Plasma Hormones and Breast Disease

by

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Chemical Abbreviations in Text

Luteinizing hormone ---------- LH

Follicular stimulating hormone ---------- FSH

Luteinizing hormone releasing hormone ---------- LHRH

Thyroid releasing hormone ---------- TRH

Thyroid stimulating hormone ---------- TSH

Tri iodo thyronine ---------- T_3

Thyroxine ---------- T_4

Human prolactin ---------- HPr

Prolactin inhibiting factor ---------- P.I.F.

Growth hormone ---------- GH

Adrenocortical trophic hormone ---------- ACTH

Oestradiol-17B ---------- E_2

Oestrone ---------- E_1

Testosterone ---------- T

Oestradiol-17B/testosterone ratio ---------- $E_{2/T}$ ratio

Sex hormone binding globulin ---------- SHBG

5α dihydrotestosterone---------- dihydrotestosterone

Dehydroepiandrosterone ---------- DHA

Dehydroepiandrosterone sulphate ---------- DHAS

Serum glutamic oxalo trasaminase ---------- SGOT

Serum glutamic pyruvate transaminase ---------- SGPT

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statistical methods for these data. Most of the detailed calculations were the work of the author, some by computer programmes written by Mrs. Raab. The rank correlations were carried out by the latter. The description of the statistical methods was written by the author to explain the methods used, but make no claim to be her original work.

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SUMMARY

The aim of this study was to conduct a detailed examination of hormone concentrations in the plasma of subjects with breast disease. In particular, women with breast cancer were investigated, and men with gynaecomastia.

Plasma oestradiol-17B, testosterone, luteinizing hormone, follicular stimulating hormone and prolactin levels were studied in women with breast cancer. All plasma hormone concentrations were within the normal range although plasma testosterone levels were higher in women with breast cancer than in normal women matched for age, parity and menstrual status.

Pituitary releasing hormones were used to induce a rise in the levels of plasma LH, FSH, growth hormone (GH), thyroid stimulating hormone (TSH) and prolactin concentrations in patients with breast cancer and the various pituitary function tests were compared. It was shown that luteinizing hormone releasing hormone (LHRH) induced a rise in the levels of plasma LH and FSH and thyrotrophic releasing hormone (TRH), a rise in plasma TSH and prolactin. The addition of TRH to LHRH did not affect the levels of plasma LH and FSH above that produced by LHRH alone. TRH led to a higher elevation of plasma prolactin levels than chlorpromazine. A test consisting of the administration of LHRH, TRH and insulin by infusion was found to be the most suitable for studying pituitary function in women with breast cancer undergoing yttrium implantation of the pituitary.

This test was then used to assess the pituitary function after yttrium implant. Response of each hormone to its own releasing hormone was used to assess the degree of pituitary ablation obtained which, in turn, was correlated with clinical response. The results showed that the more complete the ablation, the more likely there was to be a clinical response. This test of pituitary function was also used to assess the effect of quadruple chemotherapy on pituitary function. The results showed that in women with advanced breast cancer chemotherapy did not have a demonstrable effect on pituitary function.

The effect of administration of the hormonal drugs - an antioestrogen (tamoxifen) and a synthetic oestrogen (stilboestrol) on plasma FSH, oestradiol-17B, testosterone and prolactin levels were examined. Both drugs depressed plasma FSH levels, stilboestrol having a greater effect than tamoxifen. Stilboestrol also induced elevation of plasma prolactin concentrations. The effect of these drugs on the concentrations of plasma hormones was, however, not related to the clinical response of the disease.

These studies showed a pulsatile pattern of concentrations of plasma luteinizing hormone (LH) in normal young men, which was absent in both young and old men with gynaecomastia and in normal old men. In comparison to normal young men, young men with gynaecomastia had a lower plasma LH level and consequently a lowered ratio of LH to follicle stimulating hormone (FSH) LH/FSH ratio, while in comparison with normal old men older men with gynaecomastia had a normal LH/FSH

ratio, but raised values of LH and FSH. This group of older men with gynaecomastia also had higher plasma oestradiol-17B (E^2) levels and a raised oestradiol-17B testosterone ratio, when compared with normal men of a similar age.

