Journal of Steroid Biochemistry, 1973, Vol. 4, pp. 57–63. Pergamon Press. Printed in Great Britain

URINARY EXCRETION OF ESTRIOL CONJUGATES IN NORMAL PREGNANCY

MATTI J. TIKKANEN

Department of Clinical Chemistry, University of Helsinki, Meilahti Hospital, SF-00290 Helsinki, Finland

(*Received* 18 *May* 1972)

SUMMARY

The excretion of the four main urinary estriol conjugates was determined in a group of women in late pregnancy and at intervals in the course of four normal pregnancies. The proportions of the various conjugates changed progressively with advancing pregnancy in some subjects, but not in others. In late pregnancy the following pattern is normal: estriol-16-glucuronide (68·0%), estriol-3-glucuronide (22·9%), estriol-3-sulfate,16-glucuronide (6·5%) and estriol-3-sulfate (2·5%).

INTRODUCTION

A RELATIVE preponderance of urinary estriol over estrone and estradiol is characteristic of pregnancy and becomes greater as gestation advances. In late human pregnancy estriol is mainly produced in the fetoplacental unit and the sequence of events is largely as follows: Most of the estriol is formed in the placenta from neutral and phenolic precursors of fetal origin[1–4] and is transferred to the mother[5]. A minor part is conveyed to the fetus, where it is mostly converted to E3-3S*[6, 7]. Unconjugated estriol seems to be transferred across the placenta and fetal membranes to the mother at a markedly greater rate than conjugated estriol[5, 8, 9]; most of the fetal E3–3S is hydrolyzed in the arylsulfatase-rich placenta and fetal membranes, and the unconjugated steroid is released into the maternal circulation. Conjugation with glucuronic and sulfuric acids takes place mainly in the maternal liver and intestine, and the conjugates are subsequently excreted in the urine. Some authors have suspected that in certain pathological conditions the type of conjugation might be affected and that the possible advantages of determinations of particular urinary conjugate fractions in obstetric disease should be evaluated[10–12]. Both during the course of pregnancy and in late pregnancy the urinary excretion of E3–16Gl, E3–3Gl, E3–3S,16Gl and E3–3S was measured with a gas–liquid chromatographic method[13] developed for this purpose.

EXPERIMENTAL

Material

Twenty-four-h urine samples were collected from normal pregnant women. The samples were frozen immediately (−18°) unless the analysis was to be started the same day.

*The following trivial names and abbreviations have been used in this text. Estriol-3-glucuronide (E3–3Gl) = 16α,17β-dihydroxy-1,3,5(10)-estratrien-3-yl-β-D-glucopyranosiduronate; estriol-3-sulfate,16-glucuronide (E3–3S,16Gl) = 17β-hydroxy-1,3,5(10)-estratrien-3-yl-sulfate-16α-yl-β-D-glucopyranosiduronate; estriol-16-glucuronide (E3–16Gl) = 3,17β-dihydroxy-1,3,5(10)-estratrien-16α-yl-β-D-glucopyranosiduronate; estriol-3-sulfate (E3–3S) = 16α,17β-dihydroxy-1,3,5(10)-estratrien-3-yl-sulfate.

Method

A detailed description of the method, including instrumentation, solvents, reagents and procedure, has been published elsewhere[13]. Data on accuracy, specificity, precision and sensitivity were presented and the method was shown to satisfy the criteria of reliability and to be suitable for quantitative analysis of urinary estriol conjugates in human pregnancy[13]. In short, the procedure is as follows: Separation of the four estriol conjugates is achieved by sequential gel filtration* on Sephadex G-25 and chromatography on Sephadex LH-20. After hydrolysis of the conjugates with *Helix pomatia* extract, the free steroid is removed by extraction and methylated. The methylated fraction is purified by chromatography on alumina and silylated. The final determination of the bis-trimethylsilyl ether of estriol 3-methyl ether is performed with gas-liquid chromatography on two (selective and non-selective) liquid phases. The accuracy of the method is improved by using labelled E3-3Gl, E3-3S,16Gl, E3-16Gl and E3-3S as internal standards in all determinations. Nonlabelled estrone is added before silylation and serves as an internal standard for gas–liquid chromatography. The results are calculated as previously described[13] and the amounts of estriol conjugates are expressed as milligrams of estriol/24 h.

