Endogenous Steroid Concentrations in Human Breast Tumours Determined by High-Resolution Mass Fragmentography

By D. MILLINGTON, DIANE A. JENNER, T. JONES and K. GRIFFITHS
*Tenovus Institute for Cancer Research, Welsh National School of Medicine,
Heath Park, Cardiff CF4 4XX, U.K.*

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A pilot study of the endogenous steroid concentrations in human breast tumours was performed. The technique of high-resolution molecular-ion monitoring during combined g.l.c.–mass spectrometry was used to determine oestrone, oestradiol-17 β and oestriol in concentrations above 1 ng/g wet wt. of tissue, and dehydroepiandrosterone, testosterone, androsterone (3 α -hydroxy-5 α -androstan-17-one) and 3 β -hydroxy-5 α -androstan-17-one in concentrations exceeding 5 ng/g, in extracts of five primary breast tumours.

Several reports have recently appeared on a possible relationship between the presence or absence of oestradiol-17 β 'receptor' proteins in human breast tumours and the response of the patient to subsequent endocrine therapy (Engelsman *et al.*, 1973; Maass *et al.*, 1972; Brecher *et al.*, 1971). The techniques used to assay the binding of oestradiol-17 β , however, reveal the presence only of unoccupied binding sites, and no attempt has yet been made to determine the number of binding sites occupied by the endogenous oestradiol-17 β . An attempt was made by Maass *et al.* (1972) to relate plasma oestradiol-17 β concentration to the receptor capacity of human breast-tumour tissue. Although no relationship was found to exist for plasma oestrogen concentrations below 200 pg/ml, no binding capacity could be detected in patients with plasma oestradiol-17 β concentrations exceeding 300 pg/ml. Similar observations were made by Trams *et al.* (1973) for the binding of oestradiol-17 β in human uterine tissue. High plasma oestrogen concentrations were found to relate to low binding capacity.

The relationship between endogenous steroid concentrations and oestradiol-17 β receptor concentration in breast tumours has yet to be determined. As a preliminary to this investigation a technique for endogenous steroid analysis has been assessed and the results of the study are now reported. The study involved the technique of combined g.l.c.–mass spectrometry, using the highly specific and sensitive mode of single ion detection (mass fragmentography) at high resolution (10 000, 10% valley). Low-resolution mass fragmentography has previously been used for the analysis of oestrogens in urine (Maume *et al.*, 1973) and in plasma (Adlercreutz & Hunneman, 1973).

Materials and methods

Samples of primary breast-tumour tissue were obtained at mastectomy and stored at –15°C until

used. Chopped tissue was then homogenized in 50 ml of acetone, by using a Silverson tissue grinder, centrifuged (800 g, 10 min) and the acetone was decanted. The tissue was resuspended in acetone and the extraction procedure repeated twice more. The combined acetone extracts were dried under vacuum and the residue redissolved in aq. 80% (v/v) methanol (20 ml). This solution was extracted with an equal volume of light petroleum (b.p. 80–100°C) to remove lipids, and the light petroleum was then back-extracted with an equal volume of 80% methanol. The conjugated steroids were removed by redissolving the dried combined aqueous-methanol extract in ether and extracting with an equal volume of water. The water was then back-extracted twice with ether. The final ethereal solution was dried and the residue redissolved in ethanol. This solution and standard steroid solutions were stored at –20°C. Samples were prepared for g.l.c. analysis by transferring measured volumes of the stock solution into a 1 ml screw-cap vial and treating the residue after evaporation with bis(trimethylsilyl)acetamide (30 μ l) and light petroleum (b.p. 60–80°C) (20 μ l). The tightly capped vials were left overnight at 20°C before analysis.

