SUMMARY

Changes in the hormonal environment induced by endocrine ablation in women with metastatic breast cancer have been defined and compared with the clinical course of the disease.

Adrenalectomy plus oophorectomy causes more profound decreases in steroid hormone secretion and excretion than does pituitary implantation with 90-Yttrium, but does not appear to produce a higher proportion of remissions or remissions of longer duration. The preoperative excretion of one or more of these steroids is no guide to the subsequent response of the patient to treatment. In a considerable proportion of the cases, steroids of presumed adrenocortical origin are still excreted postoperatively but in a manner quite unrelated to the cause of cancer. Withdrawal of the maintenance therapy for a limited period, in order to define more clearly endogenous adrenal function, was also unable to demonstrate any significant difference between responding and non-responding patients.

The favourable outcome of treatment in about 20 per cent of cases lends support to the concept of "hormone responsiveness" of certain breast cancers, although the relationship between the disease and the endocrine system is obviously more complex than would permit the designation of these tumours as "oestrogen dependant".

Eventual relapse of the disease is however inevitable, and intensive study of the hormonal environment in the same patient when the disease was in remission and later when it was progressing again was unable to demonstrate changes which could be responsible. It must be concluded that this alteration in the behaviour of the tumour is due to changes in the nature of the cancer rather than changes in the hormonal stimulus, as far as this can be shown by the presently available assay techniques.

STEROID EXCRETION STUDIES IN BREAST CANCER

ACKNOWLEDGEMENTS

The work described in this thesis was carried out in the department of the Regius Professor of Surgery, Sir Charles Illingworth, and under the supervision of Professor J. N. Davidson of the Department of Biochemistry, to both of whom I wish to express my gratitude for their interest and encouragement. I am also indebted to Dr. R. Hobkirk for helpful discussions in the initial stages of the study.

The nature of this research involved both the medical and nursing staff of Professor Illingworth's wards in considerable additional work, and I am deeply appreciative of the skill and care with which they undertook this. I wish further to thank Mr. R. A. McAllister and members of the technical staff of the Surgery Department for their skilled assistance in the performance of several of the assays.

Finally, I wish to record my most sincere gratitude to Professor A. P. M. Forrest. In the first place his skilled surgical collaboration made the study possible, but much more than this, his deep interest in, and endless enthusiasm for this field of research was a constant source of inspiration.

The rationale for the hormonal approach to cancer rests on the assumption that the growth of particular malignant tissue is not autonomous, but retains some of the characteristics of the tissue from which it arose. In breast cancer this implies that the tumour, up to some point in its development, is dependent for its growth on the same hormones as govern the development of the normal breast parenchyma. The aim of such treatment therefore has been to define these essential hormones and to eliminate their secretion.

With the development of satisfactory procedures for the assay of many hormones and their metabolites in body fluids, the precise biochemical effects of the empirically adopted endocrine ablative procedures could be determined, and the success of the operation in terms of regression of the cancer, could be related to overall alterations induced in the hormonal status of the patient.

This thesis described studies carried out on the urinary excretion of several groups of steroid hormones and their metabolites in women with breast cancer, the alterations induced by oophorectomy, adrenalectomy and /

/and implantation of the pituitary gland with radioactive yttrium, and the relationship of these alterations to the growth of the cancer as judged by the clinical response of the patient.

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INTRODUCTION

INTRODUCTION.

The concept of endocrine influence in mammary cancer originated in the last years of the nineteenth century with clinical observations on the beneficial effects of oophorectomy in two patients with recurrent or advanced breast cancer (Beatson, 1896). Lack of consistent benefit from the operation led to oophorectomy being temporarily abandoned as a treatment of advanced cancer of the breast. In the succeeding thirty to forty years a large body of information was amassed on the induction and development of mammary tumours of all types in experimental animals (Review: Loeb, 1940). Later, with a background of the knowledge gained on the effects of ovarian, adrenal and pituitary secretions in experimental neoplasia, attempts were again instituted to modify the course of human breast cancer by hormonal means, firstly by the administration of sex hormones (Loesser, 1938; Ellis et al., 1944; Haddow, Watkinson and Paterson, 1944), and later when satisfactory substitution therapy became available, by more extensive ablation of the endocrine organs (Huggins and Dao, 1953; Luft and Olivecrona, 1953). /

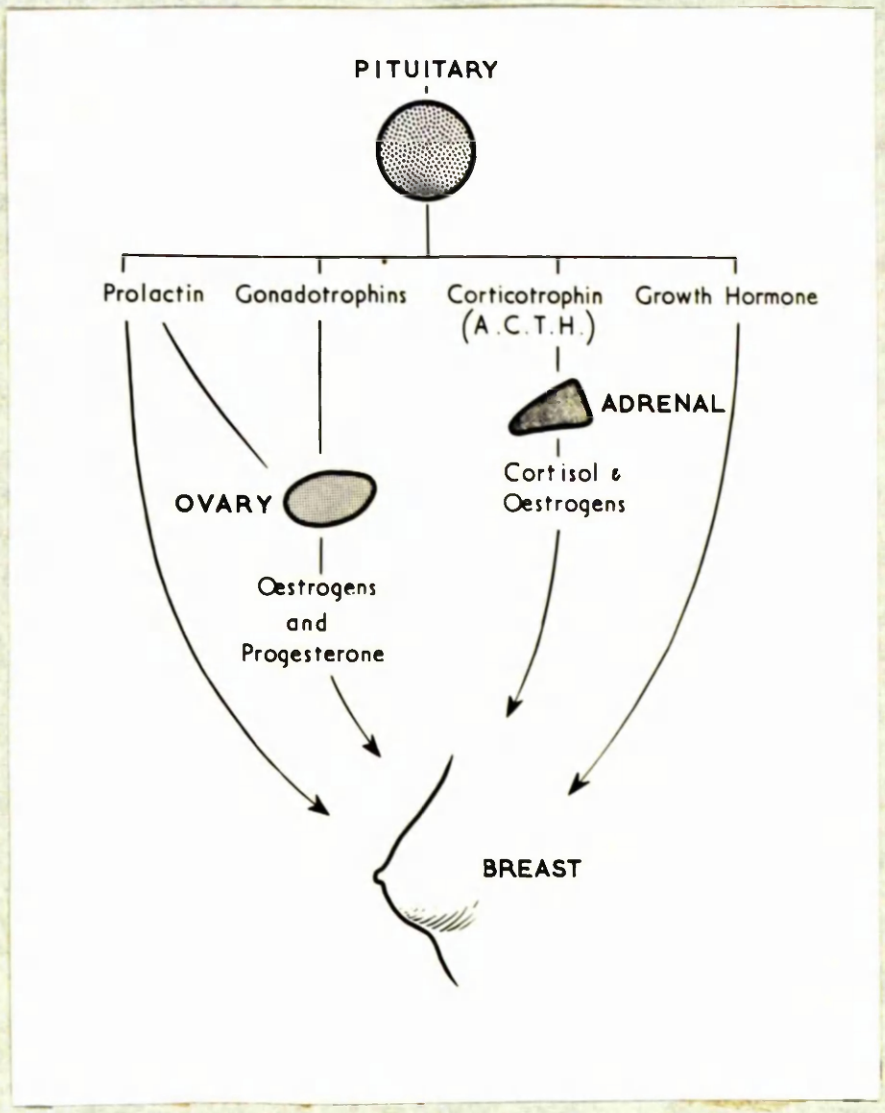

Fig. 1. Hormones concerned in normal breast development (Forrest 1957).

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Normal and neoplastic breast growth. The effect of the individual hormones of the ovaries, the pituitary and the adrenal glands on breast growth in the experimental animal, and their interrelationships and synergisms have been extensively studied over the past twenty to thrity years. (Reveiw: Cowie and Folley, 1958). It has been shown that in male and female rats deprived of some or all of their endogenous hormones by hypophysectomy, gonadectomy and in some cases also adrenalectomy, varying stages of mammary growth and development can be induced by administration of the appropriate hormones (Lyons, Johnson, Cole and Li, 1955).

The normal and pathological growth of the mammary gland was originally thought to be under the sole control of ovarian hormones. Later an overriding influence of the pituitary secretions was postulated and later still the adrenocortical hormones were also implicated. The so called "growth tetrad" of hormones, oestrone, progesterone, prolactin and growth hormone when injected in optimum proportions into hypophysectomised-oophorectomised virgin rats, will simulate full lobuloalveolar development of the /

/the breasts, characteristic of late pregnancy. Less complete development of the gland can be induced by omitting one or more of the hormones from the combination injected. This combination of hormones will not bring about lactation in these animals. This may only be initiated by administration of prolactin, growth hormone and ACTH or cortisone. The only morphological requirement for the induction of milk secretion by this "lactational triad" is the alveolus.

Most of the changes described have been reproduced on a slightly reduced scale in male rats (Lyons et al., 1955) and similar reactions have been described in weanling hypophysectomised male mice (Hadfield, 1957).

Although such direct evidence of hormonal action is lacking in the human, the hypertrophy of the breast associated with long-term oestrogen therapy in boty males and females, the changes in the breasts brought about by alterations in ovarian function, and the atrophy or failute of development caused by cessation of pituitary function, suggest that there is no reason to believe breast development and function in the human requires any less complex mechanism than that in the experimental animal. A hypothetical scheme for the interactions of the various factors is illustrated in Figure 1 (Forrest, 1957). /

Studies on the endocrine aspects of experimental carcinogenesis have mostly been carried out in mice, which, on account of their short life span and relatively high incidence of spontaneous malignant mammary tumours are the most satisfactory species available (Review: Lacassagne 1955). Rats develop, with moderate frequency, spontaneous mammary fibroadenoma, a benign lesion, which has also been studied quite extensively (Review: Noble and Cutts, 1959).

It was first observed over forty years ago that breeding females had a higher incidence of mammary cancer than virgin mice of a similar strain, and that early oophorectomy could reduce the frequency of tumour development (Lathrop and Loeb, 1916). The rôle of the ovary in the process of carcinogenesis was confirmed by the induction of mammary tumours in castrated male mice by ovarian implants (Murray, 1927). The factor of importance from the ovary was then shown to be its internal secretion when Lacassagne (1932) produced tumours in male mice of a cancer strain by repeated injection of oestrogen. Similar treatment, or subcutaneous implantation of the crystalline steroid, can also induce mammary carcinoma in rats /

/rats (Geschickter, 1939; Noble, McEuen and Collip, 1940). Although in combination with oestrogen, progesterone will produce mammary cancer in males of weak, or even cancer resistant strains, of mice, (Lacassagne, 1937; Gardner, 1939) there is no direct evidence implicating it, on its own, in mammary carcinogenesis. The presence of active corpora lutea however have been considered to be of importance by some authors (Law, 1941; Symeonidis, 1948). As evidence, they cite the low incidence of tumours in virgin mice, the greater frequency in those allowed to have a number of pregnancies, and the greatest frequency in those which had experienced successive gestations.

Strains of mice of high incidence of spontaneous tumours, in which congrol observations had shown mammary cancer to develop after three months oestrogen treatment, if hypophysectomised, develop no tumours after five months oestrogen administration (Lacassagne and Chamorro, 1939). In animals of the same strain, hypophysectomy abolished the mammary hyperplasia which had resulted from three months pretreatment with oestrogens, despite continued administration of the hormones. These findings have led to the belief that ovarian and hypophyseal secretions interact in their effect /

/effect on the mammary gland, the action of the latter probably being to prime the cells of the gland.

Contrary to early findings, more recently, Woolley, Fekete and Little (1939) have shown that in cancer strain mice oophorectomised at birth, after a lag phase, sexual development proceeded normally and mammary tumours developed, always associated with nodular hyperplasia of the adrenal cortex, which is assumed to be secreting oestrogenic hormones at a much higher than normal rate, under intense and continued stimulation by the anterior pituitary. This participation of the pituitary has been confirmed by the demonstration that hypophysectomy prevents the developments of adrenal adenomata and consequently of mammary cancer (Fergusoon and Visscher, 1953).

While the experimental animal has yielded useful information regarding breast cancer, few of the conclusions drawn from the studies appear to be applicable to the human disease and many inexplicable differences remain. This is exemplified by the fact that while, in the mouse repeated gestation increases the incidence of spontaneous mammary cancer, in women the disease is most frequent in the /

/the non-parous, the high incidence amon' nuns being an established fact (Review: Stocks, 1957).

Also in contrast to the findings in animals, oestrogens cannot be directly implicated in carcinogenesis in the human. There are reports of mammary cancer developing in males during the long term stilboestrol therapy for prostatic cancer (Darget, 1946; Howard and Grosjeau, 1949), and in women treated with oestrogens over a period of two to four years (Allabam and Owens, 1939); Parsons and McCall, 1941). These reports have however been questioned, and it is impossible on the available evidence, to condemn oestrogens as carcinogenic to humans. Notwithstanding this, the fact that the administration of oestrogens to a premenopausal woman with the established disease may cause exacerbation, and also that the administration of testosterone or the removal of ovaries and adrenal glands, or hypophysectomy, may cause a regression of the cancer, is circumstantial evidence of the important rôle of these steroid hormones in the development of human breast cancer.

Endocrine Methods of Treatment of Breast Cancer.

The responsiveness of mammary cancer to alterations in the hormonal environment of the host is an example of a /

/a species difference in the same disease.

In most strains of mice, spontaneous or induced mammary tumours are responsive to such changes only during the very earliest stages in their development, if at all (Haddow, 1938; Nathanson and Andervont, 1939; Martinez and Bittner, 1954), although a tumour in a hybrid strain of mice which only grows during pregnancy, regressing after parturition, has been described (Foulds, 1958). In the rat, hormone responsive tumours, mostly of a benign nature, have been described by Noble and Collip (1941) and by Huggins and Mainzer (1958). Shay, Aegerter, Gruenstein and Komarov (1949) showed the induction of an interesting mammary carcinoma in rats by the intragastric instillation of 3-methylcholanthrene. Huggins and his colleagues (Huggins, Briziarelli and Sutton, 1959) have subsequently confirmed these tumours to be of a hormone responsive type.

In the human disease hormonal treatment of any kind is not normally embarked upon until the cancer has reached an advanced stage. Despite what would appear to be rather unpromising circumstances, about 30 per cent. of women thus treated obtain a worthwhile remission of their disease, judged by strict objective standards. /

/standards.

Surgical oophorectomy in the premenopausal woman has been reported to give an objective remission rate of 43.7 per cent. in 191 cases (Treves, 1957) and castration in males with advanced breast cancer may also bring about a remission of the disease.

Reviewing 936 patients with mammary cancer treated with sex hormones, the Sub-committee of the American Medical Association concluded that andorgens are superior in relieving symptoms in both pre- and post-menopausal patients and for healing bone metastases. On the other hand, oestrogens are superior in causing regression of soft-tissue metastases in postmenopausal women and in inducing regression of primary tumours in postmenopausal women.

Since the introduction of bilateral adrenalectomy for the treatment of prostatic and mammary carcinoma, (Huggins and Dao, 1953) the very many reports which have been published suggest that this procedure, usually combined with oophorectomy, will lead to worthwhile objective remission in 30 - 40 per cent. of patients. Adrenalectomy plus oophorectomy is normally applied to postmenopausal women, or those who have been previously castrated by /

/by surgical or radiological means. It is unlikely that adrenalectomy influences breast cancer by a similar mechanism to oophorectomy, but by a more complete removal of the hormones concerned. This is supported by the fact that a patient who has no response to oophorectomy is extremely unlikely to benefit from subsequent adrenalectomy, whereas regression even of a transitory nature, after castration, is usually followed by further remission of disease after adrenalectomy.

The implication of the anterior pituitary hormones in breast growth in rats suggested that hypophysectomy should perform a double function in the treatment of breast cancer. As well as its effect through reducing adrenocortical secretion, the cessation of production of prolactin and growth hormone might also be expected to exert a further inhibitory effect on the tumour growth. In practice however the results obtained by both surgical hypophysectomy (Luft and Olivecrona 1953; Pearson et al., 1954) and by implantation of the pituitary gland with radioactive materials (Forrest et al., 1959) are very similar to those for adrenalectomy and oophorectomy. A report recently however (Atkins et al., 1960) comparing the results of the two procedures in an extensive series of patients, does in /

/in fact suggest that the latter is a statistically significantly superior procedure, both judged by the number of remissions obtained, and by their duration. The authors suggest that the two operations affect the cancer in quite different ways, and not through a common action on the adrenal gland as might be supposed. These conclusions however are not confirmed by the preliminary report of a joint committee of the American College of Surgeons and the American College of Physicians, which undertook a retrospective study on a series of 870 patients treated by either oophorectomy plus adrenalectomy or hypophysectomy. It is the opinion of the committee that there is no difference between the results of the two operations, that the mechanism of response must in fact be the same for both operations and that the responsive patient will benefit equally much from either operation.

Steroid Hormone Metabolism.

The main groups of steroid hormones and their metabolites which may be associated with breast cancer, the excretion of which have been studied in this investigation are

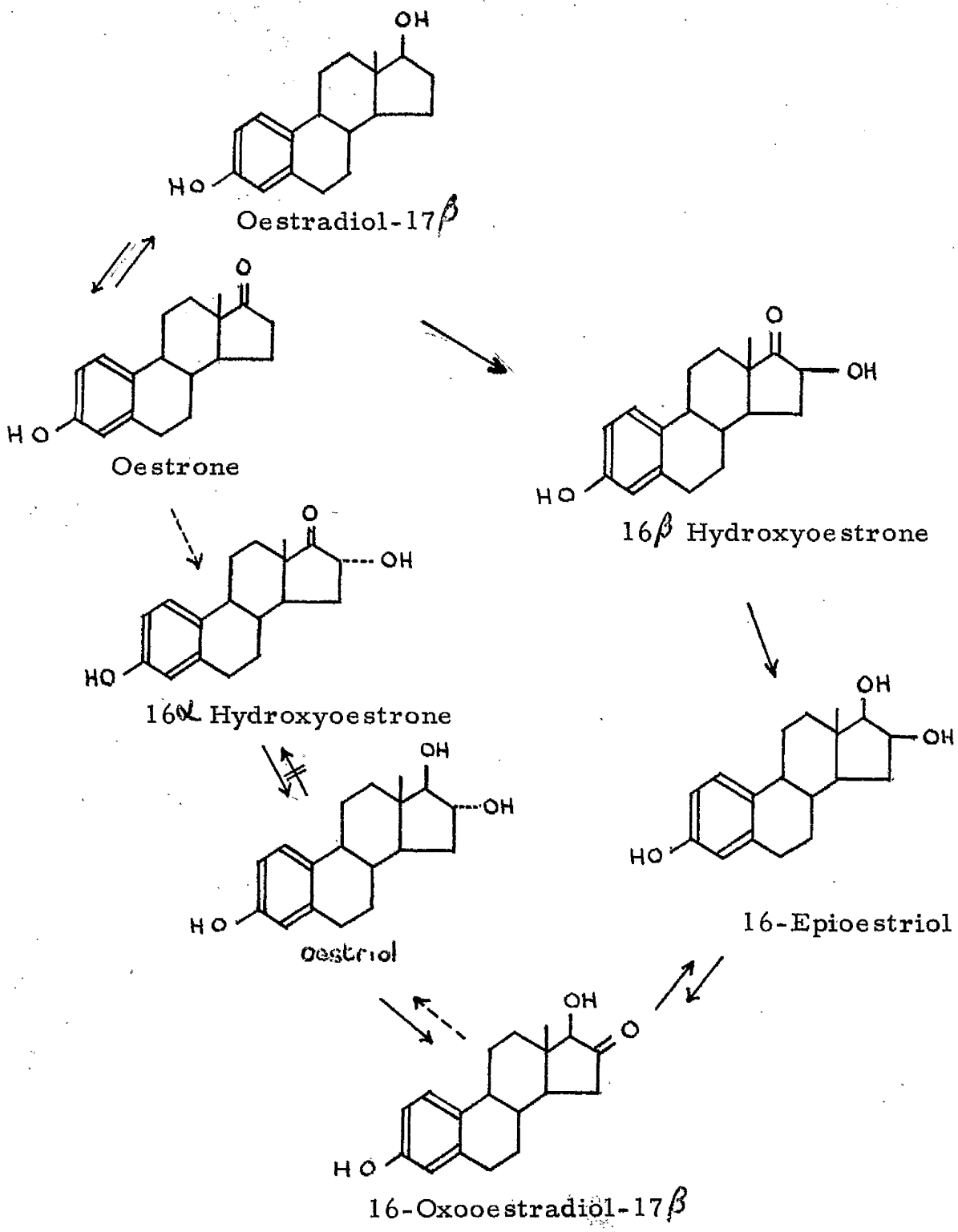
a) Oestrogens
b) 17-Oxosteroids
c) Progesterone and Pregnanediol

OBSERVATION	REFERENCE
Oestrone isolated from beef adrenal glands.	Beall 1939.
Oophorectomised mice develop adrenal cortical hyperplasia and carcinoma associated with normal development of sex organs.	Woolley et al. 1939. Fekete Wooley and Little 1941.
Oophorectomised mice with adrenal tumours excrete four times as much oestrogen as intact females of the same strain.	Dorfman and Gardner 1944.
Feminizing effect of some adrenal tumours in the human male.	Frank 1934. Simpson and Joll 1938. Roholm and Teilum 1942. Wilkins 1948. Landau Stimmel, Humphreys and Clark 1954. Staublitz, Oberkircher, Lent Bissell and Farnsworth 1954.
Administration of cortisone to patients with adrenal hyperplasia leads to a diminution of urinary oestrogen as well as 17-oxosteroid excretion.	Gardner and Migeon 1952.
In castrated females, ACTH causes a rise in urinary oestrogen excretion, which is abolished by adrenalectomy.	Brown Falconer and Strong 1959. Strong et al., 1956.
Conversion of androsten-3:17-dione (an androgen of adrenal origin) to oestrone shown in the human.	Meyer 1955. West, Damast, Sarro and Pearson, 1956.

Table 1. Evidence for an adrenal origin of oestrogens.

Oestrogens. The main site of oestrogen production in the premenopausal non-pregnant female is now generally agreed to be the theca interna of both the maturing Graafian follicle and the corpus luteum after ovulation. Although the oestrogens produced in, and secreted by, the human ovary, have not yet been identified with certainty, there is indirect evidence, obtained from studies of oestrogen excretion throughout the normal menstrual cycle, that oestradiol-17β and oestrone are the principal ovarian hormones elaborated in the human (Brown, 1955a). There have been no reports of the isolation of any oestrogen from the ovarian vein blood of a premenopausal woman.

There are numerous reports of continued oestrogen excretion by oophorectomised women, and also by post-menopausal women and normal men, and the evidence summarised in Table 1 suggests strongly that the source of this oestrogen is the adrenal cortex. Studies on adrenal venous blood have yielded no direct evidence of oestrogen secretion by this tissue although it is likely that the assay techniques employed were scarcely sensitive enough to detect the very small quantities present (Short and Forrest, 1959). /



⟶ Reactions known to occur

⟶‖ Reactions known not to occur

----> Postulated but unproved reactions

Figure 2. Metabolism of Oestradiol-17β. (Bulbrook and Strong, 1959; after Marrian 1958; Breuer, Knuppen and Nocke, 1959; and Breuer, Nocke and Bayer, 1958).

Figure 2 shows a metabolic scheme for most of the oestrogens so far isolated from human urine. Four others not included have recently been described. After the administration of oestradiol-17β-16-^{14}C to women with breast cancer, 2-methyoxyoestrone (Kraychy and Gallagher, 1957; Engel, Bagget and Carter, 1957) and 2-methoxyoestriol (Fishman and Gallagher, 1958) were isolated from the urine. 18-hydroxyoestrone (Loke, Marrian, Johnson, Meyer and Cameron, 1958) and more recently 2-methoxyoestrone and the third member of the methoxy series 2-methoxyoestradiol (Fransden, 1959) have been characterised from late pregnancy urine. The quantitative significance of these latest findings is uncertain at present, although 16β hydroxyoestrone is likely to be quantitatively as important as oestrone and perhaps more important than oestradiol-17 . In urine collected during the normal menstrual cycle, values of 1.0 - 25 Oug. per 24 hrs. have been obtained (Marrian, Loke, Watson and Panattoni, 1957). It appears however that biologically, 16-epioestriol and 16β hydroxyoestrone are much less active than oestradiol-17β in the tests at present used. /

/used.

Satisfactory quantitative relationships between production and excretion in pre- and postmenopausal women have only been established for oestriol, oestrone and oestradiol-17β. These are at present most commonly measured by the well established method of Brown (1955b) and its modification (Brown, Bulbrook and Greenwood, 1957a) which satisfy the criteria of reliability necessary for a method which is to be used in clinical studies. Recently, a method of estimating 2-methoxyoestrone, oestrone, ring Dα-ketolic oestrogens, oestradiol-17β, 16-epioestriol and oestriol in human urine has been published (Givner, Bauld and Vagi, 1960) and although it is inevitably too complex for use in routine studies, it should, when satisfactorily established, yield more complete information on oestrogen metabolism than is at present available. That more complete data are urgently required is evidenced by the facts that while only 25 per cent. of administered oestradiol-17β or oestrone appears in the urine as oestriol, oestrone and oestradiol-17β (Brown, 1958), 65 per cent. of the radioactivity of an injected dose of ^{14}C labelled oestradiol-17β appears in the urine (Beer and Gallagher, 1955). The proportion of this unaccounted radioactivity due /

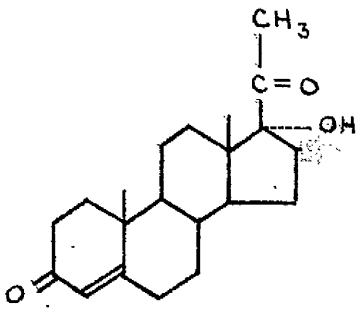
Hormone	Urinary Metabolite	Oxosteroid Fraction	3-Hydroxy-Orientation
Cortisol 11β-Hydroxy Δ^4-Androsten-3:17-dione Cortisone	11β-Hydroxyandrosterone 11β-Hydroxyaetiocholanolone 11-Oxoandrosterone 11-Oxoaetiocholanolone	11-oxy 17-oxosteroids	3α fraction
Δ^4-Androsten-3:17-dione	Androsterone Aetiocholanolone	11-deoxy 17-oxosteroids	
Dehydroepiandrosterone	Dehydroepiandrosterone		3β fraction

Table 2. Neutral 17-oxosteroid Fractions in female Urine, and their probable source.

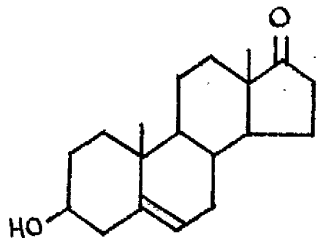
/due to the newly discovered oestrogens may be of considerable importance.

17-Oxosteroids. The neutral 17-oxosteroids in the urine of normal women, and their probable hormonal precursors, are shown in Table 2. They arise both from the oxidative removal of the 2C side-chain from the C_{21} glucocorticoids such as cortisol, and directly from the C_{19} adrenal androgens, androstene-3:17-dione and dehydroepiandrosterone. It has also been suggested (Plantin et al., 1958) from studies on urinary excretion by adrenalectomised women with intact ovaries, that a small amount of urinary aetiocholanolone may also derive from ovarian precursors.

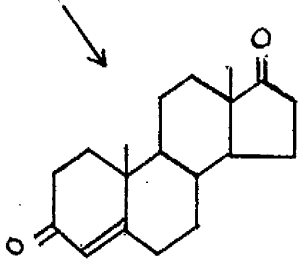
The metabolic sequence leading to the formation of androsterone and aetiocholanolone in women are summarised in Figure 3. These two steroids, along with dehydroepiandrosterone, form the 11-deoxy 17-oxosteroid fraction of the urinary 17-oxosteroids. Δ^4 Androstene-3:17-dione has been isolated from human adrenal venous blood (Romanoff, Hudson and Pincus, 1953; Short and Forrest, 1959) and it is almost certainly secreted by the gland. The isolation and characterisation by infra-red spectroscopy of dehydroepiandrosterone and 17/ hydroxyprogesterone from /



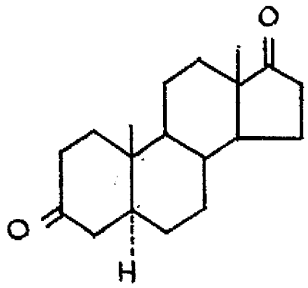
17 -α HYDROXYPROGESTERONE



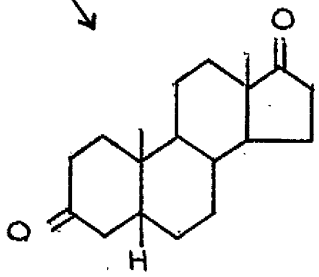
DEHYDROEPIANDROSTERONE



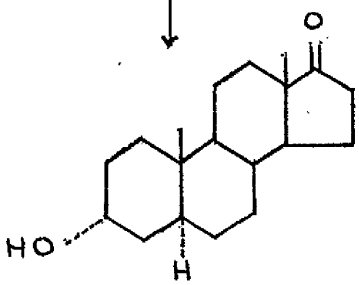
ANDROSTENE - 3 : 17 - DIONE



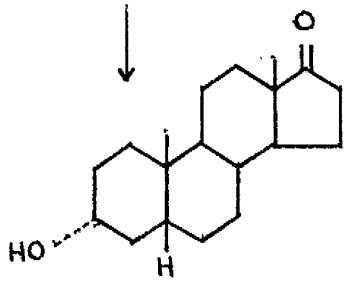
ANDROSTANE - 3 : 17 - DIONE



AETIOCHOLANE-3 : 17 - DIONE



ANDROSTERONE



AETIOCHOLANOLONE

Figure 3. The metabolism of Δ^4 androstene-3 : 17-dione. (After Dorfman and Ungar, 1953).

