Journal of Steroid Biochemistry, 1973, Vol. 4, pp. 489–501. Pergamon Press. Printed in Great Britain

MALE BREAST CANCER—II

METABOLISM OF OESTRADIOL-17β IN MEN WITH BREAST CANCER

OLE SCHEIKE, BIRGIT SVENSTRUP and V. AASTED FRANDSEN
The Radium Center, Strandboulevarden 49, 2100 Copenhagen, Denmark and The Hormone Department, Statens Seruminstitut, Copenhagen, Denmark

(*Received* 9 *November* 1972)

SUMMARY

A tracer dose of [^{3}H]-oestradiol-17β was given intravenously to 19 male patients with breast cancer. The metabolism of this tracer was examined by measuring total radioactivity, the radioactive metabolites: oestrone, 2-methoxyoestrone, oestradiol-17β and oestriol in 48h urine samples. The metabolism of the tracer was compared with that of 19 healthy men and 5 men with non-endocrine malignant diseases, all of comparable age. There was no statistical difference between the three groups of subjects. Total oestrogens were measured in the urine of the three groups of subjects and no statistical difference between the groups was found. The results of this study do not imply a specific oestrogen excretion pattern or metabolism of oestradiol-17β in men with breast cancer.

INTRODUCTION

THE THEORY that the oestrogens play an important role in the ethiology of breast cancer in the female and in the male have been discussed for many years. In 1932 Lacassagne induced mammary carcinoma in male mice by oestrogen administration[1]. Concerning male breast cancer some data have later been published which seem to substantiate the above mentioned theory.

In Egypt and certain tropical regions the male/female ratio of mammary cancer is quite high: from 3 to 10%[2–5]—whereas the ratio by most tumour registries in the western countries is reported to be approximately 1%. This high incidence of male breast cancer is by these authors attributed to an impairment of the hepatic metabolism of the oestrogens due to the fact that liver damage occurs frequently in these countries. In 1968 Symmers[6] gave a remarkable report of two transvestite males. In order to change their appearance to that of women each underwent castration; bilateral mammoplasties were performed and for a prolonged period they took oestrogens orally, by implant and by breast inunction. Each developed a breast carcinoma in their thirties, a very low age for male breast carcinoma.

Little is known of any relationship between abnormal metabolism of oestrogens and the development of breast cancer. Some studies on the metabolism of administered labelled oestrogens have been carried out both in females and in males with breast cancer[7–10].

Few authors have dealt with the metabolism of administered oestrogens in males with breast cancer. In 1966 Zumoff *et al.*[10] administered labelled oestradiol-17β to 6 men with breast cancer, 5 normal healthy men of comparable age and 9 men with other malignant diseases unrelated to the endocrine system. Intravenous injections of radioactive oestradiol-17β were given, and the

excretion of oestrone, oestradiol-17β, oestriol, 2-hydroxy-oestrone and 2-methoxyoestrone was measured in 72 h urine collections. The excretion of oestradiol-17β was similar in the three groups, but the men with breast cancer excreted higher amounts of oestriol accompanied by a considerably smaller excretion of oestrone, 2-hydroxyoestrone and 2-methoxyoestrone. These authors suggested a relationship between oestrogen biotransformation and male breast cancer.

A series of 265 cases of male mammary carcinoma has been collected from all over Denmark, using the files of the Danish Cancer Registry. It comprises all cases recorded over the period from January 1st, 1943, to July 1st, 1972. The histologic preparations from 187 of these cases have been revised and reclassified in a study to be published [11]. The opportunity to contact 19 of these patients with this disease encouraged us to investigate the metabolism of administered oestradiol-17β and the excretion pattern of the oestrogens in the urine of males with breast cancer.

EXPERIMENTAL

Clinical material

The investigations were performed on 19 men with histologically proven mammary carcinoma, being treated at the Radium centers in Copenhagen, Aarhus and Odense. The primary treatment in these patients was simple or radical mastectomy with postoperative radiotherapy. One patient was investigated before primary treatment, 3 patients were under primary treatment (postoperative radiotherapy) during the investigations. As acute stress is known to influence oestrogen excretion [12] none of the patients had major surgery less than 2 weeks prior to urine collections, most of them having had their primary treatment a month or more before the investigations. 11 patients presented no signs of recurrences, 4 had recurrences (2 locally and 2 in the bones) but all were ambulatory and feeling well (Table 1).