RESULTS† AND DISCUSSION

Serial determinations

The results obtained in the four normal pregnancies (referred to as pregnancies A–D) are presented in Figs. 1–4. The duration of pregnancy is expressed as weeks after the last menstrual period. It is probable that the estriol conjugate pattern of late pregnancy differs from that occurring in early pregnancy before and at the time when the feto–placental unit takes over the production of estrogens from the ovary. In the present study only seven determinations were made before the 20th week and the greatest variation in the proportions of the individual conjugates was observed in these determinations. Statistical analysis of the results obtained in early pregnancy as compared to late pregnancy was therefore not made. In the second half of pregnancy the order of magnitude of the four conjugate fractions was the same in all determinations: E3–16Gl (51·7–85·1%) E3–3Gl (7·2–36·9%) E3–3S,16Gl (3·1–13·9%) E3–3S (0·3–6·0%).

This is the first time the urinary excretion of estriol conjugates has been determined with a method based on gas–liquid chromatography. Few data are available for comparison, since earlier studies have often been based on nonspecific and semiquantitative methods. In the following discussion, only investigations with sufficient identification of the estriol conjugates measured are cited. Beling [10] presented evidence that the ratio of peak II estriol to peak I estriol slowly increases with advancing pregnancy. Ahmed and Kellie[11] observed a similar change between weeks 28 and 37 in one pregnant woman and also found a fall in the ratio of E3–3Gl to E3–3S,16Gl. With respect to a changing ratio of peak II to peak I, the results of the present study are not uniform; this is illustrated in Figs. 1–4, where the percentage distribution of the conjugates in pregnancies A–D is shown. An increasing ratio of E3–16Gl to E3–3Gl (and hence of peak II

*The terms *gel filtration* and *peak I* and *peak II* (indicating the two fractions of urinary estrogen conjugates obtained by this procedure) are used in this paper in the sense used by Beling[10].

†Presented in part at the 5th Meeting of Finnish Medical Societies, Helsinki, March 25–27, 1971 (Ref. 14) and at the 8th Acta Endocrinologica Congress, Copenhagen, July 4–8, 1971 (Ref. 15).

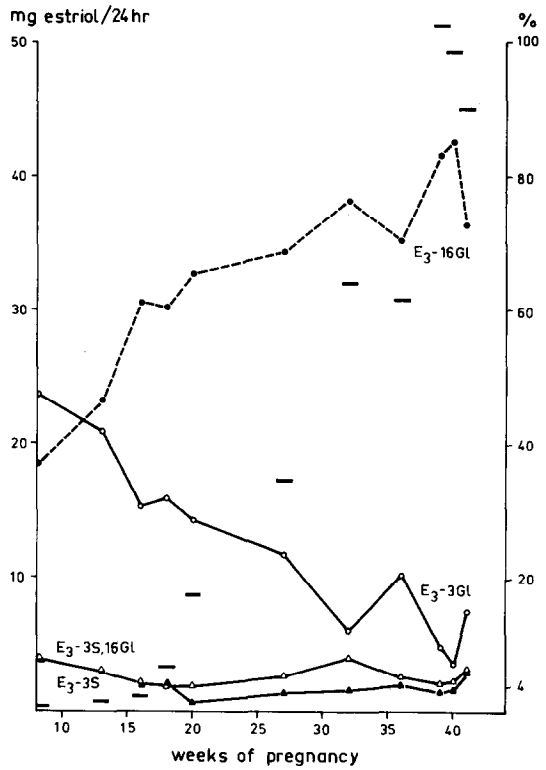

Fig. 1. Urinary excretion of estriol conjugates in pregnancy A. Note: The percentages for each conjugate were calculated as follows:

$$\frac{\text{mg of } E_3 \text{ in conjugate fraction}}{\text{mg of total urinary } E_3} \times 100.$$

Total excretion of estriol (mg) is indicated by horizontal bars; the curves depict proportions (%) of estriol conjugates. Abbreviations: E3-16Gl = estriol-16-glucuronide; E3-3Gl = estriol-3-glucuronide; E3-3S,16Gl = estriol-3-sulfate,16-glucuronide; E3-3S = estriol-3-sulfate.

