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THE QUANTITATIVE RELATIONSHIP OF URINARY OESTROGEN CONJUGATES AFTER INJECTION OF TRITIATED OESTRADIOL

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SUMMARY

Following the injection of tritiated oestradiol-17β to 6 pregnant women, the urinary oestrogen conjugates were separated by DEAE-Sephadex columns into 3-glucosiduronates, 16(or 17)-glucosiduronates and sulphates. These fractions were further divided into oestrone, oestradiol-17β and oestriol by Brown's method. In the first 24-hr specimens following the injection, oestrone glucosiduronate was quantitatively the most important fraction, followed by oestriol-3-glucosiduronate and oestriol-16-glucosiduronate, and 17β-oestradiol-17-glucosiduronate and 17β-oestradiol-3-glucosiduronate. The quantitative relationship of these 5 oestrogen conjugates changed in the second and third 24-hr urine specimens in which the proportion of the glucosiduronates of oestrone and 17β-oestradiol fell progressively, while the percentage of the two oestriol glucosiduronates increased.

INTRODUCTION

OESTROGEN metabolism has been studied extensively by methods involving the administration of radioactive labelled steroids. Ever since Beer and Gallagher[1] used ^{14}C-labelled oestrone and oestradiol for the first time in human subjects, a wealth of detailed information on the metabolic fate of oestrogens has been collected. In pregnancy the situation is complicated by the fact that both the foetus and the mother are contributing to the overall metabolism[2].

Most of the investigations have concentrated on the metabolic changes that take place on the steroid skeleton. Very few studies have been undertaken to obtain information on the mode of conjugation of the urinary metabolic products of the injected oestrogen. However, in some instances information on the conjugation of the excreted oestrogen was obtained by the method employed for hydrolysis (glucuronidase, solvolysis, for example [3, 4]). This lack of information is not surprising as until recently, methods for the estimation and isolation of individual oestrogen conjugates were not available. In 1965 a method for the separation of urinary oestrogen glucosiduronates into two fractions, namely oestrogen-3-glucosiduronates and oestrogen-16(or-17)-glucosiduronates was described[5]. Thus, by combining this method with Brown's method for separating oestrone, 17β-oestradiol and oestriol, it was possible to estimate the quantitative interrelationships between oestrone-3-glucosiduronate, oestriol-3-glucosiduronate, oestriol-16-glucosiduronate and two oestradiol glucosiduronates in normal human pregnancy urine[6]. Later, improvements extended the method to oestrogen sulphates[7]. Most recently the two 17β-oestradiol glucosiduronates have been identified[8] as 17β-oestradiol-3-glucosiduronate and 17β-oestradiol-17-glucosiduronate, previously named oestradiol glucosiduronate A and B[6].

This report deals with the quantitative relationship of the various conjugated oestrogens in urine following the injection of tritiated 17β-oestradiol to pregnant women. Throughout this paper the term "oestrogens" denotes oestrone, oestradiol-17β and oestriol.

MATERIALS AND METHODS

17β-Oestradiol-6,7-^3H dissolved in benzene–ethanol 1 : 1 was purchased from The Radiochemical Centre, Amersham, England. It had a specific activity of 285 mCi/mM (0·95 mCi/mg) and was used without further purification as preliminary tests (solvent partition, chromatography) had shown it to be >99% pure. The benzene–ethanol was evaporated in a nitrogen stream at 60°. The residue was transferred into a sterile flask with 25 ml ethanol. From this stock solution the ampoules for injection were prepared by dilution of 0·5 ml with 20 ml isotonic saline. All operations were done under sterile conditions. Random samples were taken for microbiological check and assay of radioactivity. The mean radioactivity in the ampoules was found to be $2{\cdot}70 \times 10^7$ dpm (2·71, 2·68, 2·73, 2·70).

Injection of radioactive 17β-oestradiol. Six patients who were admitted to hospital for termination of pregnancy were injected intravenously with $27{\cdot}0 \times 10^6$ dpm of oestradiol-6,7-^{3}H in 20 ml isotonic saline +0·5 ml ethanol. Syringes and ampoules used for the injection were washed with 20 ml saline and assayed for residual radioactivity. Details of the patients receiving tritiated 17β-oestradiol are given in Table 1.

Three 24-hr urine specimens were collected starting from the time of the injection. The specimens were kept in the deep freeze if not processed immediately following completion of the collection.

Scintillation mixtures. (A) Toluene scintillator: 0·1 g 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) and 4·0 g 2,5-diphenyloxazole (PPO), both scintillation grade, dissolved in toluene (A.R) to make 1000 ml. The solution was kept in a flask wrapped in aluminium foil.