Table 1. Clinical information on the male breast cancer patients studied

Subject	Age	Follow-up
BP	51	2 years and 2 months
SK	54	1 month
WJ	54	1 year and 11 months
HH	58	Recurrence (locally). After 6 years and 10 months
VG	58	Investigated during primary treatment (Radiotherapy)
GL	62	Investigated during primary treatment (Radiotherapy)
JM	62	12 years
KH	67	Investigated during primary treatment (Radiotherapy)
NV	67	9 months
JJ	70	Recurrence (bones). After 1 month
MS	73	9 months
AK	73	8 months
EB	74	4 years and 2 months
CP	75	Recurrence (locally). After 6 years
AR	76	6 years and 1 month
PP	76	Investigated before primary treatment
CR	76	2 primary malignancies: (a) 14 years, (b) 4 years and 5 months
AJ	76	7 months
LM	78	Recurrence (bones). After 6 years and 6 months

Control studies were made in 19 healthy men with a mean age of 67·1 years (age 51–80) and in 5 men with other malignant diseases unrelated to the endocrine system with a mean age of 69·2 years (age 55–78) (Table 2). The healthy men were personnel at Statens Seruminstitut and men living in a home for the aged. In all subjects studied detailed histories were taken to rule out factors that might influence the metabolism and excretion of the oestrogens. None of the subjects had kidney diseases. One of the mammary cancer patients had been treated with penicillin for years because of an endocarditis, but none of the other subjects in the project were taking any drugs, hormones included.

Table 2. Clinical information on patients with other malignant diseases. All but one (FH) were ambulatory

Subject	Age	Diagnosis	Follow-up
KE	55	Carcinoma of the larynx	Investigated during primary treatment (Radiotherapy)
FH	68	Sarcoma of the left femur	Investigated during primary treatment (Radiotherapy)
EK	70	Carcinoma of the lung	7 months. (Metastatic at the time of diagnosis)
JP	75	Carcinoma of the skin	3 months
JQ	78	Carcinoma of the rectum	2 years

Trivial names. Oestrone: 3-hydroxy-1,3,5(10)-oestratrien,17-one. Oestradiol: 1,3,5(10)-oestratriene-3,17β-diol. Oestriol: 1,3,5(10)-oestratriene-3,16α,17β-triol. 2-hydroxyoestrone: 2,3-dihydroxy-1,3,5(10)oestratriene-17-one. 2-methoxyoestrone: 3-hydroxy-1,3,5(10)-oestratrien,17-one-2-methylether.

Chemicals. Isotopically labelled oestradiol-17β [2,4,6,7-^{3}H] oestradiol (S.A. 3·49 m Ci/ng) from New England Nuclear Corporation was used without further purification. The radiochemical purity was checked by thin layer chromatography on silicagel in two systems: cyclohexane–ethylacetate (13:7, v/v) and chloroform–acetone (9:1, v/v) and was found to be $> 96\%$ pure.

Unlabelled oestrone and 2-methoxyoestrone were obtained from Ikapharm, Israel, oestriol from Mann Research Laboratories, New York and oestradiol-17β from Sigma.

All reagents were of analytical grade:

sulphuric acid, Merck; sodium hydroxide, Elektrokemiska Aktiobolaget; sodium hydrogencarbonate, Merck; benzene, Merck; Girard reagent T, Merck; acetic acid, Merck; chloroform, Merck; carbon tetrachloride, Merck; boric acid, Merck; dimethylsulphate, Merck; hydrogenperoxide, Merck; cyclohexane, Merck; acetone, Merck; ethylacetate, Merck; silica gel G nach Stahl, Merck; dioxane, Merck; Naphthalene, Merck; POPOP, (2,2'-phenylen-bis-5-(2,5-diphenyloxazol)), Merck; PPO, (2,5-diphenyloxazol), Merck; *n*-hexane, May and Baker; diethylether Ph.D.; absolute ethanol, DDSF.; aluminium oxide neutral, Woelm.

Benzene and *n*-hexane were distilled before use. Diethylether was distilled

over 1/10 vol of a 50% solution of ferrous sulphate in 5% sulphuric acid and used the same day.

Other reagents were used without purification.

Methods (*Figs.* 1 *and* 2; *Tables* 3–5)

A solution of (2,4,6,7-^{3}H) oestradiol-17β was prepared in pyrogen free propylene glycol. Each subject studied received approximately 3 μCi of oestradiol-17β, dissolved in 6 ml propylene glycol, by intravenous injection. 48-h urine samples were collected immediately after the injection. The radioactivity in the urine