From these results, it would seem that the causes of breast disease are multifactorial which will be solved only after many related aspects have been considered and discussed and further research carried out. This thesis seeks no more than to make a contribution to this discussion; nevertheless it is felt that certain aspects of breast disease have been clarified and that sampling methods have been identified to give a more accurate and realistic indication of plasma hormone levels.

Nothing discovered in the course of this work or in the study of the literature, discounts a hormonal contribution to the cause of breast cancer. It is believed that in the further refinement and investigation of the full role of these plasma hormones in male and female breast disease, will the cause of breast cancer be eventually clarified.

I

INTRODUCTION

I INTRODUCTION

Any individual woman stands a 1:14 chance of developing breast cancer during her life. It ranks as the commonest cause of death in women in the 35-40 age group and every year 12,000 women die of breast cancer in the United Kingdom. Any part of the breast may be affected by cancer but it most frequently commences in the upper outer quadrant of the breast. Spread of the disease can be local, lymphatic or blood borne.

Conventionally, cancer of the breast is graded according to severity. The method used to stage breast cancer is the Union Internationale Contre le Cancer (UICC) T.N.M. system in which tumour, nodes and metastases are each classified. From these "stages" of severity are constructed: stage I - growth confined to breast; stage II - breast plus affected mobile lymph nodes in axilla; stage III - tumours more than 5 cms in size, or tumours of any size with direct extension to chest wall or skin, or any tumour associated with fixed axillary lymph nodes, or supra or infra clavicular lymph nodes thought to be invaded; stage IV - as above plus distant metastases. An example of an advanced case (stage IV) of breast cancer with skull secondaries is seen in Figures 1 and 2.

Histologically, the commonest type of breast cancer is an invasive duct carcinoma. Various histological types are described of which a non-descript pattern is the most common (90%); histological appearance of the type is seen in Figure 3.

Fig. 1

Photograph of a patient with advanced cancer of the breast.

Fig. 2

Photograph of an X-ray of skull secondaries in the patient in Fig. 1.

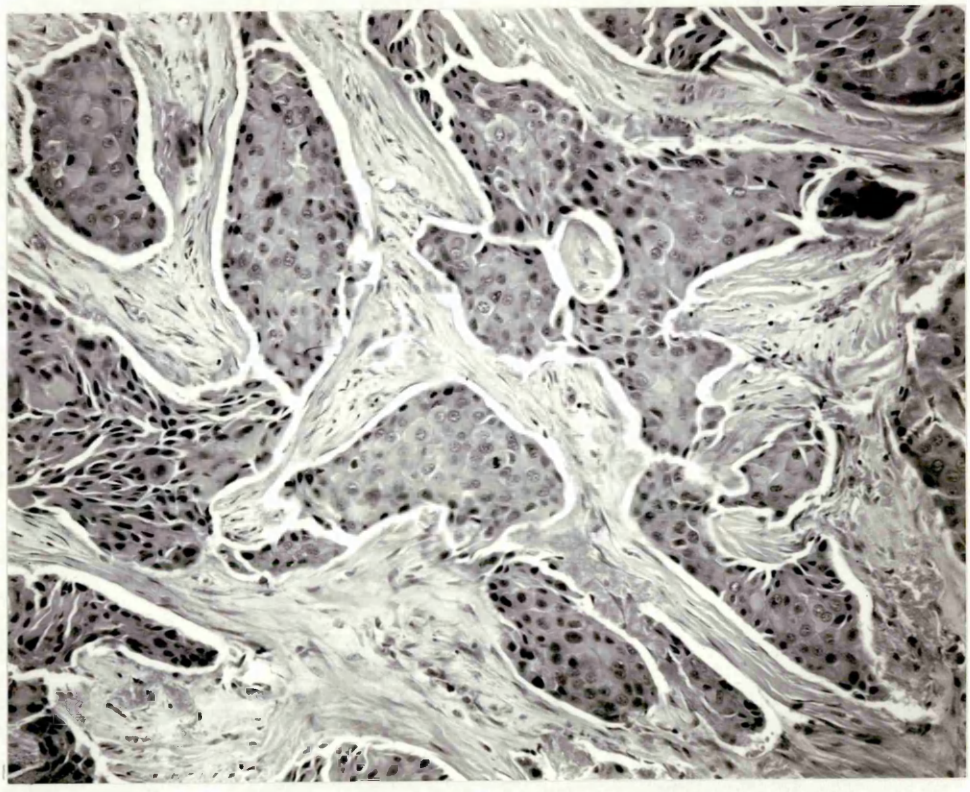


Fig. 3

Photomicrograph showing an invasive ductal carcinoma of breast.
(H & E Magnification x 150)

The results of treatment of breast cancer are poor and after 5 years (from all types of treatment) only 68% with stage I are alive, 40% of stage II and 15% of stage III and 2% of stage IV are still alive.