to peak I) is seen in pregnancy A (Fig. 1), and to a lesser extent in pregnancies B and D, but not in pregnancy C. In general, the E3-3Gl/E3-3S,16Gl ratios show a clear tendency to fall with advancing pregnancy indicating that E3-3S, 16Gl represents an increasing proportion of peak I estriol, although there is a considerable fluctuation between the ratios in consecutive determinations. The cause of these gradual changes in pregnancy is not clear but they might be explained as secondary to a slowly deteriorating enterohepatic circulation of estrogens; E3-3Gl is synthesized exclusively in the mucosal cells of the intestine [16-18] and E3-3S,16Gl is the predominant biliary estriol conjugate[19], hence diminishing biliary excretion of estriol would probably result in diversion of E3-3S,16Gl to urine and reduce the amount of estriol available for E3-3Gl formation in the intestine. As Adlercreutz and Tenhunen[20] have pointed out, some persons seem to be abnormally sensitive to the increased amounts of estrogens produced in pregnancy and develop intrahepatic cholestasis. The same authors also noted that the changes seen in normal pregnancy are qualitatively similar to

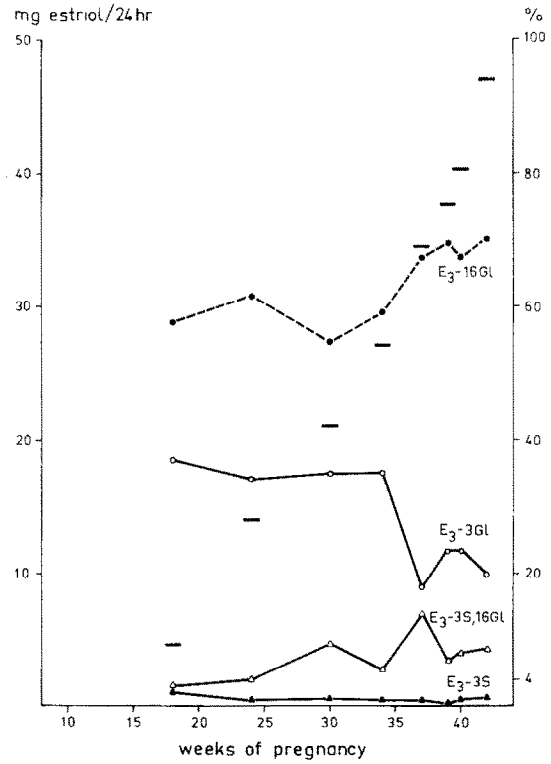

Fig. 2. Urinary excretion of estriol conjugates in pregnancy B. Note: For explanations see legend to Fig. 1.

those found in recurrent (intrahepatic cholestatic) jaundice, and that the pattern in this pathologic condition can be regarded as an aggravation of the pattern observed in some normal late pregnancies[20]. It appears, then, that the changing pattern of urinary estriol conjugates reflects a mild cholestatic condition probably due to increased estrogen action on the liver in predisposed subjects.

Determinations in late pregnancy

The results are presented in Table 1. The determinations numbered 1–7 were made in 7 healthy pregnant women between weeks 33 and 42, and the values numbered 8–11 are mean values for the corresponding weeks in the pregnancies A–D. When the means (± standard deviations) of the percentages are calculated for each of the four conjugates, the following proportions of estriol conjugates emerge as the normal pattern in late pregnancy urine:

E3–16Gl	E3–3Gl	E3–3S,16Gl	E3–3S
68·0 ± 7·4%	22·9 ± 6·7%	6·5 ± 2·7%	2·5 ± 1·2%.

Simultaneous determinations of all four conjugates have not been reported previously. In 11 normal pregnant women in the last trimester of pregnancy Adlercreutz *et al.*[21] obtained a mean percentage of 2·8 for E3–3S. In the same study the mean percentages for peak I estriol and peak II estriol were 27·3 and

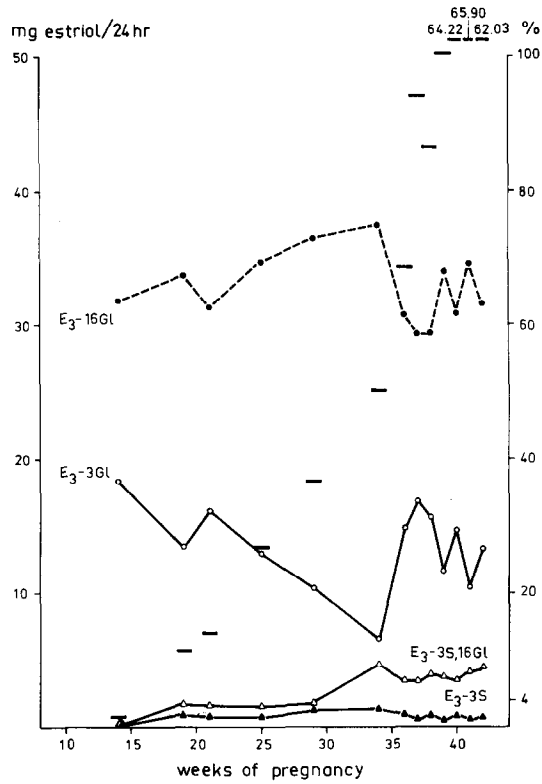

Fig. 3. Urinary excretion of estriol conjugates in pregnancy C. Note: For explanations see legend to Fig. 1.