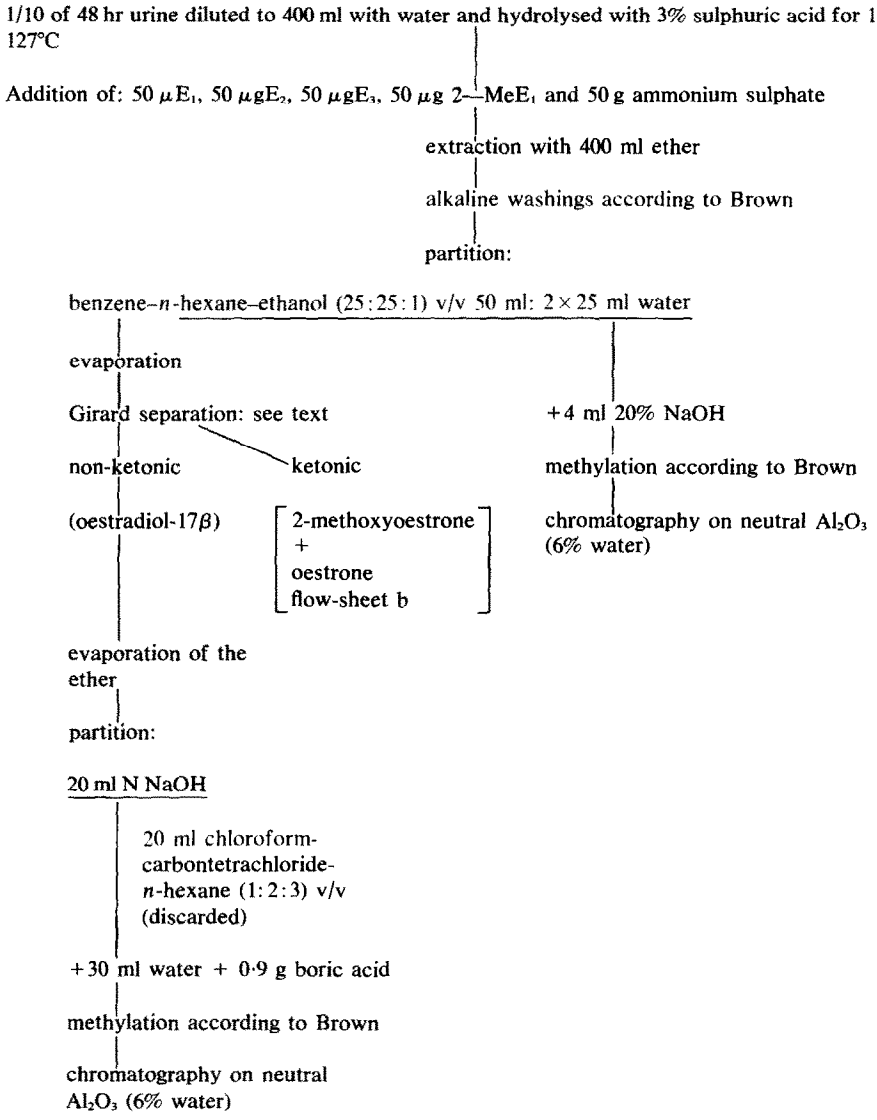

Fig. 1. Flow-sheet of the method (a).

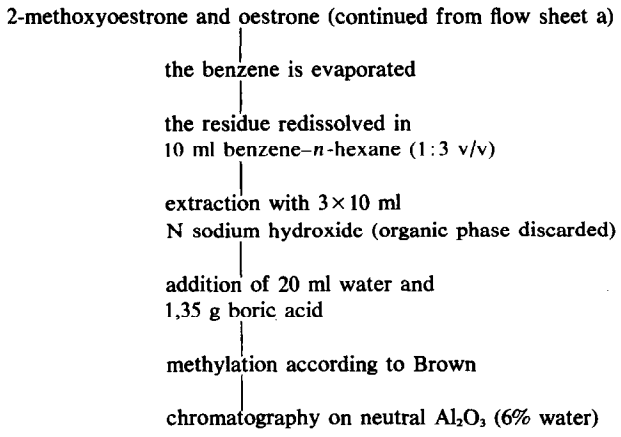

Fig. 2. Flow-sheet of the method (b).

samples was counted in a Philips liquid scintillation analyser using dioxane containing 50 mg POPOP (2,2'-phenylenbis-(5-phenyloxazol)), 7 g PPO 2,5-diphenyloxazol) and 50 g naphthalene per l as counting solution. One ml urine was counted in 10 ml counting solution. After addition of 10,000 d.p.m., all samples were counted again to correct for quenching. A correction was made for the detritiation of 2-methoxyoestrone in the C-2 position. The method used for fractionation of oestrone, oestradiol-17β, 2-methoxyoestrone and oestriol is based on the principles of the method of Brown[13]. Acid hydrolysis was performed as described by Frandsen[14]. 1/10 of the 48-h samples diluted to 400 ml with distilled water was autoclaved with 3% sulphuric acid at 127°C (1·6 kg/cm^2) for 1 h. After the hydrolysis 50 μg of each oestrogen was added to the samples to correct for the methodological loss. The oestrogens were extracted with ether and the ether extract was washed with alkaline solution as described by Brown[13]. After evaporation of the ether, oestriol was separated from the other oestrogens by solvent partition: 50 ml benzene–*n*–hexane–ethanol (25:25: by vol) and 2×25 water (oestriol). The further purification of this metabolite follows the procedure described by Brown[13].