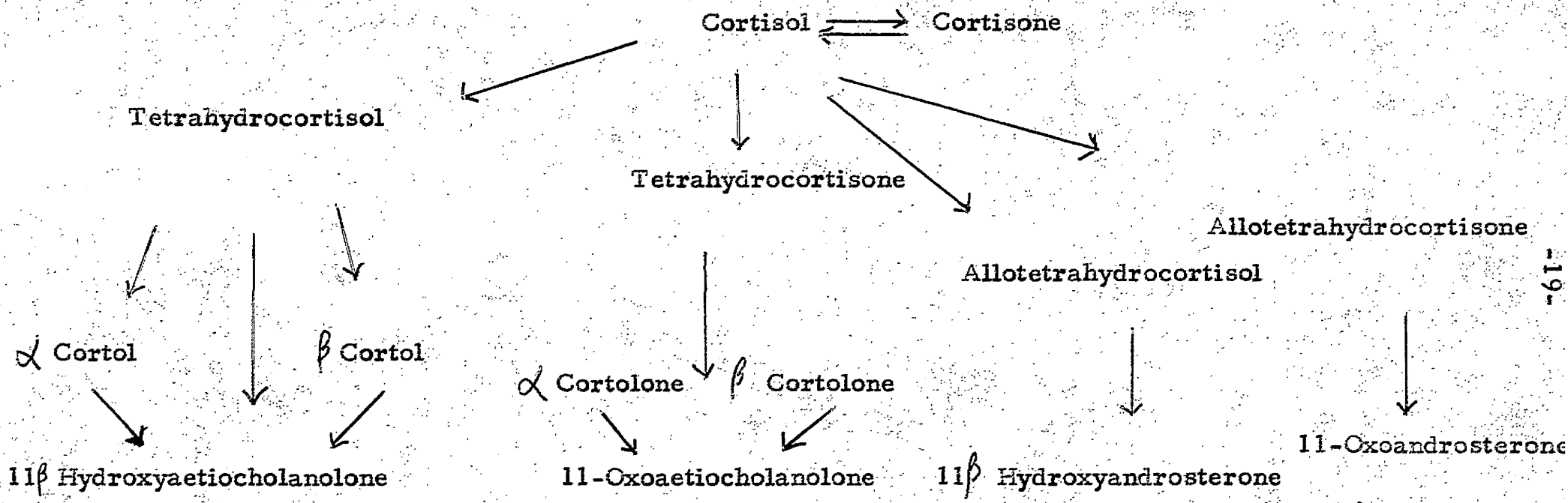


Figure 4. Metabolic formation of the 11-oxy 17-oxosteroids (after Dorfman 1956).

/from adrenal vein blood has also been reported (Lombardo, McMorris and Hudson, 1959).

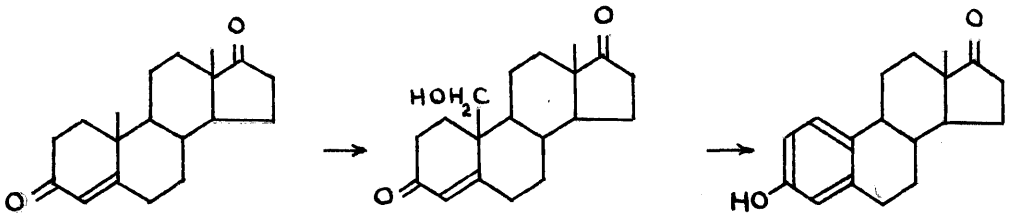
The metabolites of cortisol differ from those shown in Figure 3 by having an oxygen function at C11. Thus when cortisone is being administered to a patient, the 11-oxy 17-oxosteroids appearing in the urine are derived, in part at least, from the exogenous steroid. This assumes particular importance in the adrenalectomised and hypophysectomised patient maintained on cortisone, where only 11-deoxy 17-oxosteroid excretion gives a measure of adrenal function. The metabolic formation of the 11-oxy 17-oxosteroids is shown in Figure 4.

Much fundamental work on the fractionation of individual urinary 17-oxosteroids by chromatographic methods was performed by Dingemanse, Huis In't Veld and de Laat (1946) and by Dobriner, Lieberman and Rhoads (1948). Procedures similar to that of Dingemanse, employing adsorption chromatography on alumina columns, have been developed on a smaller scale by many workers (Zygmuntowicz, Wood, Christie and Talbot, 1951; Pond, 1951; Johnsen, 1956). Gradient elution chromatography, designed to sharpen the separation of fractions achieved, was introduced by Kellie and Wade (1957). /

/

Chromatography on paper has been used by other workers (Savard, 1953; Rubin, Dorfman and Pincus, 1954) and the method employed in the present study (Hobkirk, 1958) involves enzymic hydrolysis of the separated steroid glucuronides and sulphates and rigorous purification of the extract, prior to paper chromatography.

Estimations of urinary 17-oxosteroids are in themselves important in the study of human breast cancer, in view of their derivation from androgenic compounds, which are known to exert an effect on the development of the disease. Early reports (Dobriner, Lieberman, Hariton, Sarett, and Rhoads, 1947) associated 11β hydroxyaetiocholanolone with malignant disease, since a compound related to it, $\triangle^{9,11}$ aetiocholanolone (likely a hot acid dehydration artefact) could only be found in the urine of persons with some form of cancer. Later studies using superior methods however failed to confirm this. (Plantin and Birke, 1955). Additional point has been given recently to urinary 17-oxosteroid assay in breast cancer by the observation that the human ovary can convert testosterone to oestradiol-17β (Bagget, Engel, Savard and Dorfman, 1956), and the observations by several workers that androstene-3: 17 dione can be /



Δ^4 Androsten-3:17-dione 19-Hydroxy Δ^4 Androsten-3:17-dione Oestrone

Figure 5. Postulated pathway of conversion of androgens to oestrogens.

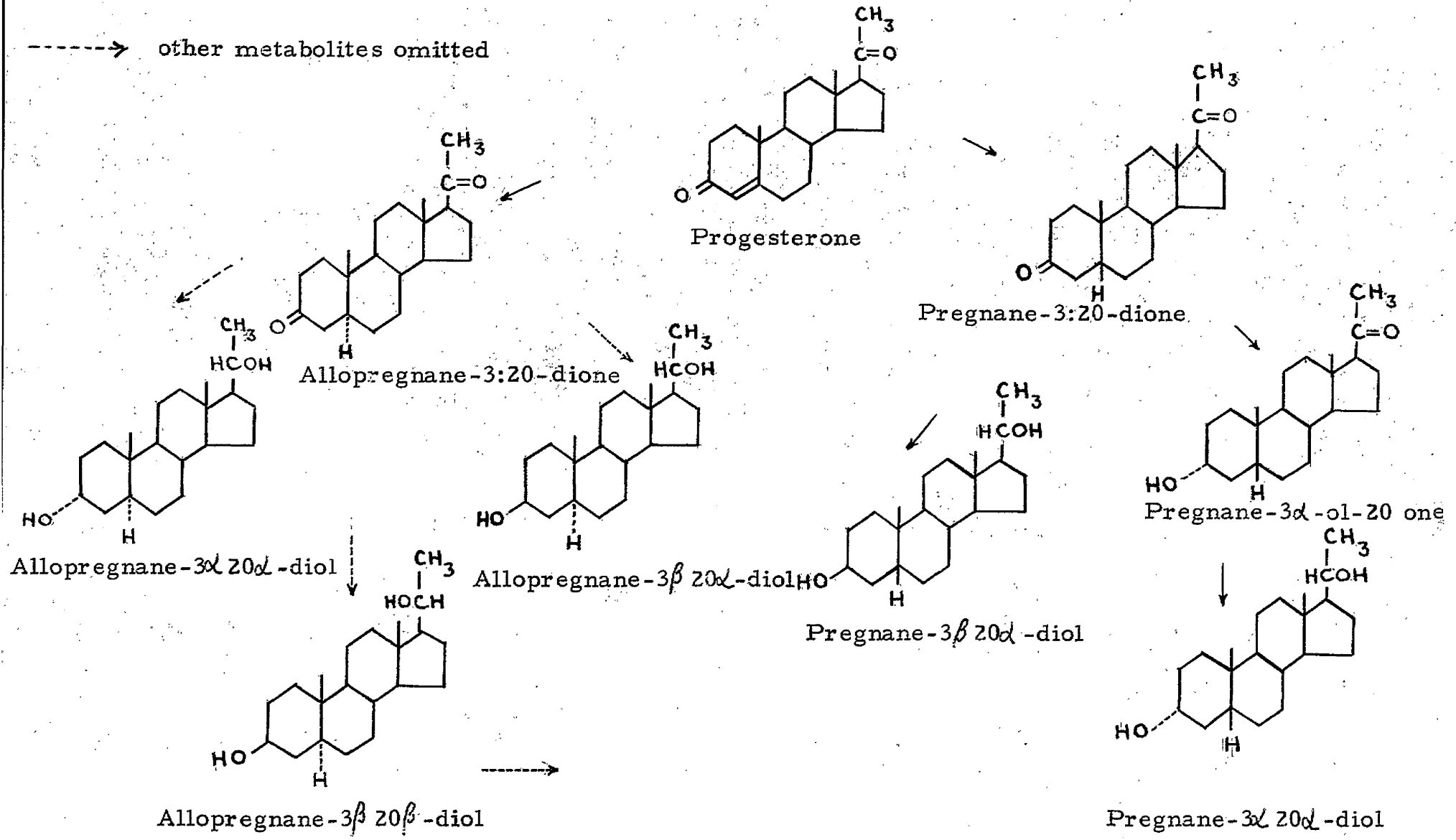


Figure 6. Metabolism of Progesterone (Bulbrook and Strong, 1959; after Dorfman, 1955).

/be converted to oestrone by way of a 19-hydroxylated intermediate in good yield (Meyer, 1955; Ryan, 1958), Fig. 5.

Progesterone and Pregnanediol. In the non-pregnant woman progesterone is secreted by the granulosa lutein cells of the corpus luteum and probably also by the adrenal cortex (Loraine, 1958) and is largely metabolised according to the reactions shown in Fig. 6.

Pregnane-3α 20α-diol is the only metabolite of those shown which can be determined in the urine in a reliable manner. This can be done by the chromatographic method of Klopper, Michie and Brown (1955), and although its excretion represents only a small and somewhat variable proportion of metabolised progesterone - 5.0 to 22.0 per cent. of an injected dose (Klopper and Michie, 1956) - it provides the only practicable index of its metabolism.

Other factors which must be considered along with the alternative pathways shown in Fig. 6 are firstly, the as yet undetermined role of two recently isolated steroids, 6β-hydroxyprogesterone and 6-oxoprogesterone (Dorfman, 1957) and secondly, the fact that the metabolism of 11-deoxycorticosterone (DOC) can also contribute to /

/to urinary pregnanediol output.

That pregnanediol excretion can give a measure of progesterone secretion by the adrenal gland has been demonstrated by a sharp rise in the level of excretion in oophorectomised and postmenopausal women when given intravenous ACTH (Klopper, Strong and Cook, 1957). It has been suggested from these findings that urinary pregnanediol assay might be a useful means of assessing adrenocortical function in hypophysectomised and possibly incompletely adrenalectomised women, since the procedure is not influenced by the administration of cortisone. The methods available however are not yet sensitive enough to detect the low level of excretion likely to be found in such women. In view of the apparently important role of progesterone in experimental mammary cancer (Lyons, Li and Johnson, 1958; Huggins, Torralba and Mainzer, 1956) further studies of progesterone metabolism with more sensitive assay methods will be of considerable interest.

METHODS OF ANALYSIS

METHOD OF ANALYSIS

General Considerations.

The determination of steroid hormones of adrenal and ovarian origin and their metabolites, in the urine of women who have undergone some form of endocrine ablation, presents particular methodological difficulties since in the absence of the main hormone producing tissues, urinary excretion of these steroids will be minimal. The methods normally employed for the assay of urinary oestrogens, 17-oxosteroids, pregnanediol, and 17-hydroxycorticosteroids were devised for use on the urine of normal subjects or patients with intact endocrine glands. In these cases, the presence of small amounts of interfering materials in the final extracts used for colorimetry was of little importance compared with the large amounts of steroid present. In urines from adrenalectomised or hypophysectomised women, however, the proportion of interfering material might be quite considerable, and an important factor in reducing the reliability of an assay.

Certain essential requirements must be fulfilled by a hormone assay procedure before it can be used in a study of the present type. These "criteria of reliability" were /

/were described by Borth (1952) as accuracy, precision, specificity and sensitivity.

ACCURACY may be defined as the nearness with which a given analytical result approaches the "true" result, and is usually determined by estimating the percentage recovery of pure steroids added to a urine sample before analysis. Ideally, since all steroids excreted in the urine are conjugated, either with glucuronic acid or sulphuric acid, the steroid glucuronides or sulphates should be added to the urine. Very few such compounds are available as yet, and, at present, recovery experiments are carried out using pure crystalline steroids. Although this is not the ideal procedure it does serve to give a reasonable indication of the losses which are liable to occur during the course of an estimation. Marrian (1955) has stated that a method could be considered quantitatively satisfactory if 75% or more of the added steroid is recovered. Since the accuracy of a published method may vary very considerably in different laboratories, Marrian has stressed the importance of this always being determined before an assay is adopted for investigations in the clinical field.

The PRECISION of a method is concerned with its reproducibility and is expressed as the standard deviation /

/deviation from the mean in replicate experiments. The acceptable degree of precision in any assay is difficult to define, but Marrian (1955) has suggested that for steroid methods the best that can reasonably be expected is a standard deviation of ± 10% at optimal steroid concentrations and ± 25 - 30% sub-optimal concentrations.

The term SPECIFICITY as applied to a chemical assay can be defined as the determination of one chemical entity to the exclusion of all others (Lorraine, 1958). In some quantitative steroid methods this can be extremely difficult to establish absolutely since the hormones or metabolites under consideration may be excreted in amounts so small as to preclude the classical methods of isolation and characterisation, such as melting point determinations and direct spectroscopy. In these cases the specificity normally depends on the accumulation of several types of indirect evidence which, taken all together, are satisfactory proof that the method measures what it is claimed to measure, and nothing else.

The SENSITIVITY of an assay method is usually understood as defining the minimum amount of substance which can be detected by that method. From theestimate of /

/of the standard deviation 's' of results from their means from duplicate determinations it is possible to work out:-

(a) The quantitative sensitivity of a method. This is the least amount of a substance measured which is distinguishable from zero, and the maximum percentage error at that level.

(b) The quantitative sensitivity of a method. This is the least amount of a substance which can be measured with not more than a specific error.

OESTROGENS.

The method used for estimating urinary oestrone, oestradiol-17β and oestriol was that of Brown (1955b) and its modification, Brown et al., (1957a).

In principle, the procedure involves the acid hydrolysis of the oestrogen conjugates in the urine, ether extraction of the free steroids, and separation of the phenolic fraction by alkali extraction of the ether. The phenolic fraction is partitioned between benzene, petroleum ether, alkali and water, followed by quantitative methylation of the phenolic groups of the oestrogens, fractionation of the methyl ethers by absorption chromatography on alumina columns and finally colorimetric assay of the separated oestrogen methyl ethers by an improved Kober reaction /

Oestrogen	Number of Assays	Oestrogen Added µg/l	Mean Recovery µg/l	Range µg/l	Mean Percentage Recovery
Oestrone	4	5.0	4.2	4.1 - 4.3	84.0
Oestriol	6	7.2	6.2	6.1 - 6.3	86.0
Oestradiol-17β	6	6.8	5.7	5.6 - 5.7	83.8

Table 3. Recovery of crystalline oestrogens added to acid hydrolysed urine.

+ from Brown, Bulbrook and Greenwood (1957b).

* $'s' \sqrt{\frac{\Sigma d^2}{2N}}$ where d the difference between duplicate determinations
N the number of determinations performed

Oestrogen	Present Study		C.E.R.U.+		I.C.R.F.+	
	Range estimated	's' *	Range estimated	's'	Range estimated	's'
Oestrone	0.0 - 3.9	0.37 (44)	0.0 - 4.9	0.35	0.0 - 2.4	0.33
	4.0 - 15.0	0.57 (11)	10.0 - 19.9	0.44	5.0 - 40.0	0.59
Oestriol	0.0 - 3.9	0.39 (28)	0.0 - 4.9	0.44	0.0 - 2.4	0.37
	4.0 - 15.0	0.45 (16)	10.0 - 19.9	0.53	5.0 - 40.0	0.96
Oestradiol	0.0 - 8.5	0.39 (35)	2.5 - 4.9	0.37	2.5 - 4.9	0.51

Table 4. Estimates of precision of the oestrogen assay, expressed as estimates of the standard deviation ('s') of the results from their means (from duplicate determinations). The figures in brackets indicate the number of duplicate estimations performed.

/reaction using the Allen colour correction to make allowance for interfering chromogenic material. This method was shown to be subject to interference from high concentrations of metabolites of cortisone and from the administration of certain drugs. In order to overcome this, an additional step was introduced into the analysis in which the phenolic fraction is saponified with sodium hydroxide before methylation. This reduces the impurities in the final extract by over 50 per cent.

The accuracy of this method was checked by measuring the recovery of oestrone, oestriol and oestradiol-17β added to acid-hydrolysed urine. The results obtained are shown in Table 3. It is generally accepted that a further 10 - 20 per cent. loss of oestrogen occurs during acid hydrolysis, so the overall recovery of oestrogen achieved is 60 - 75 per cent., a figure generally regarded as satisfactory for this method.

All determinations were carried out in duplicate and an estimate of the precision of the method can be made by calculating 's' which is an estimate of the standard deviation worked out from the difference between duplicate determinations in a series of assays (Snedecor, 1955). The results are shown in Table 4 in which the values obtained for /

/for 's' are compared with those published by two other groups of workers using the same method of assay (Brown et al., 1957b) and are shown to be in good agreement with them.

Evidence obtained from several sources suggests that Brown's method is specific for oestrone, oestriol and oestradiol-17β. This evidence can be summarised as follows:

a) the known high specificity of the Kober reaction.

b) countercurrent distribution studies on the oestrogen methyl ether fractions obtained from urine (Diczfalusy, 1955).

c) parallel chemical and bioassay studies (Bulbrook, Greenwood and Williams, 1960).

d) comparison with radioisotopic studies (Gallagher, Kraychy, Fishman, Brown and Marrian, 1958).

From a statistical analysis of a large number of determinations (Brown et al., 1957b), it has been concluded that the lowest levels (µg per 24 hrs.) distinguishable from zero are oestrone 0.64, oestriol 0.84, oestradiol-17β 0.60. For convenience, in this laboratory, the lowest limit of sensitivity is regarded as 1.0 µg of each oestrogen per 24 hours.

17-OXOSTEROID FRACTIONATION

The principal urinary 17-oxosteroids were measured by the method of Hobkirk (1958).

The urine is saturated with ammonium sulphate and the steroid glucuronides and sulphates extracted with an ether-ethanol mixture. After complete removal of the solvent the conjugates are successively hydrolysed by limpet β-glucuronidase and acid (pH 1) at room temperature for 48 hours. Neutral ketonic fractions are prepared with a micro-Girard technique and separated into 3α and 3β fractions by a micro-digitonin precipitation. The 3β fraction is measured without further purification, while the 3α fraction is submitted to paper partition chromatography against steroid standards in a ligroin: propylene glycol system. The individual compounds separated and their probable sources are shown in Table 2. They are eluted from the paper with ethanol and assayed by the Zimmermann reaction, modified by the substitution of an organic base tetramethyl ammonium hydroxide, for the alcoholic potassium hydroxide normally used. The organic base is more stable and gives lower reagent blanks. /

/ blanks.

The accuracy of the method has been investigated by measuring the recovery of androsterone added to the urine before hydrolysis and extraction. The results are shown in Table 5.

Owing to the length of time required for the performance of one assay (3 - 4 weeks) and the impracticability of carrying out more than three assays simultaneously, it was impossible to carry out duplicate determinations.

Although the Zimmermann colour reaction used in the final quantitation is not specific for 17-oxosteroids, three types of evidence suggest that the method does measure the compounds shown in Table 2. These are,

a) the rigorous purification of the α fraction.

b) the behaviour of the α fraction on paper chromatograms.

c) the identity of the sulphuric acid absorption curves of eluates from the paper with those of pure crystalline steroids.

The limiting factor in the sensitivity of this method is the sensitivity of the final Zimmermann colour reaction. Using this method, amounts of individual steroids as low as 0.01 mg. per 24 hrs. may be detected, but quantitative significance is doubtful below approximately 0.10 mg. per 24 hrs.

Androsterone Added mg/1	Number of Assays	Mean Percentage Recovery and Range
3.6	2	70.1 (67.7 - 72.5)
3.1	1	73.3

Table 5. Recovery of crystalline Androsterone added to female urine.

Pregnanediol Added mg/1	Number of Assays	Mean % Recovery ± S.D.
0.80	6	94.1 ± 18.0
4.16	7	73.9 ± 5.1

Table 6. Recovery of crystalline Pregnanediol added to male urine.

Method of Assessment	Number of Assays	Range mg/24 hr.	Mean ± S.D.	's'
Replicate Assays on One Pool	8	0.61-0.83	0.72 ± 0.077	
Duplicate Assays	76	0.0 -4.0		0.064

Table 7. Assessment of precision of pregnanediol assay.

PREGNANEDIOL.

Urinary pregnanediol was measured by the method of Klopper et al., (1955). Acid hydrolysis of the urine is followed by toluene extraction, alkali washing to remove phenolic steroids and permanganate oxidation. The free pregnanediol is then separated from related pregnanetriols and other oxidation products, by absorption chromatography on alumina columns, acetylated and rechromatographed. Pregnanediol diacetate is then estimated colorimetrically with concentrated sulphuric acid.

Recovery experiments, in which free pregnanediol was added to urine before hydrolysis gave satisfactory results, shown in Table 6.

Multiple determinations were performed on one pool and the Standard Deviation (S.D.) calculated from the results obtained. In Table 7 this is compared with the value of the function 's' as previously defined, calculated from the differences between duplicate assays in a large series of determinations.

The specificity of the method depends on the purity and identity of the final product, or on the absence of any substance other then pregnane-3α :20α-diol which gives /

/gives a colour with concentrated sulphuric acid. The sulphuric acid spectrum and the i.r. spectrum or the urinary residues have been shown to correspond closely to those of crystalline pregnanediol diacetate (Klopper et al., 1955; Coyle, Mitchell & Russell, 1956). The urinary residues also have chromatographic properties similar to those of pure pregnanediol and its diacetate.

Since the sulphuric acid chromogenic material from the urine appears to be pregnanediol diacetate only, the sensitivity of the colour reaction governs the sensitivity of the method. It is likely that values below 0.5 mg. per 24 hours are not quantitatively significant.

Method of Assessment	Number of Assays	Range mg./24 hrs.	Mean ± S.D.	's'
Replicate Assays of One Pool	8	6.90 - 8.63	8.0 ± 0.571	
Duplicate Assays	18	5.10 - 30.0		0.597

Table 8. Assessment of precision of 17-Hydroxycorticosteroid assay.

TOTAL 17-HYDROXYCORTICOSTEROIDS.

The method of Appleby, Gibson, Norymberski and Stubbs (1955) was employed. In this, urinary 17:20 ketols are reduced to the corresponding glycols, which, along with the 17:20 glycols originally present, are estimated by the Zimmermann reaction, after oxidative removal of the side chain

The accuracy of this method as determined by recovery experiments is difficult to assess due to the varying chromogenicity of the 17-oxosteroids derived from the different urinary corticosteroids.

All estimations have been performed in duplicate, and data concerning the precision of the assay are given in Table 8.

It has been shown that the method will measure, in urine, only 17-oxosteroids derived from urinary C_{21} compounds, since 17-oxosteroids added to the urine before assay do not interfere with the analysis.

The same considerations apply to the sensitivity of this method as do to the 17-oxosteroid fractionation. The urines assayed by this method all came from patients with intact adrenal glands so the question of lower limits of sensitivity has not been investigated. /

TOTAL 17-OXOSTEROIDS.

These were measured by the method advised by the Clinical Endocrinological Committee, of the Medical Research Council (1951).

Because of the well defined nature of the assay and the chemically heterogeneous residue obtained for measurement, no recovery experiments were attempted.

HORMONE ASSAY IN HUMAN BREAST CANCER

HORMONE ASSAY IN HUMAN BREAST CANCER.

The extensive circumstantial evidence already described implicating the hormones of the ovaries, adrenal glands and pituitary gland in mammary carcinogenesis made accurate quantitative studies of the hormonal status of breast cancer patients an urgent requirement, to set clinical empiricism on a basis of scientific fact.

The relative merits of blood and urine determinations in assessing the hormonal environment have been reviewed by Marrian (1955). Only small and somewhat variable amounts of an administered dose of various steroid hormones can be recovered in the urine in the form of unchanged hormone or recognisable metabolites, and in these cases it must be assumed that urinary steroid assays will similarly measure only a small amount of the hormone secreted in the body. While it has definite disadvantages, Marrian concludes that a urine determination will yield a value which may be accepted as bearing some approximate proportionality to the total amount of hormone secreted during a certain period of time - usually from 8 hrs. to 24 hrs. Blood determinations which have been urged by some authorities on account of /

/of this, provide information about the levels of a hormone or metabolite at one instant only - the instant of withdrawal of the sample. While this may well be as valuable as, or in some cases more valuable than, a urinary assay, it is information of a different kind and the two types of assay should be regarded as supplementary sources of information rather than alternatives (Marrian 1955). Moreover, urinary assays are at present generally more reliable than methods available for estimating corresponding substances in the blood.

Aims of hormone assay in breast cancer. These have recently been summarised by Strong (1958) as

a) To define the environment in which the disease evolves.

b) To determine the hormonal effects of endocrine treatment of the disease.

c) To seek a method of predicting the response of patients to the forms of palliation at present available.

a) The Environment of the Disease.

The establishment of a relationship, if any exists, between an abnormal hormonal environment and the development of mammary cancer in human subjects is /

/is a task of formidable dimensions. It would involve estimating the daily urinary excretion of a series of hormones and metabolites throughout one or more complete menstrual cycles in a population of women sufficiently large to yield ultimately, an adequate number of cases of breast cancer. Such a study has not so far been attempted although a large scale survey is at present being undertaken by the Imperial Fund for Cancer Research in an island community in the Channel Isles. An alternative to this would be the study of the hormonal status in precancerous disorders such as chronic cystic mastitis (Nathanson, 1944). It is however by no means generally accepted that recognisable precancerous conditions do exist in this disease, and clarification of this point must precede any hormone studies. A second alternative to a comprehensive survey is to compare the hormone excretion in untreated patients in whom the disease is established, with normal subjects of comparable menstrual status (Strong, 1958). Studies of this type have been reported recently by Kellie (1954), Loraine, Strong and Douglas (1957) and Brown (1958). It is generally agreed that no abnormality of hormone excretion characteristic of breast cancer can be demonstrated, although Brown has shown an increase /

Status before Oophorectomy	Number of Patients	Assay	Conclusion	Reference
	6		Several cases had elevated levels after oophorectomy	Bret, Bardiaux & Milinier (1955)
Pre- and post-menopausal	14	Chemical	Oophorectomy reduced oestrogen excretion in premenopausal women but had no effect after the menopause. In all cases excretion continued	Bulbrook & Greenwood (1957)
Premenopausal Menopausal Postmenopausal	46	Chemical	Excretion continued after operation in all cases	Bulbrook, Greenwood, Hadfield & Scowen (1958)
Premenopausal	11	Biological	Oestrogen reduced to undetectable amounts in 72 hrs. after oophorectomy and 140 days by ovarian irradiation	Block, Vial & Pullen (1958)
Premenopausal	7	Chemical	No reduction in excretion of oestrone or oestradiol-17β compared with follicular phase. Compared with luteal phase, oestradiol also reduced	Birke et al., (1958)

Table 9. Effect of Oophorectomy on Urinary Oestrogen Excretion

Status before Oophorectomy	Number of Patients	Assay	Conclusion	Reference
Postmenopausal	4	Chemical	Oestrogen levels not significantly altered by oophorectomy	Brown et al., (1959)
Premenopausal	17	Chemical	Ovarian irradiation caused marked drop in oestrogen excretion. The decrease in oestriol excretion double that in oestrone and oestrodiol-17β. Oophorectomy did not further decrease excretion	Diczfalusy, Notter, Edsmyr and Westman (1959)
Premenopausal	15	Chemical	Significant fall in oestrogen excretion only in six cases. Mainly in oestriol fraction	Swyer, Lee and Masterton (1961)

Table 9. Effect of Oophorectomy on Urinary Oestrogen Excretion. (cont'd)

/increase in the excretion of oestriol and in the ratio oestriol : total oestrogen in postmenopausal patients before treatment.

b) The Effects of Treatment on Hormone Excretion.

Most of the work carried out in recent years, using reliable hormone assay methods has been directed at defining more clearly the effects of the ablation of certain endocrine organs on steroid hormone production and excretion, and correlating them wherever possible with the subsequent behaviour of the disease.

(1) Oophorectomy and Ovarian Irradiation: The results of oestrogen assays carried out after one or other of these procedures are summarised in Table 9.

