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Short Communications

Steroid Metabolism by Human Breast Tumours

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Extensive studies relating urinary 11-deoxy-17-oxo steroids and prognosis in patients with advanced breast carcinoma (Bulbrook, 1965) have directed attention to the major source of urinary C_{19} steroids, DHA* sulphate, which is secreted by the adrenal and is present in plasma in high concentration (Yamaji & Ibayashi, 1969). Its metabolism by extra-adrenal tissue is therefore of particular interest. Adams & Wong (1968*a*, 1969) demonstrated in breast tumour tissue the presence of steroid-metabolizing enzymes capable of converting cholesterol into pregnenolone, 17α-hydroxyprogesterone into androstenedione (androst-4-ene-3,17-dione) and testosterone into oestriol [oestra-1,3,5(10)-triene-3,16α,17β-triol]. This communication now presents results from studies similar to those of Adams & Wong (1968*a*), together with preliminary data from the perfusion of [7α-^{3}H]DHA sulphate through human breast tumours *in situ*. Important differences, however, were found in the nature of some of the metabolites isolated, which bear fundamentally on the concept of hormone action in the breast.

Tumour tissue was maintained at 0°C after removal from the patient until prepared for incubation 30min later. The tissue was thinly sliced, the slices were chopped into smaller segments with a razor blade and portions were incubated with radioactive steroids (Table 1) in Krebs–Ringer bicarbonate–glucose medium (Cohen, (1957) (12.5 ml/g of tissue), shaking at 37°C in an atmosphere of O_2+CO_2 (95:5) for 2h without addition of cofactors. Purity of incubated precursors was checked by isotope-dilution analysis before incubation. Reactions were stopped by addition of acetone, and known quantities in the range 300–500μg each of the non-radioactive carrier steroids, shown in Table 1, added in ethanol. Mixtures were homogenized in a Silverson mixer and separated into neutral, phenolic and conjugated fractions as described by Fahmy, Griffiths, Turnbull & Symington (1968).

Perfusion studies were performed on tumours localized in the medial half of the breast, supplied predominantly by the internal mammary artery, which was exposed during exploration of the second or third intercostal space for lymph-node biopsy. A ligature was tied proximal to the point of injection, and [7α-^{3}H]DHA sulphate (approx. 33nmol, specific radioactivity 0.36μCi/nmol) administered as a single injection within 30s. After removal of the breast, the tumour was dissected from surrounding tissues, maintained at 0°C until transferred to the laboratory, and sliced into 20ml of acetone, to which 300μg of carrier non-radioactive steroids was then added (Table 1). The mixture was homogenized, and the steroids were fractionated as before and separated on thin layers of silica gel $HF_{254/366}$ (E. Merck A.-G., Darmstadt, Germany) (Fahmy *et al.* 1968). The following solvent systems were used, the proportions being by volume: I, chloroform–acetone (37:3); II, cyclohexane–ethyl acetate (7:3); III, benzene–ethyl acetate (9:1); IV, cyclohexane–ethyl acetate–ethanol (9:9:2); V, hexane–ethyl acetate (1:1); VI, cyclohexane–ethyl acetate (9:11); VII, ethyl acetate–2-methylpropan-2-ol–5M-NH_3 (50:41:20); VIII, chloroform–methanol–water (187:12:1). Steroids were measured after elution and derivatives were prepared as described by Griffiths, Grant, Browning, Cunningham & Barr (1966). Radioactivity was measured by a Nuclear–Chicago liquid-scintillation spectrometer (model 6860). The mean of the specific radioactivities was used to calculate the percentage conversion from the precursor incubated or perfused.

16α-Hydroxy-DHA was analysed by g.l.c., after conversion into the bistrimethylsilyl ether, on a 150cm column of 1.5% QF-1 on Supasorb AW-HMDS (100–120 mesh) (British Drug Houses Ltd., Poole, Dorset, U.K.) at 183°C. 5α-Dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) and its derivatives were determined on a 150cm column of 1% XE-60 on Gas-Chrom Q (Applied Science Laboratories, State College, Pa., U.S.A.) at 200°C.

Evidence for the identification of the metabolites formed in the incubation and the percentage conversions are given in Table 1. Extensive metabolism of DHA sulphate was shown to occur,

* Abbreviation: DHA, dehydroepiandrosterone.

Table 1. *Evidence for the identification of metabolites formed during the incubations*

Experimental details are given in the text. Percentages of the total radioactivity incubated are given in parentheses. The perfusion studies were carried out (*a*) with 12.5μCi of DHA sulphate and (*b*) with 11.8μCi of DHA sulphate. Specific radioactivities are given as d.p.m./μmol. Values in parentheses indicate the percentages of the original precursor perfused found in the metabolite; values in square brackets refer to the percentages of radioactivity taken up by the tumour that was found in the metabolite.