Table 1. Excretion of urinary estriol conjugates in late pregnancy expressed as estriol mg/24 h

Subjects	E3-3Gl	E3-3S,16Gl	E3-16Gl	E3-3S	Total E3
1	6·77	2·75	19·05	0·53	29·10
2	3·51	0·95	10·80	0·48	15·74
3	14·79	4·68	21·42	1·35	42·42
4	2·59	1·62	18·12	1·16	23·49
5	4·19	0·45	12·04	0·47	17·15
6	4·53	0·66	10·51	0·39	16·09
7	6·86	0·86	20·26	0·31	28·30
8A	5·43	2·18	34·77	1·77	44·15
9B	8·67	3·23	25·10	0·33	37·33
10C	13·01	3·86	31·42	0·74	49·03
11D	4·51	1·36	14·37	0·49	20·73

71·6. The corresponding values in the present investigation are 29·4 and 70·5. The present results are also broadly in accord with those recently reported by Ahmed and Kellie[11], who did not measure E3-3S but obtained values for the other three estriol conjugates. They reported slightly lower proportions for E3-3Gl and slightly higher proportions for E3-3S,16Gl than those of the present

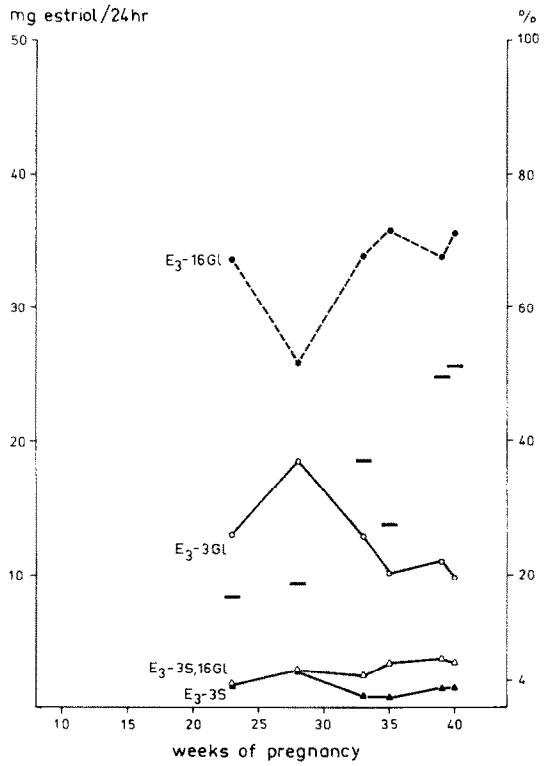

Fig. 4. Urinary excretion of estriol conjugates in pregnancy D. Note: For explanations see legend to Fig. 1.

investigation. Thus the mean ratio of E3–3Gl to E3–3S,16Gl as calculated from Table 1 is 4·3, as compared to their 2·2 (calculated from Ref. 11). The excretion of these two conjugates is of special interest, since their quantitative relationship was shown to change in the course of pregnancy (see above) and to be reversed in pregnancies complicated by recurrent intrahepatic cholestasis[22]. Previous investigations on the urinary excretion of endogenous estriol conjugates have shown that E3–16Gl constitutes the largest fraction, followed by E3–3Gl[10, 21, 23]. The results obtained after administration of labelled precursors at midpregnancy[24–27] are consistent with this concept. However, the relationship and normal excretion pattern of the two sulfated estriol conjugates has not been established previously.

From the new results presented here, and from those obtained by others, it can be concluded that:

(1) Despite the great variation in the proportions of the different conjugates in late pregnancy urine, a distinct normal pattern can be established, approximately as follows:

$$\text{E3–16Gl : E3–3Gl : E3–3S,16Gl : E3–3S} = 9 : 3 : 0{\cdot}9 : 0{\cdot}3$$

(2) Gradual changes in the proportions of the different conjugates take place in the course of pregnancy, but the occurrence of these changes seems to depend

on the individual sensitivity of the secretory function of the liver to the increasing estrogen levels in pregnancy.