(B) Bray scintillator[9]: 0·2 g POPOP, 4·0 g PPO, 60 g naphthalene (BDH, "for molecular weight determinations"), 20 ml ethyleneglycol (BDH, extra pure),

Table 1. Details of patients receiving tritiated 17β-oestradiol

No.	Patient	Age	Weeks gestation	Comments	Endogenous oestrogen excretion in 24 hr (mean of duplicates)		
					Oestrone	17β-Oestradiol	Oestriol
1	HOL	20	23	Inevitable abortion	130 μg (low)	44 μg (low)	4250 μg (normal)
2	SAR	19	20	Psychiatric reasons	455 μg (normal)	147 μg (normal)	5390 μg (normal)
3	MIC	40	9	Schizophrenia	25 μg (normal)	13·3 μg (normal)	59 μg (normal)
4	BUR	33	12(?)	Psychiatric reasons	12 μg (low)	8 μg (low)	66 μg (low)
5	MIN	38	8	Rubella infection	37 μg (normal)	33 μg (normal)	42 μg (normal)
6	DOU	18	34	Anencephalic foetus	425 μg (low)	187 μg (low)	3227 μg (low)

100 ml methanol (A.R), dissolved in dioxan (A.R) to make 1000 ml. The solution was kept in a flask wrapped in aluminium foil.

Tritiated standards for estimation of quenching. Standard tritiated toluene (Packard), $2{\cdot}28 \times 10^6$ dpm/ml, was diluted 1 : 10 with toluene (A.R).

Measurement of radioactivity. All urine specimens and aqueous solutions were counted in Bray scintillator (1 ml aqueous solution + 10 ml scintillator). All non-aqueous extracts were evaporated to dryness and the residues redissolved in toluene scintillator for counting. Samples were counted until sufficient disintegrations were recorded to give a counting error of not more than ±5% in most cases, but for some very low counts the error was up to ±10%. Efficiency of counting was determined by addition of tritiated standards to the counting vials and re-counting.

DEAE-Sephadex chromatography[7]. 20–25 ml of urine were diluted with 10 ml distilled water and applied to a DEAE-Sephadex column (50 ml burette, 11 mm internal diameter, height of DEAE-Sephadex filling approximately 500 mm at the start of the experiment), prepared in water. When the urine had percolated into the bed material, gradient elution was started. The gradient for elution was obtained by pouring into each of the nine identical chambers of a Buchler Varigrad 95 ml of the following aqueous solutions: 2 *M*-NaCl in chambers 8 and 9; 0·3 *M*-NaCl in chamber 3 and 0·05 *M*-NaCl in chambers 1, 2, 4, 5, 6, and 7. After completion of this gradient the DEAE-Sephadex column was further eluted with 70 to 150 ml 2 *M*-NaCl. The column effluent was collected in fractions of 100 drops each (approximately 12·5 ml).

1 ml samples of all fractions were taken and transferred into counting vials. After addition of Bray scintillation mixture the radioactivity was measured in a liquid scintillation spectrometer.

The remainders of the fractions were combined in sets of 5(1–5, 6–10, 11–15 etc.) in order to cope with the large numbers, and subjected to Brown's method.

Estimation of oestrogens. For the separation of oestrone, 17β-oestradiol and oestriol and their quantitative estimation Brown's method was used[10]. However, after alumina chromatography of the methylated oestrogens and evaporation of the solvents the residues were not subjected to the Kober reaction. Instead, the residues were dissolved in toluene scintillator, transferred to counting vials and the radioactivity measured. Endogenous oestrogens were estimated in aliquots of the first 24-hr urine specimens by the same method, using a modified Kober reaction[11].

RESULTS AND DISCUSSION

1. *Recovery of radioactivity in urine following injection of tritiated 17β-oestradiol to pregnant women*

The excretion of radioactivity in the urine following the intravenous injection of 17β-oestradiol-6,7-^{3}H is shown in Tables 2 and 3. Between 52 and 84% (mean 64%) of the injected radioactivity was recovered in the urine in the 72 hr following the injection. Of this total amount an average of 40% (from 26 to 70%) is present in the first 24-hr specimen, 17% (from 10 to 23%) in the second, and 7% (from 4 to 9%) in the third 24-hr specimen. As between 1 and 3·3% (mean 2·1%) of the radioactivity in the injection fluid was recovered from the syringes and vials after injection, the true recoveries in urine are somewhat higher than the figures given in Tables 2 and 3.

