The following proportions of patients in different groups underwent objective remission: Whole series, 32/75 (43%). Those with osseous metastases, 27/52 (52%); without osseous metastases, 5/23 (22%). Pre-menopausal patients, 13/21 (62%). Post-menopausal patients, 19/54 (35%). Those who had their operations within six months of the disease becoming uncontrolled, 23/45 (51%); more than six months, 9/30 (30%). Pre-menopausal patients with osseous metastases, 13/17 (76%).

Previous endocrine therapy influenced the response in various ways.

Age, the total duration of the disease, and the histological structure of the tumour did not influence the result.

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OESTROGEN EXCRETION OF PATIENTS WITH BREAST CANCER*

BY

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By means of the more sensitive assay techniques now available it has been established that oestrogens may persist after oophorectomy and adrenalectomy in the urine of patients suffering from breast cancer. Jessiman (1958), using the bio-assay technique of Smith and Smith (1952), reported the presence of oestrogens after oophorectomy and adrenalectomy, while Dao (1953), also measuring biological activity, had found a fall in oestrogen levels after castration but complete disappearance after adrenalectomy. Chemical determinations by the Brown method (Bulbrook and Greenwood, 1957; Greenwood and Bulbrook, 1957; Bulbrook, Greenwood, Hadfield, and Scowen, 1958) indicated the persistence of oestrone (OE), oestradiol (OD), and oestriol (OT) in urine after both these operations and also after hypophysectomy. Although Diczfalussy and his colleagues (1958) questioned the nature of the small quantities of oestrogen-like chromogens

measured by the Kober reaction used in Brown's method, parallel chemical and biological assays by Bulbrook, Greenwood, and Williams (1958) confirmed the presence of oestrogenic activity in urine extracts from post-operative patients where small amounts of these substances had been determined chemically.

Since it was suggested many years ago that the continued growth of mammary carcinoma might be dependent on oestrogenic hormones and improvement could be achieved by removal of the ovaries, many comparisons have been made between the quantities of oestrogens excreted by patients with breast cancer and their clinical condition, but no correlation has yet become apparent.

The aim of this present investigation was to study oestrogen excretion in conjunction with subsequent clinical progress to see if pre-operatively it might be possible to predict from the hormone picture a patient's response to oophorectomy or adrenalectomy. The post-operative progress was compared with any alteration in amounts or pattern of hormone excretion caused by the operation; and in addition the histology of the primary tumour was reviewed in connexion with oestrogen levels and response to treatment.

Methods

The methods used were those of Braunsberg, Osborn, and Stern (1954) and Braunsberg, Stern, and Swyer (1954) for OE and OD, utilizing partition chromatography and fluorimetry; OT was measured by Brown's method, including the additional purification step (Brown, 1955; Brown, Bulbrook, and Greenwood, 1957).

Assays were made on 24-hour urine specimens collected before operation and, in most cases, within three weeks afterwards. It proved very difficult to obtain additional specimens once the patients had left hospital, so only the results of immediate post-operative determinations were available for comparison, and it was not known what further changes might have occurred during the subsequent course of the disease.

Vaginal smears were usually taken on the same day as the urine collection and stained with Shorr's stain. The percentages of cornified and basal cells were recorded.

Fifteen pre-menopausal women, whose ages ranged from 37 to 50, with an average of 45, were investigated before and after oophorectomy. Five others, aged between 37 and 51, who had previously been castrated, underwent adrenalectomy. A further four patients aged 38 to 66 had a combined operation in two stages—namely, bilateral oophorectomy and unilateral adrenalectomy, followed 14 days later by removal of the second adrenal.

Assessment of the effect of hormone ablation was often very difficult and depended on clinical impression rather than definite evidence. Patients showing signs of regression of bone metastases, even temporarily, disappearance of skin metastases, or alleviation of bone pain were classed as "improved." Also described as improved were cases where there was some indication that life had been prolonged in a state reasonably free from distress. In a number of patients the clinical response was either equivocal, in which case they were grouped with the "not improved," or it was too early after the operation for assessment to be made.

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Results

All the 15 menopausal women showed a fall in "total" oestrogen levels—that is, the sum of OE, OD, and OT—after operation, although in only six of them was the decrease significant ($P < 0.05$). In three instances the value for P was between 0.1 and 0.5, and in the remaining six cases there were not enough assays to test for significance. This drop was due mainly to decreased OT excretion, shown by all patients to some extent and being significant ($P < 0.05$) in four. OE and OD fell less obviously and in a few cases even rose slightly post-operatively. One post-menopausal and two menopausal patients who were also studied showed similarly reduced oestrogen values after castration.