These results confirm earlier reports based on bioassay, that oestrogen excretion continues, perhaps at a lower level, after oophorectomy, ovarian irradiation and after the natural menopause (Dao, 1953; Smith and Emerson, 1954; Struthers, 1956; Diczfalusy et al., 1959). No correlation has been found between the level of preoperative oestrogen excretion and the subsequent response of the patient, or differences between the excretion levels postoperatively in the responding and non-responding groups. The lower level of oestrogen excretion after the /

Steroids measured	Assay	Number of Patients	Conclusions	Reference
Oestrogens	Biological	12	Adrenalectomy abolished excretion	Dao (1953)
	Biological	1	Excretion continued	Dao (1957)
	Chemical	10	Excretion continued in 9	Greenwood & Bulbrook (1956)
	Chemical	17	Excretion continued in 13	Bulbrook & Greenwood (1957)
	Chemical	49	Excretion continued in 30% of cases	Bulbrook & Greenwood (1958)
	Chemical	13	Excretion continued in 6	Bulbrook, Greenwood, Hadfield & Scowen (1958b)
	Biological	30	Excretion continued in 60% of cases	Bulbrook, Greenwood & Williams (1958)
	Chemical	13	Excretion continued in all cases	Strong et al (1956)
	Chemical	11	Excretion continued in all cases	Brown et al (1959)
	Chemical	10	"Kober Chromogens" excreted in 2 cases	Hellström et al (1957)
	Chemical or Biological	4	Excretion continued in only one case	West et al (1958)
	Chemical	9	Excretion continued in all cases and increased in one	Swyer et al (1961)

Table 10. Steroid Excretion after Adrenalectomy

Steroids measured	Assay	Number of Patients	Conclusions	Reference
17-Oxosteroids	Chemical	2	Excretion of androsterone and aetiocholanolone continued in both	Kellie & Wade (1957)
	Chemical	7	Excretion of androsterone in 1: aetiocholanolone in 6.	Hobkirk (1958)
	Chemical	-	Androsterone rarely found: aetiocholanolone in most cases: Dehydroepiandrosterone in 1.	Bulbrook, Greenwood & Thomas (1958)
Pregnanediol	Chemical	2	Excretion continued in both	Zimmerman, Bloch & Oseid (1954)
	Chemical	13 8	Excretion continued in all cases Excretion continued in all cases	Strong et al (1956) Klopper, Strong & Cook (1957)

Table 10. (Cont'd) Steroid Excretion after Adrenalectomy

/the menopause is scarcely affected by oophorectomy which may explain the very poor results obtained when patients with breast cancer who are past the menopause are treated in this way (Treves and Finkbeiner, 1958). Ovarian irradiation is less effective than surgical castration in suppressing the ovarian oestrogen secretion. This is suggested by a further decrease in oestrogen excretion following oophorectomy, in patients with irradiated ovaries (Smith and Emerson, 1954), although this finding is not confirmed by Diczfalusy et al., (1959). The small amount of aetiocholanolone shown by Plantin et al., (1958) to arise from ovarian precursors is unlikely to have much bearing on the clinical course of the disease in response to oophorectomy.

(2) Adrenalectomy: Oestrogens. In view of the evidence shown in Table 1 that the adrenal is the likely source of oestrogens secreted after oophorectomy, it would be anticipated that oophorectomy plus adrenalectomy would abolish the excretion not only of oestrogens, but also of 17-oxosteroids which in the female are derived from precursors of adrenal origin.

Table 10 summarises the results published of /

/of hormone assays performed after adrenalectomy. In contrast to the first report by Dao using a bioassay, all subsequent investigations have shown continued oestrogen excretion after adrenalectomy. The latter results were mostly obtained using chemical methods of estimation. Hellström and his colleagues have reservations about the nature of the compounds measured by the Kober colour reaction in urine from adrenalectomised patients, although reports of cornified vaginal smears (Struthers, 1956) and parallel bioassay and chemical assay by the method of Brown, Bulbrook and Greenwood (Bulbrook, Greenwood and Williams, 1960) suggest that biologically active oestrogen is present. Persistent, though erratic, oestrogen excretion over long periods of time after adrenalectomy has also been reported (Bulbrook and Greenwood, 1957).

From a rather limited series of thirteen patients, Bulbrook et al., (1958) formed the impression that in general, persistent oestrogen excretion after adrenalectomy was associated with failure of the patient to respond to the operation, although it did not preclude regression of the tumour. In a later short series (Bulbrook, Greenwood and Williams, 1960) these same authors were unable to detect by bioassay (0.02 µg per day) any oestrogen excretion in /

/in patients whose cancer did not respond to adrenalectomy. Brown et al., (1959) were also unable to demonstrate any differences in oestrogen excretion between patients who responded to the operation and those who did not.

17-Oxosteroids: Most interest in this group of steroids has centred on the possibility of continued excretion of the 11-deoxy 17-oxosteroids - androsterone, aetiocholanolone and dehydroepiandrosterone - after bilateral adrenalectomy. Kellie and Wade (1957) have reported the excretion of both androsterone and aetiocholanolone in two patients, and Hobkirk (1958) has detected aetiocholanolone in six patients and androsterone in only one of these, after adrenalectomy. Similar findings have been reported by Bulbrook, Greenwood and Thomas (1958). Plantin et al., (1958) could only detect $\triangle^{9,11}$ aetiocholanolone in this type of urine, however this is probably a dehydration artefact arising from aetiocholanolone during hot acid hydrolysis of the urine.

In the only clinical correlation reported, Hobkirk could show no connection between the clinical state of the patient and the excretion of these two compounds.

Pregnanediol: There are only three studies reported on pregnanediol excretion by adrenalectomised patients. In all cases (see Table 10) excretion was reported to continue /

Steroids measured	Assay	Number of Patients	Conclusions	Reference
Oestrogens	Chemical	11	Excretion continued in 9	Greenwood & Bulbrook (1956)
	Chemical	11	Excretion continued in 9	Greenwood & Bulbrook (1957)
	Chemical	16	Excretion continued in 9	Bulbrook, Greenwood, Hadfield & Scowen (1958c)
	-	17	Positive vaginal smears in 9	Hortling, Bjorkesten & Husi-Brummer (1957)
	-	-	Positive vaginal smears in some patients	Klaus (1957)
	Chemical	-	Excretion continued in some cases after pituitary implant of 90 Y or radon	Forrest et al (1958)
	Chemical	3	Excretion continued in all patients after Proton-beam irradiation	Cleveland (1958)
	Biological	50	Excretion detectable in only 2 patients a fter 9 months	Notter (1959)
	Biological	12	Oestrogenic activity returned in 6 cases	Jessiman (1958)

Table 11. Steroid Excretion after Hypophysectomy

Steroids measured	Assay	Number of Patients	Conclusions	Reference
17-Oxosteroids	Chemical	10	Excretion of aetiocholanolone in 10 and androsterone: no dehydroepiandrosterone	Holliday, Kellie & Wade (1958)
	Chemical	9	Androsterone in 6: aetiocholanolone in 7: fraction in 4 after pituitary implant ^{90}Yttrium	Hobkirk (1958)
Total 17-hydroxy-corticosteroids	Chemical	22	Normal levels of blood 17 hydroxycorticosteroids in 4 cases after cortisone withdrawal	Baron, Gurling & Radley Smith (1958)

Table 11 (Cont'd). Steroid Excretion after Hypophysectomy

/continue after operation although the actual levels reported (0.05 to 0.55 mg per 24 hrs.) are at, or below the sensitivity limits of the methods employed.

(3) Hypophysectomy: Table 11 summarises the steroid excretion studies reported in patients after hypophysectomy.

In contrast to the post-adrenalectomy findings of a consistent decrease in the level of oestrogen excretion, without necessarily its abolition, after surgical hypophysectomy excretion may decrease, remain virtually unchanged or even increase. Such findings have been reported in a small series of cases by Greenwood and Bulbrook (1956, 1957) and by Bulbrook et al., (1958). Oestrogenic vaginal smears have been reported in hypophysectomised patients by Hortling et al., (1957) and Klaus (1957), and the biological activity of the oestrogens excreted has been confirmed by parallel chemical and bioassay (Bulbrook, Greenwood and Williams, 1960). Holliday, Kellie and Wade (1958) found 17-oxosteroids in the urine of surgically hypophysectomised patients. Androsterone and aetiocholanolone were present in considerably reduced amounts and were, surprisingly, further reduced by withdrawal of maintenance cortisone. Baron, Gurling and Radley Smith (1958) reported normal plasma /

/plasma 17-hydroxycorticosteroid levels 72 hrs. after cortisone withdrawal in four out of twenty-two hypophysectomised patients, which was assumed to indicate normal adrenal function unaltered by the removal of the pituitary.

Implantation of the pituitary gland with 90-Yttrium and Radon did not abolish oestrogen excretion (Forrest et al., 1958) and in some cases excretion of androsterone and aetiocholanolone also continued (Hobkirk, 1958). As in Holliday, Kellie and Wade's cases, withdrawal of cortisone also occasionally led to a decrease in the excretion of these 11-deoxy 17-oxosteroids.

Clinical Correlations: Hortling et al., (1957) claimed that in all their patients who improved after hypophysectomy oestrogenic effects disappeared from the vaginal smear, and Bulbrook et al., (1958) were led to the general impression, as after adrenalectomy, that high postoperative oestrogen excretion was associated with relapse of cancer, or a failure to benefit from the operation. None of the other workers, however, (see Table 5) could show any correlation between the levels of excretion of oestrogens or 17-oxosteroids after hypophysectomy or radioactive implantation of the pituitary and the clinical response of the patient. /

/patient.

c) Prediction of Clinical Response to Endocrine Surgery.

The response of patients, even within apparently identical clinical groups, to endocrine surgery is at present unpredictable. Although it may be possible to reach a choice by relatively simple tests, for example the change in the urinary calcium level induced by the administration of an oestrogen or cortisone (Pearson et al., 1954; Emerson and Jessiman, 1956) the value of such tests is limited to patients with extensive bone metastases and may well involve provoking an exacerbation of disease only to demonstrate that the form of treatment used is contra-indicated (Strong, 1958).

Allen, Hayward and Merivale (1958) have suggested that in patients who subsequently benefited from endocrine surgery, the ratio of the preoperative excretion of 11-deoxy 17-oxosteroids to 11-oxy 17-oxosteroids was greater than 1 and the pattern approximated to that found in normal subjects, whereas in those who did not benefit, the ratio was less than 1 and the excretion pattern closely resembled that found after operation, although Plantin et al., (1958) and Hobkirk (1958) could not confirm this.

More recently Bulbrook, Greenwood and Hayward, /

/Hayward, (1960) have estimated the urinary excretion of aetiocholanolone and total 17-hydroxycorticosteroids in untreated breast cancer patients. From these two results they calculate a figure which they term a discriminant, the value of which gives a guide to the probable response of the patient to either oophorectomy and adrenalectomy or hypophysectomy. A more recent report by the same authors (Bulbrook, Hayward and Thomas, 1961) suggests that the usefulness of this function in the selection of patients for endocrine surgery decreases with increasing age, and that its usefulness may be very limited indeed. Confirmation of the original findings of these workers is still awaited in order to judge whether the considerable work involved in performing the assays is justified by the results obtained. Such empirical ratios are at present hard to reconcile with any known facts concerning the physiology of steroid metabolism in breast cancer.

RESULTS

1. The Effect of Endocrine Ablation on the Urinary Excretion of Neutral 17-Oxosteroids and Oestrogens by Women with Advanced Metastatic Breast Cancer.

Estimations of urinary neutral 17-oxosteroids and oestrogens have been carried out in a series of breast cancer patients with the following aims.

a) to compare the effects of different forms of endocrine surgery on the excretion of these steroids.

b) to obtain information from preoperative studies which might assist in the choice of the appropriate form of treatment for the patient.

c) to correlate the effect of endocrine surgery on the cancer, with the postoperative alterations in steroid excretion.

The significance of differences between mean excretions has been determined by Students 't' test (Fisher, 1930) using both the Arithmetic and Logarithmic values of the excretions.

Mean Excretion of Total Neutral 17-Oxosteroids (mg per day) in 12 Patients with Breast Cancer Before and After Adrenalectomy and Oophorectomy

Time of Test	Maintenance Therapy (mg per day)	Benefit	No Benefit	Total
		6 Patients	6 Patients	
Before Adrenalectomy	0 mg Cortisone	4.5 ± 0.53	4.3 ± 1.01	4.4 ± 0.55
After Adrenalectomy	50 mg Cortisone	3.1 ± 0.35	2.9 ± 0.46	3.0 ± 0.28
After Adrenalectomy	50 mg Cortisone + 120 u ACTH	2.7 ± 0.42	3.1 ± 0.44	
Mean Difference		-0.4 ± 0.6	+0.2 ± 0.5	1.4 ± 0.6
P				< 0.05

Table 12

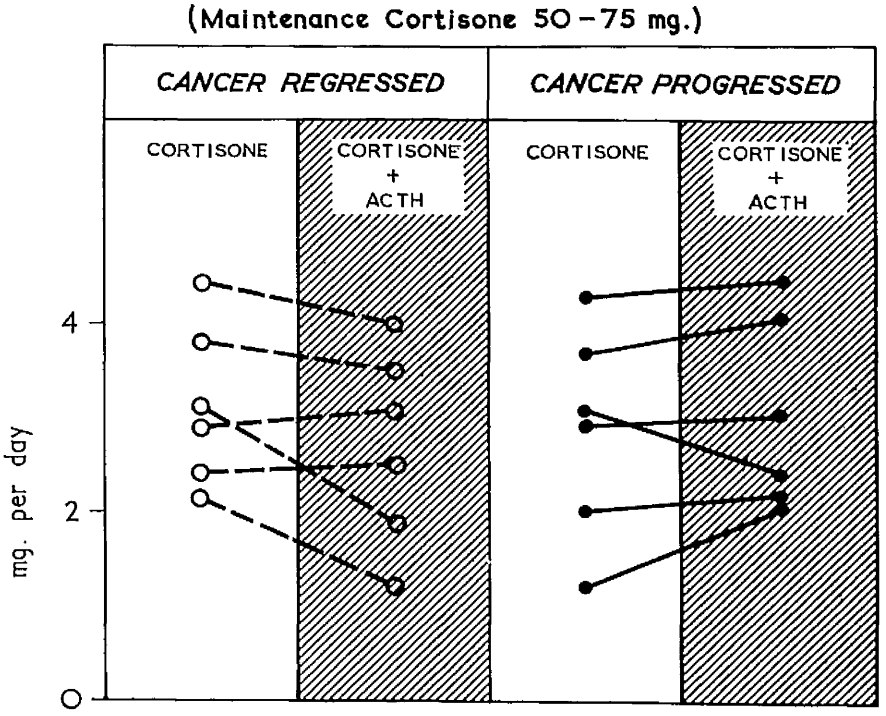


Fig. 7. Effect of ACTH administration on urinary total 17-oxosteroid secretion after adrenalectomy.

17-OXOSTEROID STUDIES.

Total Neutral 17-Oxosteroids.

Adrenalectomy and Oophorectomy. Total neutral 17-oxosteroids were estimated in at least three 24 hr. collections of urine made before, and in at least three collections made one to three months after adrenalectomy and oophorectomy, in twelve postmenopausal women with breast cancer. Postoperatively the patients received 50 mg. cortisone per day, and during one 5-day period 120 units corticotrophin (ACTH) gel daily, by intramuscular injection.

The removal of adrenals and ovaries significantly reduced the excretion of total neutral 17-oxosteroids (Table 12). The mean fall was identical in both the responding and non-responding groups of patients. ACTH administration did not cause a significant increase in oxosteroid excretion in either group (Fig. 7).

Pituitary implantation. Daily estimations of total neutral 17-oxosteroids were made before and after implantation of the pituitary with 90-Yttrium. Of the eighteen patients studied, nine had regression of cancer as a result of the pituitary destruction.

Preoperatively, three to six determinations were /

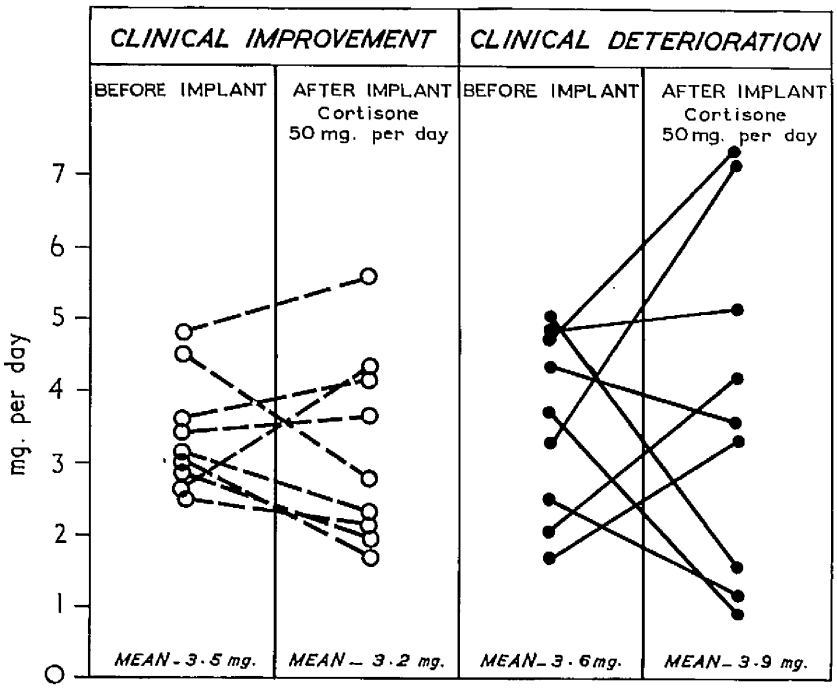


Fig. 8. Effect of pituitary implantation with 90-Yttrium on urinary total 17-oxosteroid excretion.

Mean Excretion of Total Neutral 17-Oxosteroids (mg per day) in 18 Patients

Before and After Pituitary Implantation with 90 Yttrium

Time of Test	Maintenance Therapy (mg per day)	Benefit	No Benefit	Total
		9 Patients	9 Patients	
Before	0 mg Cortisone	3.5 ± 0.29	3.6 ± 0.42	3.5 ± 0.25
After	50 mg Cortisone	3.2 ± 0.45	3.9 ± 0.88	3.5 ± 0.49
Mean Difference		-0.3 ± 0.41	+0.3 ± 0.89	
P		> 0.4	> 0.7	

Table 13

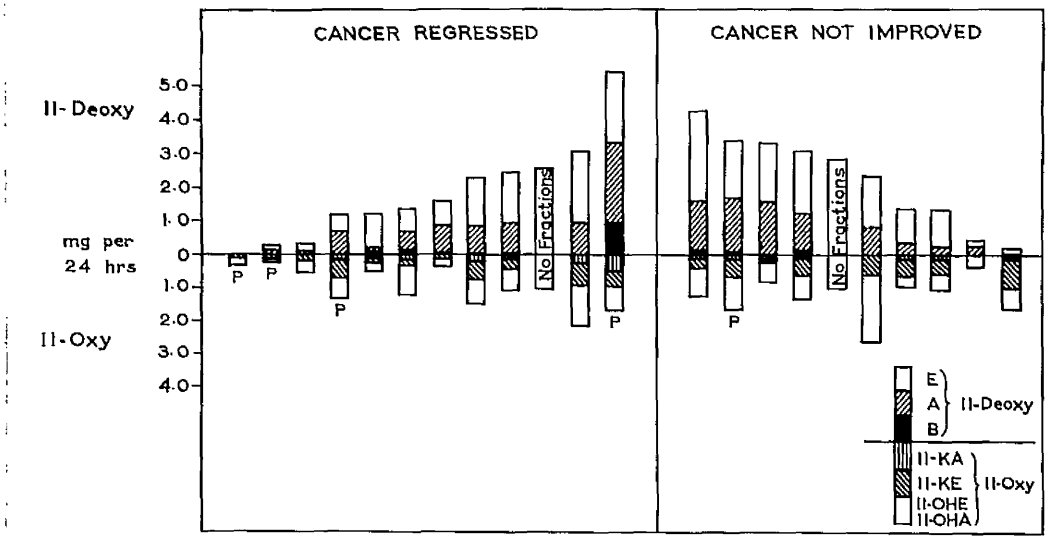


Fig. 9. Preoperative excretion of 11-oxy and 11-deoxy 17-oxosteroids in 22 women with breast cancer.

Mean Excretion of Urinary 17-Oxosteroids in 20 Women with Breast Cancer

before Endocrine Surgery

Response to Endocrine Surgery	Number of Patients	11-oxy 17-oxosteroids (mg per 24 hours)			
		11 OH E + 11 OH A	11 KE	11 KA	Total
Benefit	11	0.53 ± 0.11	0.30 ± 0.06	0.10 ± 0.03	0.93 ± 0.19
No Benefit	9	0.74 ± 0.17	0.44 ± 0.09	0.08 ± 0.02	1.26 ± 0.21

11 OH E : 11β Hydroxyaetiocholanolone
11 OH A : 11β Hydroxyandrosterone
11 KE : 11 Oxoaetiocholanolone
11 KA : 11 Oxoandrosterone

Table 14

Mean Excretion of Urinary 17-Oxosteroids in 20 Women with Breast Cancer

before Endocrine Surgery

Response to Endocrine Surgery	Number of Patients	11-deoxy 17-oxosteroids (mg per 24 hours)			
		E	A	β Fraction	Total
Benefit	11	0.85 ± 0.24	0.69 ± 0.21	0.11 ± 0.09	1.65 ± 0.49
No Benefit	9	1.31 ± 0.27	0.87 ± 0.21	0.03 ± 0.02	2.22 ± 0.47

E : Aetiocholanolone
A : Androsterone

Table 14

(Cont'd)

Comparison of the mean preoperative excretion of 17-oxosteroids in 12 patients who had benefit and 10 patients who had no benefit from endocrine surgery

Response to Endocrine Surgery	Number of Patients	11-oxy 17-oxosteroids (mg per 24 hrs.)		11-deoxy 17-oxosteroids (mg per 24 hrs.)	
		mg per 24 hrs.	log. mg per 24 hrs.	mg per 24 hrs.	log. mg per 24 hrs.
Benefit	12	0.94 ± 0.17	0.945 ± 0.120	1.74 ± 0.46	0.982 ± 0.170
No Benefit	10	1.23 ± 0.19	1.038 ± 0.080	2.28 ± 0.43	1.236 ± 0.130
Mean Difference		0.29 ± 0.26	0.093 ± 0.148	0.54 ± 0.64	0.254 ± 0.221
P		> 0.2	> 0.5	> 0.4	> 0.2

Table 15

/were performed and the postoperative results are the means of all estimations carried out within three months of implantation. In neither group of patients did pituitary implantation lead to a significant reduction in total neutral 17-oxosteroid excretion (Table 13; Figure 8).

Neutral 17-Oxosteroid Fractionation.

Preoperative Estimations. Table 14 shows the mean excretion (mg per day) of individual neutral 17-oxosteroids in 20 patients before endocrine surgery. In a further two patients, separation into 11-oxy and 11-deoxy fractions only was carried out. The values in the eleven patients whose cancer regressed subsequent to endocrine surgery are quantitatively similar to those in the nine who did not benefit. This similarity is demonstrated graphically in Figure 9.

In Table 15 the mean excretion of the 11-oxy 17-oxosteroids in the responding and non-responding groups of patients are compared statistically by Student's "t" test using both the arithmetic values of, and (x 10) the logarithms of the outputs. The total 11-deoxy 17-oxosteroid excretions are similarly analysed. No significant differences are revealed between patients who subsequently benefit from /

PREOPERATIVE ESTIMATIONS OF URINARY 17-OXOSTEROIDS IN PATIENTS WITH

Behaviour of Cancer	Case Number	Endocrine Surgery	mg per day			Mean Ratio
			11-deoxy 17-oxosteroids	11-oxy 17-oxosteroids	Ratio : 1	
Cancer not Improved (10 Patients)	Ys 25	Pituitary Implant	0.26	1.59	0.2	2.1:1
	S 1	Oophorectomy + Pit. Implant	2.36	2.56	0.9	
	Ys 9	Pituitary Implant	1.38	1.00	1.4	
	As 41	Oophorectomy + Adrenalectomy + Pituitary Implant	0.44	0.32	1.4	
	Ys 12	Pituitary Implant	1.43	0.94	1.5	
	Ys 11	Oophorectomy + Pit. Implant	3.39	1.56	2.2	
	Ys 18	Pituitary Implant	3.07	1.29	2.4	
	As 5	Adrenalectomy + Pit. Implant	2.85	0.95	2.9	
	Ys 15	Pituitary Implant	4.29	1.22	3.5	
	Ys 10	Adrenalectomy + Pit. Implant	3.35	0.82	4.1	

Table 16

PREOPERATIVE ESTIMATIONS OF URINARY 17-OXOSTEROIDS IN PATIENTS WITH BREAST CANCER TREATED BY ENDOCRINE ABLATION

Behaviour of Cancer	Case Number	Endocrine Surgery	mg per day			Mean Ratio
			11-deoxy 17-oxosteroids	11-oxy 17-oxosteroids	Ratio : 1	
Cancer Regressed (12 Patients)	O x 22	Oophorectomy	0	0.26	0.7	2.2:1
	S 20	Pituitary Implant	0.34	0.49	0.9	
	Ys 16	Oophorectomy + Pit. Implant	1.20	1.33	0.9	
	Ys 10	Pituitary Implant	1.39	1.15	1.2	
	S 39	"	0.27	0.19	1.4	
	Ys 13	"	3.10	2.06	1.5	
	Ys 14	"	2.33	1.45	1.6	
	S 44	"	2.47	0.97	2.5	
	As 6	Adrenalectomy + Oophorectomy	2.63	1.03	2.6	
	Ys 27	Pituitary Implant	1.24	0.48	2.6	
	O x 1	Oophorectomy	5.48	1.62	3.4	
	As 38	Adrenalectomy + Pit. Implant	1.55	0.27	5.7	

Table 16 (Cont'd).

/from endocrine surgery and those who do not.

Prediction of Response to Endocrine Surgery.

From Tables 14 and 15 it is obvious that grossly abnormal steroid excretion is not associated with either group of patients and as a consequence preoperative measurement of any single 17-oxosteroid is no guide to the likely response of a patient to endocrine surgery. Ratios of the excretion values of 17-oxosteroids have been reported by certain authors as being of value to them in selecting patients likely to benefit from endocrine treatment (see p.47). In Table 16 preoperative values of the ratio of 11-deoxy to 11-oxy 17-oxosteroids are shown in twenty-two patients whose subsequent response to endocrine surgery was known. The mean ratio in the twelve patients whose cancer regressed is almost identical to that in the ten patients who had no benefit, and the individual ratios were of no value in forecasting the response to treatment.

In a further series of twenty-two patients, the preoperative excretion of total 17-hydroxycorticosteroids and aetiocholanolone were determined. The individual levels, and the ratio of 17-hydroxycorticosteroid: aetiocholanolone in each patient are shown in Figures 10 and 11. Patients who failed to respond to treatment did not show /

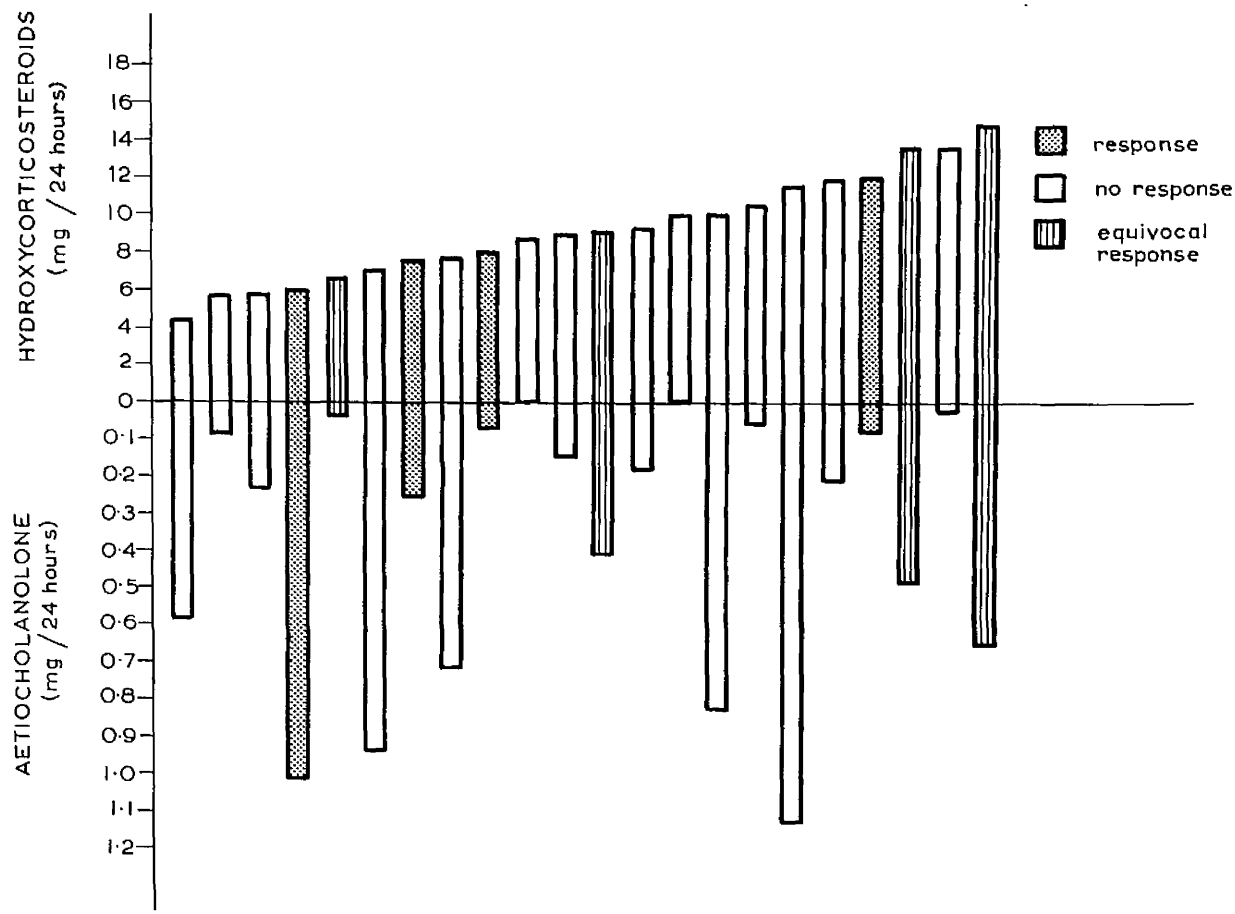


Figure 10. The preoperative excretion of total 17-hydroxy-corticosteroids and aetiocholanolone in twenty-two patients with breast cancer.