Compound isolated and derivatives formed	Solvent system used for purification	Radioactive steroids incubated and specific radioactivities of isolated carrier steroids (d.p.m./nmol)							
		[7α-^{3}H]DHA sulphate		[7α-^{3}H]DHA		[7α-^{3}H]Androstenedione		[4-^{14}C]Testosterone	
DHA sulphate	VII								
3β-Acetoxyandrost-5-en-17-one	I	34008							
DHA	III	36473	(38.3%)						
Androstenediol	V	37400							
DHA	I	26717		77606					
3β-Acetoxyandrost-5-en-17-one	III	25316	(38.9%)	77502	(78.5%)				
Androstenediol	V	25719		76342					
Androstenedione	I	128.5		597.2		49878			
Testosterone	I	124.7	(0.19%)	580.0	(0.61%)	49378	(55.6%)		
Testosterone acetate	II	126.7		584.1		48322			
Testosterone	I	1.2		5.6		160.3			
Testosterone acetate	II	1.3	(0.002%)	5.8	(0.006%)	150.7	(0.17%)		
Androstenedione	I	1.2		6.2		155.4			
Androstenediol	V	46.9	(0.07%)						
Androstenediol diacetate	II	48.7							
16α-Hydroxytestosterone	IV	0		2.9	(0.003%)	3.5	(0.003%)		
16α-Hydroxytestosterone diacetate	I	0		3.1		3.4			
Oestrone	VI	0				5.8			
Oestrone 3-methyl ether	II	0		0.51	(0.0005%)		(0.005%)		
Oestrone acetate	II	0		0.59		5.7			
5α-Dihydrotestosterone	I							176	
5α-Androstanedione	I							169	(5.1%)
5α-Androstane-3β,17β-diol	I							173	
Perfusion studies		(*a*) 16.2g of tumour tissue extracted, providing 260000 d.p.m. in acetone fraction				(*b*) 14.2g of tumour tissue extracted, providing 49600 d.p.m. in acetone fraction			
DHA	I	8100	(0.03%)			7800	(0.03%)		
3β-Acetoxyandrost-5-en-17-one	III	8200	[3.27%]			7600	[16.25%]		
Androstenediol	V	8200				8300			
Androstenedione	I	270	(0.001%)			230	(0.001%)		
Testosterone	I	290	[0.11%]			250	[0.51%]		
Testosterone acetate	II	280				240			

although androstenedione and testosterone were formed only in low yields, confirming the rather more tentative evidence of Adams & Wong (1968*a*), who demonstrated the release of labelled sulphate ion on incubation of DHA [^{35}S]sulphate with homogenates of breast tumours. No evidence was obtained for 16α-hydroxy-DHA sulphate or androst-5-ene-3β,17β-diol sulphate formation. DHA and androstenedione were converted into 16α-hydroxytestosterone, although no evidence for oestriol synthesis was obtained even after incubation with testosterone. The isolated carrier oestriol was found labelled after extensive chromatography, but the label disappeared on methyl ether and acetate formation. Some evidence for oestrone formation was found. This is contrary to the observation of Adams & Wong (1968a) using microsomal preparations of breast tumour, although they did not prepare derivatives of their oestriol, and it was suggested by Dao (1969) that contamination of the carrier oestriol with labelled androst-5-ene-3β,16α,-17β-triol might account for their results. 16α-Hydroxy-DHA was added as carrier to the incubation of tumour tissue with DHA sulphate. Extensive chromatography in systems I, IV and VIII with radioscanning indicated the carrier to be labelled. It was then acetylated, run in systems I and II, hydrolysed and re-run, and the specific radioactivity was determined and a conversion value of 0.02% was calculated from this. Testosterone was transformed into 5α-dihydrotestosterone in relatively large yields by the human breast tumour tissue, a conversion demonstrated in rat mammary fibroadenoma by King, Gordon & Helfenstein (1964). 5α-Dihydrotestosterone, currently of interest in relation to its androgenic action in prostatic tissue (Bruchovski & Wilson, 1968; Baulieu, Lasnitski & Robel, 1968), also inhibits the growth of the oestradiol-17β-dependent rat mammary fibroadenoma (Huggins & Mainzer, 1957).

The preliminary perfusion studies also showed a limited formation of DHA and androstenedione from DHA sulphate *in vivo*. The suggestion of Adams & Wong (1968*b*) that breast tumour metabolism of plasma steroids may account for the abnormal patterns of urinary steroid metabolites received little support from these studies, although such metabolism may provide a specific local steroid environment within the tumour cells on which growth of the tumour is dependent. It is of note that patients with advanced localized breast cancer have lower concentrations of urinary aetiocholanolone than have patients with primary or the advanced generalized type of disease (Gleave *et al.* 1970).

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