Girard separation. The other oestrogens were subjected to a Girard-separation, separating ketonic from non-ketonic compounds[15]. After evaporation of the benzene–*n*-hexane–ethanol, the residue was redissolved in 0·5 ml acetic acid, 0·1 g Girard Reagens T was added and placed in a boiling water bath for 20 min. After cooling in ice, 3·75 ml 2N sodium hydroxide and 15 ml cold water were added. The non-ketonic fraction containing oestradiol-17β was isolated by extraction with 2×20 ml ether. To the ketonic fraction 4·5 ml 20% sulphuric acid was added and left for 2 h at room temperature. The oestrogens: 2-methoxyoestrone and oestrone were extracted with 25 ml benzene and the benzene extract washed with 2 ml 8% sodium hydrogencarbonate solution and with 2 ml water. The benzene was evaporated to dryness.

The neutral steroids were eliminated from the non-ketonic fraction by solvent partition between N sodium hydroxide and chloroform–carbon tetrachloride–*n*-hexane (1:2:3 by vol) and from the ketonic fraction by solvent partition between N sodium hydroxide and benzene–*n*-hexane (1:3 v/v). The organic phases were

discarded and the oestrogens in the N sodium hydroxide were methylated and purified through chromatography on columns of aluminium oxide according to Brown[13]. One fifth of each fraction was subjected to Kober reaction to correct for methodological losses and 4/5 counted in a solution of 4 g omnifluor, NEN chemicals per 1 toluene. All countings were corrected for quenching.

Chromatography of 2,3-dimethoxyoestrone (ketonic fraction). This was performed as described for 3-methyl-oestradiol-17β (Brown, 13): A 2 g column of partially deactivated aluminium oxide (6% water) was packed in *n*-hexane and the *n*-hexane extract was transferred to the column. It was then eluted with respectively:

(a) 10 ml benzene–*n*-hexane (4:6, v/v)
(b) 20 ml benzene–*n*-hexane (45:55, v/v)
(c) 10 ml benzene–*n*-hexane (45:55, v/v)
(d) 25 ml benzene.

Fraction a and c were discarded. Fraction b contained 3-methyloestrone and fraction d 2,3 dimethoxyoestrone.

Estimation of the methodological losses of 2,3-dimethoxyoestrone (Kober reaction). 1·9 ml 70% sulphuric acid with 2% hydroquinone was added to each tube and after mixing, placed in a boiling water-bath for 15 min. The tubes were cooled and returned to the boiling water-bath for 10 min, after addition of 1·1 ml distilled water. The colour developed was measured at the following wavelengths: 515–550–585 nm. The corrected densities at 550 were calculated according to the equation: Corr. D 550 = 2 × D 550 − (D 515 + D585).

Reliability of the method. This point was examined according to the principles given by Borth[16]. They comprise accuracy, precision, specificity and sensitivity.

The reliability of the method of Brown 1955[13] has been investigated by several authors[13,17] and found satisfactory, consequently we have restricted the examination of the reliability of the method to the metabolite 2-methoxyoestrone.

The accuracy was examined through recovery experiments. Five and 10 μg of 2-methoxyoestrone were added to hydrolysed urines containing very little oestrogen by biological estimations. The values were corrected for endogeneous content of 2-methoxyoestrone. The results are given in Table 3.

The destruction of 2-methoxyoestrone during acid hydrolysis was estimated in

Table 3. Recovery of 2-methoxyoestrone added to hydrolysed urines. The values were corrected for the endogeneous content of 2-methoxyoestrone

Number of estimations	10	10
Amounts added	5 μg	10 μg
Recovery (μm)	4·05	8·19
Recovery (%)	81	82
S.D. (μg)	0·20	0·17
S.D. % of mean	4·9	2·1

the following way: 10 μg of 2-methoxyoestrone was added to male urine, before and after acid hydrolysis and the losses due to the hydrolysis were 8·2%, with a S.D. of $\pm$ 3·2% (N = 8).

The precision was examined through 23 single estimations on a pregnancy urine pool. The mean value obtained was 550 μg per 24 h, and the standard deviation was 28 μg equivalent to $\pm$5%.