It is a disease which disseminates early but recurs late. Recent evidence suggests that even 30 years after the initial treatment of early cancer, women still have an excess mortality from metastatic disease. "Statistical" cure has not been demonstrated. However, some 26% of women have 'personal cure' in that they die from some other cause before their metastatic disease becomes evident (Brinkley & Haybittle, 1984). If the disease is to be cured, there must be an understanding of the basic mechanism causing the cancer.

NORMAL BREAST DEVELOPMENT

The breast is a hormone target organ.

Studies in endocrine ablated rats indicate that oestrogen and progesterone are required for ductal development, the added effect of prolactin for alveolar formation and for full breast development (comparable to that found in late pregnancy), four hormones are necessary:oestrogen, progesterone, prolactin and growth hormone (Lyons et al 1955, Cowie & Folley, 1958). Evidence that these same hormones are necessary for the development of the human breast is less direct. However, the coincidence of breast development with the start of ovarian function during puberty, the effect of oestrogenic hormones on male breast development (Fitzsimmons, 1944),

the great hypertrophy of the breast during pregnancy and the atrophy of the female breast which occurs after the menopause or in hypopituitary states (Sheehan & Sommers, 1949) suggest that similar mechanisms may apply in the human breast.

Breast Cancer

There is considerable experimental evidence that the hormones which are so intimately concerned with the normal growth and function of the female breast may also affect the development and progression of mammary carcinoma. The most striking evidence that hormones are involved in the occurrence of breast cancer in humans was demonstrated by a Glasgow surgeon, George Beatson. In 1896, he demonstrated a case of a 33 year old woman who had local recurrence of tumour in her mastectomy scar which healed when the ovaries had been removed. With the discovery of cortisone, Charles Huggins in Chicago, in 1952, reported that a similar effect in postmenopausal women could be achieved by adrenalectomy. Evidence that the effect of castration on human breast cancer was due to deprivation of oestrogens was first reported by Pearson and his colleagues. They measured urinary calcium in women with metastatic bone disease during several menstrual cycles, following oophorectomy and after administration of oestradiol and demonstrated a cyclical pattern of urinary calcium during the menstrual cycle which was abolished by oophorectomy (Pearson et al, 1954). The dramatic fall in the level of urinary calcium following oophorectomy was believed to be due to reversal of bone breakdown as a result of removal of oestrogen

stimulation, a belief strengthened by reversal of this effect by the administration of ethinyl oestradiol. Further evidence for the involvement of oestrogen in the growth of human breast cancer was provided by the demonstration by Jensen and his colleagues (1971) that the cytosol of 70% of breast cancers contained the same high affinity binding protein for oestrogens as did other oestrogen sensitive tissue. Initially believed to reside in the cytoplasm, it is now suggested that oestrogen binding protein normally is situated in the nucleus. The complex formed by oestrogen and its receptor acts on the genome to stimulate cell growth and division and other events. Breast cancers which do not possess this oestrogen mechanism are insensitive to the action of the hormone.

A model for the study of hormonal effects on breast cancer is provided by the MCF-7 cell line which was originally cultured from ascitic breast cells. This is sensitive to oestrogenic stimulation and in the absence of the hormone, or following the addition of an anti-oestrogen (tamoxifen) to the culture medium, growth is suppressed. Similar effects have also been demonstrated in transplants of human breast cancer into immune deprived (nude) mice (Shafie, 1980, Seibert et al, 1983).

Similar evidence to implicate other hormones, eg progesterone, testosterone, growth hormone and prolactin in the growth of human breast cancer is less convincing.

Source of Oestrogens

In women the two major oestrogens secreted from the ovary are oestrone and oestradiol 17B. In premenopausal women the majority of the oestrogens are secreted, ie from the ovaries, the cyclical secretion from them produces high circulating levels of oestrone and oestradiol 17B at various periods during the menstrual cycle. After the menopause the circulating levels of oestrogens are much diminished and are only present in small amounts. Most of the oestrone and oestradiol 17B is not derived from ovary or adrenal but indirectly by their synthesis at peripheral sites (liver, muscle and fat) from aromatization of the androgen C19 steroids - Δ^4 androstenedione and dehydroepiandrosterone (Nimrod & Ryan, 1975).