Table 2. Recovery in urine of injected radioactivity. 1,2,3: 1st, 2nd and 3rd 24-hr urine specimen following the injection

Patient	Urine vol. (ml)	Urine corrected for blank (cpm/ml)	Percentage of total radioactivity excreted in 72 hr	Counting efficiency	Percentage of injected radioactivity ($2{\cdot}70 \times 10^7$ dpm) excreted in urine
HOL 1	1330	366	63·1%	4·5%	40·1%
HOL 2	1865	112	27·1%	4·8%	16·1%
HOL 3	2360	32	9·8%	4·4%	6·4%
					62·6%
SAR 1	1750	225	51·8%	5·7%	25·6%
SAR 2	1490	168	33·0%	5·3%	17·5%
SAR 3	1070	108	15·2%	5·0%	8·6%
					51·7%
MIC 1	1550	454	54·6%	7·9%	33·0%
MIC 2	1310	287	29·2%	8·9%	15·6%
MIC 3	1165	179	16·2%	8·3%	9·3%
					57·9%
MIN 1	1600	538	52·5%	9·6%	33·2%
MIN 2	1455	414	36·8%	9·7%	23·0%
MIN 3	935	187	10·7%	9·5%	6·8%
					63·0%
BUR 1	560	995	60·4%	5·2%	39·7%
BUR 2	910	273	26·9%	5·2%	17·7%
BUR 3	1390	84	12·7%	5·3%	8·2%
					65·6%
DOU 1	1720	771	83·2%	7·0%	70·2%
DOU 2	1760	112	12·4%	7·0%	10·4%
DOU 3	1740	41	4·5%	7·0%	3·8%
					84·4%

These figures compare well with others reported in the literature. Fishman *et al.*[12], for instance, recovered between 48% and 76% of the administered radioactivity in the urine of 5 pregnant women (gestation 6–9 months). They also showed that the excretion after the third day contributed very little to the total. Sandberg[3] found that the excretion of radioactivity in 2 pregnant women was lower (approximately 40% following oestrone injection, approximately 65% after oestradiol injection), than in non-pregnant women (approximately 80%). On the other hand our mean values of 6 pregnant women correspond very closely with Hobkirk's[13] figures in a non-pregnant woman who had received 17β-oestradiol, and who excreted 37, 16, 6 and 4% on the 1st, 2nd, 3rd and 4th day, respectively (total 63%).

Table 3. Recovery (means) in urine of injected radioactivity

	Recovery	
	% of injected radioactivity	% of total radioactivity excreted in 72 hr
1st 24 hr	40·3%	60·9%
2nd 24 hr	16·7%	27·6%
3rd 24 hr	7·2%	11·5%
Total in 72 hr	64·2%	100%

It is interesting to note that in one patient (DOU) with an anencephalic foetus, the recovery of radioactivity in the urine was considerably higher than in the other subjects. It is particularly obvious in the 1st 24-hr specimen.

2. *Patterns of radioactivity in DEAE-Sephadex chromatograms of urine*

The overall recovery of radioactivity after DEAE-Sephadex chromatography of the urines was 90% on the average (80–106%). This confirms our previous experience that DEAE-Sephadex chromatography is eminently suitable for quantitative work[5].

Figure 1 shows the pattern of radioactivity that is obtained after DEAE-Sephadex chromatography of urine following the injection of tritiated 17β-oestradiol to pregnant women. The pattern was obtained by superimposing (after correction for small variations in the position of the peaks) the chromatograms of the first 24-hr urine specimens of all 6 patients. All chromatograms clearly showed 3 peaks (numbered 3, 4 and 6) corresponding in their positions to endogenous oestrogen-3-glucosiduronates, oestrogen-16(or 17) glucosiduronates and oestrogen sulphates found in pregnancy urine[6]. In some of the individual chromatograms several additional peaks which are not clearly distinguished from the major peaks in Fig. 1, were easily discernible and well separated.

In the first 24-hr specimens most of the radioactivity (48%) was found in fractions 11–30 with the maximum in fraction 23. An average of 29% of the radioactivity was present in fractions 31–50 (maximum in fraction 39), and the remaining 23% was contained in fraction 1–10 (5%) and 51–end (18%).

3. *Estimation of oestrone, 17β-oestradiol, and oestriol in fractions of DEAE-Sephadex chromatograms*

Of the total urinary radioactivity applied to the DEAE-Sephadex columns an average of 14% (from 10 to 23%) was recovered in the oestrone + 17β-oestradiol + oestriol fractions (Table 4). These figures are similar to results reported in the literature. Fishman *et al.*[12] recovered about 12% of the 17β-oestradiol administered to 4 pregnant women in the oestrone, 17β-oestradiol and oestriol fractions. Gurpide *et al.*[14] in one pregnant patient found 19% of the injected dose in "phenolic extracts" of the urine. A similar figure (oestrone + oestradiol + oestriol about 12%) is also obtained using the results of 4 pregnant women studied by Hobkirk[4].