There was no significant difference between the pre-operative oestrogen levels of the "improved" and "not improved" groups; nor did these two classes show any difference in the amounts by which oestrogen excretion decreased after operation. However, the proportions of the three oestrogens measured pre-operatively showed some variation, the OE plus OD:OT ratio being lower in the group which was considered to have responded favourably to the treatment. After oophorectomy the greater fall observed in OT levels compared with those of OE plus OD, led to a rise in the ratio in both categories of patients so that there was no longer any obvious difference between them (see Table I).

Gonadotrophin assays were done by Dr. June East on four of the pre-menopausal women. Urine specimens were extracted chromatographically by a modification of the method of Johnsen (1955) and assayed in immature female T.O. mice by the uterus-weight technique (Klinefelter, Albright, and Griswold, 1943). Estimation of the renal enzyme β -glucuronidase in mature male mice was also used as an index of gonadotrophic activity as proposed by East (1959). Three patients showed some elevation of gonadotrophin titre after oophorectomy, corresponding with a fall in oestrogen levels. In the fourth, whose oestrogens decreased only slightly, there was no change in gonadotrophin output. Although this patient responded dramatically to operation she soon relapsed again, but the other three have maintained their improvement for more than a year.

TABLE I.—Pre-operative Excretion of Oestrogens, and Changes Due to Oophorectomy in Patients who Did and Did Not Improve

	Improved		Not Improved and Equivocal		P
	Mean (μ g./24 Hours)	S.D.	Mean (μ g./24 Hours)	S.D.	
Pre-oophorectomy levels:					
OE + OD + OT	27.83	21.44	24.20	19.18	0.8-0.7
OT	20.21	18.23	14.38	15.92	0.5-0.4
OE	5.02	2.55	7.22	4.14	0.2-0.1
OD	2.58	1.15	2.58	1.19	>0.9
Decrease:					
OE + OD + OT	15.25	17.86	19.37	23.27	0.8-0.7
OT	13.96	16.74	15.58	16.83	0.9-0.8
OE	1.33	2.24	3.62	5.81	0.4-0.3
OD	-0.06	1.43	1.08	1.04	0.2-0.1
Pre-oophorectomy:					
OE + OD	0.56	0.30	1.91	2.12	0.1-0.05
OT	(Range 0.24 to 1.06)		(Range 0.24 to 5.76)		
Post-oophorectomy:					
OE + OD	2.11	2.21	2.84	2.13	0.6-0.5
OT					

Adrenalectomy alone, performed on five previously castrated women, resulted in diminished oestrogen excretion in three, little change in one, and an increase in the fifth. The average pre-operative values were similar whether there was any subsequent clinical improvement or not. Likewise, post-operatively there was apparently no connexion between response and oestrogen levels. In four patients the OE plus OD:OT ratio before adrenalectomy was high, suggesting that, as expected, the amount of OT excreted had fallen owing to oophorectomy, although no determinations were done at that time. The other patient, who alone experienced no improvement, had a lower ratio. OT was most affected by adrenalectomy, and in all cases the ratio rose post-operatively.

Combined oophorectomy and adrenalectomy was done on four patients. This procedure resulted in decreases in the amounts of OE and OT measured, but had least effect on OD. One of the pre-menopausal women improved but her oestrogen values and OE plus OD:OT ratio were within the range of the other two patients, whose conditions remained unchanged. Ablation of ovaries and adrenals seemed to make little difference to the hormone levels found in the urine of a 66-year-old woman who was 20 years post-menopausal, yet she benefited from this treatment.

Histology

When relating the histological type of tumour with oestrogen levels and with the subsequent progress of the breast cancer, the original primary histology was divided between the extremes of differentiated and undifferentiated. In general the histological diagnosis was quite

TABLE II.—Comparison of Response to Operation Shown by Patients with Differing Histology of the Primary Tumour

	Differentiated			Undifferentiated		
	Total No.	Improved	Equivocal or Not Improved	Total No.	Improved	Equivocal or Not Improved
Oophorectomy	7	4	3	8	3	5
Oophorectomy and adrenalectomy	3	3	0	5	2	3

clear: the growth was described either as anaplastic and therefore undifferentiated or as well differentiated. In two cases the histology was inconclusive, as in one the material was from a drill biopsy, and in the other there were areas of differentiated and undifferentiated tissue in the same growth.

There was no significant difference between the absolute amounts or the ratios of oestrogens excreted by women with the two types of tumour.

