Short Communication

**OESTRADIOL SYNTHESIS FROM C19 STEROIDS BY
HUMAN BREAST CANCERS**

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THE REGRESSION of advanced breast cancer which can follow ovarian ablation is believed to be due to reduction in the levels of circulating oestrogen. The benefit which may follow adrenalectomy in oophorectomized and in post-menopausal women cannot be explained on a similar premise. In post-menopausal women plasma oestrogens are already low (England *et al.*, 1974) and the adrenal cortex secretes only trace amounts of oestrogen. The main sex hormones secreted by the adrenal cortex are C19 steroids (Cameron *et al.*, 1969) which we and others have shown to be metabolized by breast tumours (Adams and Wong, 1968; Jones *et al.*, 1970; Jenkins and Ash 1972; Miller *et al.*, 1973). Recently we gave unequivocal evidence that the C19 steroid, testosterone could be utilized by a human breast cancer to synthesize oestradiol-17 β (Miller and Forrest, 1974). The aim of this study was to determine whether this effect was reproducible in other tumours.

MATERIALS AND METHODS

Patients.—Thirteen patients with proved cancer of the breast were studied. Eight subjects were at least 5 years postmenopausal, a further 2 were less than 5 years postmenopausal and 2 more were experiencing regular menstrual periods at the time of investigation. The remaining patient had been oophorectomized 2 years before the study.

Tumour processing and incubation.—Following excision, the tumours (11 primary

and 2 secondary recurrences from the chest wall) were put on ice in the operating theatre. Sufficient tissue was removed by a pathologist for histological diagnosis and the remainder of the tumour was finely sliced and incubated for 2 h at 37°C in Krebs Ringer phosphate buffer pH 7.4 (10 ml/g tissue), containing an NADPH generating system and 45 μ Ci 7 α -³H testosterone. The metabolism of testosterone was then determined by measuring the percentage of incorporation of ³H into the various purified metabolites. Details of the methodology used for steroid purification, characterization by chemical derivatives and measurement have been described previously (Miller, Forrest and Hamilton, 1974). Identification of oestradiol-17 β fractions was based on the following criteria: (a) the fractions on acetylation and methylation formed compounds which, on thin layer chromatography, moved with the same mobility as authentic oestradiol diacetate and oestradiol-3-methyl ether respectively; (b) consistent specific radioactivity was maintained throughout derivative formation.

RESULTS

The percentage radioactivity found in the various metabolites investigated is shown in Table I.

All tumours metabolized testosterone but with considerable variation (17–54%). The presence of 5 α reductase activity was demonstrated in all tumours and both 5 α dihydrotestosterone and 5 α androstenediol were identified as metabolites. The level of production of 5 α dihydrotestosterone invariably exceeded that of 5 α andros-

TABLE I.—*Metabolism of $7\alpha^3H$ Testosterone by Human Breast Carcinomata*

	% Metabolism	% Conversion to			
		5α Dihydrotestosterone	5α Androstanediol	$\Delta 4$ Androstenedione	Oestradiol- 17β
EC	17.03	1.67	0.85	4.71	0.37
JC	39.35	1.93	0.69	6.76	0.22
E.Cr	53.79	0.65	0.13	38.49	0.07
A.R.	24.55	2.79	1.18	7.83	0.06
ES	24.48	0.37	0.09	0.39	0.05
CMcD	19.92	0.41	0.09	2.03	0.04
CR	28.94	0.72	0.16	6.90	neg?
GM	26.93	0.58	0.21	3.84	neg?
G.A.	33.36	2.61	1.18	2.91	neg?
MMcN	29.59	0.91	0.38	4.83	neg?
J.M.	27.66	3.04	1.50	0.60	0
MR	27.66	3.04	1.50	0.60	0
JR	27.10	0.44	0.15	0.65	0

neg? = low inconsistent specific radioactivity.

TABLE II.—*Evidence for the Identification of Oestradiol 17β*

	Derivative	Specific activity d/min/nmol	% Conversion
E.C.	Oestradiol free	211	
	Oestradiol diacetate	225	0.37
	Oestradiol methyl ether	229	
J.C.	Oestradiol free	126	
	Oestradiol diacetate	118	0.22
	Oestradiol methyl ether	121	
E.Cr.	Oestradiol free	44.7	
	Oestradiol diacetate	45.5	0.08
	Oestradiol methyl ether	41.6	
A.R.	Oestradiol free	35.3	
	Oestradiol diacetate	33.8	0.06
	Oestradiol methyl ether	34.9	
E.S.	Oestradiol free	31.7	
	Oestradiol diacetate	31.8	0.05
	Oestradiol methyl ether	29.2	
CMcD	Oestradiol free	17.8	
	Oestradiol diacetate	19.1	0.04
	Oestradiol methyl ether	18.2	

tanediol. All tumours converted testosterone to $\Delta 4$ androstenedione and, in most, this steroid represented the single greatest metabolite identified.

Unequivocal evidence (Table II) for the synthesis of oestradiol- 17β was found in 6 tumours, in 2 of which the amounts were substantial. In 7 tumours oestradiol- 17β was not identified. Although in 4 of these small amounts of radioactive label were incorporated in the crude oestradiol fraction, consistent specific radioactivity was not obtained in the derivatives.

COMMENT

These findings confirm that all human breast cancers can metabolize C19 steroids.

Furthermore, all tumours studied had 5α reductase activity and were able to convert testosterone into its 2 active 5α reduction products, 5α dihydrotestosterone and 5α androstanediol.

In contradistinction, not all tumours could synthesize oestradiol- 17β and we conclude that the possession of the aromatizing system is specific to certain types of tumour. To date, we have not uncovered any particular difference between those tumours which have oestradiol synthesizing capacity and those which do not.

Since biologically approximately half of all human breast cancers do show some degree of hormone dependence and one-third markedly so, it is tempting to believe

that the possession of aromatizing enzymes may be of importance in this regard. In this event, the tumours which were capable of transforming C19 steroid into oestrogen could be those which are dependent on the adrenal cortical source of C19 steroids.

We have already suggested that the beneficial effects of adrenalectomy and hypophysectomy could be due to reduction of circulating C19 precursor steroids such as DHA sulphate (Miller and Forrest, 1974). The results we now report are further evidence of such a possibility.

Studies are now in progress to determine the relationship of possession of this synthetic pathway to oestrogen receptor activity and to the clinical response to adrenalectomy and hypophysectomy.

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