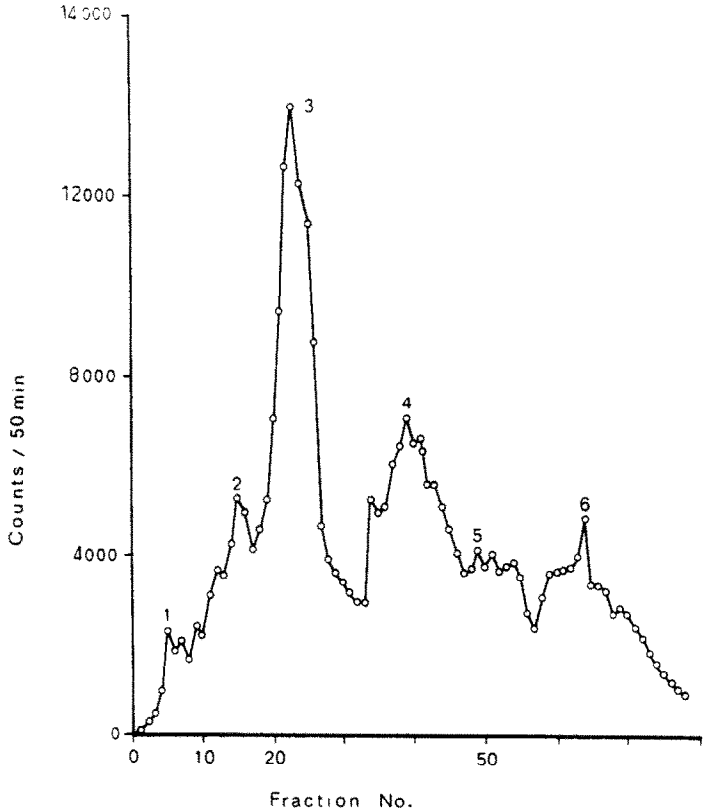

Fig. 1. Pattern of radioactivity in composite DEAE-Sephadex chromatogram of 6 urine specimens (1st 24 hr following injection of tritiated 17β-oestradiol).

The percentage contributions of oestrone, 17β-oestradiol and oestriol are also shown in Table 4. In the first 24-hr urine specimens an average of 51% was present in the oestrone, 21% in the 17β-oestradiol and 28% in the oestriol fractions. In one case (HOL), the oestrone and oestriol values differ considerably from the mean. In addition, in this patient oestrone amounts to only about half the value of oestriol, while in all other cases studied oestriol is lower than oestrone in the first 24-hr urine specimen, following the oestradiol injection. These differences may be a reflection of the clinical condition, in which the placenta presumably was separated.

Results by Fishman *et al.*[12] on the conversion of 17β-oestradiol to oestrone and oestriol by pregnant women are similar to the ones reported here. In one case the percentages of the 3 oestrogens in the first 24-hr urine specimen were about 56, 15 and 29% for oestrone, oestradiol and oestriol, respectively, very closely resembling our mean values. In another case of Fishman's, the results (oestrone 24%, oestradiol 15%, oestriol 61%) are in agreement with the results of patient HOL in this paper (26% oestrone, 15% oestradiol, 58% oestriol).

For two patients Table 4 also shows the changing proportions of oestrone, 17β-oestradiol and oestriol in the three 24-hr urine specimens following the administration of tritiated oestradiol. In both cases the proportions of oestrone

Table 4. Recovery (in % of applied dose and of total oestrogens) of radioactivity after subjecting the DEAE-Sephadex fractions to Brown's method

Patient	Total oestrogens (oestrone + 17β-oestradiol + oestriol)	Oestrone % of		17β-Oestradiol % of		Oestriol % of	
		Applied dose	Total oestrogens	Applied dose	Total oestrogens	Applied dose	Total oestrogens
HOL 1	13·9	3·7	26·5	2·2	15·8	8·0	57·7
HOL 2	13·7	1·3	9·8	0·8	5·6	11·6	84·6
HOL 3	12·2	0·9	7·4	1·0	7·8	10·3	84·8
DOU 1	9·9	4·4	44·7	1·7	17·0	3·8	38·3
DOU 2	11·9	2·0	17·0	0·9	7·5	9·0	75·5
DOU 3	12·8	0·9	7·0	0·5	3·8	11·4	89·2
SAR 1	19·1	11·6	60·9	2·8	14·5	4·7	24·6
MIC 1	9·6	4·9	51·2	2·2	23·3	2·4	25·5
MIN 1	23·4	14·0	59·9	6·3	27·1	3·0	13·0
BUR 1	15·1	9·4	62·2	4·0	26·8	1·7	11·0
Mean of 1st 24 hr	15·2	8·0	50·9	3·2	20·8	3·9	28·4
Mean of all	14·2	—	—	—	—	—	—

and 17β-oestradiol diminished while that of oestriol increased with time. These changes were most pronounced for oestrone, the proportion of which decreased to about a quarter or a sixth, respectively, on the third day. The percentage of 17β-oestradiol decreased to one half, or one quarter, respectively, of the first-day figures. The contribution of oestriol on the third day was 1·5 or 2·3 times, respectively, that on the first day following the injection. Similar changes during the first 3 days in the relative amounts of urinary oestrone, 17β-oestradiol and oestriol can also be found in one of the patients of Fishman *et al.*[12].