The specificity was examined by comparing values obtained by the Kober-reaction with values obtained when thin layer chromatography was performed before the Kober-reaction and with values obtained after gas chromatography.

20 pregnancy urines mixed in 5 pools were used for the investigation. The 5 pools were carried through the procedure and divided into 4 aliquots, two for Kober-reaction, one for chromatography on thin layer and one for gas chromatography.

The plates were coated with 0·5 mm silica gel G (Merck) and the solvent system used was chloroform–ethanol (99:1, v/v).

The gas chromatography was performed on a chromatograph with a flame ionisation detector (Pye). 100–120 mesh gas chrome Q coated with 2% S.D. 30 was used as support. The column length was 5 feet, the carrier gas nitrogen had a flow of 75 ml/min and the temperature was 210°C. 3-methoxyoestrone, which had a retention time of 4·35 min was used as internal standard. The retention time of 2,3 dimethoxyoestrone was 7·45 min. The results are shown in Table 4.

Based on the experiments it can be concluded that the compound estimated was identical with 2-methoxyoestrone.

Sensitivity. Investigating the specificity (Table 4) the unspecific substances amount to 3 μg/24 h after t.l.c. and 9 μg/24 h after g.l.c. It is therefore assumed that values below 20 μg/24 h are unreliable.

Radiochemical purity. In 8 urine samples, 3 from normal men, 3 from men with breast cancer and 2 from men with other malignant diseases, the radiochemical purity of the 4 oestrogen fractions was controlled by thin layer chromatography in two systems. Oestrone, oestradiol-17β and 2-methoxyoestrone in chloroform–ethanol (99:1)v/v and in cyclohexane–ethylacetate (2:1 v/v). The solvent systems used for oestriol were benzene–ethanol (8:2 v/v) and ethylacetate–cyclohexane–ethanol (45:45:10 by vol). The results are seen in Table 5.

Table 4. The specificity of the estimation of 2-methoxyoestrone examined by thin-layer and gas chromatography. See text. The values are given in μg/24 hr

Extract number	Kober	TLC	GLC
1	210	204	203
2	304	304	254
3	285	265	278
4	229	249	253
5	275	269	270
Mean	261	258	252

Table 5. Recovery of radioactivity in the 4 oestrogen fractions after thin-layer chromatography in two systems

	E_1*	E_2	E_3	2-MeE_1
Number of estimations	16	16	16	16
Recovery ± S.D. (%)	94·8±2·2	95·0±2·8	95·3±1·9	94·3±2·9
Range (%)	91·7–98·0	89·1–99·2	91·8–99·4	90·0–99·1

*Abbreviations: E_1: oestrone, E_2: oestradiol-17β, E_3: oestriol, 2-MeE_1: 2-methoxyoestrone.

The total oestrogens were estimated according to the method described by Brown *et al.*[18].

RESULTS

The following steroids were estimated in 48 h urine samples: oestrone, oestradiol-17β, oestriol and 2-methoxyoestrone.

The total oestrogens were estimated in all subjects.

Recovery of radioactivity (Tables 6–8). The percentage of the administered radioactivity recovered in the urine did not show any significant difference between the three groups of men, neither did the percentage of administered radioactivity recovered as oestrone+oestradiol-17β+oestriol+2 methoxyoes-

Table 6. Recovery of radioactivity in the urine after intravenous administration of tritiated oestradiol. Healthy men*

Subject	Per cent of administered radioactivity recovered in the urine: Totally	As E_1+E_2+E_3+2-MeO–E_1	Per cent of E_1+E_2+E_3+2-MeO–E_1 in the urine recovered as: E_1	E_2	E_3	2-MeO–E_1
KP	72	12	21	21	47	11
CL	50	9	37	18	19	25
BC	52	9	19	16	58	6
HK	62	13	46	17	19	18
HM	64	16	30	12	41	18
CN	50	10	18	16	41	26
VM	63	12	38	16	34	13
VS	62	15	14	11	70	5
HW	51	12	28	13	48	11
VA	25	5	25	3	71	2
EC	43	9	30	11	47	13
CA	46	9	24	16	36	24
HP	49	11	24	13	43	19
FS	51	11	30	8	48	14
JR	56	18	16	8	70	6
EG	54	13	18	27	50	6
AS	47	13	19	1	70	10
JC	55	12	32	15	43	11
SS	57	16	18	9	69	4
Mean±S.D.	53±10	12±3	26±9	13±6	49±16	13±7

*Abbreviations, oestrone, E_1; oestradiol-17β, E_2; oestriol, E_3; 2-methoxyoestrone, 2-Me–O_1.