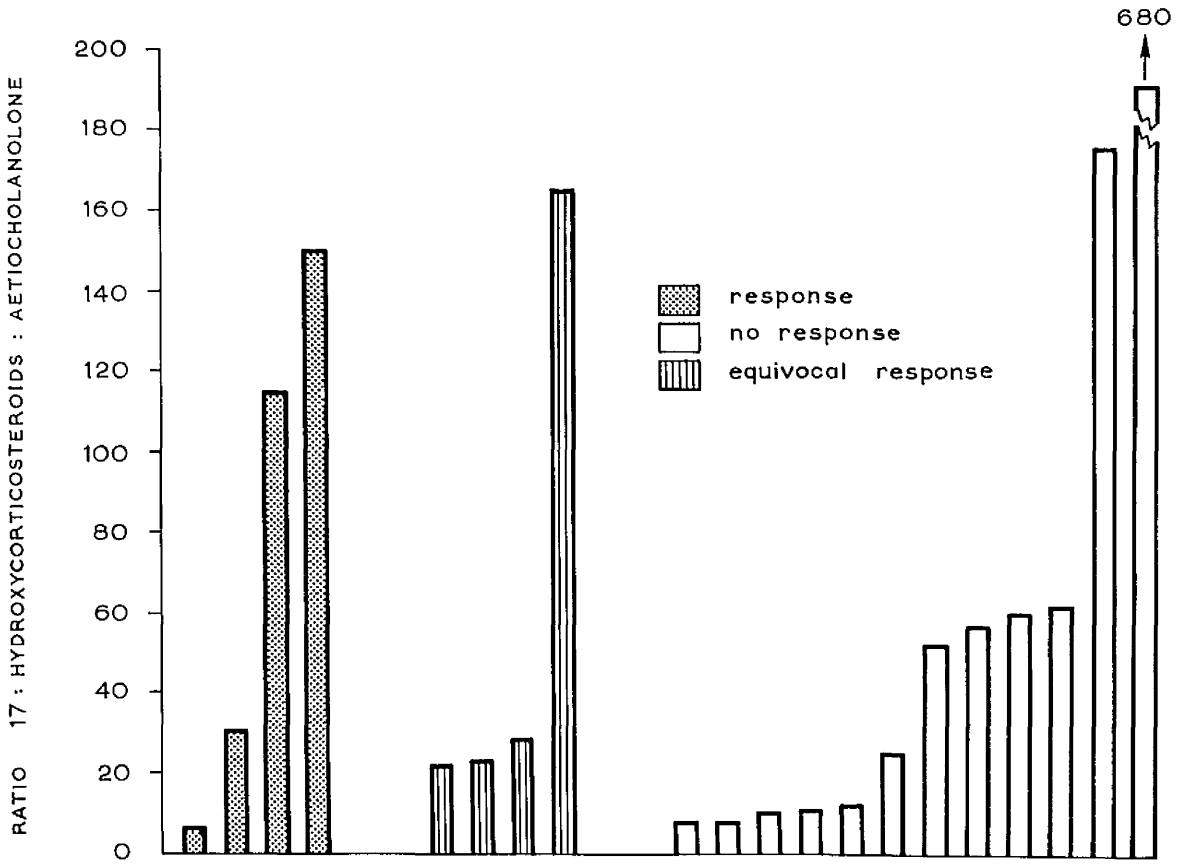


Figure 11. The ratio of the preoperative excretion of total 17-hydroxycorticosteroids to that of aetiocholanolone in twenty patients with breast cancer.

/show decreased aetiocholanolone and increased 17-hydroxycorticosteroid excretion, and these ratios also appear to be of little value as an aid in choosing patients most likely to benefit from endocrine surgery.

MEAN EXCRETION OF URINARY 17-OXOSTEROIDS AFTER ADRENALECTOMY AND OOPHORECTOMY IN 16 WOMEN WITH BREAST CANCER.

Response of Cancer	Number of Patients	11-oxy 17-oxosteroids (mg per 24 hours)			
		11 OH E 11 OH A	11 KE	11 KA	TOTAL
Benefit	10	0.58 ± 0.13	0.92 ± 0.30	0.02 ± 0.01	1.52 ± 0.35
No Benefit	6	0.49 ± 0.22	0.46 ± 0.17	0	0.95 ± 0.36

Table 17

MEAN EXCRETION OF URINARY 17-OXOSTEROIDS AFTER ADRENALECTOMY AND OOPHORECTOMY IN 16 WOMEN WITH BREAST CANCER.

Response of Cancer	Number of Patients	11-deoxy 17-oxosteroids (mg per 24 hours)			
		E	A	β Fraction	Total
Benefit	10	0.01 ± 0.005	0	0	0.01 ± 0.005
No Benefit	6 *	0.03 ± 0.026	0.03 ± 0.026	0	0.06 ± 0.052

* Only one patient excreted 11-deoxy 17-oxosteroids

Table 17 (Cont'd).

COMPARISON OF THE MEAN POST OPERATIVE EXCRETION OF 11-OXY 17-OXOSTEROIDS IN 10 PATIENTS WHO RESPONDED AND 6 WHO DID NOT RESPOND TO ADRENALECTOMY PLUS OOPHORECTOMY.

Nature of Endocrine Surgery	Response of Cancer	Number of Patients	Mean 11-oxy 17-oxosteroid excretion	
			Mg per 24 hours	Log mg per 24 hours
Oophorectomy and Adrenalectomy	Benefit	10	1.52 ± 0.35	1.063 ± 0.346
	No Benefit	6	0.95 ± 0.36	0.770 ± 0.656
	Mean Difference		0.57 ± 0.54	0.293 ± 0.212
	P		> 0.3	> 0.1

Table 18.

THE EXCRETION OF URINARY 11-DEOXY 17-OXOSTEROIDS AFTER ADRENALECTOMY IN 7 WOMEN WITH BREAST CANCER. IN ANOTHER 9 PATIENTS STUDIES NO 11-DEOXY 17-OXOSTEROIDS WERE PRESENT.

Response of Cancer	Case Number	11-deoxy 17-oxosteroids (mg. per 24 hrs.)		
		E	A	β-Fraction
Benefit	As 17	0.03	0	0
	As 9	0.01	0	0
	As 7	0.04	0	0
	As 38	0.01	0	0
	As 26	0.01	0	0
	Ax 4	0.02	0	0
No Benefit	As 5	0.16	0.15	0

Table 19.

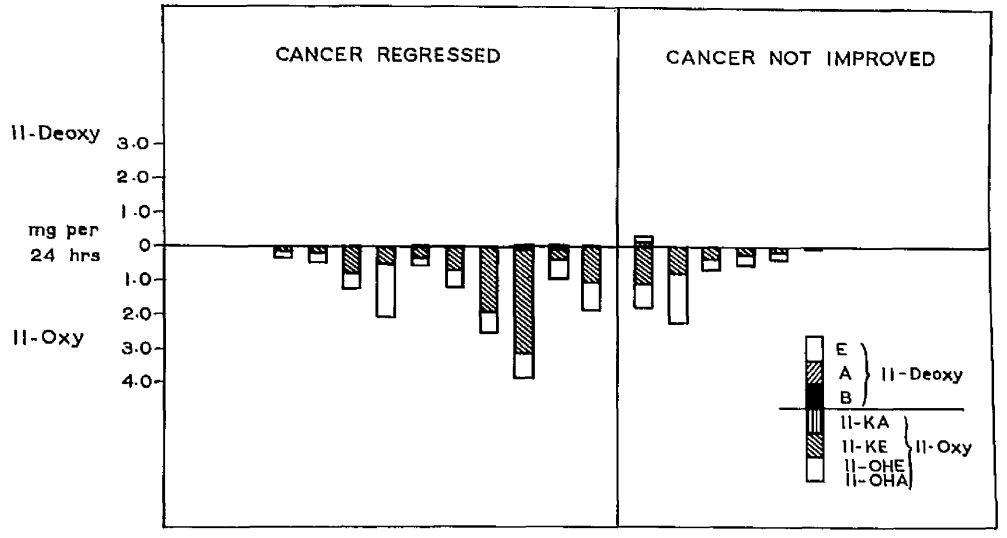


Fig. 12. Excretion of 11-oxy and 11-deoxy 17-oxosteroids after adrenalectomy and oophorectomy in 16 women with breast cancer.

MEAN EXCRETION OF URINARY 17-OXOSTEROIDS AFTER PITUITARY IMPLANTATION WITH YTTRIUM-90 IN 22 WOMEN WITH BREAST CANCER.

Response of Cancer	Number of Patients	11-oxy 17-oxosteroids (mg per 24 hours)			
		11 OH E 11 OH A	11 KE	11 KA	Total
Benefit	12	0.73 ± 0.23	0.59 ± 0.17	0.06 ± 0.03	1.38 ± 0.42
No Benefit	10	0.53 ± 0.19	0.39 ± 0.14	0.04 ± 0.03	0.96 ± 0.24

Table 20

MEAN EXCRETION OF URINARY 17-OXOSTEROIDS AFTER PITUITARY IMPLANTATION WITH YTTRIUM-90 IN 22 WOMEN WITH BREAST CANCER.

Response of Cancer	Number of Patients	11-deoxy 17-oxosteroids (mg per 24 hours)			
		E	A	β Fraction	Total
Benefit	12	0.16 ± 0.07	0.05 ± 0.03	0.06 ± 0.03	0.27 ± 0.11
No Benefit	10	0.23 ± 0.17	0.10 ± 0.07	0.02 ± 0.02	0.35 ± 0.24

Table 20 (Cont'd).

Postoperative Estimations.

Adrenalectomy and Oophorectomy. The mean excretion of individual 17-oxosteroids after adrenalectomy and oophorectomy in sixteen women with breast cancer is shown in Table 17 and is unrelated to the clinical course of the disease. (Figure 12). The 11-oxy 17-oxosteroids present in the urine of all patients (11β hydroxyandrosterone, 11β hydroxyaetiocholanolone and 11-oxoaetiocholanolone) are metabolites of maintenance cortisone and differences between the mean excretions of the responding and non-responding groups are not significant (Table 18). Adrenalectomy and oophorectomy led to the anticipated disappearance of 11-deoxy 17-oxosteroids from the urine of nine of the sixteen patients. Of the remaining seven patients six who responded to surgery excreted trace amounts of aetiocholanolone only, while in one patient who failed to benefit, small but significant amounts of aetiocholanolone and androsterone were present (Table 19).

Pituitary Implantation. Table 20 shows the mean excretion of individual 17-oxosteroids in twenty-two women with breast cancer after pituitary implantation with 90-Yttrium. As after adrenalectomy and oophorectomy, the level of steroid excretion bears no relation to the clinical response /

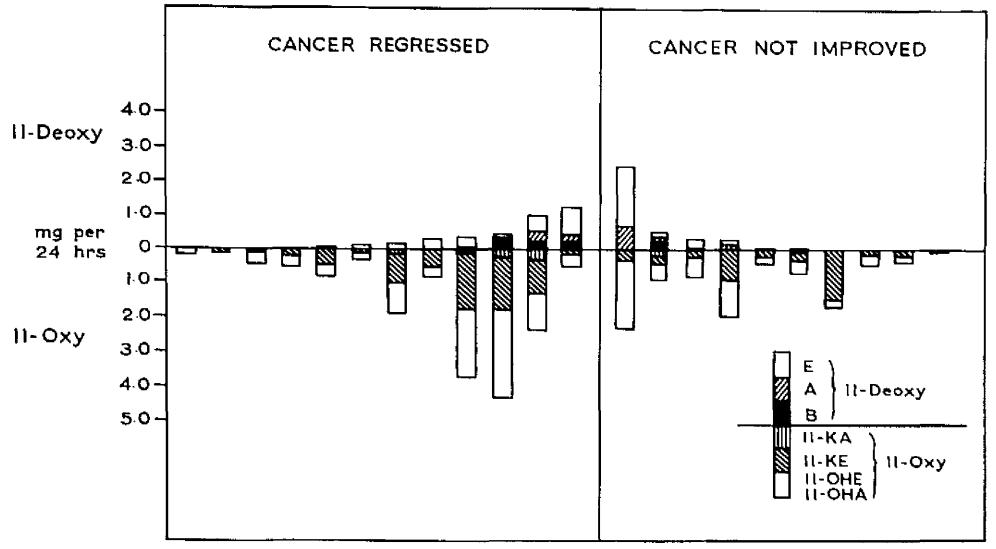


Fig. 13. Excretion of 11-oxy and 11-deoxy 17-oxosteroids after pituitary implantation with 90-Yttrium in 22 women with breast cancer.

THE EXCRETION OF INDIVIDUAL 11-DEOXY 17-OXOSTEROIDS AFTER PITUITARY IMPLANTATION WITH YTTRIUM 90 IN 13 WOMEN WITH BREAST CANCER.

Response of Cancer	Case Number	11-deoxy 17-oxosteroids (mg. per 24 hrs.)		
		E	A	β fraction
Benefit	Ys 14	0.20	0	0.10
	S 3	0.83	0.19	1.20
	Yu 10	0.26	0	0
	Ys 6	0.45	0.35	0.14
	Ys 3	0.07	0.02	0.27
	Ys 1	0.09	0	0
	Ys 22	0.04	0	0.01
No Benefit	Ys 15	0.16	0.11	0
	Ys 10	1.72	0.68	0
	Ys 25	0.28	0	0.01
	S 37	0.02	0.04	0
	S 66	0.12	0.19	0.17
	Ys 23	0	0	0.01

Table 21.

COMPARISON OF MEAN EXCRETION OF 11-DEOXY 17-OXOSTEROIDS IN 12 PATIENTS WHO RESPONDED AND 10 PATIENTS WHO DID NOT RESPOND TO PITUITARY IMPLANTATION.

Response of Cancer	Number of Patients	Mean Excretion of 11-deoxy 17-oxosteroids	
		Mg. per 24 hours	Log mg. per 24 hours
Benefit	12	0.27 ± 0.11	0.292 ± 0.118
No Benefit	10	0.35 ± 0.24	0.295 ± 0.145
Mean Difference		0.08 ± 0.25	0.003 ± 0.184
P		> 0.7	> 0.9

Table 22.

COMPARISON OF THE MEAN POST OPERATIVE EXCRETION OF 11-OXY 17-OXOSTEROIDS IN 12 PATIENTS WHO RESPONDED AND IN 10 PATIENTS WHO DID NOT RESPOND TO PITUITARY IMPLANTATION.

Nature of Endocrine Surgery	Response of Cancer	Number of Patients	Mean 11-oxy 17-oxosteroid excretion	
			Mg. per 24 hours	Log mg. per 24 hours
Pituitary Implantation	Benefit	12	1.38 ± 0.42	0.916 ± 0.436
	No Benefit	10	0.96 ± 0.24	0.839 ± 0.424
	Mean Difference		0.42 ± 0.51	0.077 ± 0.191
	P		> 0.4	> 0.6

Table 23.

/response of the patient (Figure 13). Continuing adrenal function after pituitary implantation is suggested by the presence of variable amounts of one or more of the 11-deoxy 17-oxosteroids in the urine of thirteen out of the twenty-two patients studied. The amounts are considerably greater than those present after adrenalectomy and the values are shown in Table 21. The mean outputs of total 11-deoxy 17-oxosteroids are compared statistically in Table 22, in patients who responded, and in those who failed to improve after pituitary implantation with 90-Yttrium. No significant difference was present, and a similar result was obtained for the difference between the postoperative 11-oxy 17-oxosteroid output in the two groups (Table 23).

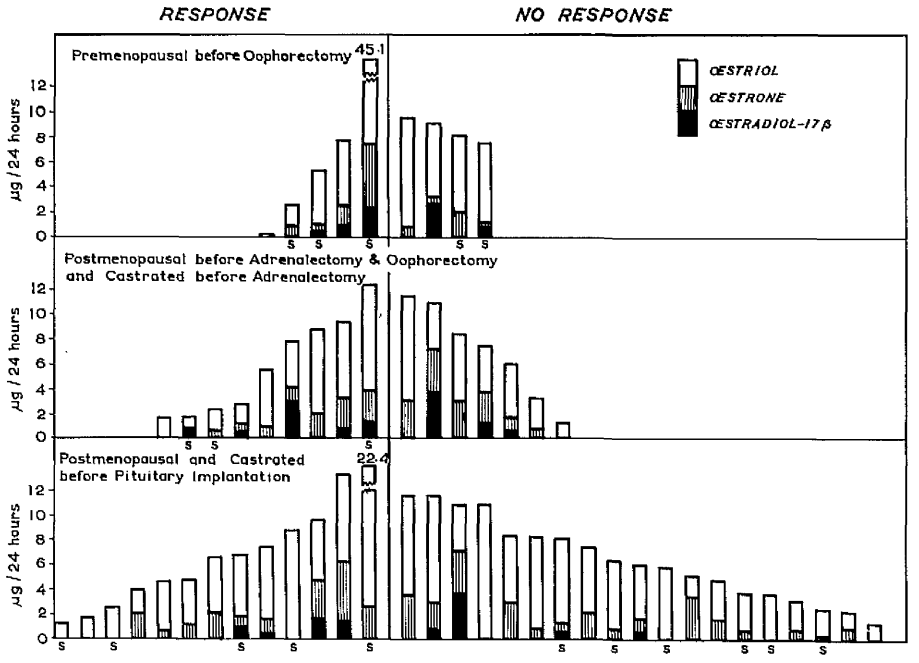


Figure 14. Preoperative oestrogen excretion by 48 women with breast cancer.

PREOPERATIVE Excretion of Oestrogens in Women treated by ADRENALECTOMY plus OOPHORECTOMY and by PITUITARY IMPLANT.

Response of Cancer		No.	µg/24 hrs.			
			Oestriol	Oestrone	Oestradiol	Total
Ax	Benefit	9	3.9 ± 0.89	1.1 ± 0.33	0.7 ± 0.31	5.7 ± 1.28
	No Benefit	7	4.1 ± 0.85	1.9 ± 0.5	0.7 ± 0.5	6.7 ± 1.41
Hx	Benefit	13	5.4 ± 1.34	1.4 ± 0.40	0.3 ± 0.16	7.1 ± 1.58
	No Benefit	19	4.7 ± 0.61	1.3 ± 0.28	0.3 ± 0.19	6.3 ± 0.74

Table 24

Preoperative Excretion of Oestrogens in Women treated by Adrenalectomy and Oophorectomy.

Response of Cancer	Number of Patients	Mean excretion of oestriol, oestrone and oestradiol	
		µg per 24 hrs.	log. µg per 24 hrs.
Benefit	9	5.7 ± 1.28	0.648 ± 0.122
No Benefit	7	6.7 ± 1.41	0.734 ± 0.130
Mean difference		1.0 ± 1.92	0.086 ± 0.173
P		> 0.6	> 0.7

Table 25

Preoperative Excretion of Oestrogens in Women treated by Pituitary Implant

Response of Cancer	Number of Patients	Mean excretion of oestriol, oestrone and oestradiol	
		µg per 24 hrs.	log. µg per 24 hrs.
Benefit	13	7.1 ± 1.58	0.732 ± 0.100
No Benefit	19	6.3 ± 0.74	0.730 ± 0.006
Mean difference		0.8 ± 1.58	0.002 ± 0.110
P		> 0.5	> 0.9

Table 26

OESTROGEN STUDIES.

Preoperative Estimations. The mean preoperative excretion of oestriol, oestrone and oestradiol-17β in forty-eight women with breast cancer before either adrenalectomy and oophorectomy or pituitary implantation is shown in Table 24, divided into those who did, and those who did not benefit as a result of treatment. The results are illustrated graphically in Figure 14, in which they are arranged in three groups (1) estimations in premenopausal women before oophorectomy (2) estimations in castrated or postmenopausal women before adrenalectomy or adrenalectomy and oophorectomy and (3) estimations in castrated or postmenopausal women before pituitary implantation with 90-Yttrium. It is clear that the response to endocrine surgery bears no relation to the preoperative levels of excretion or any or all of these steroids. The means of the arithmetic and logarithmic values of total oestrogen excretion are compared statistically in responding and non-responding patients in Table 25 and before pituitary implantation in Table 26. The mean total oestrogen levels in those patients whose cancer regressed did not differ significantly from those in whom no improvement occurred. /

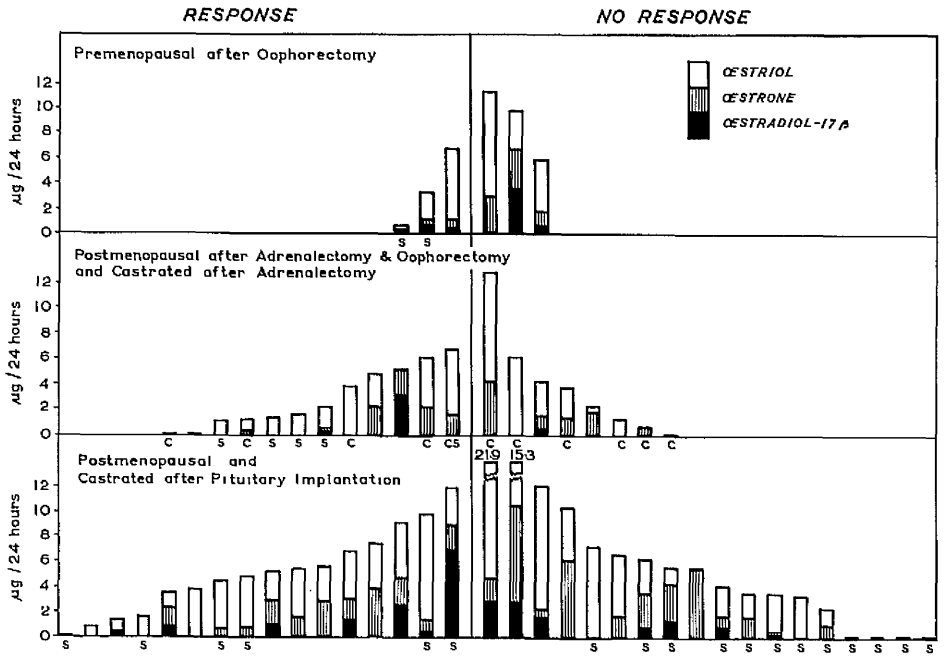


Figure 15. Oestrogen excretion by 54 women after endocrine surgery.

Post Operative Excretion of Oestrogens in Women treated by Adrenalectomy plus Oophorectomy and by Pituitary Implantation.

Response of Cancer		Number of Patients	µg per 24 hours			
			Oestriol	Oestrone	Oestradiol	Total
Ax	Benefit	12	1.9 ± 0.49	0.8 ± 0.29	0.3 ± 0.27	3.0 ± 0.69
	No Benefit	8	2.7 ± 1.12	1.2 ± 0.51	0.1 ± 0.06	4.0 ± 1.48
Hx	Benefit	16	3.0 ± 0.49	1.3 ± 0.29	0.8 ± 0.45	5.1 ± 0.83
	No Benefit	18	3.6 ± 1.03	1.8 ± 0.55	0.5 ± 0.21	5.9 ± 1.37

Table 27

Postoperative Excretion of Oestrogens in Postmenopausal or Castrated Women treated by Adrenalectomy.

Response of Cancer	Number of Patients	Mean excretion of oestriol, oestrone or oestradiol	
		µg per 24 hrs.	log. µg per 24 hrs.
Benefit	12	2.9 ± 0.69	0.372 ± 0.095
No Benefit	8	4.0 ± 1.48	0.451 ± 0.141
Mean difference*		1.1 ± 1.47	0.079 ± 0.164
P		> 0.4	> 0.6

Table 28

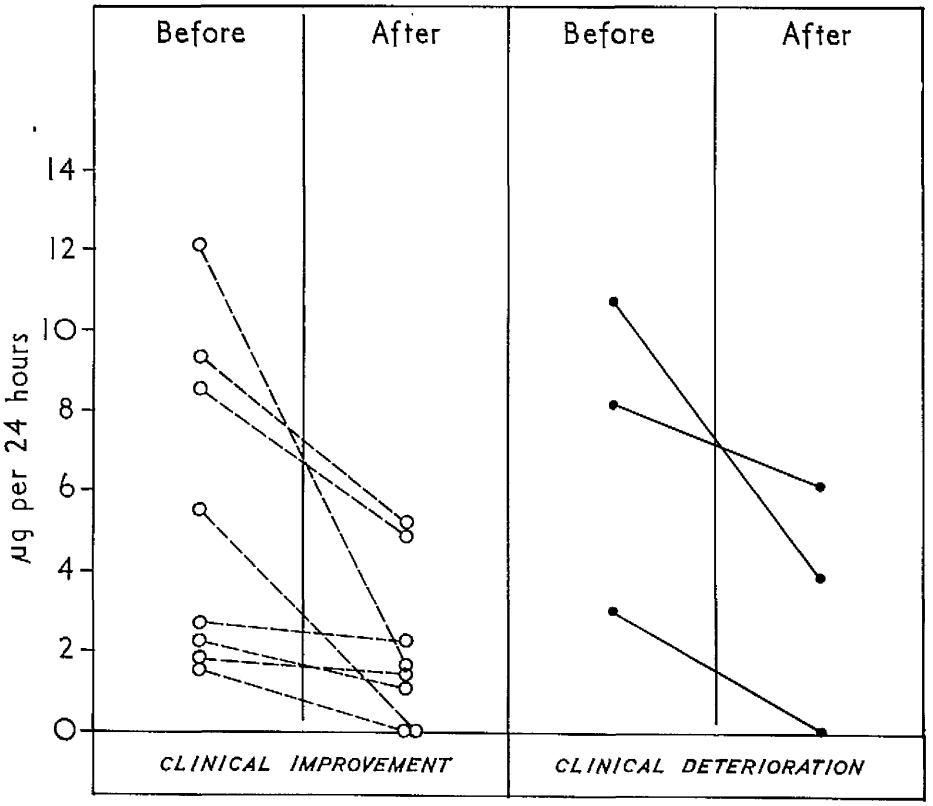


Figure 16. Total oestrogen excretion in 11 patients before and after adrenalectomy and oophorectomy for breast cancer.

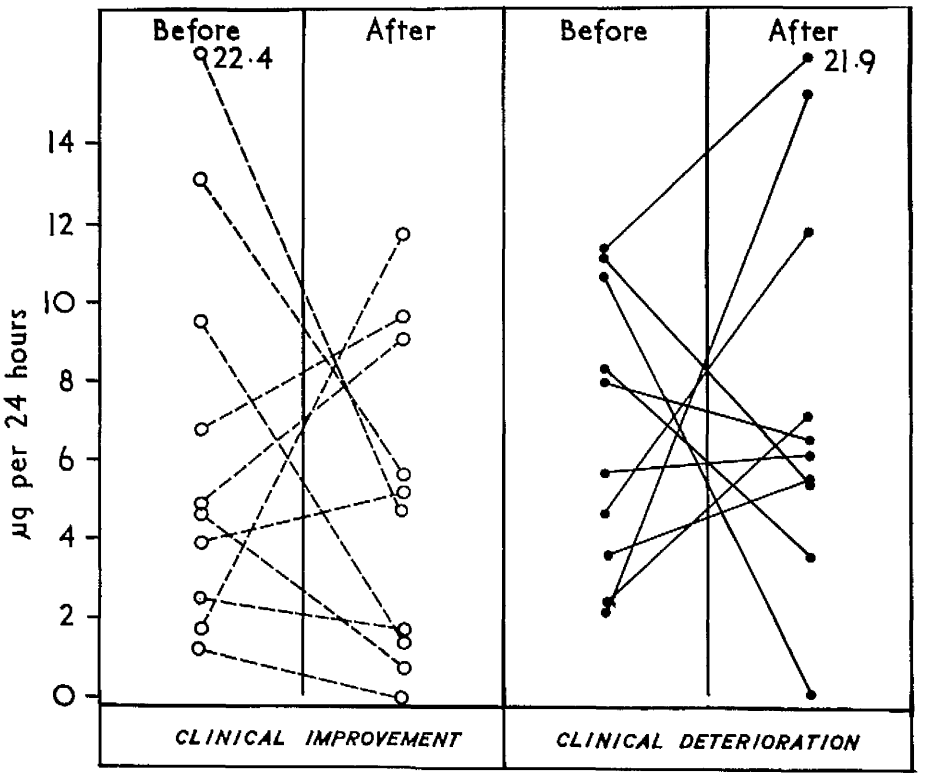


Figure 17. Total oestrogen excretion in 20 patients before and after pituitary implantation with 90-Yttrium for breast cancer.

Postoperative Excretion of Oestrogens in Postmenopausal or Castrated Women treated by Pituitary Implantation.

Response or Cancer	Number of Patients	Mean excretion of oestriol, oestrone and oestradiol	
		µg per 24 hrs.	log. µg per 24 hrs.
Benefit	16	5.1 ± 0.83	0.606 ± 0.01
No Benefit	18	5.9 ± 1.37	0.617 ± 0.01
Mean difference		0.8 ± 1.66	0.011 ± 0.130
P		> 0.6	> 0.9

Table 29

Comparison of Excretion of Oestriol, Oestrone and Oestradiol-17β after Adrenalectomy plus Oophorectomy and after Pituitary Implantation

Endocrine Surgery	Number of Patients	Mean excretion of oestriol, oestrone and oestradiol-17β	
		μg per 24 hours	log μg per 24 hours
Adrenalectomy and Oophorectomy	20	3.3 $\pm$ 0.71	0.404 $\pm$ 0.079
Pituitary Implantation	34	5.5 $\pm$ 0.82	0.612 $\pm$ 0.064
Mean difference		2.2 $\pm$ 1.19	0.208 $\pm$ 0.103
P		$<$ 0.1	$<$ 0.05

Table 30

Mean Excretion of Oestriol, Oestrone and Oestradiol-17β in 11 Patients Before and After Adrenalectomy plus Oophorectomy

Response of Cancer	Number of Patients	Time of Test	μg per 24 hrs.			
			Oestriol	Oestrone	Oestradiol	Total
Benefit	8	Before	4.0	1.1	0.4	5.5
		After	1.0	0.6	0.4	2.0
No Benefit	3	Before	3.8	2.3	1.2	7.3
		After	2.9	0.5	0	3.4

Table 31

Mean Excretion of Oestriol, Oestrone, Oestradiol-17β in 20 Patients Before and After Pituitary Implantation

Response of Cancer	Number of Patients	Time of Test	µg per 24 hrs.			
			Oestriol	Oestrone	Oestradiol	Total
Benefit	10	Before	5.1	1.5	0.4	7.0
		After	2.8	1.1	1.1	5.0
No Benefit	10	Before	5.7	1.0	0.1	6.8
		After	5.1	2.3	0.9	8.3

Table 32

/occurred. The preoperative estimation of oestrogen excretion is, therefore, not of value in the selection of patients for treatment by endocrine methods.