(3) These changes in the urinary estriol conjugate pattern mainly reflect the function of the maternal liver; hence simultaneous determination of several estriol conjugates is of little value in the assessment of obstetric disease. This does not, however, rule out the possible advantage of measuring a single conjugate fraction (e.g. E3–16Gl) instead of total urinary estriol.

ACKNOWLEDGEMENTS

This investigation was carried out during tenure of a fellowship granted by the National Research Council for Medical Sciences in Finland and the present report is partly based on the author's doctoral thesis (M. J. Tikkanen: Urinary estriol conjugates in human pregnancy. University of Helsinki 1971, 77 p.). I am indebted to Mrs. Diana Godenhjelm and Mr. Henrik Brunholm for skilful technical assistance. The expenses of this work were defrayed by grants from the Nordisk Insulinfond and the Sigrid Jusélius Foundation.

REFERENCES

1. Schwers J., Eriksson G. and Diczfalusy E.: *Acta Endocr. (Kbh.)* **49** (1965) 65.
2. Magendantz H. G. and Ryan K. J.: *J. clin. Endocr.* **24** (1964) 1155.
3. Colás A., Heinrichs W. L. and Tatum H. J.: *Steroids* **3** (1964) 417.
4. Bolté E., Wiqvist N. and Diczfalusy E.: *Acta Endocr. (Kbh.)* **52** (1966) 583.
5. Levitz M., Condon G. P., Dancis J., Goebelsmann U., Eriksson G. and Diczfalusy E.: *J. clin. Endocr.* **27** (1967) 1723.
6. Mikhail G., Wiqvist N. and Diczfalusy E.: *Acta Endocr. (Kbh.)* **42** (1963) 519.
7. Mikhail G., Wiqvist N. and Diczfalusy E.: *Acta Endocr. (Kbh.)* **43** (1963) 213.
8. Goebelsmann U., Wiqvist N., Diczfalusy E., Levitz M., Condon G. P. and Dancis J.: *Acta Endocr. (Kbh.)* **52** (1966) 550.
9. Diczfalusy E., Tillinger K. -G., Wiqvist N., Levitz M., Condon G. P. and Dancis J.: *J. clin. Endocr.* **23** (1963) 503.
10. Beling C. G.: *Acta Endocr. (Kbh.)* Suppl. **79** (1963).
11. Ahmed J. and Kellie A. E.: *J. steroid Biochem.* **3** (1972) 31.
12. Klopper A.: In *Foetus and Placenta* (Edited by A. Klopper and E. Diczfalusy). Blackwell Scientific Publications, Oxford and Edinburgh (1969) pp. 471–555.
13. Tikkanen M. J. and Adlercreutz H.: *J. steroid Biochem.* **3** (1972) (in press).
14. Tikkanen M. J. and Adlercreutz H.: *Scand. J. clin. Lab. Invest.* **27**, suppl. **116** (1971) 39.
15. Tikkanen M. J.: *Acta Endocr. (Kbh.)* Suppl. **155** (1971) 123.
16. Dahm K. and Breuer H.: *Z. klin. Chem.* **4** (1966) 153.
17. Dahm K., Lindlau M. and Breuer H.: *Acta Endocr (Kbh.)* **56** (1967) 403.
18. Støa K. F. and Levitz M.: *Acta Endocr. (Kbh.)* **57** (1968) 657.
19. Emerman S., Twombly G. H. and Levitz M.: *J. clin. Endocr.* **27** (1967) 539.
20. Adlercreutz H. and Tenhunen R.: *Amer. J. Med.* **49** (1970) 630.
21. Adlercreutz H., Svanborg A. and Ånberg Å.: *Amer. J. Med.* **42** (1967) 341.
22. Adlercreutz H. and Tikkanen M. J.: *Eur. J. clin. Invest.* **1** (1971) 361.
23. Hähnel R.: *J. Endocr.* **38** (1967) 417.
24. Goebelsmann U., Eriksson G., Wiqvist N. and Diczfalusy E.: *Acta Endocr. (Kbh.)* **50** (1965) 273.
25. Goebelsmann U., Cooke I., Wiqvist N. and Diczfalusy E.: *Acta Endocr. (Kbh.)* **52** (1966) 30.
26. Goebelsmann U., Wiqvist N. and Diczfalusy E.: *Acta Endocr. (Kbh.)* **59** (1968) 595.
27. Goebelsmann U. and Jaffe R. B.: *Acta Endocr. (Kbh.)* **66** (1971) 679.