Table 7. Recovery of radioactivity in the urine after intravenous administration of tritiated oestradiol. Men with breast cancer

Subject	Per cent of administered radioactivity recovered in the urine: Totally	As $E_1+E_2+E_3$+2-MeO–E_1	Per cent of $E_1+E_2+E_3$+2-MeO–E_1 in the urine recovered as: E_1	E_2	E_3	2-MeO–E_1
BP	47	9	23	31	34	13
SK	64	11	20	13	64	4
WJ	78	16	31	10	46	13
HH	61	12	23	10	59	9
VG	49	7	30	13	32	25
GL	60	14	22	16	52	11
JM	53	15	45	20	19	16
KH	51	12	35	9	48	9
NV	28	11	36	11	45	9
JJ	61	15	24	6	50	21
MS	60	17	26	13	52	9
AK	49	11	36	14	35	15
EB	59	13	16	12	66	6
CP	27	5	22	7	61	10
AR	61	14	30	13	45	12
PP	58	11	21	13	32	34
CR	45	8	32	14	37	18
AJ	51	12	25	13	50	13
LM	61	7	21	14	57	9
Mean±S.D.	54±12	11±3	27±7	13±5	46±13	13±7

Table 8. Recovery of radioactivity in the urine after intravenous administration of tritiated oestradiol. Men with other malignant diseases

Subject	Per cent of administered radioactivity recovered in the urine: Totally	As $E_1+E_2+E_3$+2-MeO–E_1	Per cent of $E_1+E_2+E_3$+2-MeO–E_1 in the urine recovered as: E_1	E_2	E_3	2-MeO–E_1
KE	57	12	38	21	29	12
FH	49	11	23	18	46	13
EK	54	15	5	3	90	2
JP	50	11	23	31	30	16
JQ	77	24	29	8	56	7
Mean±S.D.	57±11	15±6	24±12	16±11	50±25	10±6

trone ($P>0{\cdot}05$).* This indicates that there was no difference in the urinary metabolites of the administered oestradiol-17β.

The percentage of oestrone+oestradiol-17β+oestriol+2-methoxyoestrone recovered in the urine as the individual metabolites: oestrone, oestradiol-17β, oestriol and 2-methoxyoestrone is shown in Tables 6–8. There was no significant difference between the three groups of men ($P>0{\cdot}05$).*

The excretion of total oestrogens was estimated in the three groups. The

*Statistical method employed: The Wilcoxon Test for two samples.

following results were obtained (μg/24 h): Men with breast cancer, mean value: 14 (range 0–29), healthy men, 13 (6–21), men with other malignant diseases, 13 (10–18). The mean values of the two groups of patients did not deviate significantly from those of the healthy men. ($P > 0{\cdot}05$).†

DISCUSSION

The results in the study of Zumoff *et al.*[10] which showed an abnormality in the transformation of administered oestradiol-17β in men with breast cancer, could not be confirmed in this study. This could be due to various factors in the two studies, A: Differences in the patients, B: Different results obtained from the healthy control groups.

Ad A: Studying the clinical information on the 6 male breast cancer patients in the work of Zumoff *et al.*[10] it appears that 3 of the 6 patients were marked by their disease with weight loss, dyspnoe etc., and half of these patients had congestive heart failure probably because of myocardial infarction. All our 19 breast cancer patients were ambulatory and feeling well and none of our patients presented signs of myocardial infarction. As previously mentioned, we have ruled out factors which may influence the metabolism of the oestrogens. Some of the factors so far reported are liver disease[19], dysthyroidism[20], myocardial infarction[21], acute stress[12,39] ACTH therapy[12] and carcinoma of the bronchus complicated with osteoarthropathy[22].

Ad B: Few authors have studied the excretion of the individual oestrogens in normal men in the elder age group. This might be due to the fact that these men excrete the individual metabolites in so small amounts that the reliability of the methods employed becomes dubious. Nevertheless some authors, using modifications of the earlier analytic methods have reported some data. In 1961 Pincus[23] investigated the excretion of oestrone + oestradiol-17β and oestriol in the urine samples from 308 normal healthy men (age 28–71). He found a decline with age in the excretion of oestrone + oestradiol-17β but a much less marked decline in the oestriol excretion. Consequently the ratio oestriol/oestrone + oestradiol-17β was higher in the elderly men than in the young and greater than unity in all ages.

In 1962 Morse *et al.*[24] investigated the excretion of oestrone, oestradiol-17β and oestriol in the urine of 7 men (mean age 26 years) and of 16 men (mean age 71 years). In this study the ratio oestriol–oestrone was higher in elderly men than in the young. Morse *et al.*[24] suggested a greater proportionate conversion of oestrone to oestriol in elderly men. In 1964 Hobkirk and Nilsen[25] investigated the excretion of oestrone, oestradiol-17β and oestriol in the urine of 15 men (age 17–72). The mean ratio oestriol–oestrone + oestradiol-17β was greater than unity in these subjects.