There is also evidence that tumours possess several enzymes and can convert C19 steroids into active oestrogens (Miller et al, 1982). Studies show that there are a lot of C19 steroids available in the breast which contains high concentrations of dehydroepiandrosterone sulphate and other androgen conjugates and can produce oestrogenic hormones.

Risk of Breast Cancer

If the causes of breast cancer were fully understood then the risk factors could be identified and preventative treatment of the women at risk carried out. Hormones may also influence the risk of the development of breast cancer.

Dominant risk factors can be considered in two groups. (1) geographical variations of the incidence of the disease and (2) risk factors which occur within a given population. There are striking differences in the incidence of breast cancer and its natural history in different geographical areas. Most marked is the difference in the incidence of breast cancer in Europe and the USA and Japan, in which the incidence is eight times lower (Wynder et al, 1963). There is now evidence that this incidence is rising, an effect also seen in Japanese who migrate to the West, which suggests the importance of environmental factors (Stocks, 1957, MacMahon, 1970). It is suggested that hormonal effects may explain these differences, possibly by the effect of diet on the synthesis of metabolism of oestrogens. Interest has, therefore, been expressed in studies of the hormonal background of such women.

Estimations of urinary steroids were reported in populations of normal women known to have a high or low risk of breast cancer. Young Japanese women (low risk group) excreted a lower level of urinary 11-deoxy-17-oxo-steroids than young British women (high risk group) while in the older age group the reverse was true (Bulbrook et al, 1964; 1967).

In a study of the normal women from Guernsey, Wang and his colleagues (1976) measured plasma dehydroepiandrosterone sulphate, androsterone sulphate and $\Delta 4$ androstenedione levels in these normal British (high risk group) and Japanese women (low risk group) and found that plasma dehydroepiandrosterone sulphate and $\Delta 4$

androstenedione concentration was lower in the Japanese than the British women. Plasma androsterone sulphate levels were lower in all groups of Japanese women but not significantly so. These results were contrary to expectation.

A study of the plasma oestrogen concentration was also carried out in normal British and Japanese postmenopausal women (Bulbrook et al, 1976). These workers found that the concentrations of plasma oestrogens in these two races were not significantly different except in an adolescent group. They concluded that the difference in the incidence rates in breast cancer in Britain and Japan did not appear to be related to difference in plasma oestradiol-17B or oestrone levels.

Within the population of women, whether at low risk or high risk of developing breast cancer, women who have their first child at a younger age are less susceptible to the risk of developing breast cancer than those whose first full time pregnancy occurs later (MacMahon, 1970). Thus, women who have borne their first child before the age of 25 have about half the risk of developing breast cancer compared to women who have their first child over the age of 30. Nulliparous women also are more at risk but less so than women who have their first pregnancy after the age of 35 (MacMahon, 1970, 1973). It has been suggested that the number of menstrual cycles which occur before the first full time pregnancy is the important determinant of risk so that pregnancy with full stimulation of breast development early in reproductive life is protective. In

other words, it is confinements during the first ten years of reproductive life which protect against the late development of breast cancer (Smithers et al, 1952).

Attempts to define whether the hormone environment predisposes to the development of breast cancer has been studied on the Island of Guernsey (Bulbrook and Hayward, 1967; Bulbrook et al, 1969; Bulbrook et al, 1971). Twenty four hour samples of urine were collected from five thousand women over five and a half years and stored. As patients with breast cancer emerged in the population their urine was matched by those of ten to twelve controls and all were then fully analysed. In the first report of women studied, twenty-seven women had developed breast cancer and the mean excretion of aetiocholanolone and androsterone in these women were then compared with that in one hundred and eighty-seven matched controls and in one thousand five hundred and six women in the population who had not developed breast cancer. A group of these Guernsey women in the high risk category - (old at menarche, old at first child, family history of breast cancer and a low urinary aetiocholanolone level) were later studied further (Wang et al, 1979). Plasma samples were taken five years after the urine samples from three hundred and eighty-six of these normal high risk women (aged 30-69).

