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SHORT COMMUNICATION

EXTRACTION OF OESTROGEN CONJUGATES FROM URINE

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SUMMARY

Of five methods tested for the quantitative recovery of conjugated oestradiol-17β from pregnancy urine, extraction with ethyl acetate at pH 1 to 2 (after making the urine 20% with respect to NaCl) gives the best results. Data for the recovery of conjugated oestrone and oestriol by the various methods used are also presented.

VARIOUS methods for the isolation of conjugated oestrogens from pregnancy urine were tested with the aim of finding the one which gives the best recovery for 17β-oestradiol conjugates. The presence of two oestradiol glucuronides in pregnancy urine has been demonstrated earlier[1], but not enough material could be obtained for their more detailed identification at the time.

The following methods have been included in the investigation: Sephadex extraction[2], extraction with ether–ethanol[3], extraction with n-butanol[4], extraction with ethylacetate[5], and precipitation with ammonium sulphate[6]. The recent method of Bradlow[7], based on the use of Amberlite XAD-2 could not be included in this study, as the resin was not available to us at the time. For quantitative estimation of oestrone, oestradiol-17β and oestriol the method of Brown was used[8]. The results are summarised in Tables 1–5 and are based on duplicate estimations of the three oestrogens in normal pregnancy urine and the extracts. The recoveries are given in per cent of the oestrogen content of the original urine.

Table 1. Isolation of oestrogen conjugates from pregnancy urine using the method of Gupta and Goodwin[2]

	Oestrogens in urine	Oestrogens removed by Sephadex treatment	Oestrogens in Sephadex eluate		Overall recovery in Sephadex eluate
			in μg	in % of oestrogens removed by Sephadex	
Oestrone	31·7 μg	22·6 μg (71·3%)	17·4 μg	77·0%	54·9%
Oestradiol 17β	9·0 μg	6·0 μg (66·7%)	3·4 μg	56·7%	37·8%
Oestriol	97·0 μg	66·75 μg (68·8%)	41·7 μg	62·5%	43·0%

Table 2. Recovery of oestrogen conjugates from pregnancy urine by one extraction with ether–ethanol (3 : 1)[3]

	pH					
	4	5	6	7	8	9
Oestrone	87·7	82·3	73·1	73·7	71·7	72·4
Oestradiol-17β	—	81·1	86·9	83·0	81·8	86·9
Oestriol	66·2	66·1	61·6	66·6	58·4	66·6

Table 3. Recovery of oestrogen conjugates from pregnancy urine containing 10% NaCl by one extraction with n-butanol[4]

	pH				
	3	4	5	6	7
Oestrone	68·4	57·9	69·1	65·6	61·1
Oestradiol-17β	43·6	51·0	46·9	53·6	54·6
Oestriol	87·0	85·4	—	84·3	85·2

Table 4. Recovery of oestrogen conjugates from pregnancy urine containing 20% NaCl by one extraction with ethylacetate[5]

	pH					
	1	2	3	4	5	6
Oestrone	104·6	94·9	86·1	46·5	37·9	21·2
Oestradiol-17β	104·1	102·7	87·3	81·1	64·2	35·1
Oestriol	86·9	78·7	53·6	22·6	4·4	3·3

Table 5. Recovery of oestrogen conjugates from pregnancy urine by precipitation with ammonium sulphate[6]

	pH							
	2	3	4	5	6	7	8	9
Oestrone	94·2	92·9	91·0	86·5	72·5	75·8	73·8	78·2
Oestradiol 17β	72·1	75·4	69·2	72·1	71·3	71·1	—	69·1
Oestriol	90·5	91·4	90·5	85·1	85·6	83·8	83·8	80·2

Sephadex extraction as described by Gupta and Goodwin[2] for the isolation of C_{19}- and C_{21}-steroid conjugates is not suitable for the quantitative recovery of C_{18}-steroid conjugates from pregnancy urine. Only about 70% of the oestrone, oestradiol-17β and oestriol present in the urine are removed by treatment with the Sephadex (Table 1). Removal of the conjugated oestrogens bound to the Sephadex by elution with methanol–ethanol was also not quantitative, so that the overall

recovery by the method amounted to only 55% for oestrone, 38% for oestradiol-17β and 43% for oestriol.

In our hands extraction of pregnancy urine with ethylacetate at pH 1 or 2 gives the best results for oestradiol conjugates, *i.e.* a quantitative extraction of all oestradiol present in the urine by a single extraction. Similarly, Burstein and Lieberman[5] achieved a quantitative extraction of dehydroepiandrosterone sulphate by ethylacetate. This procedure is strongly pH-dependent and recoveries drop substantially at pH values above 2 (see Table 4). If the ethylactate extracts are to be used for isolation of conjugated steroids, prolonged standing at the low pH must be avoided to prevent solvolysis. Extraction of conjugated oestradiol by ether–ethanol[3] is independent of pH and yields about 85%. This compares well with Kellie[9], who with 3 extractions reported recoveries of about 95% of synthetic oestradiol glucosiduronates added to urine, or ammonium sulphate solutions. With the other methods used recoveries of conjugated oestradiol were significantly lower, ranging from approximately 45 to 75%.

Recoveries of conjugated oestrone and conjugated oestriol are often considerably different from each other and from those for oestradiol conjugates. Ammonium sulphate precipitation, for instance, removes about 90% of the oestriol from the urine but only 70% of the oestradiol-17β (Table 5). On the other hand, ethyl acetate extraction at pH2 recovers only about 80% of the oestriol from urine, while oestradiol-17β recovery amounts to 100% (Table 4).

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