Postoperative Estimations. The excretion of oestriol, oestrone and oestradiol-17β in twenty patients after adrenalectomy and in thirty-four patients after pituitary implantation is shown in Table 27. The results, arranged in an identical way to those before operation are illustrated in Figure 15, and again demonstrate no obvious relationship to the response to oophorectomy, adrenalectomy or pituitary implantation. The mean difference in oestrogen excretion between the responding and non-responding groups is not statistically significant after either adrenalectomy (Table 28) or after pituitary implantation (Table 29). Comparison of the results after adrenalectomy and oophorectomy with those after pituitary implantation indicates that significantly lower excretion follows the former operation (Table 30).

Estimations Before and After Operation in the same Patient.

Results of such studies are shown in Tables 31 and 32 and are illustrated more clearly in Figures 16 and 17. Statistical analysis confirms that adrenalectomy and oophorectomy is followed by a decisive drop in oestrogen /

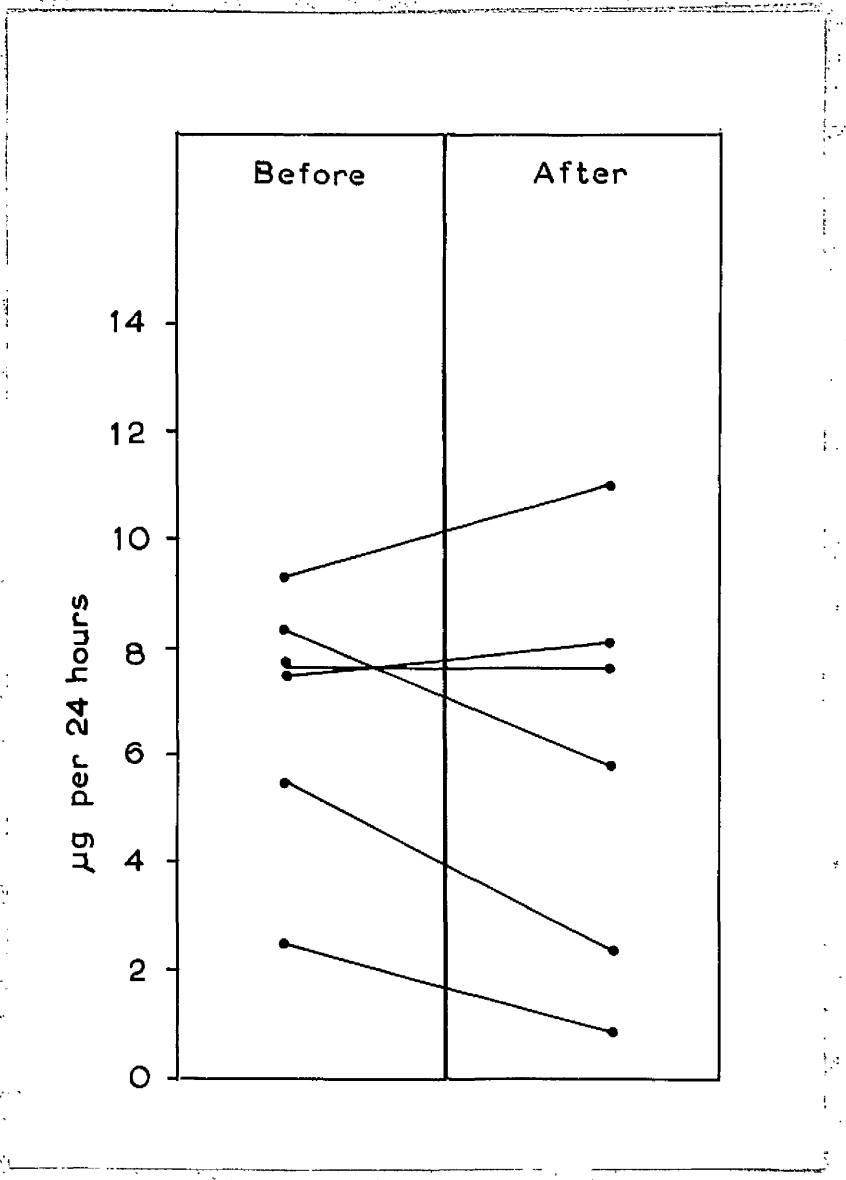


Figure 18. The effect of oophorectomy alone on the total oestrogen excretion by 6 premenopausal women with breast cancer.

Mean Excretion of Oestrogens in 11 Patients before and after Adrenalectomy plus Oophorectomy

Time of Test	Oestriol, Oestrone, and Oestradiol µg per 24 hrs.
Before	5.9
After	2.4
Mean difference	-3.5 ± 0.92
P	< 0.01

Table 33

Mean Excretion of Oestrogens in 20 Patients before and after Pituitary Implantation

Time of Test	Oestriol, Oestrone, and Oestradiol µg per 24 hrs.
Before	6.93
After	6.66
Mean difference	-0.27 ± 1.71
P	> 0.8

Table 34

Mean Excretion of Oestriol, Oestrone and Oestradiol-17β in 6 Patients before and after Oophorectomy alone and Comparison

Number of Patients	Time of Test	µg per 24 hours			
		Oestriol	Oestrone	Oestradiol	Total
6	Before	5.2	1.0	0.6	6.8
	After	4.5	0.8	0.7	6.0
	Mean difference				-0.8±0.7
	P				> 0.3

Table 35

Status	Patient	Control level µg/day	ACTH Admin. µg/day	% Increase	Average % Increase
Intact Adrenals	1	8.5	19.0	124	148
	2	2.6	7.3	181	
	3	2.7	10.8	300	
	4	6.2	12.7	105	
	5	5.4	7.0	30	
Adrenalectomised	6	2.6	2.2	-	16
	7	6.9	7.6	10	
		1.3	2.2	69	
	8	4.3	2.9	-	
		1.3	0	-	

Table 36. The Effect of ACTH Administration on the Excretion of Oestrogens by Postmenopausal or Oophorectomised Women with and without Adrenal Glands.

/oestrogen output, but not to absolutely zero levels, whereas no consistent change results from pituitary implantation (Tables 33 and 34). The clinical response to these operations was not related to changes in oestrogen levels.

Oestrogen Excretion after Endocrine Surgery.

It has become more obvious recently, using the improved assay techniques now available, that oestrogen secretion and excretion does not cease with the menopause, and the relatively small decrease in oestrogen excretion following oophorectomy in premenopausal women (Table 35; Figure 18) is additional proof of the presence of an extra-ovarian source of oestrogen production. The main source of the precursors of urinary oestrogens excreted by post-menopausal and castrated women is generally held to be the adrenal gland (see Table 1). Further evidence of this has been obtained from studies on the effect of ACTH on urinary oestrogen excretion in five postmenopausal patients with intact adrenals, and on five occasions in a further three patients, after bilateral adrenalectomy. Table 36 summarises the results obtained and effectively demonstrates oestrogen production by the adrenal gland.

Neither adrenalectomy plus oophorectomy or /

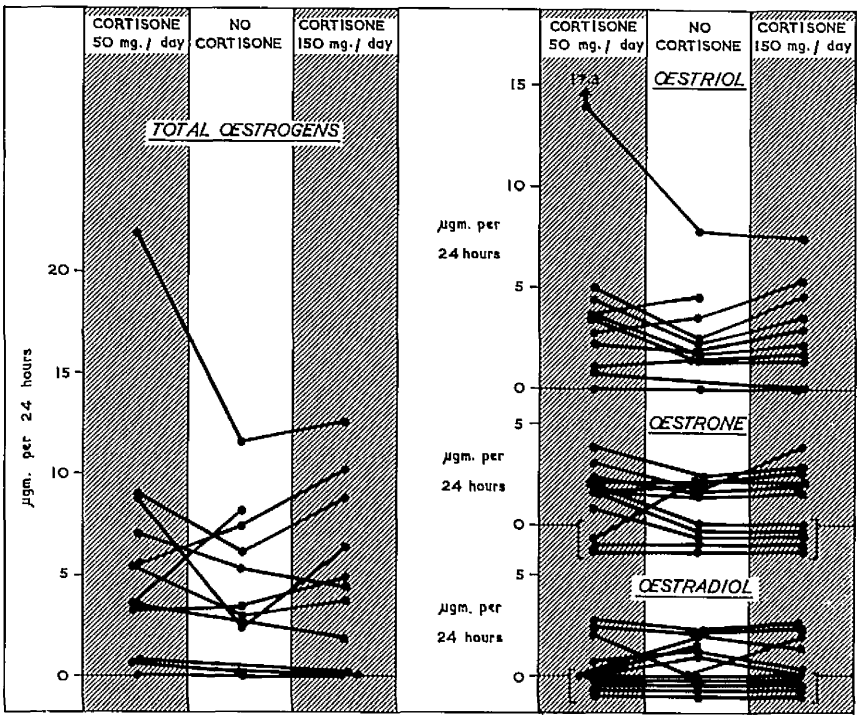


Figure 19. The effect of variation of cortisone dosage on oestrogen excretion by 12 patients after pituitary implantation with 90-Yttrium.

The Excretion of Oestriol, Oestrone and Oestradiol-17β in twelve patients after Implantation of the Pituitary with 90-Yttrium. Effect of variation of Cortisone dose.

Cortisone mg/day	Mean Oestrogen Excretion (µg/24 hrs.)			
	Oestriol	Oestrone	Oestradiol	Total
50	4.5* ± 1.4	1.5 ± 0.3	0.7 ± 0.3	6.4* ± 1.8
0	2.8 ± 0.7	1.2 ± 0.3	0.9 ± 0.3	5.1 ± 1.1
150	2.9 ± 0.8	1.5 ± 0.4	0.9 ± 0.3	5.3 ± 1.3

*These high means were caused by exceptionally high oestriol excretion in one patient (17.3 ug per 24 hrs.). The means of the other 11 patients were oestriol 1.7±0.5; total 4.9±0.9

Table 37

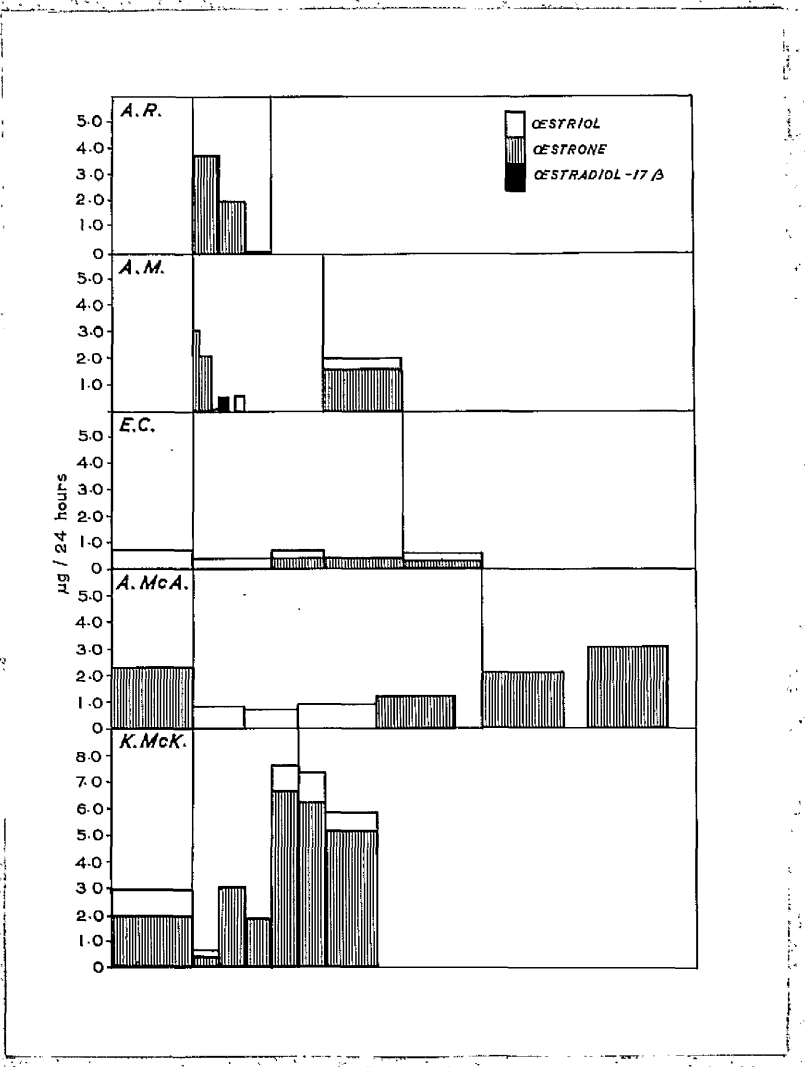


Figure 20. Oestrogen excretion by five oophorectomised-adrenalectomised-hypophysectomised women while maintained on a synthetic diet. Period between vertical bars.

/or pituitary implantation invariably completely abolishes oestrogen excretion, as has been shown by other workers, some of whom have demonstrated that the Kober chromogenic material in the urine of such patients was in fact biologically active oestrogen (see p.42) and as yet, the source of these steroids is unknown. Suggestions have included

a) the metabolism of maintenance cortisone

b) the diet

c) secretion by accessory adrenocortical tissue

d) the tumour

The first three possibilities have been investigated. Variation of the cortisone dosage, and even its complete stoppage for periods of up to five days had no regular effect on oestrogen excretion either in adrenalectomised-oophorectomised patients (Figures 26 - 31) or in patients after pituitary implantation (Table 37: Figure 19) which would be consistent with any metabolic conversion of maintenance cortisone to the oestrogen estimated in this study.

The effect on oestrogen excretion of maintaining five oophorectomised-adrenalectomised-hypophysectomised patients on a completely synthetic diet (Complan, Glaxo) of known composition for from three to fourteen days is shown in Figure 20. Although in two cases the output was reduced /

/reduced to zero, in the other three, real difference from the control levels were not demonstrated. It should be noted that in this study, the amounts of oestrogen excreted were mostly around the limits of sensitivity of the method.

Evidence concerning the possible role of accessory adrenocortical tissue in steroid secretion in general, after adrenalectomy and oophorectomy in breast cancer patients is described later (see pp. 82 et seq). No results were obtained which would suggest that the presence of such tissue could account for the observed excretion.

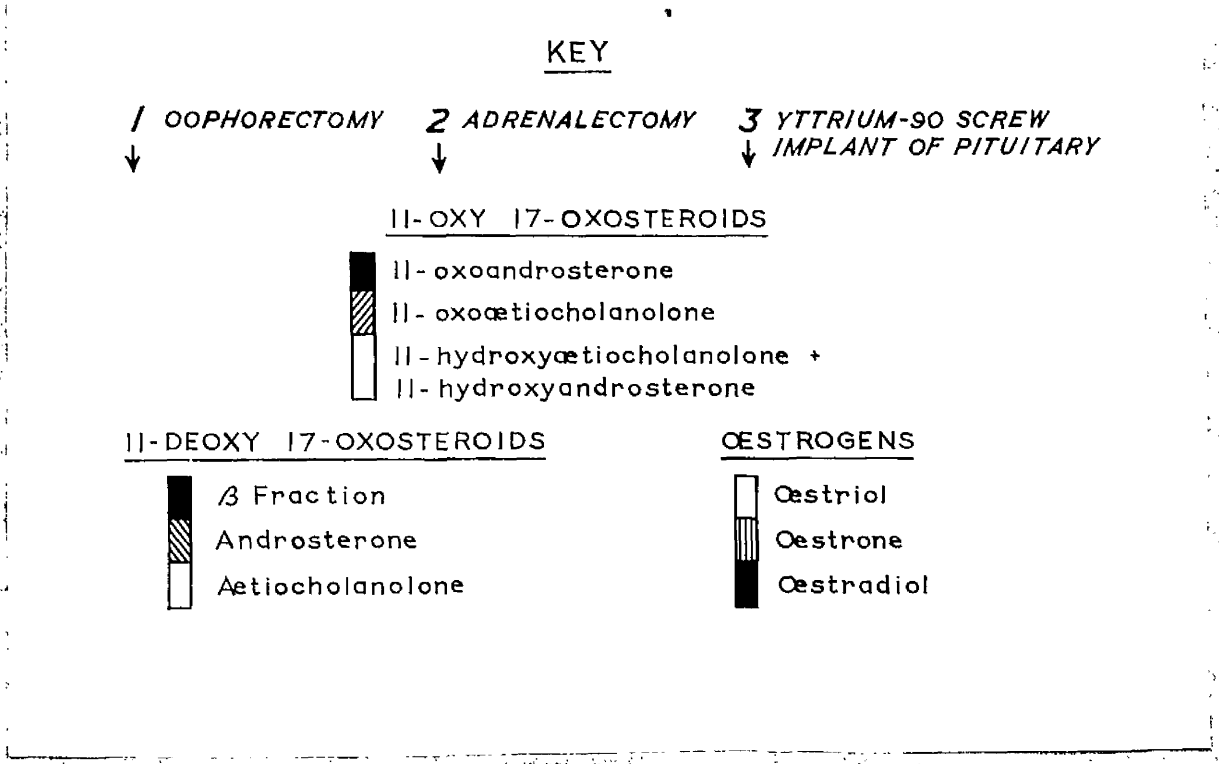
Long Term Studies of Steroid Excretion in Breast Cancer.

Since estimations of 17-oxosteroids or oestrogens limited to before and after one or other form of endocrine surgery did not demonstrate any connection between hormonal levels at that point in time, and the behaviour of the disease, it was felt that, in some cases, a broader approach to the investigation of the hormonal environment in the patient should be undertaken. To this end, urinary pregnanediol excretion was determined, along with the other assays already described, on three-day pools of urine at as regular intervals as possible throughout treatment. This type of study was completed in four patients with breast cancer, one of whom was a male, and is still proceeding, after four years, in a fifth. /

/fifth. In the course of the investigation, the cancer was progressing, regressing after treatment and finally progressing again. It was hoped that from this wider regular survey of the endocrine status of the patient any resurgence of hormonal activity, if it existed, would be more readily observed and could be compared with the clinical course of the cancer. The long term effects of endocrine ablation on the steroid production were also studied. The results of the regular multiple steroid assays in five patients are shown in Figures 21 - 25.

Total 17-oxosteroid and 11-oxy 17-oxosteroid excretion continued at fairly constant levels throughout the course of the study, these steroids, as already described, being derived, after operation, from the metabolism of the cortisone maintenance therapy. As would be expected 11-deoxy 17-oxosteroid excretion was reduced to trace amounts by adrenalectomy and in one case only, C.E., up to about 0.5 mg. per 24 hrs., was detected on three occasions as the disease relapsed. No other findings in this patient, however, suggested any resurgence of endogenous steroid secretion.

Small amounts of urinary pregnanediol were usually excreted, decreased by adrenalectomy, but /



Figures 21 - 25. Excretion of steroid hormones and metabolites throughout the course of treatment in four women and one man with breast cancer.

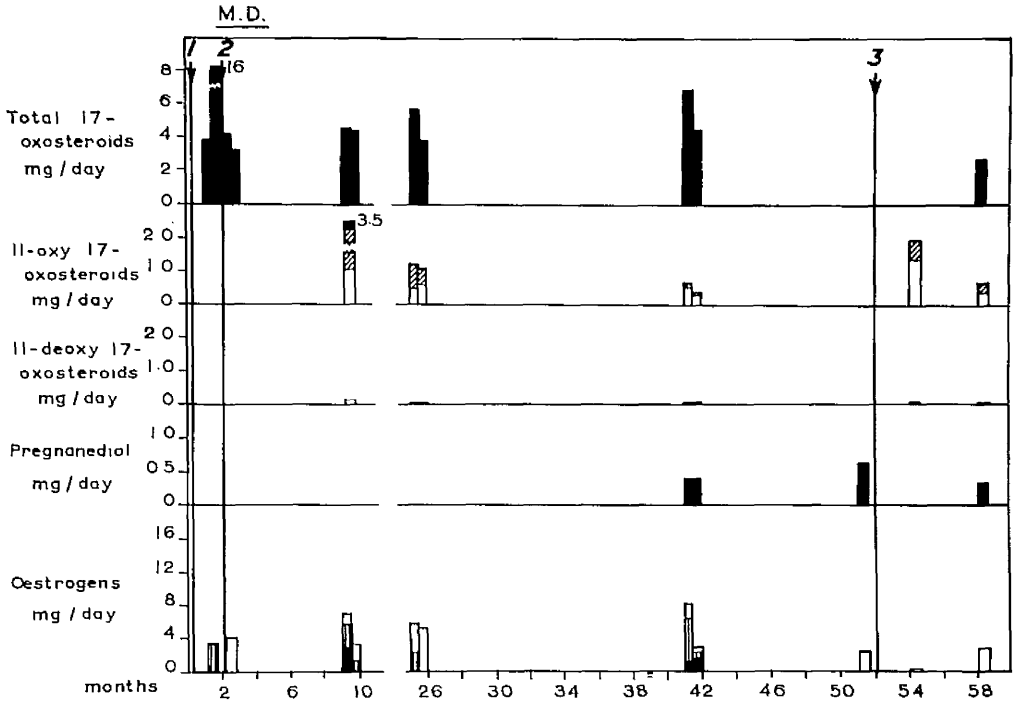


Figure 21. Excretion of steroid hormones and metabolites throughout the course of treatment in four women and one man with breast cancer.

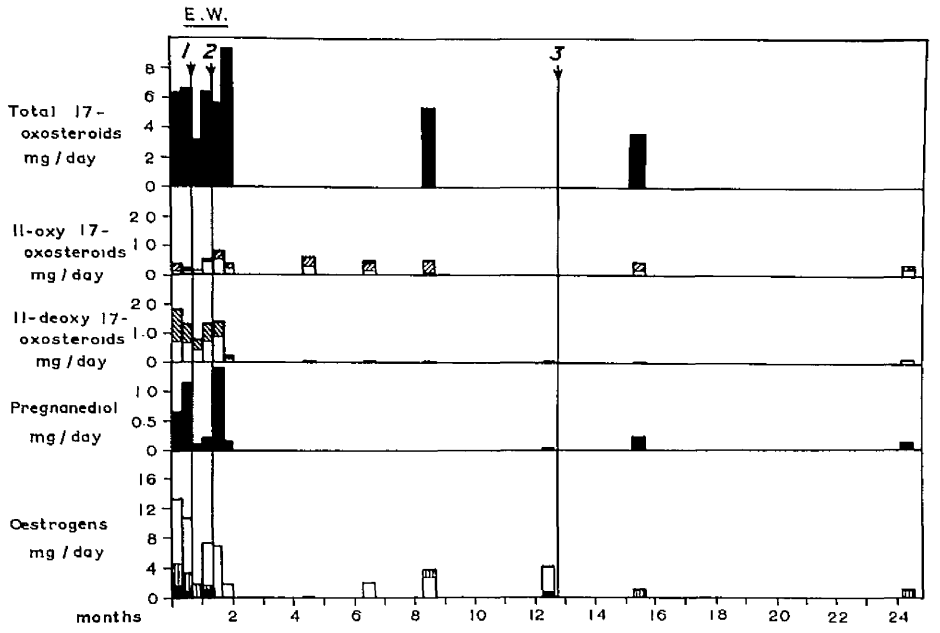


Figure 22. Excretion of steroid hormones and metabolites throughout the course of treatment in four women and one man with breast cancer.

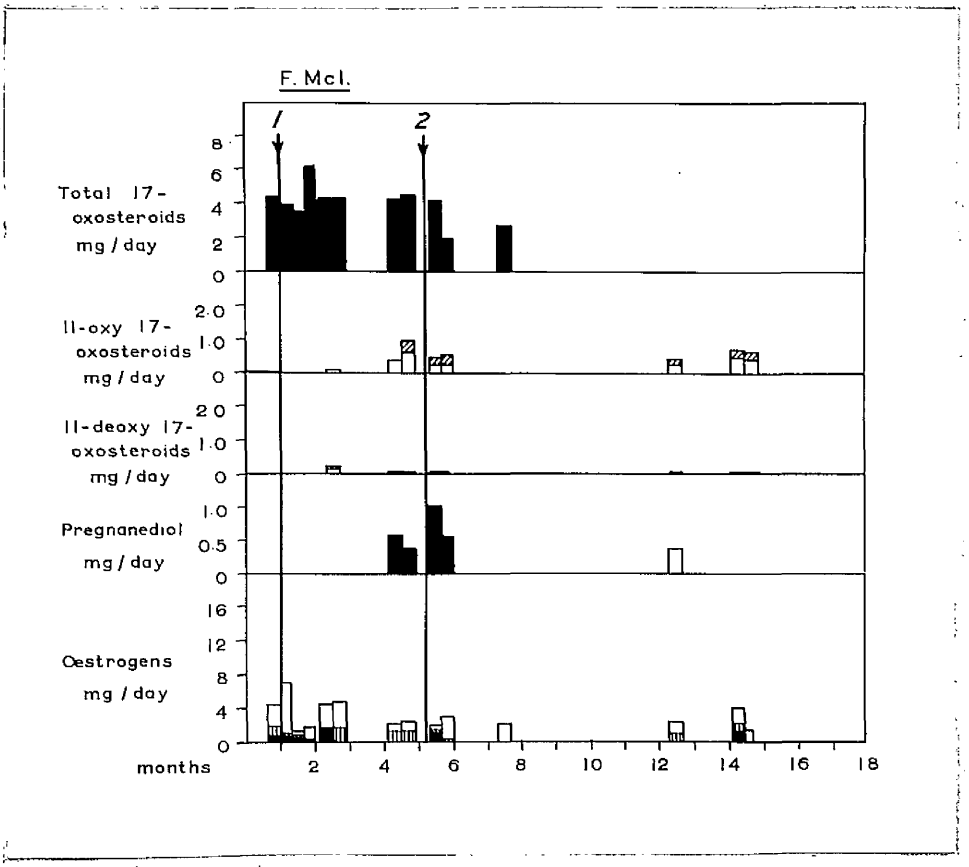


Figure 23. Excretion of steroid hormones and metabolites throughout the course of treatment in four women and one man with breast cancer.

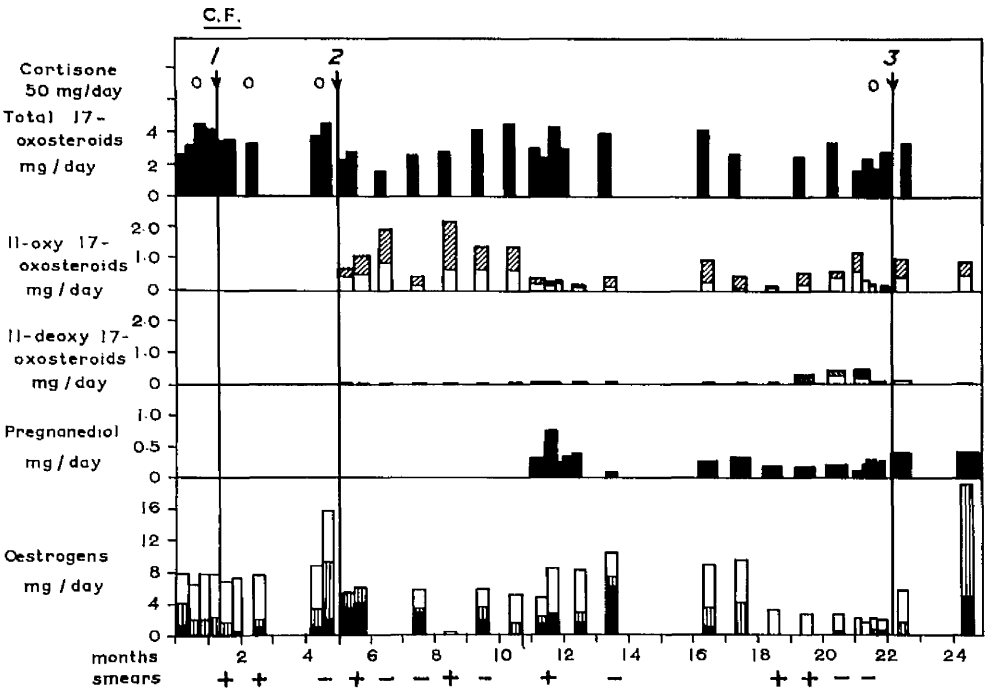


Figure 24. Excretion of steroid hormones and metabolites throughout the course of treatment in four women and one man with breast cancer.

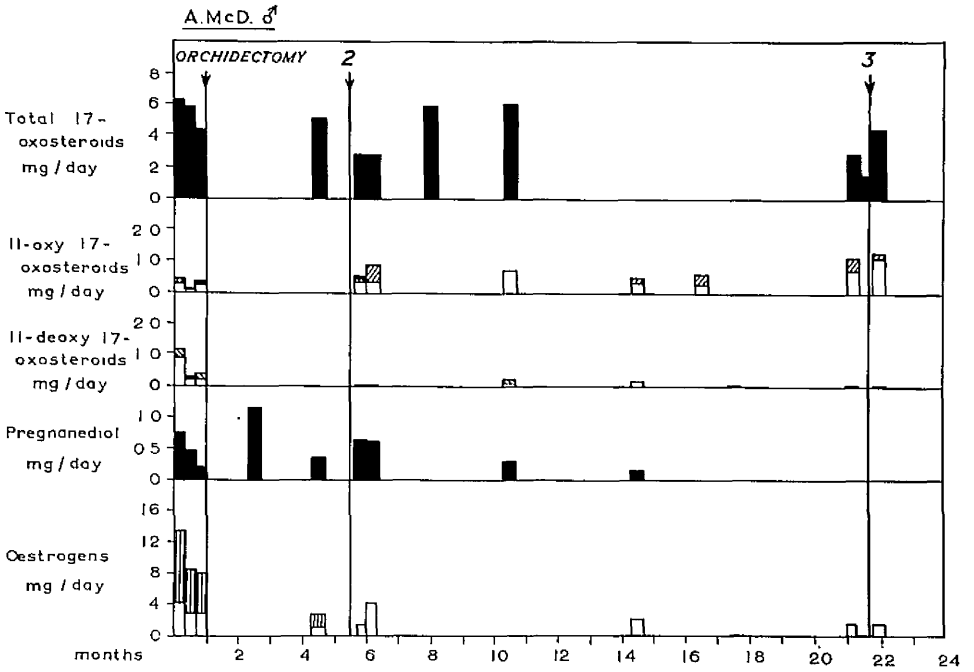


Figure 25. Excretion of steroid hormones and metabolites throughout the course of treatment in four women and one man with breast cancer.