The distribution of the radioactivity associated with oestrone, 17β-oestradiol and oestriol in the DEAE-Sephadex fractions is shown in detail for one case (patient BUR) in Table 5 and Fig. 2. It is also indicated in Table 5 which fractions were combined as 3-glucosiduronates, 16(or 17)-glucosiduronates and sulphates for the calculation of the percentage compositions. Counts in fractions where two neighbouring peaks overlap, were distributed equally between the two fractions. The individual data of all other patients were treated as shown in the example, and the results are summarized in Table 6.

The position of the maxima for oestrone, 17β-oestradiol and oestriol on the DEAE-Sephadex chromatograms varies to some extent. But on the average the positions are identical to those found previously for endogenous oestrogen conjugates of pregnancy urine[7], i.e. maximum for oestrogen-3-glucosiduronates in fractions 21–25, for oestrogen-16(or 17)-glucosiduronates in fractions 41–45, for oestrogen sulphates in fractions 66–70.

Table 5. Distribution of the radioactivity associated with oestrone (O_1). 17β-oestradiol (O_2) and oestriol (O_3) in the fractions obtained after DEAE-Sephadex chromatography of urine. Results are given in counts/10 min, corrected for background

DEAE-Sephadex fractions	BUR 1		
	O_1	O_2	O_3
1–5	11	0	3
6–10	1182	57	41
11–15	3467	206	152
16–20	2773	121	155
21–25	24831	3134	1305
26–30	26465	1692	28
31–35	544	1638	823
36–40	132	2496	1154
41–45	343	463	3983
46–50	1043	3006	2291
51–55	1675	12328	206
56–60	180	515	170
61–65	63	1093	87
66–70	112	234	545
71–75	96	104	114
76–80	71	67	102
3-Glucosiduronates	1–40	6–45	1–30
16(or 17)-Glucosiduronates	36–65	41–60	26–65
Sulphates	61–80	56–80	61–80

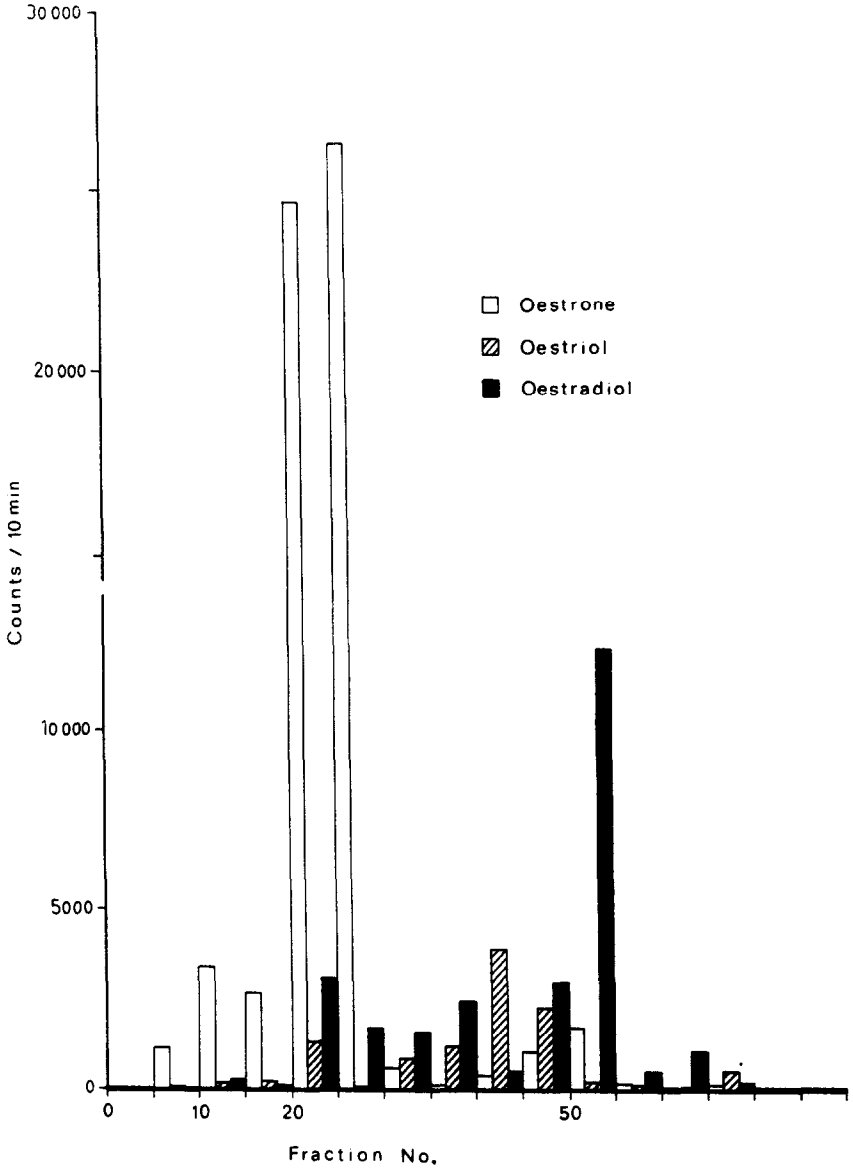

Fig. 2. Distribution of radioactivity associated with oestrone, 17β-oestradiol and oestriol in the fractions of a DEAE-Sephadex chromatogram of urine (BUR) (1st 24 hr after injection of 17β-oestradiol). DEAE-Sephadex fractions were combined in sets of 5 (1–5, 6–10, 11–15 etc.) for fractionation into oestrone, 17β-oestradiol and oestriol.