It has been suggested that the more differentiated type of neoplasm was associated with hormone dependence, and that cases of undifferentiated carcinoma were unlikely to benefit from endocrine ablation, but this was not always borne out. Several investigators of this problem have confirmed that histological classification did not give any indication of the response. In the present series, improvement after oophorectomy alone, or oophorectomy together with or followed by adrenalectomy, has been shown by patients with both types of primary tumour (see Table II).

Discussion

Evaluation of 116 vaginal smears showed that although some correlation existed between the overall cytological picture and urinary oestrogen values

estimated chemically, it was of a low order, particularly in the intermediate ranges. One may conclude that single occasional smears are not accurate enough to give a useful indication of oestrogenic activity in this group of women.

Even with sensitive chemical determinations, selection of those patients most likely to benefit from endocrine ablation is apparently not possible by means of OE, OD, and OT measurements alone. The trend shown by this small series suggests that the subjects with the larger amounts of the more active oestrogens, OE and OD, relative to the less active OT, may be the ones less likely to respond to castration. However, there is much overlapping, and the ratio OE plus OD:OT would not seem to have any practical predictive value.

In most cases the operations were carried out as therapeutic procedures when metastases had actually occurred, rather than as a prophylactic measure. It might be that by the time these women were considered for gonadectomy, tumours which were possibly once hormone-dependent had become autonomous. Another possibility is that tumour growth is dependent not on the absolute amounts of oestrogens secreted but on the balance between the various oestrogens, their metabolites, and other hormones (Bulbrook, Greenwood, and Hayward, 1960). It may be that where surgery is effective it succeeds by altering that balance.

Summary

Oestrone, oestradiol, and oestriol estimations were made pre- and post-operatively on patients with breast cancer who underwent oophorectomy or adrenalectomy, or both.

No difference was found in the pre-operative oestrogen levels of patients who improved and those who did not.

Clinical progress was not correlated with the decrease in oestrogens caused by operation.

No connexion was evident between the histology of the primary tumour and oestrogen levels or clinical response.

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THIOTEPA IN CARCINOMA OF BREAST TREATED BY BILATERAL ADRENALECTOMY AND OOPHORECTOMY

BY

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Watson and Turner (1959) described a new approach to treatment of metastatic carcinoma of the breast by combining thiotepa with testosterone. It was claimed that the combination of the cytotoxic agent with testosterone proved more effective than either agent used alone. Tumour-inhibitory effects were seen in 88% (30 out of 34 cases). Encouraged by these results, it was decided at this hospital to try the effect of thiotepa on patients with advanced carcinoma of the breast who had previously been subjected to bilateral adrenalectomy and oophorectomy. The patients selected for treatment had either failed to improve after operation or had relapsed after a period of improvement.

Testosterone was given orally as methyltestosterone in doses of 20 to 30 mg. daily. This route of administration and the dosage were chosen as the method is the one used at this hospital. Nevertheless, the drug was poorly tolerated by many patients. Hirsuties, deepening of the voice, nausea, and vomiting often occurred, and sometimes hypertension and oedema developed in patients hitherto well controlled with adrenal substitution therapy. For these reasons testosterone treatment had often to be abandoned.

Thiotepa was given by intramuscular injection, starting with 15 mg. three times a week for two weeks, and thereafter 15 mg. weekly. This scheme was chosen because most cases were treated as out-patients. White-cell counts were initially performed twice weekly, and then weekly during maintenance therapy. Treatment was continued until leucopenia (defined as a total white-cell count of fewer than 4,000/c.mm.) rendered it hazardous or until deterioration was obvious. When patients developed leucopenia thiotepa was withheld until the white-cell count rose above 6,000/c.mm.; but usually there was a further fall in white cells, and eventually treatment with thiotepa had to be stopped.

Results

Of the 12 patients studied, five had had a period of improvement after bilateral adrenalectomy and oophorectomy varying from 11 to 30 months. In the remaining seven progression of the malignant disease had continued in spite of operation. Thiotepa produced temporary improvement in two patients (Cases 5 and 7). In the remainder no effect was seen. Details of the individual cases are summarized in the Table, and the case histories of the patients who improved are given below.

Case 5.—This patient underwent a radical mastectomy for carcinoma of the left breast in 1954, followed by a course of radiotherapy. She remained well until March, 1957, when she complained of a hoarse voice and dysphagia, and was found to have supraclavicular and cervical glandular secondaries, together with nodules in the scar of the radical mastectomy. Bilateral adrenalectomy and oophorectomy, performed in April, 1957, was followed by rapid relief of the dysphagia and hoarseness, and by partial regression of the glandular and skin secondaries. In September, 1959,