/but uninfluenced by the course of the disease.

Oestrogen excretion continued in all cases, with the exception of the male patient, A. McD. The amounts were variable, and followed no pattern, either in any one patient, or from patient to patient, but were regularly high enough to come within the limits of accuracy of the method of assay used. In one case, C.F., in which vaginal smears were done concurrently with the chemical oestrogen determinations, cornified smears were found intermittently, not always associated with a high level of oestrogen excretion.

In no patient was change in the clinical condition, either improvement or deterioration, foreshadowed by, or mirrored in, any regularly demonstrable alteration in the hormonal environment.

Relapse of Breast Cancer after Response to Adrenalectomy - Accessory Adrenocortical Tissue.

Accessory adrenocortical tissue is known to occur in man, and the following series of investigations were carried out to determine whether there was evidence of returned adrenal function in patients who relapse after /

/after initial response to adrenalectomy. In four patients the tests were carried out on four occasions, firstly while the cancer was in remission, and secondly during subsequent relapse. In the remaining two women who also had regression of cancer following adrenalectomy, tests were performed only during relapse. Serial estimations of adrenal hormones and their metabolites in the urine and peripheral blood were performed before, during and after a period when cortisone maintenance therapy was stopped. Withdrawal of m aintenance therapy was necessary in order to abolish interference in certain assays by metabolites of the exogenous cortisone, and to free any remaining or accessory adrenocortical tissue from its suppressing action. The duration of the period for which therapy was withheld varied from patient to patient and depended on the appearance of clinical symptoms of cortisone lack (anorexia, vomiting, weakness and hypotension) which necessitated restarting maintenance therapy. Throughout the whole period of the study, which usually lasted about twelve days, all urine was collected and the daily output of total neutral 17-oxosteroids, individual 17-oxosteroids, oestrogens and pregnanediol measured. /

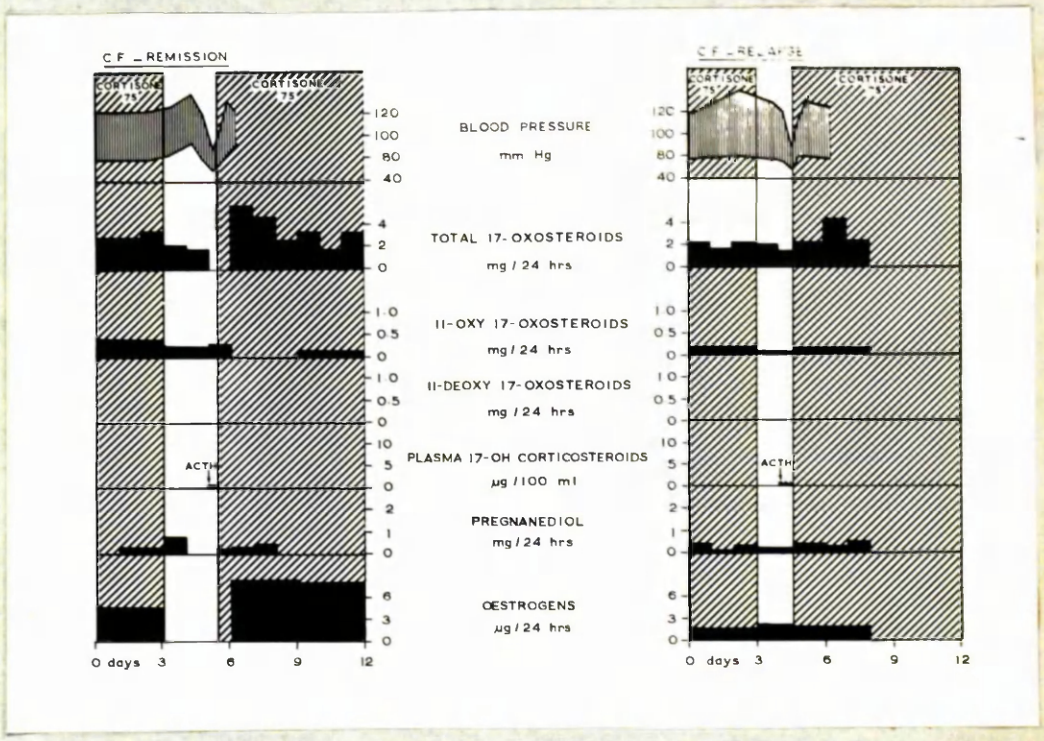


Figure 26. Results of Cortisone Withdrawal Tests in six patients after Adrenalectomy.

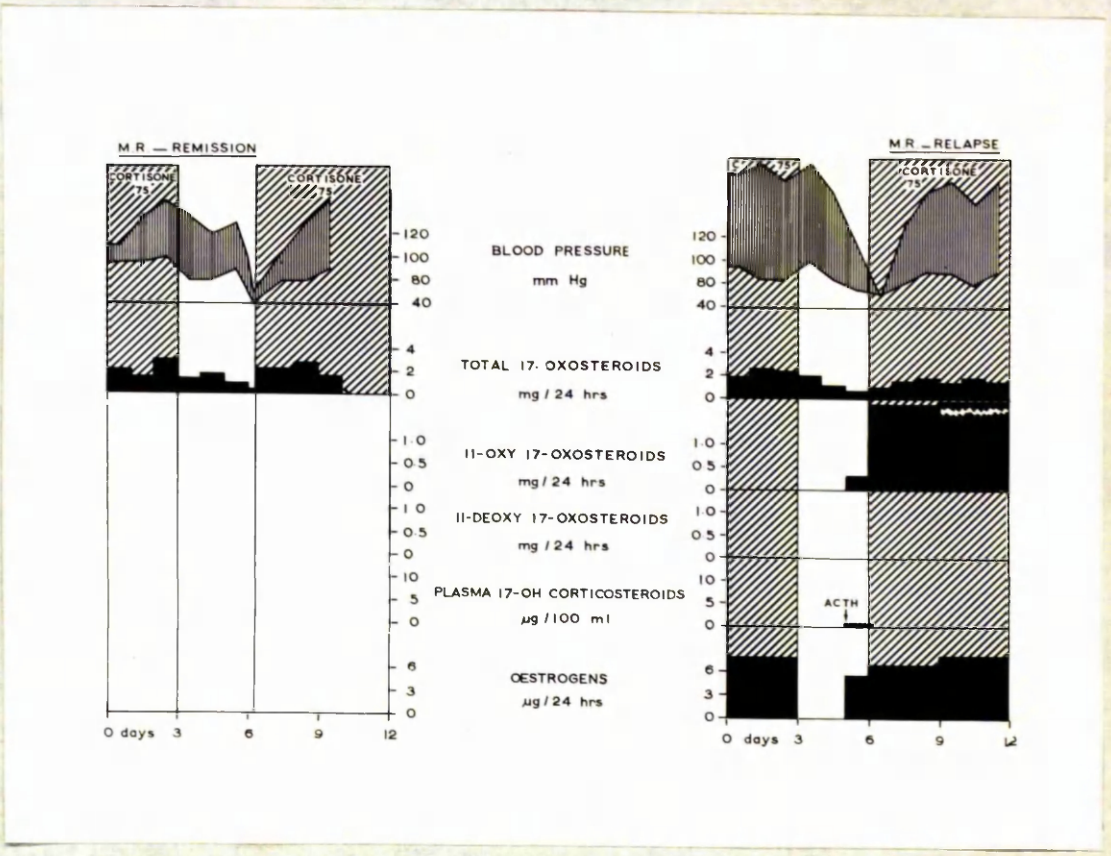


Figure 27. Results of Cortisone Withdrawal Tests in six patients after Adrenalectomy.

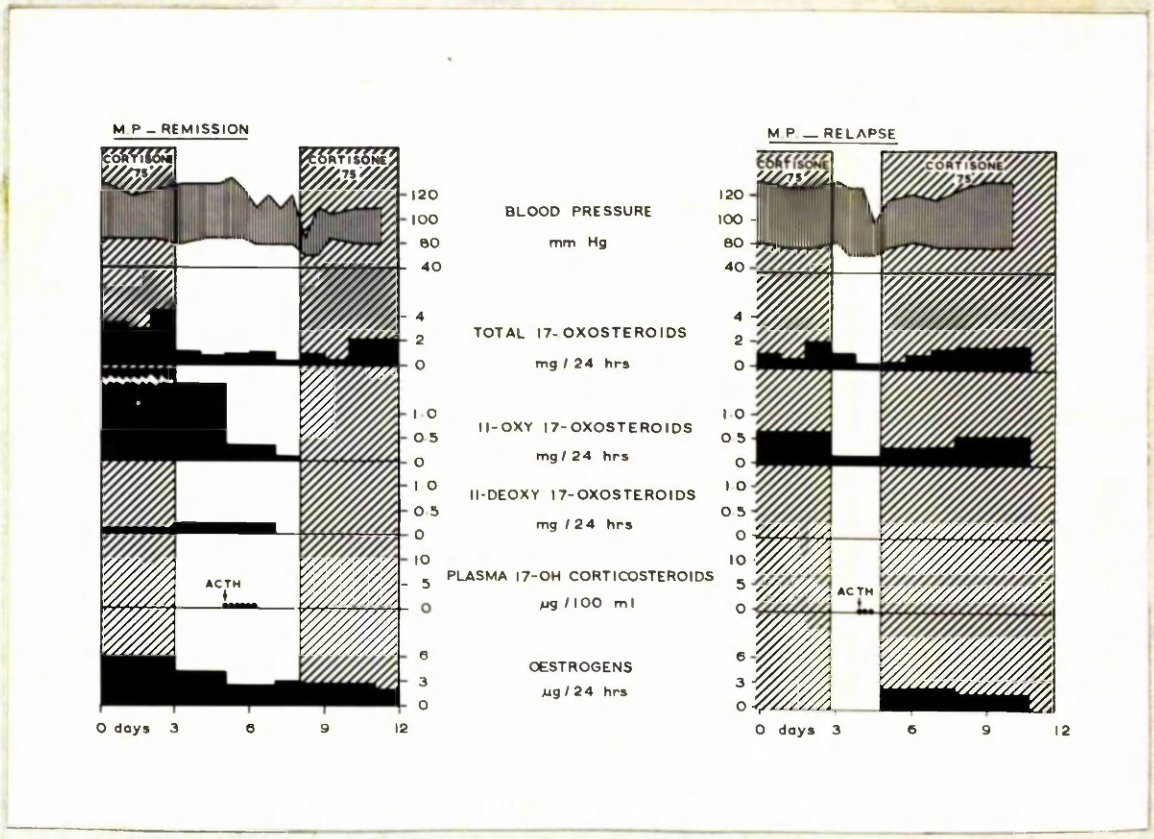


Figure 28. Results of Cortisone Withdrawal Tests in six patients after Adrenalectomy.

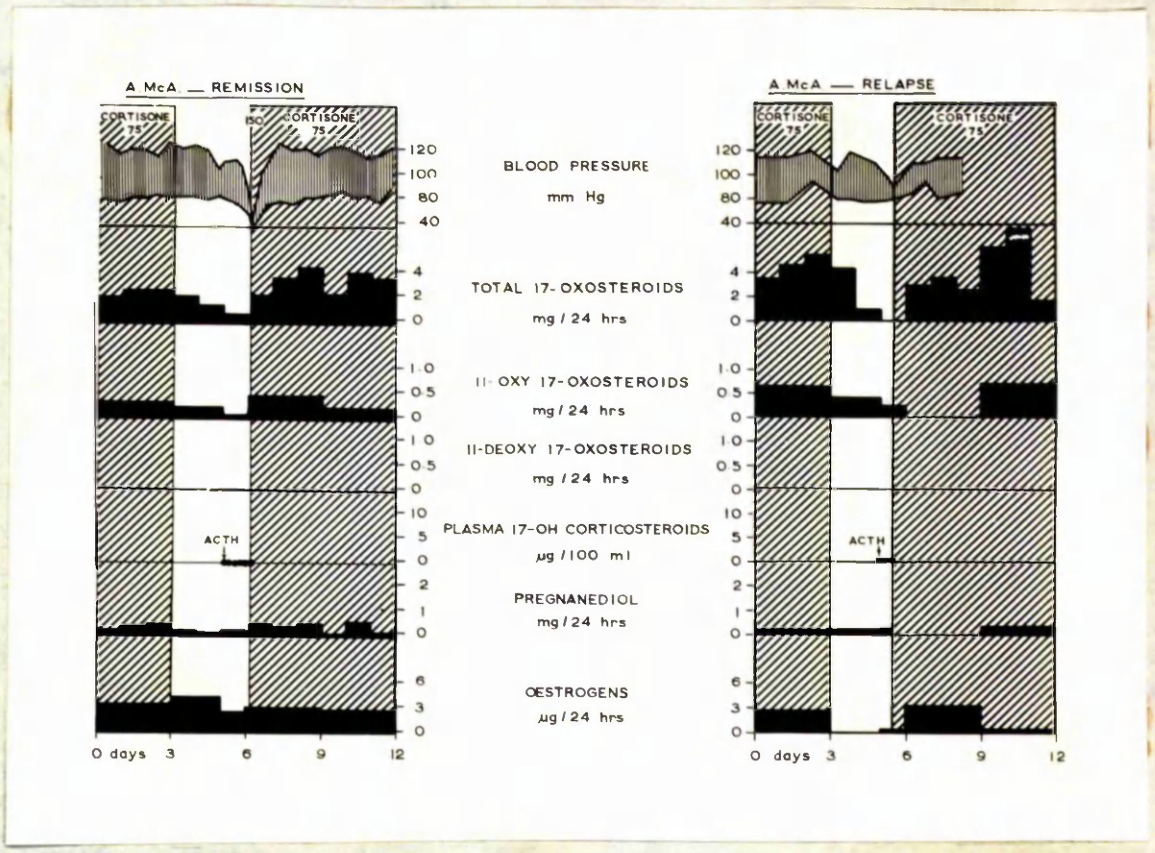


Figure 29. Results of Cortisone Withdrawal Tests in six patients after Adrenalectomy.

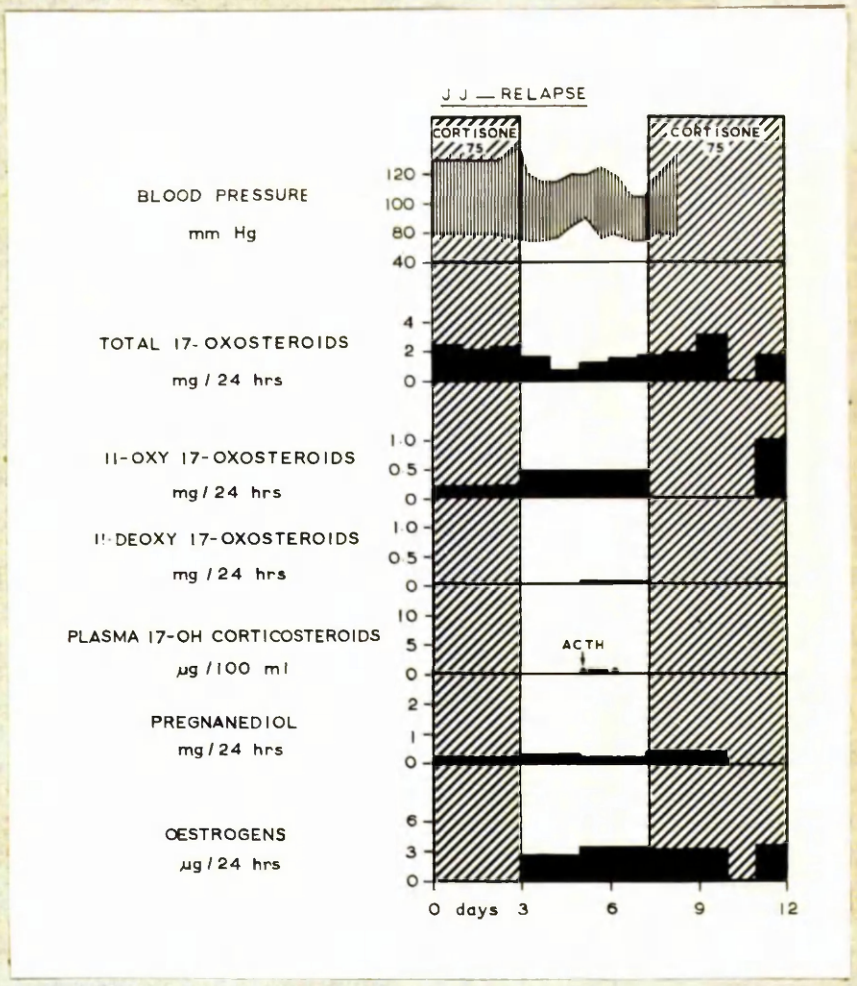


Figure 30. Results of Cortisone Withdrawal Tests in six patients after Adrenalectomy.

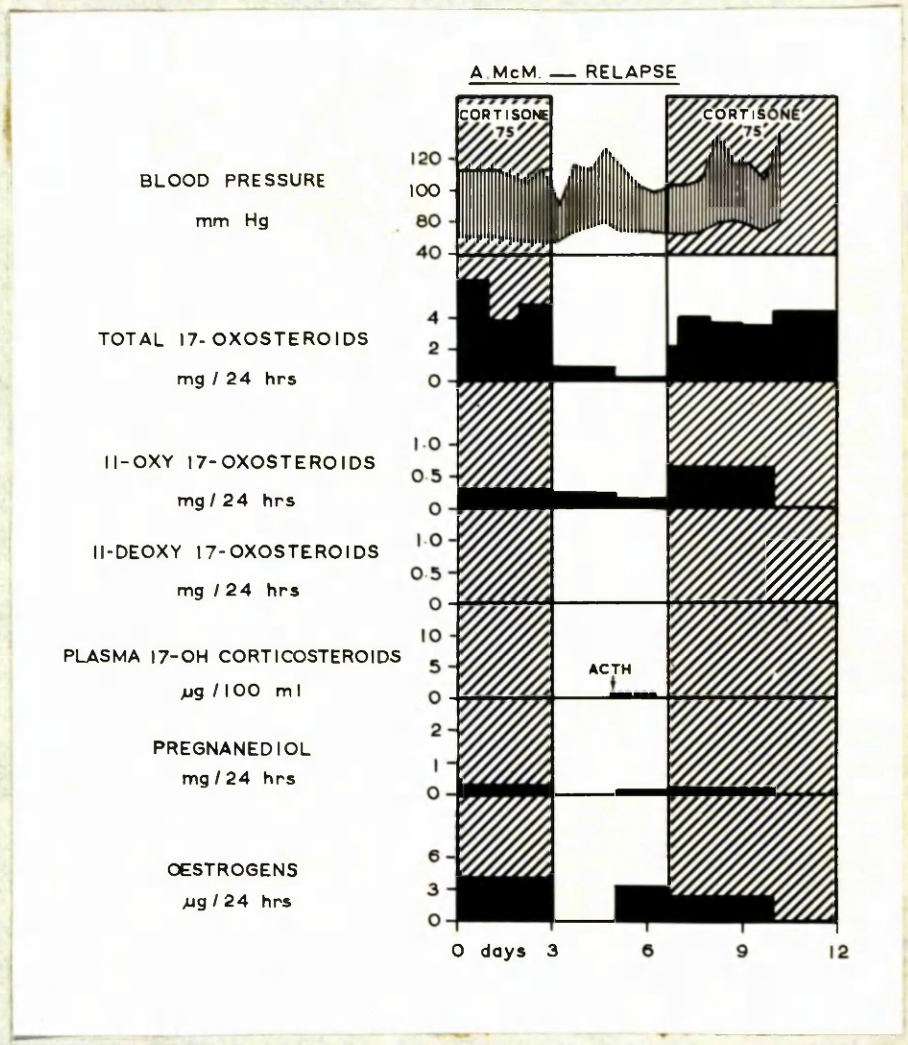


Figure 31. Results of Cortisone Withdrawal Tests in six patients after Adrenalectomy.

Number of Days Patients were able to Withstand Deprivation of Cortisone.

Patient	Days off Cortisone	
	Remission	Relapse
M. P.	$5\frac{1}{2}$	$2\frac{3}{4}$
C. F.	$2\frac{3}{4}$	$2\frac{1}{4}$
A. McA.	$3\frac{3}{4}$	3. 1/8
M. R.	$3\frac{2}{3}$	3
A. McM.		$4\frac{1}{3}$
J. J.		$4\frac{1}{3}$

Table 38

/measured. On the third day of cortisone withdrawal peripheral blood samples were taken before, and at two-hourly intervals after, the intravenous infusion of 25 units of corticotrophin (ACTH) gel in 200 ml. 0.9 per cent. saline. The concentration of free plasma 17-hydroxycorticosteroids estimated by a modification of the Porter-Silber reaction (1950) by Dr. V.J. O'Donnell of the Department of Pathology, Glasgow Royal Infirmary.

The results of the steroid estimations performed during the tests in individual patients are shown in Figures 26 - 31. In general the clinical effects of stopping cortisone were not apparent for 37-72 hours. The time each patient was able to tolerate cortisone lack is shown in Table 38. This period was not of longer duration when there was a recrudescence of tumour growth.

The pattern of steroid excretion following cortisone withdrawal was similar in all cases, irrespective of the clinical behaviour of their cancer. When cortisone was stopped, the excretion of total 17-oxosteroids and 11-oxy 17-oxosteroids in the urine, progressively fell, and reached zero or near zero levels by the end of the withdrawal period. No noticeable difference in the excretion pattern of total /

/total 17-oxosteroids and 11-oxy 17-oxosteroids was associated with relapse of cancer.

The 11-deoxy 17-oxosteroids were absent from the urine of all but one patient at all stages of the tests, both in patients in remission and in those in relapse. In the exceptional patient (J. J. : Figure 27) the amounts of these steroids detected were at the lower limits of sensitivity of the method, and are likely, when taken in conjunction with the other findings, to be of no significance.

In no patients were 17-hydroxycorticosteroids detected in the peripheral blood plasma on the third day of cortisone withdrawal either before or after ACTH stimulation.

Significant amounts of oestriol, oestrone and oestradiol-17β were frequently found, in the urine but amounts did not vary regularly with changes in cortisone dosage or with the clinical response of the patient to adrenalectomy. The same conclusions were true for the small amounts of pregnanediol found in the four patients in whose urine it was estimated.

No patient had further benefit from subsequent pituitary implant with 90-Yttrium and in the three patients in whom post-mortem examinations were carried out, no /

/no accessory adrenocortical tissue was found.

Cortisone Withdrawal Test in Patients who had no Response to Adrenalectomy.

Absence of response to adrenalectomy, which is the rule in about 70 per cent. of cases so treated, would suggest either that the gland had not been totally removed, or that the tumour was already independent of hormonal factors for its growth. Incomplete surgery, in such a large number of cases, is highly unlikely, and it is more probable that the failure of the tumour to regress is due to a different response by the tumour to the same type of change of hormonal environment as produces remissions in favourable cases.

Cortisone withdrawal tests, as previously described, carried out in three cases who did not benefit from adrenalectomy appear to confirm this suggestion. The results are shown in Figures 32 - 34. With the exception of the 17-hydrocorticosteroid results in Figure 33 (M. M.) the changes in the hormone excretion pattern are analogous to those produced by cortisone withdrawal in the patients who benefited from adrenalectomy. The unexpected 17-hydroxy-corticosteroid excretion in M. M. is inexplicable in view of the other findings, none of which indicate the presence of functioning adrenal tissue. /

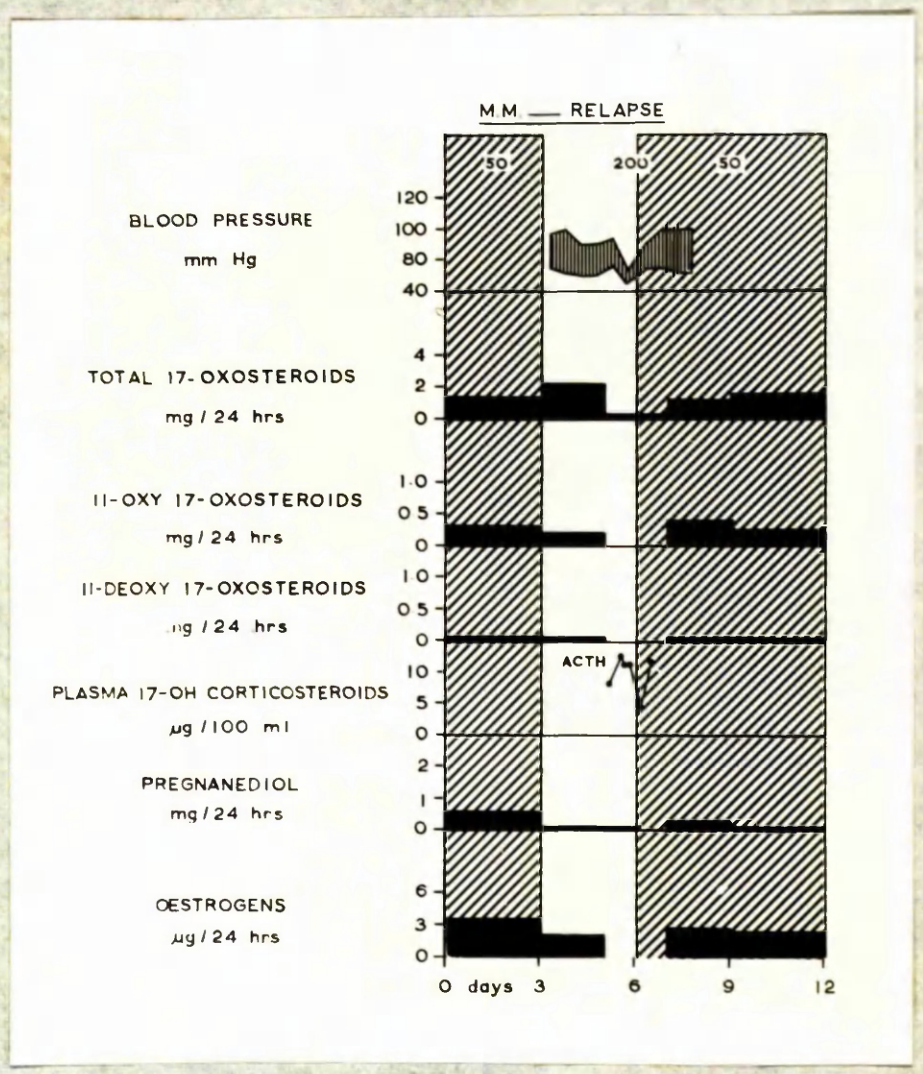


Figure 32. The Results of Cortisone Withdrawal Tests carried out in 3 Patients with breast cancer who had no benefit from adrenalectomy.

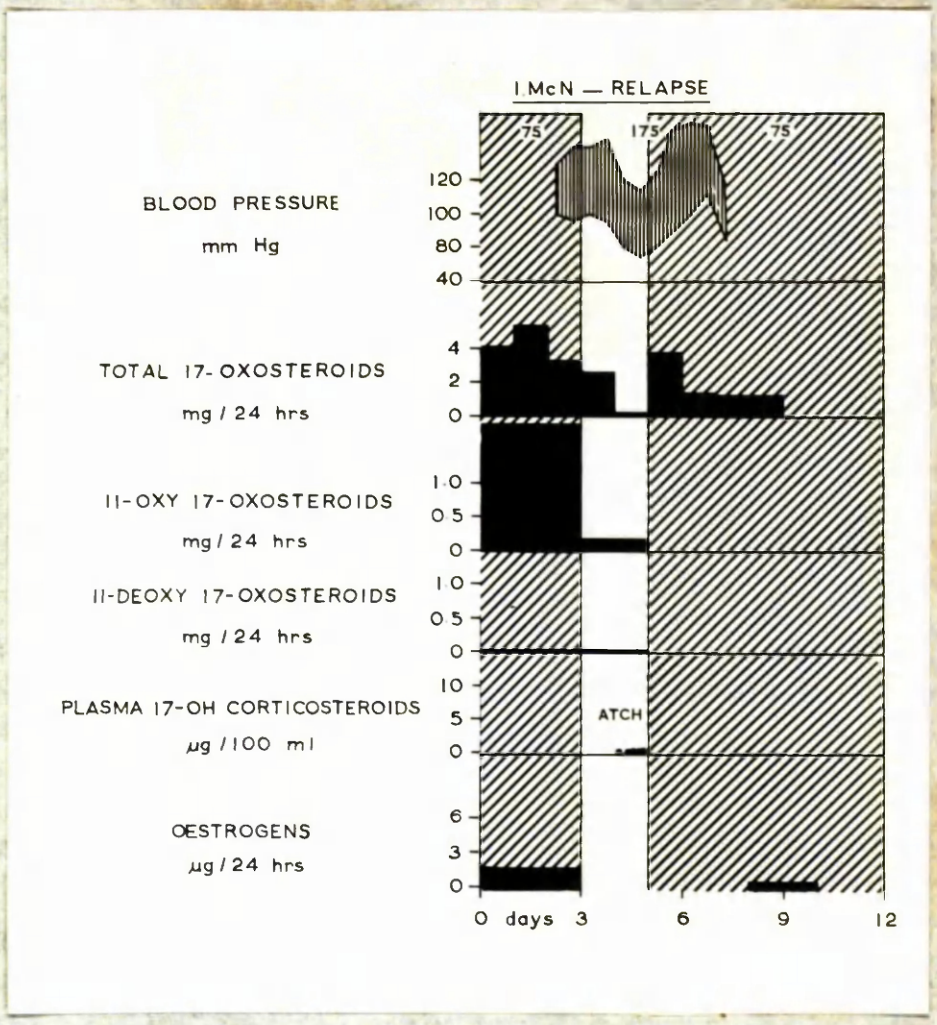


Figure 33. The Results of Cortisone Withdrawal Tests carried out in 3 Patients with breast cancer who had no benefit from adrenalectomy.