The levels of plasma androgens were found to correlate with the excretion of urinary aetiocholanolone and the levels were at the lower end of the normal range in this group, indicating that the low level of urinary aetiocholanolone five years before was not an

indicator of transient androgen production.

Other risk factors within a population include a family history of breast cancer. A woman with a first degree relative who has developed carcinoma of the breast is herself at greater risk than a woman with no family history of breast cancer (Anderson, 1971) and such families provide suitable groups for hormone studies (Wang et al, 1979). It is also relevant that the administration of oestrogens given for hormone replacement is associated with an increased risk of breast cancer (Hoover et al, 1976). Furthermore, women who are deprived of ovarian function early in life have a lower incidence of breast cancer (Herrel, 1937, Smithers et al, 1952).

In view of these facts it is not surprising that much work has concentrated on the hormonal background of patients with established breast disease, the effect of endocrine therapy on hormone levels and hormonal changes which may increase risk. Such studies initially depended on urinary estimates but with the availability of new immune assay methods of greater sensitivity, plasma estimations became available. These have now been applied and in this thesis three studies, using immunoassay methods of estimating hormones in circulating body fluids are described.

These are:

1) Detailed studies of plasma hormone levels in women with established breast cancer, sampled consecutively and compared with carefully matched controls.

2) Studies of the effect of anti-oestrogen therapy, hypophysectomy and chemotherapy on hormonal levels in patients with advanced breast cancer.

3) Comparison of plasma hormone levels in men with gynaecomastia and normal hormonal subjects.

Each study is described following a review of the relevant literature, but this has been confined to the time at which the study was performed. Published reports which have appeared in later years have been included in the discussion section which concludes each study.

The methods of hormone assay which have been used in the separate sections are all similar. These are described in a general methods section which precedes the description of the three individual studies.

II

GENERAL METHODS

1. Plasma Collections

The protocol for plasma collections was similar in all subjects with minor variations according to the study. Three types of plasma sampling were adopted.

a. Single blood samples at a fixed time each day.

b. Multiple blood samples at various intervals during the day.

c. Frequent multiple sampling in pituitary function tests.

a. Single Plasma Sampling

A single sample of blood was removed from an arm vein using an 18G needle. The blood was then heparinised and centrifuged at 2,500 rpm for 10 minutes at -4°C. Plasma was pipetted off, subsequently frozen and stored at -20°C. If follow up samples from an individual were required, these samples were taken at the same time of day as the first sample and all the plasmas from a single patient were then measured in one batch in the same assay.

b. Multiple Plasma Sampling

In certain studies blood was withdrawn at frequent intervals throughout the day. The patients investigated were allowed to walk around freely and to eat meals. An 18G teflon cannula with stilette was inserted into an arm vein through which blood was withdrawn. Plasma was prepared and stored as described previously. Fig. 4 shows a patient undergoing this procedure.

Fig. 4

Photograph of a patient with breast cancer undergoing a multiple plasma hormone study - note the cannula in situ in the right wrist.

c. Details of the sampling methods used in pituitary function studies are described in Study 3.

2. Hormone Assay Methods

For technical reasons it has been necessary to have some assays of the same hormone carried out in two different laboratories. The laboratories involved were the Tenovus Institute, Cardiff and the Immunoassay Section, Department of Clinical Chemistry, Royal Infirmary, Edinburgh.

Because of the differences in technique between the assays in the two laboratories, it should not be assumed that the numerical results obtained are strictly comparable or have the same normal range. Within any one study, however, control and other group values will have been obtained from the same laboratory by the same method and are therefore comparable.

As the determinations of plasma hormone levels constitute the major measurements within this project, it is pertinent to examine the reliability of these measurements. This can be assessed under four main headings (Borth, 1952). For an assay to be considered reliable each of these criteria should be fulfilled.

a. Reproducibility

b. Specificity

c. Accuracy

d. Sensitivity

a) Reproducibility

Reproducibility is the precision of an analytical measurement. It is usually assessed as the coefficient of variation of the analysis.

Coeff. of variation = SD/x X 100%

where SD is the standard deviation.

X is the mean measurement.

The standard deviation may be assessed either from the variance of many measurements about the mean from one sample or from the difference between duplicate estimations on many samples. In routine clinical chemistry, for example, the coefficient of variation normally runs between approximately 1-7%.

b) Specificity

The specificity of a method reflects the extent to which only the compound to be analysed contributes to the signal generated by the detector used. Specificity may be examined by various methods including:-

1) Analysis of fluids likely to be free from the hormone to be measured eg. water or plasma from a subject who has had surgical ablation of endocrine (hormone secreting) organs.