Combined g.l.c.–mass spectrometry was carried out by injecting 1 μ l of the sample solution into a Varian 2700 gas chromatograph fitted with a Pyrex column (2 m $\times$ 2 mm internal diam.) packed with a homogeneous mixture of 3% OV-225 and 1% OV-210 on Supasorb (100–120 mesh) in the ratio 1:2. The injector and detector temperatures were 250°C and 275°C respectively, and the oven temperature was kept at 255°C. The gas chromatograph was operated with a helium flow rate of 25 ml/min, in tandem with a Varian–MAT 731 mass spectrometer, via a two-stage Watson–Biemann separator at a temperature of 250°C. A glass-lined probe at 240°C completed the all-glass introduction system to the ion source, which was operated at 150°C with an electron beam energy of 70 eV (11 aJ). The assay for

each steroid derivative was then conveniently set up by monitoring the intensity of the molecular-ion peak on a potentiometric recorder at a resolution of 10 000 (10% valley). Under these conditions, the recorder response varied linearly with sample concentration in the range 0.1–10 ng/ μ l and the detection limit was approx. 10 pg/ μ l for the oestrogens and 30 pg/ μ l for the C_{19} steroids. The two parameters of g.l.c. retention time and high-resolution molecular-ion detection were sufficient to detect and quantify each component regardless of the complexity of the mixture and the presence of overlapping g.l.c. peaks.

Results and discussion

The g.l.c. trace of a standard solution of the silylated oestrogens of particular interest in these investigations is shown in Fig. 1. In each of these

derivatives, the molecular-ion peak was also the most intense ion in the mass spectrum under the conditions used, and therefore was the most convenient for single ion monitoring. Fig. 1 also shows the mass fragmentograms obtained by monitoring the signal at m/e 416.257, corresponding to the molecular weight of the bis(trimethylsilyl) ether of oestradiol-17 β , in a blank solution (b) and in one of the breast tumours (c). Each of the tumour extracts was successively analysed in this way for oestradiol-17 β ; then the process repeated for the determination of oestrinol and oestrone, by high-resolution monitoring of the ions at m/e 504.291 and 342.201 respectively. The isomeric C_{19} steroids dehydroepiandrosterone and testosterone were well separated on the gas chromatograph after trimethylsilylation and were measured simultaneously by monitoring m/e 360.248, and androsterone (3 α -hydroxy-5 α -androstan-17-one, 3-epiandrosterone (3 β -hydroxy-5 α -androstan-17-one) and

Fig. 1. G.l.c.-mass spectrometry of steroids

(a) G.l.c. separation of oestradiol-17 β [as bis(trimethylsilyl) ether] (i), oestrinol [as tris(trimethylsilyl) ether] (ii) and oestrone (as trimethylsilyl ether and ketone) (iii). Experimental details are described under 'Materials and methods'. (b) and (c) Mass fragmentograms of oestradiol-17 β , monitoring m/e 416.257 at a resolution of 10 000 (10% valley) in a blank solution (b) and in the extract of sample 6 (c) respectively.

Table 1. Concentrations of endogenous steroids in breast tumours

Results are given in ng/g wet wt. of tissue. In all cases, the concentration of 5 α -dihydrotestosterone was below 5 ng/g.

Tumour	Dehydroepiandrosterone	Testosterone	3 α -Hydroxy-5 α -androstan-17-one	3 β -Hydroxy-5 α -androstan-17-one	Oestrone	Oestradiol-17 β	Oestrinol
1. M.B.	35	55	75	38	8	500	<1
2. R.L.	15	25	10	10	3	16	<1
3. M.H.	30	100	55	60	20	15 $\times$ 10 ³	22
4. E.D.	25	25	50	<5	17	50	3
5. E.J.	<5	30	100	38	<1	10	<1
6. L.B.	70	<5	100	20	6	45	30

5 α -dihydrotestosterone (17 β -hydroxy-5 α -androstane-3-one) were also assayed simultaneously by monitoring *m/e* 362.264. Peak heights were determined for all steroids isolated from tumour tissue and related to the peak height of steroid standards from similar fragmentograms.

Results are given in Table 1 for the analysis of the endogenous steroid content of five primary breast tumours removed from postmenopausal women. Sample 6 was a sample of normal breast tissue.

The results of the present observations do not take into account the errors introduced by the extraction procedures. It is clear, however, that the technique of high-resolution mass fragmentography is ideal for the determination, not only of the endogenous oestradiol-17 β concentrations in breast tissue, but also of the other steroids that make up the hormonal milieu of the tumour.

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