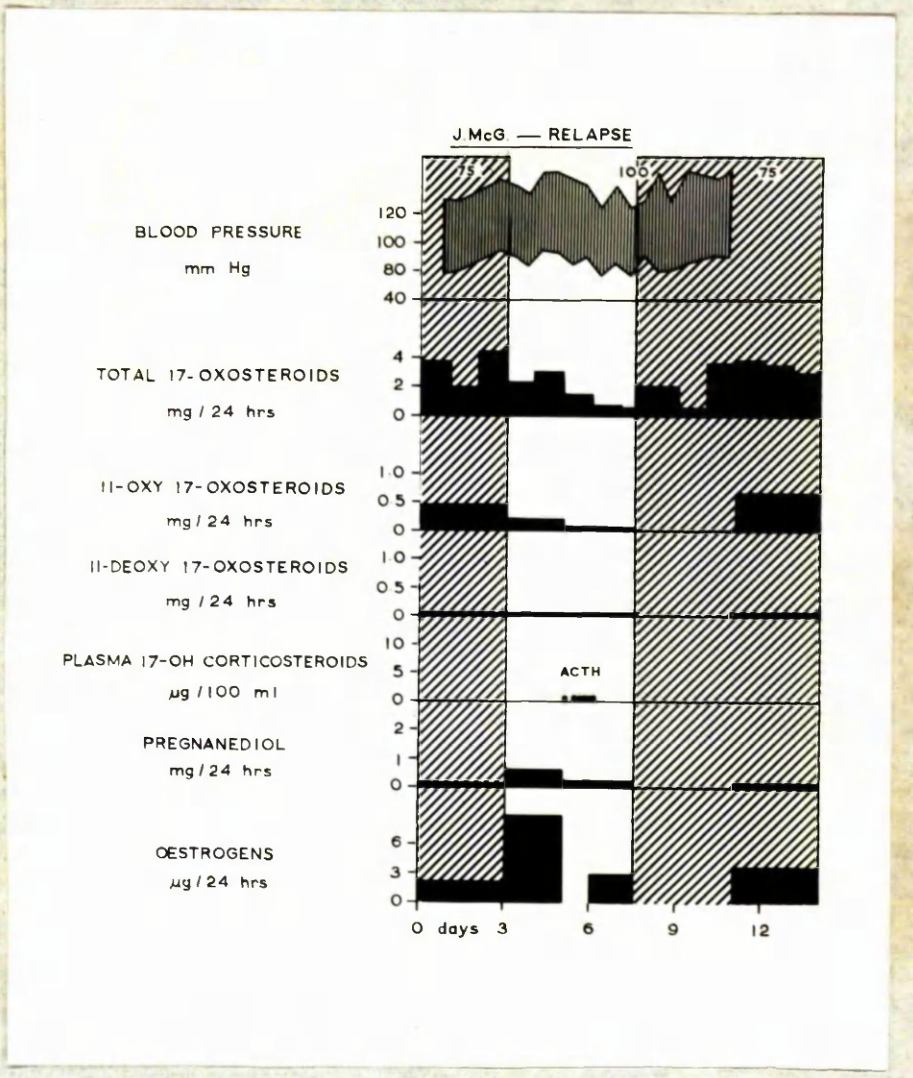


Figure 34. **The Results of Cortisone Withdrawal Tests carried out in 3 Patients with breast cancer who had no benefit from adrenalectomy.**

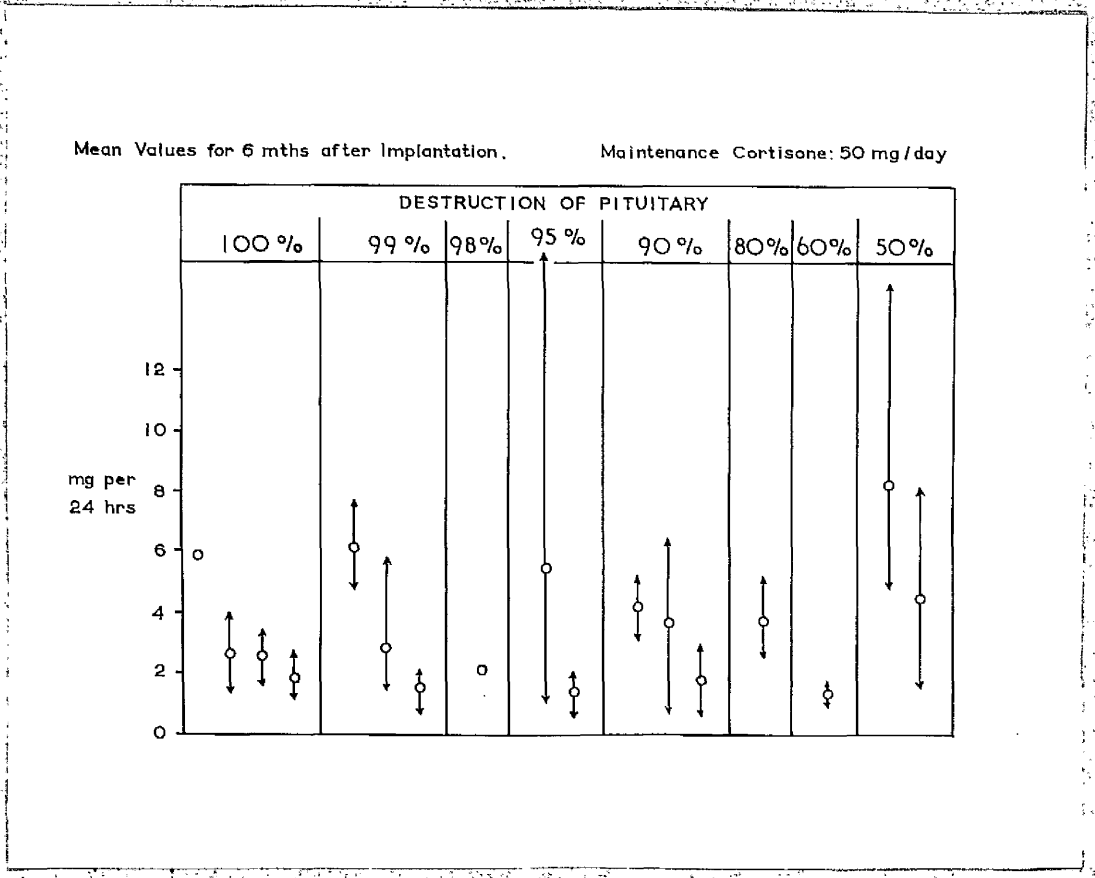


Figure 35. The relation of mean total 17-oxosteroid excretion to pituitary destruction in seventeen patients after implantation with 90-Yttrium.

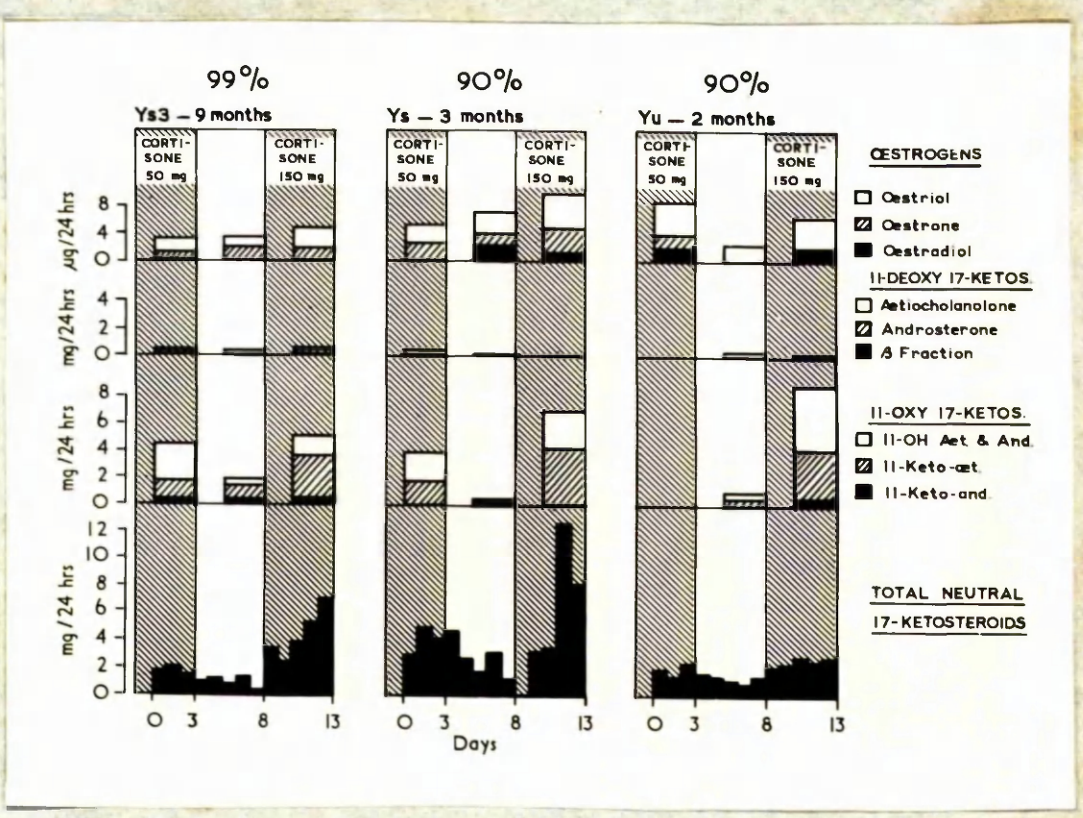


Figure 36. The effect of withdrawing cortisone maintenance therapy on total 17-oxosteroid excretion after pituitary implantation with 90-Yttrium.

Assessment of Pituitary-Adrenal Function after Implantation of the Pituitary Gland with 90-Yttrium.

As the urinary steroids which have been estimated in this study are of ovarian origin, the amounts excreted in the absence of maintenance cortisone must bear a direct relationship to adrenal and ovarian function. Thus in postmenopausal patients, the 17-oxosteroids, pregnanediol and almost all the oestrogen in the urine arise from precursors secreted by the adrenal glands. The relationship between endogenous steroid production and the extent of destruction of the pituitary is less well defined and information is lacking about the degree of functional impairment of the target organs after even complete hypophysectomy.

In view of this, the usefulness of urinary steroid estimations as a measure of pituitary destruction was investigated. This was done by determining the urinary steroid excretion after 90-Yttrium implantation of the pituitary in a series of patients and correlating this with the extent of destruction of the hypophysis subsequently found at post mortem histological examination.

The mean value of total 17-oxosteroid excretion /

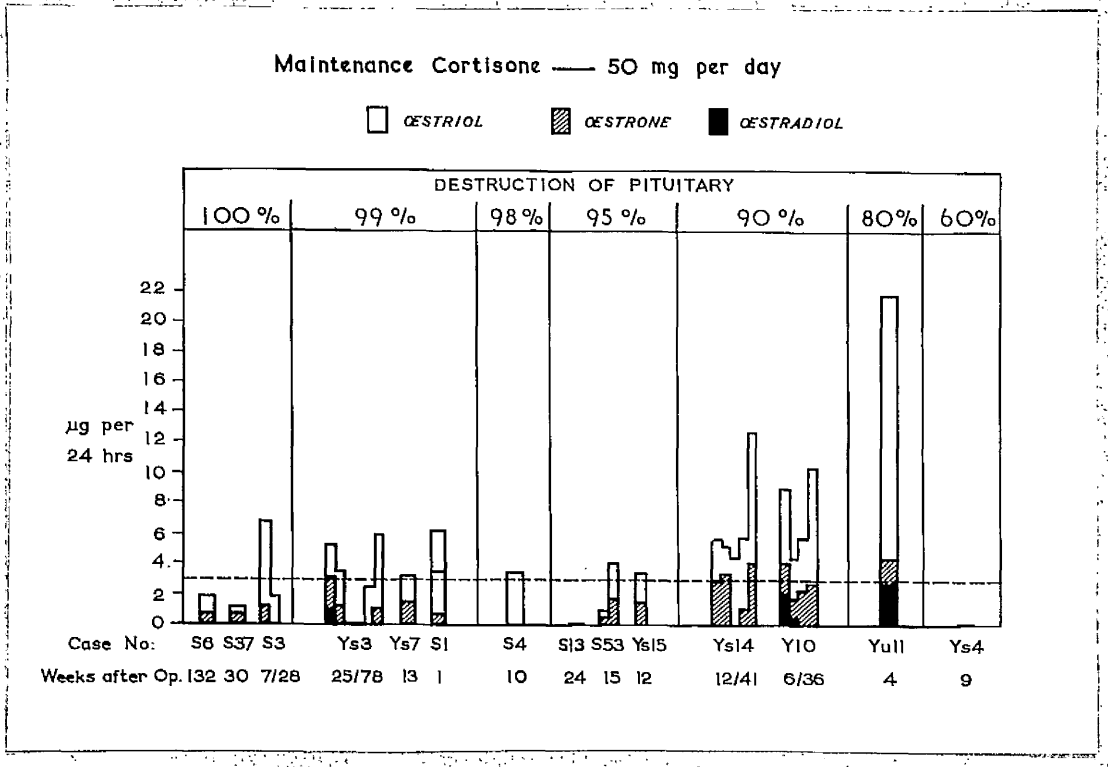


Figure 37. The relation of mean oestrogen excretion to pituitary destruction in fourteen patients after implantation with 90-Yttrium.

The Relation of Mean Total 17-Oxosteroid Excretion to Pituitary Destruction in Seventeen Patients after Implantation with 90-Yttrium.

Patient	% Destruction	Mean total 17-oxosteroid excretion + range (mg/24 hrs.)
S3	100	5.9
S6	100	2.6 (1.7 - 3.4)
S37	100	1.8 (1.4 - 2.6)
S10	100	2.6 (1.4 - 3.8)
S1	99	6.1 (4.8 - 7.6)
Ys 7	99	1.5 (0.7 - 2.0)
Ys 3	99	2.8 (1.5 - 5.7)
S4	98	2.1
S53	95	1.3 (0.9 - 1.7)
Ys 15	95	5.4 (1.1 - 15.7)
Ys 14	90	4.2 (3.1 - 5.0)
Yu 16	90	1.7 (0.5 - 2.6)
Ys 11	90	3.6 (0.6 - 6.3)
Yu 11	80	3.6 (2.5 - 5.0)
Ys 4	60	1.2 (1.0 - 1.5)
Ys 10	50	8.1 (4.8 - 14.5)
Yu 13	50	4.3 (1.6 - 7.9)

Table 39

The Relation of Mean Oestrogen Excretion to Pituitary Destruction in 14 Patients after Implantation with 90-Yttrium.

Patient	% Destruction	Weeks after Implant	Oestrogen Excretion (µg per 24 hrs.)			
			Oestriol	Oestrone	Oestradiol-17β	Total
S3	100	7	5.6	1.2	0	6.8
		28	1.9	0	0	1.9
S6	100	132	1.2	0.6	0	1.8
S37	100	30	0.4	0.6	0	1.0
S1	99	1	2.6	2.8	0.7	6.1
Ys 7	99	13	1.7	1.5	0	3.2
Ys 3	99	25	2.3	2.0	0.9	5.2
		40	2.2	1.2	0	3.4
		42	3.0	1.0	0	4.0
		77	5.0	0.9	0	5.9
		78	8.0	2.1	0	10.1
S4	98	10	3.3	0	0	3.3

Table 40

The Relation of Mean Oestrogen Excretion to Pituitary Destruction in 14 Patients after Implantation with 90-Yttrium.

Patient	% Destruction	Weeks after Implant	Oestrogen Excretion (µg per 24 hrs.)			
			Oestriol	Oestrone	Oestradiol-17β	Total
S13	95	24	0	0	0	0
S53	95	15	0.4	0.4	0	0.8
		15	2.3	1.7	0	4.0
Ys 15	95	12	2.0	1.5	0	3.5
Ys 14	90	12	2.7	2.9	0	5.6
		13	5.3	3.4	1.4	10.1
		40	4.7	1.0	0	5.7
Yu 16	90	6	5.0	2.0	2.0	9.0
		7	4.6	0	2.0	6.6
		18	2.6	1.3	0.5	4.4
		34	3.6	2.1	0	5.7
		36	7.7	2.7	0	10.4
Yu 11	80	4	17.3	1.9	2.7	21.9
		5	7.4	2.7	2.4	12.5
Ys 4	60	9	0	0	0	0

Table 40 (Cont'd)

/excretion for each patient was not related to the extent of pituitary destruction as determined after death (Table 39; Figure 35). As in the adrenalectomised patients, the total 17-oxosteroids are largely made up of 11-oxy 17-oxosteroids. Clear evidence that these derive from the metabolism of maintenance cortisone, came from three patients, subsequently shown to have respectively 99, 90 and 90 per cent. destruction of the gland, in whom withdrawal of the maintenance therapy for a period of five days, resulted in a fall to low levels in the excretion of total 17-oxosteroids and 11-oxy 17-oxosteroids (Figure 36).

Detectable amounts of oestriol, oestrone and oestradiol-17β, were present in the urine after pituitary implantation. The levels were roughly inversely proportional to the degree of destruction of the gland. However in three patients with 99 per cent. destruction, and in one with complete destruction, significant quantities were still detected, while in a patient with 40 per cent. of the gland intact, none was detected (Table 40, Figure 37).

After implantation the amounts of 11-deoxy 17-oxosteroids detected in the urine were low in six of the eight patients in whom 90 - 100 per cent. of the gland was /

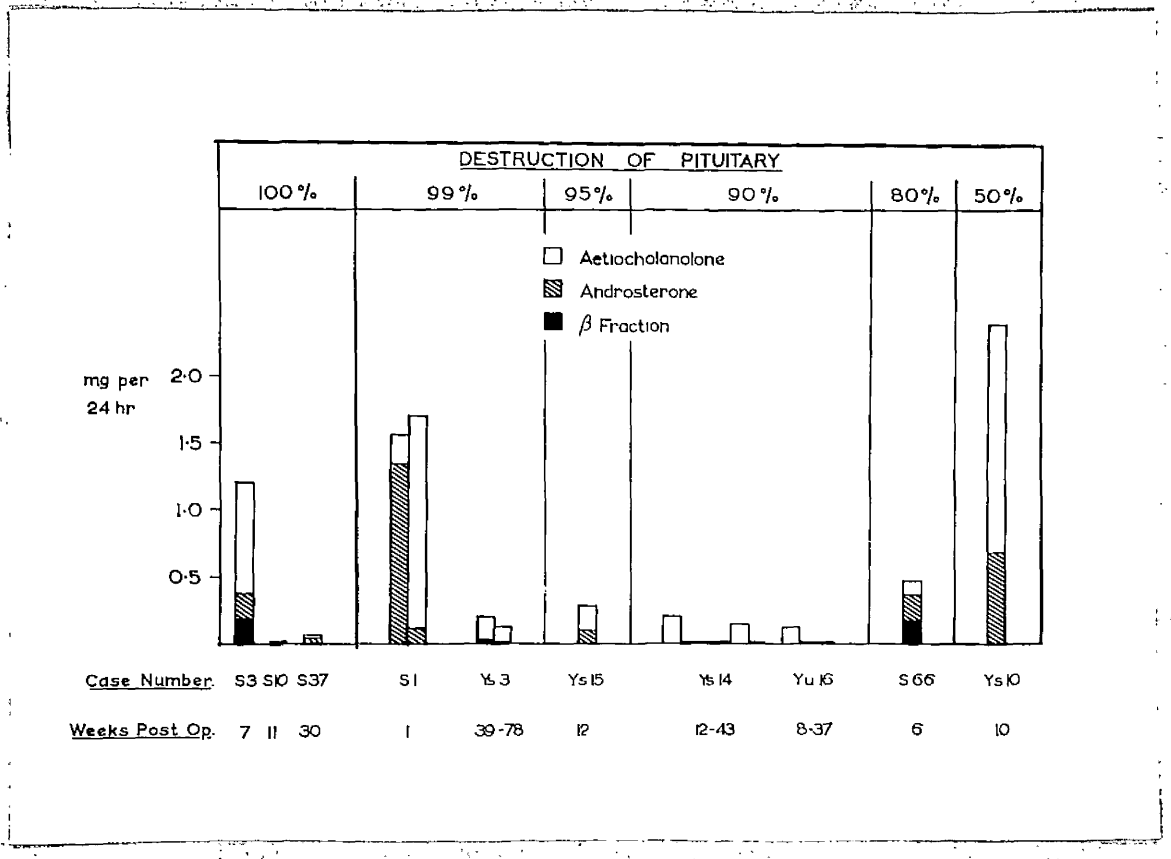


Figure 38. The relation of 11-deoxy 17-oxosteroid excretion to pituitary destruction in ten patients after implantation with 90-Yttrium.

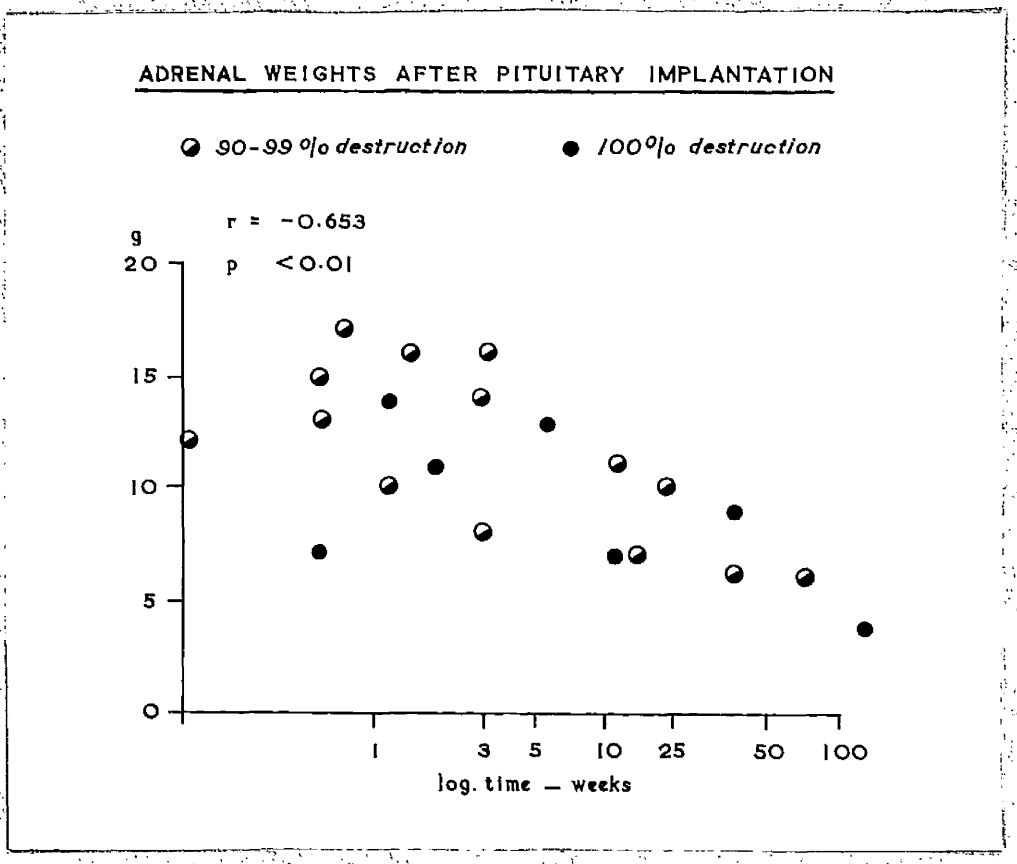


Figure 39. Correlation between post mortem adrenal weights, and the time elapsing between pituitary implantation and death.

The Relation of 11-Deoxy 17-Oxosteroid Excretion to Pituitary Destruction in 10 Patients after Implantation with 90-Yttrium.

Patient	Percentage Destruction	Weeks after Operation	Excretion of 11-deoxy 17-oxosteroids (mg/24 hrs.)			
			1.	2.	3.	4.
S3	100	7	0.83	0.19	0.18	1.20
S37	100	30	0.02	0.04	0	0.06
S10	100	11	0	0	0	0
S1	99	0	0.22	1.35	0	1.57
		1	1.60	0.10	0	1.70
Ys 3	99	77	0.17	0	0.02	0.19

1. Aetiocholanolone
2. Androsterone
3. β Fraction
4. Total 11-Deoxy 17-Oxosteroids

Table 41

The Relation of 11-Deoxy 17-Oxosteroid Excretion to Pituitary Destruction in 10 Patients after Implantation with 90-Yttrium.

Patient	Percentage Destruction	Weeks after Operation	Excretion of 11-deoxy 17-oxosteroids (mg/24 hrs.)			
			1.	2.	3.	4.
Ys 15	95	12	0.16	0.11	0	0.27
Ys 14	90	12	0.20	0	0	0.20
		13	0	0	0	0
		40	0	0	0	0
		41	0	0	0	0
		42	0.14	0	0.01	0.15
		43	0	0	0	0
Yu 16	90	8	0.13	0	0	0.13
		36	0	0	0	0
		37	0	0	0	0
S66	80	6	0.12	0.19	0.17	0.48
Ys 10	50	10	1.72	0.68	0	1.40

1. Aetiocholanolone
2. Androsterone
3. β Fraction
4. Total 11-Deoxy 17-Oxosteroids

Table 41
(Cont'd).

/was destroyed, while when 20 per cent. of the gland was spared, approximately 0.5 mg. per day was excreted, and with 50 per cent. remaining, the level was over 2.0 mg. per day (Table 41, Figure 38). Of the two patients with significant 11-deoxy 17-oxosteroid output in the former group, one with total ablation of the gland had all three steroids present in the urine. The estimation was carried out seven weeks postoperatively, and full functional depression of the organ might not have taken place by then. A similar situation existed in the case of the other patient in whom the estimation was performed one week after implantation.

Physical atrophy of the adrenal glands after radiation hypophysectomy is a slow process, and a significant correlation between the post mortem adrenal weight and the logarithm of the time elapsing between implantation and death has been shown (Figure 39). It is reasonable to assume therefore that functional atrophy of the adrenal even after complete destruction of the pituitary will also take place quite slowly.

Notwithstanding this, adrenal function is obviously markedly depressed by effective implantation of the pituitary, but since there is only a rough correlation /

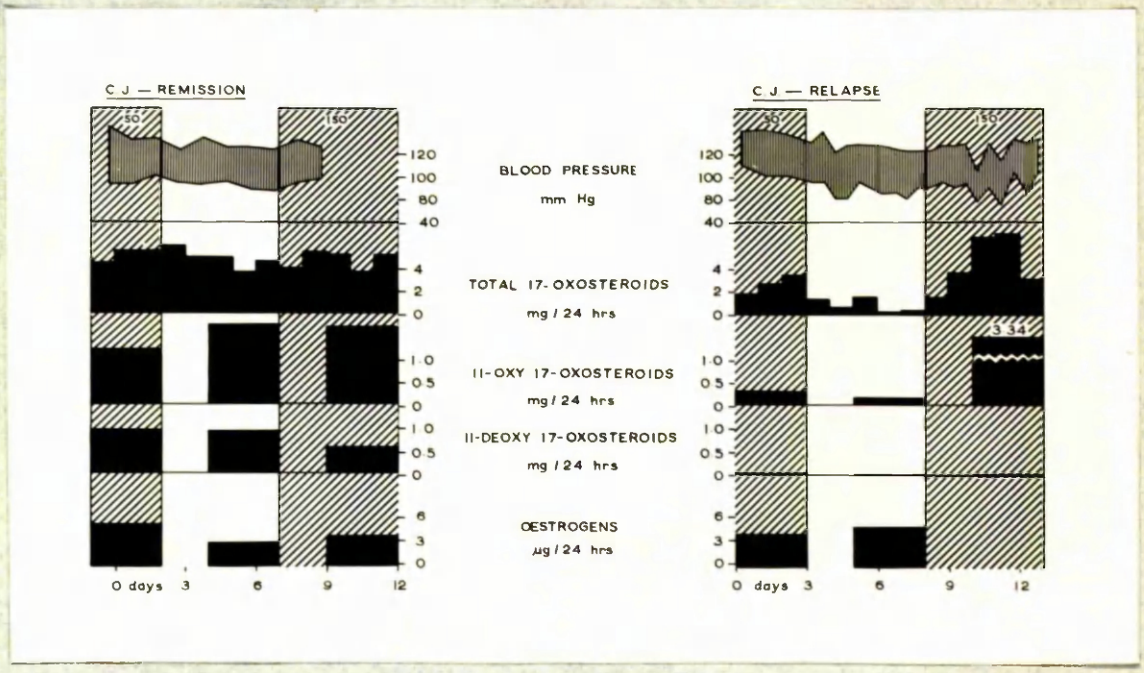


Figure 40. Results of Cortisone Withdrawal Tests in 4 patients after pituitary implantation.