In an attempt to obtain some information on the nature of the radioactivity that was not associated with oestrogens in Brown's method, the difference between the total dpm's and the dpm's accounted for as oestrogens was calculated for the DEAE-Sephadex fractions of the 1st 24-hr urine specimens following oestradiol-17β injection. The results show that the main part of the unidentified radioactivity is found in fractions 16–30 (about 40% of unidentified radioactivity), and in

Table 6. Quantitative relationship of urinary oestrogen conjugates following the injection of tritiated 17β-oestradiol

(1) Fractionation of conjugates by DEAE-Sephadex Chromatography	(2) Fractionation of oestrogens by Brown's method	HOL 1st (%)	HOL 2nd (%)	HOL 3rd (%)	DOU 1st (%)	DOU 2nd (%)	DOU 3rd (%)	SAR 1st (%)	MIC 1st (%)	MIN 1st (%)	BUR 1st (%)	Means of 1st 24-hr specimens (%)
3-Glucosiduronates	Oestrone	24·6	7·5	4·2	37·7	16·0	3·8	47·4	45·6	58·3	58·6	45·4
	17β-oestradiol	8·2	2·8	3·6	9·8	3·8	2·1	7·8	7·0	13·6	9·5	9·3
	Oestriol	32·8	53·2	51·1	23·6	41·7	39·1	15·0	8·6	1·9	1·6	13·9
16(or 17)-Glucosiduronates	Oestrone	1·5	0·9	1·8	3·5	0·0	3·2	12·4	3·9	0·0	3·3	4·1
	17β-oestradiol	6·5	2·2	3·7	5·5	3·1	1·0	6·4	14·3	12·6	15·6	10·2
	Oestriol	21·6	26·7	34·2	14·0	26·3	44·7	9·3	13·6	9·1	8·6	12·7
Sulphates	Oestrone	0·4	1·5	0·3	3·0	1·0	0·0	1·2	1·7	1·6	0·3	1·4
	17β-oestradiol	1·1	0·5	0·0	1·4	0·5	0·5	0·2	2·1	0·8	1·7	1·2
	Oestriol	3·3	4·5	1·0	1·4	7·6	5·5	0·4	3·2	2·0	0·8	1·9

fractions 31–45 (about 24% of unidentified radioactivity). The location of these peaks of unidentified radioactivity coincides with the position of the oestrogen-3-glucosiduronates and 16(or 17)-glucosiduronates, respectively. However, unidentified radioactivity in relation to radioactivity associated with oestrogens is lowest in these fractions (Table 7): while the average amount of radioactivity associated with oestrogens was 14% (Table 4), as much as 30% of the radioactivity was associated with oestrogens in the DEAE-Sephadex fractions 26–30. On the other hand, of the small amounts of radioactivity in fractions 1–10 and 56–70, less than 5% was identifiable by Brown's method.

4. *Quantitative relationship of conjugated oestrogens in urine following the administration of tritiated 17β-oestradiol*

The quantitative relationship of conjugated oestrogens in urine following the injection of tritiated 17β-oestradiol to pregnant women is shown in Table 6. It should be pointed out that these results depend on the specificity of Brown's method. This method does not separate all the known oestrogens; it effectively separates the major oestrogens of normal urine into three fractions containing mainly oestriol, oestrone and 17β-oestradiol, respectively. However, it is well known for instance, that the oestriol fraction contains not only oestriol (16α, 17β) but also the other three oestriol epimers (16-epi-oestriol, 17-epi-oestriol, 16,17-epi-oestriol). Likewise, the oestrone and oestradiol fractions contain other oestrogens; 2-methoxy-oestrone, for instance, in the method of Brown, is found in the oestradiol fraction, its amounts ranging from 0 to 30% of the oestradiol[12].

