modified this method and estimated the pregnanediol colorimetrically: the method was further developed by Morrow & Benua (1946) and by McCormack (1946).

The author's original intention was to use Guterman's method for the assay of pregnanediol. It is sufficiently rapid for routine determinations, but it was found impossible completely to precipitate the pregnanediol with an aqueous solution of sodium hydroxide. When working with very small amounts of pregnanediol the loss in recovery has a comparatively large effect on the accuracy of the results. Furthermore, it was found that the gravimetric determination of the precipitated pregnanediol was far more accurate than the colorimetric. Guterman's method (with some technical improvements) was highly satisfactory as regards the mode of extraction and the subsequent removal of the acidic and phenolic compounds, but for the isolation of the pregnanediol a chromatographic adsorption method was employed that allowed the very nearly complete recovery of the total amount of pregnanediol in a pure crystalline state.

METHOD

A. Hydrolysis and extraction

A sample of 100 ml. of urine from a 24 hr. specimen, 50 ml. toluene, and 10 ml. conc. HCl are refluxed for 15 min. The mixture is allowed to cool to room temperature and transferred to a separating funnel. The lower layer of urine is drawn off, the upper layer, consisting of toluene and emulsion is washed twice with 15 ml. of 0.1N-NaOH and subsequently twice with 15 ml. of distilled water.

B. Removal of phenolic and acidic compounds

The washed solution is boiled until the water is completely removed by evaporation. After cooling the solution to 30–40°, a solution of 10 ml. 1.5% (w/v) NaOH in absolute methanol is added and the mixture evaporated until it has assumed a pale yellow to green-yellow colour. The hot toluene solution is filtered through a Pyrex glass filter (porosity G 4) with slight suction. The precipitate is washed with 15 ml. hot toluene and the combined filtrates are evaporated to dryness by heating on a hot plate in a gentle stream of air. Care must be taken not to exceed a temperature of 120° and the last traces of toluene must be removed.

C. Isolation of pregnanediol

To the residue 4 ml. benzene are added and the solution obtained is passed through a column (dimensions 10 × 120 mm.) of Merck alumina, standardized according to

Brockmann. The column is washed twice with 3 ml. benzene and then four times with 3 ml. benzene containing 20% (v/v) ether. The crystalline product obtained by evaporation *in vacuo* of the combined four benzene-ether extracts is washed with 1 ml. absolute ether, and is weighed. In a typical experiment the product had m.p. 232–236°. After recrystallization from methanol the purified substance melted at 234–236°. Admixture with an authentic specimen did not depress the melting-point.

RESULTS

Accuracy of the method. Pregnanediol was determined in pregnancy urine and menopause urine. A second series of assays was carried out after the addition of authentic pregnanediol to the same urine specimens. The results obtained are shown in Table 1.

Table 1. *Pregnanediol determination in various urines, before and after the addition of authentic pregnanediol to the specimens*

Material	Pregnanediol (mg./100 ml.)	Pregnanediol added (mg./100 ml.)	Total pregnanediol found (mg./100 ml.)
Menopause urine	0.0	1.2	1.1
	0.0	1.0	0.9
	0.0	0.6	0.5
Pregnancy urine	1.2	2.0	3.2
	1.3	0.7	1.9
	1.5	1.0	2.5
	1.5	0.6	2.0

It is apparent that the method developed in our laboratory allows very nearly quantitative recovery of the pregnanediol even when only small amounts are present. A loss of about 10% is inevitable, because it is hardly practicable to elute the last traces of pregnanediol from the adsorption column.

The complete determination can be effected in 3.5 hr. Four to six tests can be made in one day by one person.

Excretion of sodium pregnanediol glucuronide subsequent to injection of progesterone. The excretion of sodium pregnanediol glucuronide in the urine may result from the metabolism of progesterone derived from activity of the corpus luteum, or may result from conversion of deoxycorticosterone and related compounds secreted by the adrenals. Investigations of various authors have shown that 5–11% of administered progesterone is excreted in the urine as sodium pregnanediol glucuronide, in the human menopause (Westphal, 1940) or in normal female rabbits (Westphal, 1941, 1942a; Heard, Bauld & Hoffmann, 1941). Pregnanediol has also been found in the urine of normal men and hysterectomized women after administration of progesterone (Buxton & Westphal, 1939). The conversion of deoxycorticosterone into pregnanediol was established

by Westphal (1941, 1942*a*, 1942*b*). The urine of normal male and female rabbits after injection of deoxycorticosterone contained pregnanediol in amount corresponding to 7–20 % of the deoxycorticosterone administered.

Fig. 1. Excretion of pregnanediol following the injection of 100 mg. progesterone in menopause women (5 cases).

For our purposes it was important to investigate the type of pregnanediol excretion following the injection of progesterone to menopause patients. Five normal menopause patients received each an injection of 100 mg. progesterone. Pregnanediol was

determined in the five urine specimens before the injection and on each of 7–8 days after the administration of progesterone.

The graphs in Fig. 1 show that the rate of the excreted progesterone in the metabolized form of pregnanediol has a maximum level 1 day after the administration of progesterone. The rate of excretion drops to a low level during the following 5 or 6 days and the excretion of pregnanediol ceases on the 6th or 7th day. The graphs are very similar to those obtained in the urinary excretion of oestrogens after injection of oestradiol and oestrone to ovariectomized women.

SUMMARY

1. A method is described for the quantitative isolation of free pregnanediol from normal pregnancy and non-pregnancy urine of women, which allows the recovery of small amounts (0.5 mg. %).

2. Pregnanediol was determined in the urine of menopause patients following the injection of progesterone, and it is shown that the administered hormone is partially excreted as the conversion product pregnanediol, over a period of 5–6 days.

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