The data reported by these authors are in good agreement with our results in normal men. In all four studies the ratio oestriol–oestrone is greater than unity in men in the elder age group. Other authors have performed similar investigations in men of younger age group[26, 27]. In these studies the ratio oestriol–oestrone is less than unity.

Investigations into the metabolism of administered labelled oestradiol-17β in normal elderly men are few. In 1968 Zumoff *et al.*[28] studied the excretion pattern

†Statistical method employed: Student's *t*-test.

of the urinary metabolites of labelled oestradiol-17β administered to a group of 6 healthy young men (aged 22–33) and compared the results from this group with the results from the group of healthy elderly men used as controls to the patients with male breast cancer in the study from 1966[10]. Both groups of healthy men showed oestriol–oestrone ratios below 1. The ratio oestriol–oestrone was in our 19 healthy men greater than unity in 16 cases and less than unity in 3 cases. The basis for the evaluation of the oestrone and the oestriol excretion in the patients with male breast cancer is, as can be seen, widely different in the two studies.

Studies on the oestrogen excretion in the urine of female patients with breast cancer have been performed by several authors.

Concerning the excretion of "total oestrogens" (oestrone + oestradiol + oestriol) no excretion pattern specific for breast cancer patients has been reported[29]. In premenopausal women with breast cancer the ratio oestriol–oestrone + oestradiol is reported to be subnormal by some authors[30, 31], other authors have reported a ratio above normal[32, 33]. In postmenopausal women a ratio below normal has been reported by Schweppe *et al.*[34]. Persson and Risholm[32] however report opposite results. In 1958 Brown[35] showed that postmenopausal women with breast cancer after injection of 2,5 mg oestradiol-17β intramusculary excreted "total oestrogens" in amounts similar to normal women, but the ratio oestriol–oestrone + oestradiol was above normal.

Some investigations on the metabolism of administered small tracer doses of labelled oestradiol-17β have been performed in female breast cancer patients, the results are however conflicting. In 1965 Crowley *et al.*[7] reported a study of the urinary excretion pattern of the metabolites of oestradiol-4-^{14}C administered to a group of 38 postmenopausal breast cancer patients and a group of 5 postmenopausal women with benign mammary dysplasia. In both groups the ratio oestriol–oestrone + oestradiol was below normal. Hellman *et al.* (1967)[8] studied the transformation of ^{3}H-oestradiol in a group of 11 postmenopausal women with breast cancer compared with 5 healthy postmenopausal women. No difference was found between the two groups. In a later report[9] the same authors performed the same study in a group of 23 women of varying age with breast cancer including the women previously reported on. In this larger study an increase in oestriol glucosiduronate and a decrease in oestrone and 2-methoxyoestrone was found in the patients with breast cancer compared with a group of 12 healthy women of varying age.

A prospective study by Bulbrook and associates[36,37] indicates that the majority of women who subsequently develop breast cancer excretes subnormal amounts of androgens and corticosteroids. The results of Aldercreutz *et al.* [38] and Bulbrook[39] suggests that the excretion of oestrogens in women follows the same pattern as that of the androgens. If this correlation exists in the majority of women and in patients with breast cancer, Bulbrook[39] advances the hypothesis that cancer of the breast might arise more frequently in women excreting subnormal amounts of oestrogens, androgens and corticosteroids in the preclinical phase of the disease.

Lemon[40] suggests that oestriol plays a significant anticarcinogenic role in female breast cancer: as an "impeded" non carcinogenic oestrogen it competes with oestradiol-17β for oestrogen receptor proteins of the breast. By displacing oestradiol-17β from these receptor proteins it may protect the target organ from the possible carcinogenic effect of oestradiol-17β.

The following conclusion can be drawn from our study:

The excretion pattern of oestrone, oestradiol-17β, oestriol and 2-methoxyoestrone following the administration of oestradiol-17β has been found to be the same in men with breast cancer compared with healthy men and men with other malignant diseases.

ACKNOWLEDGEMENTS

Miss Ellen Green provided excellent technical assistance. The work was supported by a grant from the National Anti-Cancer League.