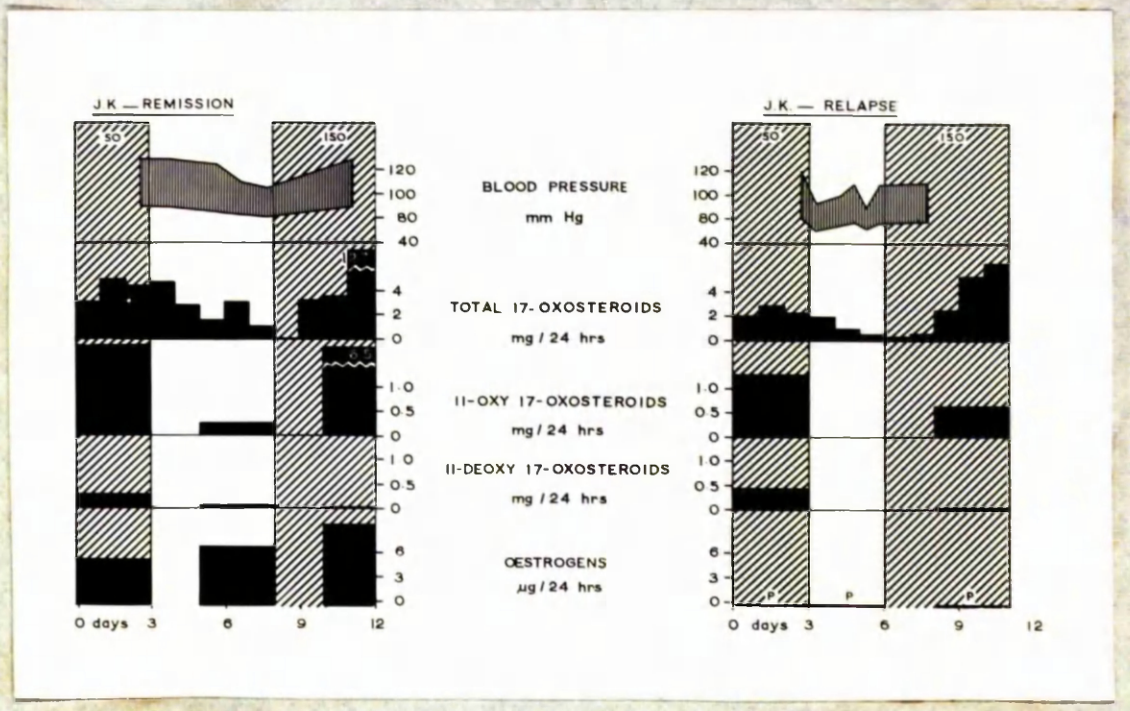


Figure 41. Results of Cortisone Withdrawal Tests in 4 patients after pituitary implantation.

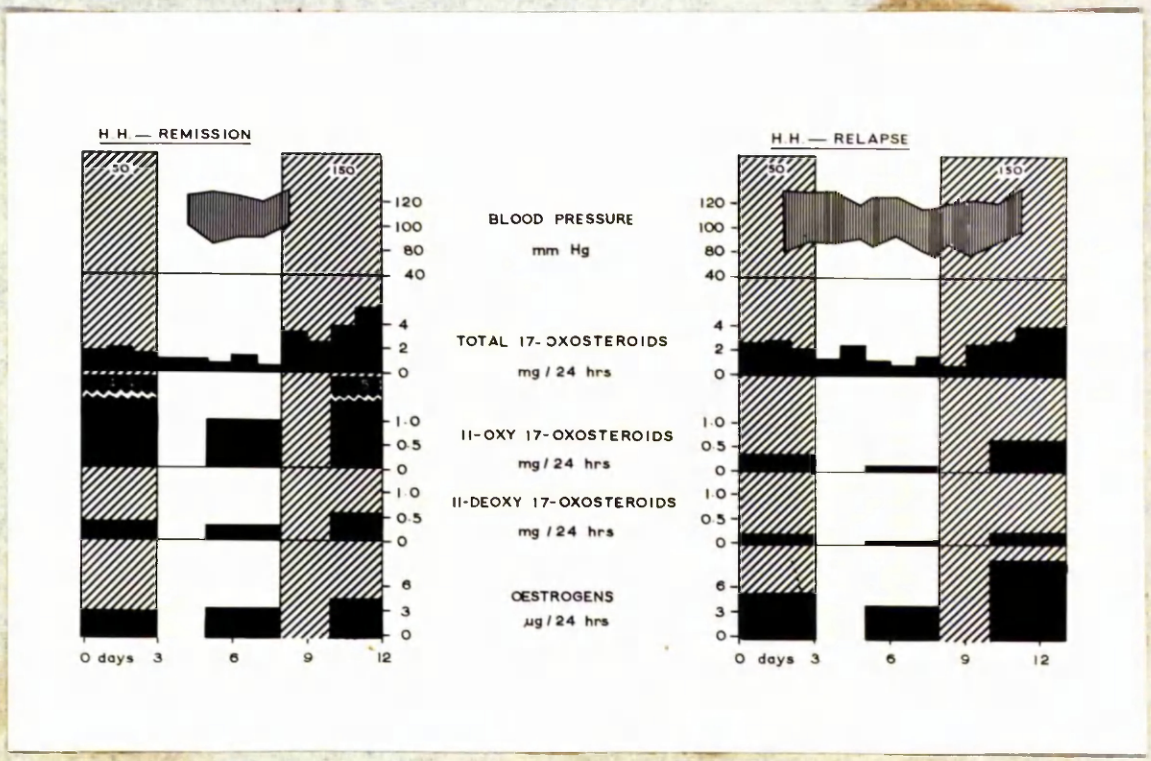


Figure 42. Results of Cortisone Withdrawal Tests in 4 patients after pituitary implantation.

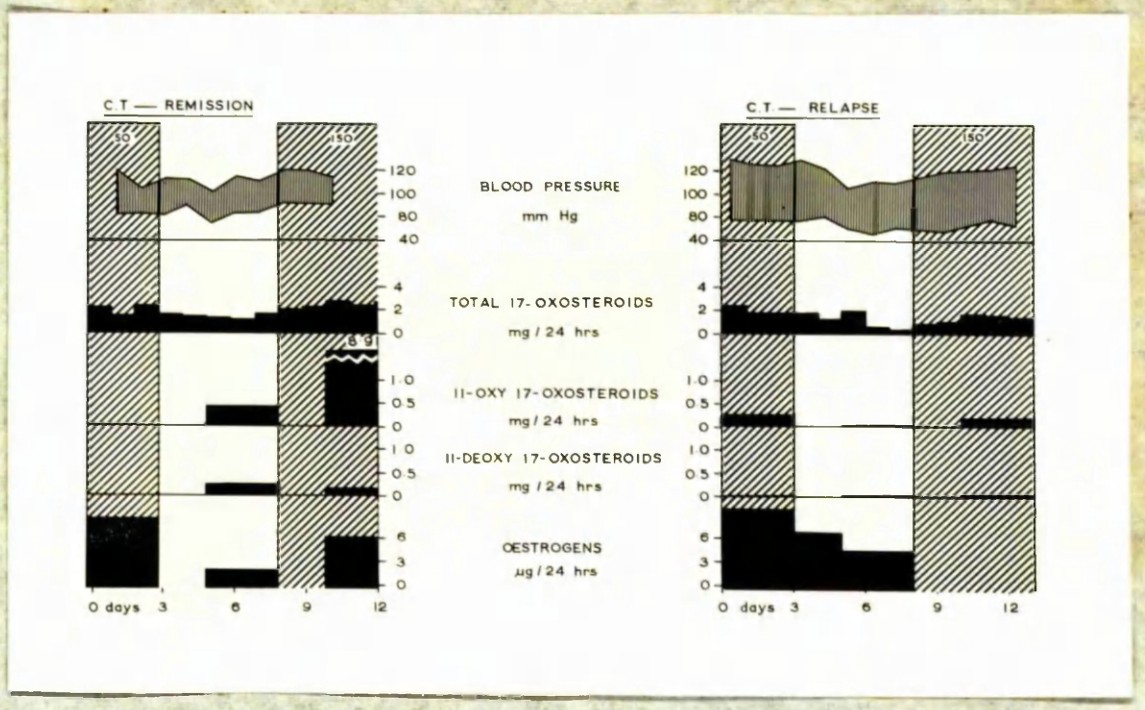


Figure 43. Results of Cortisone Withdrawal Tests in 4 patients after pituitary implantation.

/correlation between increasing percentage destruction and decreasing steroid excretion, these tests cannot be used to differentiate the completely destroyed gland from one in which a few foci of histologically normal cells remain.

Cortisone Withdrawal Tests after Pituitary Implantation.

The Cortisone Withdrawal Test, as described previously, was performed in four patients in remission after pituitary ablation and after subsequent relapse. The results are shown in Figures 40 - 43.

Each patient was able to tolerate the five days without maintenance therapy, both when the disease was in remission and when it was later progressing again. The gradual atrophy of the adrenal glands in the absence of ACTH secretion and under the influence of exogenous cortisone, is further confirmed by the observation that the level of excretion of all the steroids during the period of cortisone lack was higher when the cancer had regressed and the ability of the gland to respond to cortisone withdrawal had considerably lessened by the time the test was repeated during relapse of disease. Throughout the period of both tests oestrogen excretion continued at significant levels.

DISCUSSION

DISCUSSION.

The results of an investigation into the hormonal status of women with metastatic breast cancer, into the alterations induced by endocrine ablation and into whether any relationship existed between these alterations and the subsequent clinical course of the disease, have been described. The hormonal status was defined by multiple assays of several urinary steroids. The reasons for this approach have been discussed.

17-OXOSTEROID AND OESTROGEN STUDIES.

Preoperative Results.

It has been shown in this study that there is no statistical difference in the preoperative excretion of total oestrogen 11-oxy 17-oxosteroid and 11-deoxy 17-oxosteroids between responding and non-responding patients, and no obvious difference in the excretion of any individual oestrogen or 17-oxosteroid between the two groups. These preoperative estimations are therefore of no value in differentiating between patients likely to benefit from endocrine surgery, and those who will not. /

/not. The results of the oestrogen studies are at variance with the impressions formed by Huggins and Dao (1954) based on the bioassay of urinary oestrogens, but they do confirm the conclusions of Birke et al., (1958) and of Swyer et al., (1961), both using chemical methods of estimation.

The similarity shown in the preoperative mean 17-oxosteroid excretion pattern between the responding and non-responding groups of patients is in close agreement with the findings of Plantin et al., (1958) although these workers employed a different form of chemical assay.

As described earlier (p. 48) claims have been made regarding the usefulness of ratios of levels of preoperative excretion of certain neutral steroids or groups of steroids, in predicting the response to subsequent endocrine ablation. In twelve patients whose cancer regressed, the ratio of 11-deoxy: 11-oxy 17-oxosteroids was 2.2:1 and in ten patients who had no benefit it was 2.1:1. These results are again in agreement with those of Plantin et al., (1958) but do not confirm the original report of Allen et al., (1958).

The recent report of Bulbrook et al., (1960) described the excretion of total 17-hydroxycorticosteroids /

/17-hydrocorticosteroids and aetiocholanolone, in fifty patients before any endocrine treatment. The results of a similar study on a smaller number of patients did not confirm the reported elevated 17-hydrocorticosteroid excretion and decreased aetiocholanolone excretion in patients who failed to respond to subsequent endocrine ablation, and were unable to provide any reliable information which could be of value in the selection of patients likely to benefit from endocrine surgery. It should be noted, however, that the methods of determining aetiocholanolone used in the two studies differed in certain essentials.

Postoperative Studies.

Subsequent to endocrine surgery it was sought to establish:

(a) How the pattern of steroid excretion established after adrenalectomy plus oophorectomy compared with that established after radioactive implantation of the pituitary gland.

(b) If after either operation the same pattern of steroid excretion was established in all patients irrespective of the clinical effectiveness of treatment, or if there was a particular pattern of excretion in any group, indicative of a hormonal environment either favourable or unfavourable to the continued growth of the cancer. /

/cancer.

(a) Comparison of Steroid Excretion after Adrenalectomy plus Oophorectomy with that after Pituitary Implantation.

Oestrogens.

Although the proportion of patients not excreting any oestrogens postoperatively was very similar for both procedures - 3 out of 20 or 15.0 per cent. after adrenalectomy, and 5 out of 34 or 14.7 per cent. after pituitary implantation - the results obtained show that adrenalectomy plus oophorectomy invariably resulted in a pronounced fall in oestrogen excretion, the mean level after operation in eleven cases being significantly lower ($P<0.01$) than before treatment. In contrast, pituitary implantation caused no significant change ($P>0.8$) in the mean oestrogen excretion in twenty patients, in some cases even causing an increase. Cleveland (1958) similarly found continued oestrogen excretion in three patients after proton beam irradiation of the pituitary while Hortling et al., (1957) and Klaus (1957) found positive vaginal smears in a high proportion of breast cancer patients who had been submitted to surgical hypophysectomy. Despite this difference the overall remission rates for the two /

/two operations in a large series of cases, has been shown to be very similar (Forrest pers. comm.). From Tables 9 - 11 it can be seen that continued excretion in quite a high proportion of cases, after both procedures, is the experience of other workers, but comparable studies, before and after operation in the same patient are only available for adrenalectomy plus oophorectomy (Swyer et al., 1961). These workers, investigating five castrated women undergoing adrenalectomy, found a decrease in three, no change in one, and an increase in one after surgery. In a further four cases undergoing combined adrenalectomy plus oophorectomy, a fall in total oestrogen excretion always occurred.

17-Oxosteroids.

Only total neutral 17-oxosteroid estimations were performed on a series of patients both before and after endocrine ablation. The results obtained bore a similarity to those of the comparable oestrogen studies, the mean excretion being significantly reduced ($P < 0.05$) by adrenalectomy plus oophorectomy in twelve patients, but remaining unchanged in eighteen patients after pituitary implantation. A similar effect of adrenalectomy /

/adrenalectomy on total neutral 17-oxosteroid excretion in eleven cases has been reported by Birke et al., (1958).

11-deoxy 17-oxosteroid excretion has been shown in this study to continue after adrenalectomy plus oophorectomy in seven out of sixteen or 44 per cent. of the patients studied. Six of the patients, however, only excreted aetiocholanolone, and in every case the amounts were so small as to be of doubtful quantitative significance. It is unlikely, however, that the extremely low excretion detected, less than by Kellie and Wade (1957) is due to loss of aetiocholanolone due to artefact formation (Plantin et al., 1958) since all hydrolyses were carried out at room temperature.

Considerably greater 11-deoxy 17-oxosteroid excretion was found after pituitary implantation. Thirteen out of twenty-two, or 59 per cent. of the patients treated, continued to excrete these steroids. Aetiocholanolone, androsterone and β fraction were detected in four cases, aetiocholanolone and androsterone only, in three, aetiocholonaolone and β fraction only in three, aetiocholanolone only in two, and in one a trace of β fraction only. In contrast to the post adrenalectomy findings the /

/the amounts excreted were appreciable, in ten of the thirteen cases being well within the limits of the assay method used. Holliday, Kellie and Wade (1958) were able to detect aetiocholanolone and androsterone in all ten patients studied after surgical hypophysectomy, and in the six of these studied while receiving cortisone maintenance, the amounts were similar to those found in the thirteen cases described here. These workers could not detect dehydroepiandrosterone in any case and suggested that this might be a criterion of completeness of hypophysectomy. The extent of radiation destruction of the pituitary achieved in the thirteen patients studied is not known and 17-oxosteroid excretion as a criterion of pituitary destruction is discussed later (see p. 127).

It can therefore be concluded that adrenalectomy is superior to hypophysectomy in suppressing steroid hormone secretion and excretion and that the two procedures do not establish a similar hormonal environment in the patient. Notwithstanding this, the incidence and duration of remission of cancer, arising from the two operations, are very similar although it has been claimed recently that hypophysectomy may in fact be slightly more effective clinically (Atkins et al., 1960). /

(b) Correlation between the Pattern of Steroid Excretion Established by Endocrine Surgery and the Response of the Patient.

Oestrogens.

The mean excretion of total oestrogen by twelve patients who benefited from adrenalectomy was compared with that in eight who did not by Students 't' test, and a significant difference was not demonstrated. It should be noted that while the amounts excreted in many cases were approaching the lower limits of sensitivity of the method, zero levels and sustained high levels of excretion were commonly found, in both clinical groups. These results are in general agreement with those described in pp. 43-44 but do not support the view of Bulbrook et al., (1958), that remission after adrenalectomy tended to be associated with low or zero levels of oestrogen excretion.

A similar analysis of oestrogen excretion, in thirty-four patients after pituitary implantation also failed to show any significant difference between the responding and non-responding groups and in this case the larger amounts generally excreted would tend to make the assays more reliable. This finding differs from that /

/that reported by Bulbrook et al., (1958) in which those patients who responded to surgical hypophysectomy had lower postoperative values than those patients who failed to benefit and that of Hortling et al., (1957) who found that oestrogenic effects disappeared from the vaginal smears of eight patients who responded to hypophysectomy.

Attempts to correlate the behaviour of the disease with the amount of oestrogen excreted after endocrine ablation have not been successful and no such simple relationship seems to exist. However the oestrogens measured in this study represent only one quarter of the total oestrogen excreted and until it is possible to investigate the rôle of the other more recently discovered members of this group of hormones, it can only be concluded that the response of the disease after endocrine ablation is not related to any change in the excretion of oestriol, oestrone or oestradiol-17β

17-Oxosteroids.

It has been shown by Sandberg, Chang and Slaunwhite (1957) from studies with ^{14}C-cortisol that a significant amount of administered cortisone is converted to 17-oxosteroids of the 11-oxy aetiocholanolone /

/aetiocholanolone group, which account for the continued excretion of 11-oxy 17-oxosteroids after both adrenalectomy and hypophysectomy. They are of no value in indicating the possible persistence of adrenocortical function after either operation, and it is not surprising therefore that they bore no relationship to the clinical course of the disease.

The 11-deoxy 17-oxosteroids, in females, are metabolites of the adrenal androgens. $\triangle^4$-androstene-3: 17-dione, and dehydroepiandrosterone, and as such are unlikely to include steroids derived from the metabolism of maintenance cortisone. Small amounts of the 11-deoxy 17-oxosteroids are excreted after both adrenalectomy and pituitary implantation. The amounts are often at the limits of the sensitivity of the colour reaction used, and it is quite clear that the presence of such small amounts does not preclude benefit from the operation, and that there is no correlation between clinical response of patients to pituitary implantation, and the amounts of these steroids excreted postoperatively.

These conclusions, concerning the lack of any correlation between steroid excretion and the clinical course of breast cancer after endocrine surgery are /

/are further borne out by the results shown in Figures 21 - 25. This more comprehensive study of steroid excretion throughout the course of treatment in five patients reveals no pattern of excretion associated either with success or failure of the operation and does not in any way suggest that a re-establishment of the preoperative hormonal environment, resulting from the steroid secretion by either accessory adrenocortical tissue or any other tissue such as the tumour itself (Daniel, 1957), is an important factor in the relapse of the disease, after a favourable response.

Oestrogen Excretion after Endocrine Ablation.

There emerged in the course of these studies, the fact that no form, or combination of forms of endocrine ablation, could consistently abolish completely, oestrogen excretion. The lack of correlation between changes in oestrogen excretion and the response of the disease, suggests that this group of hormones, or at least those members of it, at present able to be accurately estimated in the urine, are less important than was originally thought. Oestrogen excretion continuing after endocrine ablation does not preclude a favourable response to the operation, but the source and nature of these oestrogens is of considerable interest. /

/interest.

Oophorectomy alone led to a relatively minor fall in oestrogen excretion in the premenopausal woman, although in considering this, account must be taken of the fact that the stage of the menstrual cycle in which the preoperative estimations were performed is not known, and this has been shown to be of considerable importance in the premenopausal woman (Brown, 1956). The accepted ability of the adrenal to secrete oestrogen was further demonstrated by the effect of ACTH injection on oestrogen excretion in adrenalectomised women, and in oophorectomised women, with intact adrenals. In the latter group, this is shown to produce a mean increase of 148 per cent. in oestrogen excretion, as compared with 16 per cent. in the former group.

In the absence of pituitary and adrenals, as well as ovaries, there is no proven source of oestrogen production but four possible sources may be considered.

Metabolism of maintenance cortisone: No conversion of maintenance cortisone to oestrogens has been demonstrated in this study. Varying the cortisone dosage and even withdrawing it completely in patients after 90-Yttrium implantation of the pituitary, has no pattern of effect, /

/effect, consistent with the source of the steroids being the administered cortisone. This, however, is not unexpected since conversion of the cortisone to oestriol, oestrone or oestradiol-17β would involve the "in vivo" removal of an oxygen function from the 11-position in the steroid nucleus, a reaction which has never been previously demonstrated. The conversion of maintenance cortisone may still take place, however, giving rise to a series of oestrogens not previously described viz. those with an 11-oxygen function. Very recently the first report has been published of the isolation of 11β hydroxyoestrone and 11β hydroxyoestradiol-17β from the urine of women with breast cancer after the injection of ^{14}C-labelled cortisone (Chang and Dao, 1961). Such compounds being considerably more polar than oestriol, oestrone and oestradiol-17β, and having markedly different chromatographic properties, would not be estimated by Brown's method. A similar conversion, however, could not be shown after the injection of ^{14}C-labelled cortisol, and the overall quantitative significance of the transformation is not yet known, not is the oestrogenicity of these compounds in the tests at present used.

It may be that the oestrogens in the urine of patients after endocrine ablations are derived from the diet. /

/diet. It has been known for many years that green plants and other foodstuffs may contain a considerable amount of oestrogenic material of unspecified nature (Waller and Janney, 1930: Vague, Garrigues, Berthet and Favier, 1957) and the current practice of treating cattle with synthetic oestrogens may result in high concentrations in some cuts of meat. Recently Hudson (1958) has reported that a normal hospital diet in the United States contained about 22 µg of oestrogenic substance. Although synthetic oestrogen is outside the scope of the present study the possibility of some of the exogenous material being steroid oestrogen detectable by Brown's method was not discounted. The result, in terms of excretion of oestriol, oestrone and oestradiol-17β, of maintaining five adrenalectomised-hypophysectomised women on a purely synthetic diet in this study, was that in only two cases did the oestrogen excretion fall to zero levels. In the other three cases being unaffected or even increased. On this limited evidence, it can only be concluded that exogenous oestrogen is not an important factor in the oestrogen excretion after endocrine ablation.

It has been suggested on many occasions that accessory adrenocortical tissue may be stimulated into a /

/a state capable of steroid secretion, after adrenalectomy. Oestrogen excretion during cortisone withdrawal in adrenalectomised patients, however, does not conform to the pattern which would be expected in the presence of functioning adrenocortical rests.

The possibility of the breast tumour itself secreting oestrogen was proposed by Daniel (1957) when, using Brown's method, he was unable to detect significant amounts of oestrogen in the urine of ten patients with hypopituitarism but no breast cancer. Biologically active oestrogens have been isolated from breast cancer tissue, but this does not necessarily imply their formation by that tissue, since the uptake of radioactive oestrone by mammary cancer is greater than that by normal tissue (Lewison, Levi, Jones, Jones and Silberstein, 1951). Were the tumour the source of the oestrogens in the urine of patients with breast cancer, their levels should decrease during regression of the cancer and increase when the tumour later becomes active again. The results of the long term studies (Figures 21 - 25) show that this pattern is not commonly found. It is difficult to believe that a relatively undifferentiated tissue, such as a rapidly growing breast tumour should be capable of the /

/the complicated processes of steroid hormone synthesis and secretion.

The mechanism of the response to adrenalectomy and oophorectomy, shown by some patients with breast cancer is obscure, although it is reasonable to assume that endocrine surgery deprives the tumour cells of a hormonal factor essential for active growth. In the same way, it has frequently been suggested that eventual relapse may be due to the development of an accessory source of adrenal-ovarian hormones, which restore optimum conditions for tumour proliferation. Such accessory adrenocortical tissue is known to occur in man (Graham, 1953; Falls, 1955). The results have been described of a series of investigations conducted to determine whether there was evidence of returned adrenal function in patients relapsing after initial regression, or of continuing adrenal function in patients who derived no benefit from operation.

An accurate assessment of residual adrenocortical function after adrenalectomy can only be made when the intake of maintenance cortisone is stopped. The Cortisone Withdrawal Test used in these circumstances has been described (p.83). /

/

The clinical response to the withdrawal of maintenance therapy indicates the patients' ability to survive without it, and furthermore, during its deprivation any accessory adrenal tissue will be released from the suppressing action of exogenous cortisone, although the time required for the return of adrenal function after long term steroid therapy is uncertain (Fredell, Johnson, Krupp, Engleman and McGrath, 1955; Geyer, 1958; Vermeulen, 1958). The observation that adrenalectomised patients can tolerate cortisone lack for variable periods ranging from two to five days agrees with that of Lipsett, West, Maclean and Pearson (1957) and Birke, Duner, Franksson, Liljedahl and Pernow (1960). Relapse of cancer was not associated with prolongation of the time patients could withstand withdrawal of maintenance therapy, nor was failure to show any benefit from endocrine surgery. The pattern of steroid excretion during the withdrawal period was also consistent with an absence of functioning adrenocortical tissue.

These findings, in patients who had no benefit and in those whose cancer was progressing again after /

/after initial favourable response, were identical to those obtained when the tumour had regressed, and indicate that the unfavourable courses of the disease are not associated with the development of functioning accessory adrenocortical tissue.

At present an accurate assessment of the completeness of hypophysectomy resulting from surgery or from radioactive implantation of the pituitary gland in patients with breast cancer, can only be made by post-mortem examination of the contents of the sella turcica. Consequently some method of estimating, during the postoperative period, the degree of destruction achieved, would be of considerable value in planning further treatment.

The response of the cancer, although in some cases obvious and dramatic, has been shown to bear no relation to the condition of the gland as seen at autopsy, so other parameters such as the persistence of measurable amounts of pituitary hormones and of the pituitary conditioned functions of peripheral endocrine glands have been studied. The measurement of changes in excretion of anterior pituitary hormones after /

/after operation, the most direct and potentially most satisfactory parameter is at present limited to gonadotrophin estimations, the only member of the group, for which a satisfactory assay procedure is at present available.

Although changes in adrenal function can be determined by satisfactory analytical procedures, the results obtained must be interpreted with caution in terms of alterations in pituitary function, especially in the very ill patient, since widespread malignant disease may influence such changes.

Having regard to these limitations, the levels of excretion of several different urinary steroids of adrenal origin after radioactive implantation of the pituitary were compared with the degree of destruction of the gland as found at subsequent postmortem. The results obtained show no correlation which would permit deduction as to the degree of destruction of the gland from the levels of excretion of either total 17-oxosteroids 11-deoxy 17-oxosteroids or oestrogens. In some cases appreciable excretion coexists with histologically complete destruction.

Two points must be borne in mind however when /

/when interpreting these results.

Firstly, it may be that after radioactive implantation, a pituitary with up to ten to fifteen per cent. of the cells still histologically demonstrable, may be, in terms of function, no different from a completely destroyed gland in view of the intense irradiation absorbed by the residual tissue. Consequently it may not be justifiable to expect to find significant differences in adrenal function in patients with more than eighty-five per cent. destruction of the pituitary as demonstrated histologically.

Secondly, the time elapsing between the operation and the determination of adrenal activity should be considered. This is obviously of importance in some of the multiple estimations in the same patient and the only significant correlation shown in the study was that between the adrenal weight at death and the time elapsing between the pituitary implantation and death. This confirms that adrenal atrophy after pituitary destruction is a slow process, after an initial sharp decrease to lowered levels. All the hypophysectomised patients in the present study could withstand five days without maintenance therapy. Adrenal function is obviously not so intimately dependant /

/dependant on uninterrupted hypophyseal stimulation as to be a sensitive indicator of very small alterations in pituitary function.

The present finding of 11-deoxy 17-oxosteroids in the urine of female patients with complete pituitary destruction suggests that androgens of adrenal origin may be added to aldosterone (McLean, Lipsett, Li, West and Pearson, 1957) and cortisol, already shown to be secreted by the adrenal in the absence of ACTH stimulation. Further evidence of this has recently been reported by Wilson, Lipsett and Butler (1960).

The results of the Cortisone Withdrawal Tests carried out in four patients after pituitary implantation, suggest no reason for the relapse of the disease after favourable response to this treatment. As after adrenalectomy the results of these tests are substantially similar both during remission and relapse, although after hypophysectomy, the levels of excretion during the second withdrawal period are generally lower. Müller (1957, 1958) has suggested that in patients after hypophysectomy the pharyngeal pituitary may develop the capacity to produce pituitary hormones and so /

/so defeat the object of the operation. His claim is based on histological changes which can be demonstrated in the pharyngeal pituitary after damage to, or destruction of, the adenohypophysis. Also, his patients only required substitution therapy for eight weeks after hypophysectomy, after which adrenal hormone secretion reverted to normal. A similar picture has been reported for pituitary transplants in mice (review Bielschowsky and Horning, 1958) which are thought to secrete exclusively prolactin and ACTH. The results of the Cortisone Withdrawal Tests carried out in this study are at variance with these reports, and in no case can reactivation of the disease be ascribed to a restitution of the preoperative hormonal status. Unlike Müller's cases, however, cortisone maintenance therapy was instituted immediately after implantation and maintained throughout the postoperative period.

SUMMARY.

Changes in the hormonal environment induced by endocrine ablation in women with metastatic breast cancer have been defined and compared with the clinical course of the disease.

Adrenalectomy plus oophorectomy causes more profound decreases in steroid hormone secretion and excretion than does pituitary implantation with 90-Yttrium, but does not appear to produce a higher proportion of remissions or remissions of longer duration. The preoperative excretion of one or more of these steroids are no guide to the subsequent response of the patient to treatment. In a considerable proportion of cases, steroids of presumed adrenocortical origin are still excreted post-operatively but in a manner quite unrelated to the cause of cancer. Withdrawal of the maintenance therapy for a limited period, in order to define more clearly endogenous adrenal function was also unable to demonstrate any significant difference between responding and non-responding patients.

The favourable outcome of treatment in about /

/about 20 per cent. of cases, lends support to the concept of "hormone responsiveness" of certain breast cancers, although the relationship between the disease and the endocrine system is obviously more complex than would permit the designation of these tumours as "oestrogen dependent."

Eventual relapse of the disease is however inevitable, and intensive study of the hormonal environment in the same patient when the disease was in remission and later when it was progressing again was unable to demonstrate changes which could be responsible. It must be concluded that this alteration in the behaviour of the tumour is due to changes in the nature of the cancer, rather than changes in hormonal stimulus as demonstrable by the present methods of steroid hormone assay.

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