The results shown in Table 6, therefore, do not refer to oestrone-3-glucosiduronate, 17β-oestradiol-3-glucosiduronate, etc., in the chemical sense but rather to a mixture, containing mainly the compounds mentioned, but possibly including other compounds behaving similarly both in DEAE-Sephadex-gradient elution and Brown's method. With these limitations in mind it can be seen from Table 6 that in the first 24-hr specimen following the injection of tritiated oestradiol most of the urinary radioactivity associated with oestrogens was found in the oestrone-3-glucosiduronate fraction, i.e. from 25 to 59% (mean 45%). This fraction was in all cases studied the most important one quantitatively. Approximately equal amounts of oestriol-3-glucosiduronate and oestriol-16-glucosiduronate were recovered if the means are taken for comparison (13–14%). However, individual variations were much greater for these two fractions (2–33% for oestriol-3-glucosiduronate, 9–22% for oestriol-16-glucosiduronate), sometimes the one being more abundant, sometimes the other. 17β-Oestradiol-3-glucosiduronate and 17β-oestradiol-17-glucosiduronate were also present in about equal amounts (10%) varying from 7 to 14% for 17β-oestradiol-3-glucosiduronate, and from 6 to 16% for 17β-oestradiol-17-glucosiduronate. These five fractions accounted for about 92% of all conjugated oestrogens listed in Table 6. Of the remaining 8% about half were found in the sulphate fraction and the other half in the "oestrone-16(or -17)-glucosiduronate" fraction. Obviously this last fraction is not an oestrone conjugate but represents a compound which behaves like oestrone in Brown's method and is conjugated with glucuronic acid at ring D. Its identity has not been established as yet.

The quantitative relationship of the five oestrogen conjugate fractions following the injection of tritiated 17β-oestradiol, changed with time. This is shown in Fig. 3, in which the quantitative relationship of the conjugates in the first, second

Table 7. Radioactivity associated with oestrogen in per cent of total radioactivity in fraction concerned

Patient	1–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	51–55	56–60	61–65	66–70	Above 70
HOL	1·6	1·3	8·8	9·1	19·8	16·1	11·5	7·4	14·8	24·2	22·9	1·2	4·5	3·4	11·7
DOU	3·8	0·3	1·5	2·0	4·3	16·4	10·3	29·2	8·2	15·6	11·8	6·0	2·2	1·6	25·7
SAR	4·1	16·5	6·7	6·8	27·8	22·4	29·7	17·0	17·5	23·7	21·3	7·0	5·3	2·6	3·2
MIC	1·1	1·4	4·7	5·1	17·1	23·6	12·1	11·8	16·8	12·5	2·5	4·1	3·5	15·3	14·7
MIN	—	0·7	12·1	15·6	51·1	55·4	48·0	53·0	30·7	18·9	3·0	3·2	9·2	—	—
BUR	0·2	5·4	6·8	6·4	30·0	44·4	7·2	5·9	5·4	15·7	31·1	3·0	3·6	5·8	12·2
Mean	2·2	4·3	6·8	7·5	25·0	29·7	19·8	20·7	15·7	18·4	15·3	4·1	4·7	5·7	13·5

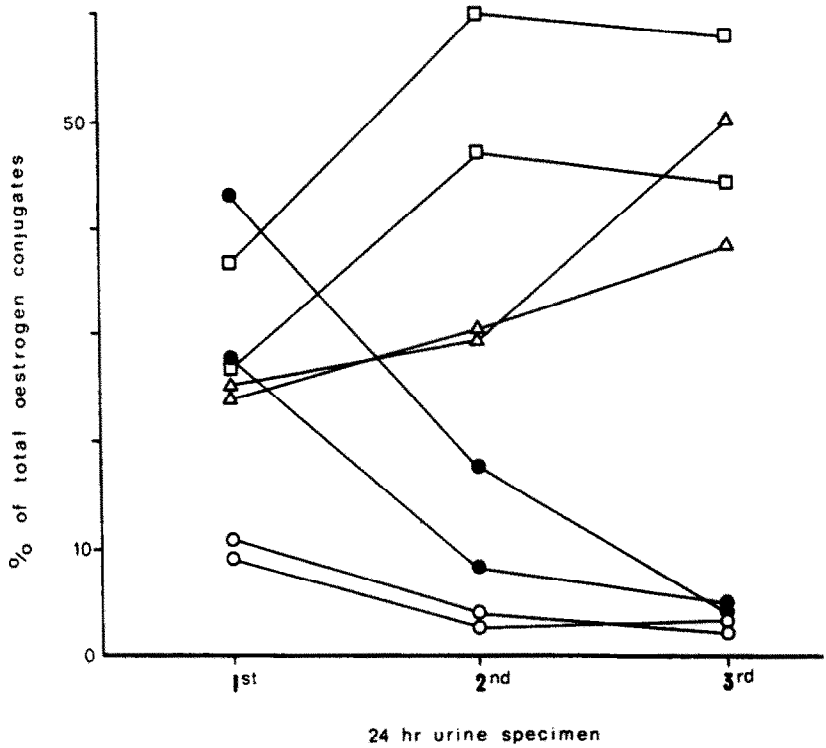

Fig. 3. Quantitative relationship of 4 oestrogen glucosiduronates in the 1st, 2nd and 3rd 24-hr urine specimens after injection of 17β-oestradiol. □——□, Oestriol-3-glucosiduronate; △——△, Oestriol-16-glucosiduronate; ●——●, Oestrone-3-glucosiduronate; ○——○, 17β-Oestradiol-3-glucosiduronate (17β-Oestradiol-17-glucosiduronate showed a very similar excretion pattern).

and third 24-hr urine specimens after administration is compared. The proportions of oestrone-3-glucosiduronate, 17β-oestradiol-3-glucosiduronate and 17β-oestradiol-17-glucosiduronate fell progressively in the 72 hr following administration. During the same period the proportions of oestriol-3-glucosiduronate and of oestriol-16-glucosiduronate increased. While some of these changes were already indicated by the changing pattern of oestrone, 17β-oestradiol and oestriol, regardless of conjugation (see section 3 of results), study of the quantitative relationship of the oestrogen conjugates revealed more detailed aspects of the changing patterns.