REFERENCES

1. Lacassagne A.: *C.R. Acad. Sci. (Paris)* **195** (1932) 630.
2. El-Gazayerli M. M. and Abdel-Aziz A. S.: *Br. J. Cancer* **17** (1963) 566.
3. Davies (1950) (quoted by Sinner W.): *Strahlentherapie* **115** (1961) 524.
4. Demoix (1950) (quoted by Sinner W.): *Strahlentherapie* **115** (1961) 524.
5. Marsden A. T. H.: *Schweiz. Z. allg. Path. Bakt.* **18** (1955) 728.
6. Symmers W. St. C.: *Brit. med. Jl.* **2** (1968) 83.
7. Crowley L. G., Demetriou J. A., Kotin P., Donovan A. J. and Kushinsky S.: *Cancer Res.* **25** (1969) 371.
8. Hellman L., Fishman J., Zumoff B., Cassouto J. and Gallagher T. F.: *J. clin. Endocr.* **27** (1967) 1087.
9. Hellman L., Zumoff B., Fishman J. and Gallagher T. F.: *J. clin. Endocr.* **33** (1971) 138.
10. Zumoff B., Fishermann J., Cassouto J., Hellman L. and Gallagher T. F.: *J. clin. Endocr. Metab.* **26** (1966) 960.
11. Visfeldt J. and Scheike O.: *Male Breast Cancer—I.* Histological Typing and Grading of 187 Danish Cases, in press.
12. Brown J. B., Falconer C. W. A. and Strong J. A.: *J. Endocr.* **19** (1959) 52.
13. Brown J. B.: *Biochem. J.* **60** (1955) 185.
14. Frandsen V. A.: *The Excretion of Oestriol in Normal Human Pregnancy.* Munksgaard, Copenhagen (1963).
15. Pincus G. and Pearlman W. H.: *Endocrinology* **29** (1941) 413.
16. Borth R.: *Ciba Found. Endocrinology* **2** (1952) 45.
17. Bulbrook R. D., Greenwood F. C. and Williams P. C.: *J. Endocr.* **20** (1960) 220.
18. Brown J. B., MacLeod S. C., MacNaughtan C., Smith M. A. and Smyth B.: *J. Endocr.* **42** (1968) 5.
19. Zumoff B., Fishmann J., Gallagher T. F. and Hellman L.: *J. clin. Invest.* **47** (1968) 20.
20. Gallagher T. F., Fukushima D. K., Noguchi S., Fishman J., Bradlow H. L., Cassouto J., Zumoff B. and Hellman L.: *Recent. Prog. Horm. Res.* **22** (1966) 283.
21. Bauld W. S., Givner M. L. and Milne I. G.: *Can. J. Biochem.* **35** (1957) 1277.
22. Ginsburg J. and Brown J. B.: *Lancet* **2** (1961) 1274.
23. Pincus G.: *Growth in Living Systems* pp. 407–20. Basic Books Inc., New York (1961).
24. Morse W. I., Clark A. F., MacLeod S. C., Ernst W. A. and Gosse C. L.: *J. clin. Endocr.* **22** (1962) 678.
25. Hobkirk R. and Nilsen M.: *Steroids* **3** (1964) 453.
26. Bersohn I. and Oelofse P. J.: *S. Afr. med. J.* **32** (1958) 979.
27. Preedy J. R. K. and Aitken E. H.: *J. biol. Chem.* **236** (1961) 1300.
28. Zumoff B., Fishman J., Cassouto J., Gallagher T. F. and Hellman L.: *J. clin. Endocr.* **28** (1968) 937.
29. Lemon H. M.: *Cancer* **23** (1969) 781.
30. Bacigalupo G. and Schubert K.: *Eur. J. Cancer* **2** (1966) 75.
31. Lemon H. M., Wotiz H. H., Parsons L. and Mozden P. J.: *J. Am. med. Ass.* **196** (1966) 112.
32. Persson B. H. and Risholm L.: *Acta endocr., Copenh.* **47** (1964) 15.
33. Marmorston J., Crowley L. G., Myers S. M., Stern E. and Hopkins C. E.: *Am. J. Obstet. Gynec.* **92** (1965) 460.
34. Schweppe J. S., Jungman R. A. and Lewin I.: *Cancer* **20** (1967) 155.
35. Brown J. B.: *Endocrine Aspects of Breast Cancer*, pp. 213–220. E. and S. Livingstone, Edinburgh (1958).

36. Bulbrook R. D. and Hayward J. L.: *Lancet* **1** (1967) 519.
37. Bulbrook R. D., Hayward J. L. and Spicer C. C.: *Lancet* **2** (1971) 395.
38. Adlercreutz H., Luukkainen T. and Svanborg A.: *Annls Med. exp. Fenn.* **45** (1967) 227.
39. Bulbrook R. D.: *Adv. steroid Biochem. Pharmac.* **1** (1970) 387.
40. Lemon H. M.: *Cancer* **25** (1970) 423.