Although it is not possible to generalize on the basis of the results of 2 patients only, it might be significant that the changes in the mode of conjugation of oestriol and 17β-oestradiol as time elapsed after the injections, were different. Whereas both 17β-oestradiol glucosiduronates decreased similarly to about one half of the first day figure on the second day, and to about one third on the third day, oestriol-3-glucosiduronate and oestriol-16-glucosiduronate behaved differently from one another. The proportion of oestriol-3-glucosiduronate reached a maximum on the second day and decreased slightly on the third day. On the other hand the proportion of oestriol-16-glucosiduronate continued to increase and reached its proportionally highest values on the third day.

The patterns of urinary oestrogen conjugates following the injection of labeled oestradiol to pregnant women have not been studied previously. However, the quantitative relationships between (endogenous) oestrone glucosiduronate, 17β-oestradiol-3-glucosiduronate, oestriol-3-glucosiduronate, 17β-oestradiol-17-glucosiduronate and oestriol-16-glucosiduronate in human pregnancy urine [6] are known and lend themselves to comparison (Table 8). In normal pregnancy urine oestriol-16-glucosiduronate constitutes the majority (about 60%) of the oestrogen glucosiduronates, followed by oestriol-3-glucosiduronate (27%), oestrone glucosiduronate (10%) and the two oestradiol glucosiduronates (2% each). These figures were obtained as means of 20 patients (32–40 weeks pregnant). In some respects a similar pattern was found after injection of oestradiol and analysis of the urinary oestrogen conjugates: the two oestradiol glucosiduronates were present in about equal amounts in all of the three 24-hr specimens following the injection and their percentages (being about 10% in the first 24-hr specimens)

Table 8. Comparison of quantitative relationship of endogenous urinary oestrogen glucosiduronates and of urinary oestrogen conjugates found after injection of labeled oestradiol. n = Number of patients studied

Compound	Normal pregnancy urine $n = 20$	Urinary excretion following injection of labeled oestradiol		
		1st 24 hr $n = 6$	2nd 24 hr $n = 2$	3rd 24 hr $n = 2$
Oestrone glucosiduronate	10 ± 4·5%	45·5%	12%	4%
17β-oestradiol-3-glucosiduronate	2 ± 0·8%	9·3%	3·3%	2·8%
Oestriol-3-glucosiduronate	27 ± 9%	13·9%	47%	45%
17β-oestradiol-17-glucosiduronate	1·7 ± 0·7%	10·2%	3%	2·3%
Oestriol-16-glucosiduronate	59·9 ± 10%	12·7%	26%	39%

approached those of endogenous oestradiol glucosiduronates in the third 24-hr specimen. The two oestriol-glucosiduronates, however, show a different pattern. In late pregnancy urine oestriol-16-glucosiduronate was always the major endogenous oestrogen conjugate. In the first 24-hr urine specimen following the injection of labeled oestradiol to pregnant women, oestriol-16-glucosiduronate and oestriol-3-glucosiduronate were on the average found in about equal amounts. In some cases the one was in excess and sometimes the other. The percentage contribution of the sum of oestriol-16-glucosiduronate and oestriol-3-glucosiduronate was considerably higher in normal pregnancy urine (the two representing on the average 87% of total glucosiduronates) than those of their labeled counterparts in the first 24-hr urine specimen, (oestriol-16-glucosiduronate 13%, oestriol-3-glucosiduronate 14% on the average).

Of all radioactive oestrogen conjugates oestrone glucosiduronate was the most prevalent in 5 out of 6 cases and averaged 45% in the first 24 hour specimen after injection of oestradiol. It decreased rapidly on the second and third day. Endogenous oestrone glucosiduronate of pregnancy urine constituted on the average only 10% of the total glucosiduronates, but the individual variation from patient to patient was large[6].

Obviously much remains to be done: a high percentage of the radioactivity

is still unidentified; more specific methods for measuring oestrogens must be used to include not only the three classical compounds, but also the more important of the newer oestrogens, such as 16-hydroxy oestrone, 16-oxo-17β-oestradiol, 2-methoxy oestrone and 16-epi-oestriol, which are known to occur in pregnancy urine in substantial amounts; finally, the oestrogen conjugates should be definitely identified by recrystallization to constant specific activity.

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