2) Comparison of the apparent concentrations of a hormone in plasma before and after the addition of such compounds as might interfere with the assay.

3) Comparison of the apparent hormone concentrations found on analysis of different sizes of plasma samples.

4) Examination of the apparent hormone concentration of plasma found after purification of the sample to varying degrees.

c) Accuracy

Accuracy is an assessment of the proximity of the measurement to the true value (e.g. as measured by an ideal method). The accuracy of an analytical method can be determined by measuring the percentage recovery of known amounts of a pure compound added to the sample before analysis (theoretically, after correction for any losses, accuracy should be 100%).

d) Sensitivity

The sensitivity of a method for the determination of a substance may be defined as the smallest mass of that substance which can be distinguished with a given degree of certainty from zero. Sensitivity is, therefore, particularly important when dealing with measurements at low levels. It is frequently calculated from the formula $ts.\sqrt{n}$ where t=student's t value for $p<0.01$, s = standard deviation at low levels, n = no. of measurements. Under certain circumstances, sensitivity can be shown to be numerically equal to approximately twice the standard deviation of the method blank. Sensitivity may also be influenced by other factors in addition to the precision of the blank such as the size of the sample analysed, the manipulative loss incurred prior to detection and the fraction of the sample submitted for detection.

In the sections which follow, the assays were carried out by two different laboratories each of which have examined the reliability criteria for their own assay(s); where possible, these data have been included.

i. Plasma Gonadotrophins - LH and FSH

Females (Tenovus Institute, Cardiff)

The assay methods for LH and FSH were those described by Groom et al (1977). The anti-sera for LH (F87) and FSH (M-93) were gifts from Professor W. R. Butt, Birmingham Hospital for Women, the purified LH for labelling (IRC-2) was from Dr. A Stockell Hartree, University of Cambridge and the purified FSH for labelling (CPDS-13) from Professor Butt. The non-radioactive LH standard was 63/15 while the FSH standard was 2nd IRP HMG. All incubations were carried out at 4°C.

Briefly, hormone standards and samples were pipetted in 100 ul solution with 200 ul water and 200 ul of first antibody solution (diluted 1:240,000 for LH and 1:500,000 for FSH) on Day 1. The tubes were mixed and left overnight at 4°C. The following day, the ^{125}I labelled hormone (approx 1ng/ml) was added in 100 ul solution and the tubes were re-incubated overnight. On the third day, the second antibody was added in 200 ul solution and then left to precipitate the double antibody complex overnight at 4°C. On Day 4, the precipitate was collected by centrifugation, the tubes were decanted and drained and the precipitate was counted in a gamma counter. The specificity of these assays has been examined by

assay of standard pituitary preparations supplied by the Medical Research Council: reasonable agreement was found between the nominal and determined values for LH and FSH (Groom et al, 1971), and discrepancies could be accounted for by known contamination of the standard used. The within assay CV for each gonadotrophin assay was less that 10% at the levels under study and all samples assayed in any one study were analysed within a single assay. The lowest detectable level for LH was 0.5 u/l and that for FSH was 1 u/l. The range of values found in normal subjects were 0.5 - 80 u/l in pre-menopausal women, rising to 200 u/l in postmenopausal women.

Males (Immunoassay Section, Dept. of Clinical Chemistry, Royal Infirmary, Edinburgh)

LH and FSH were measured by radioimmunoassay (Hunter et al, 1974) by methods similar to those described above. The assay for plasma LH employed anti-sera (raised in guinea pigs) to the LH preparation DEAE 2 obtained from Dr. A Stockell Hartree, University of Cambridge, and the assay for FSH employed anti-sera to the crude FSH containing fraction CM-1 obtained from Professor W R Butt, Birmingham Hospital for Women. LH for iodination was fraction IRC-2 (Hartree) distributed as preparation 71/53 from MRC National Institute for Biological Standards and Control (NIBSC). FSH for iodination was fraction CPD 5/6 from Dr. Butt. The non-radioactive standards used were for the LH assay preparation 68/40, and for FSH 68/39 both from NIBSC. Separation of bound from free peptides was by double antibody technique using goat or rabbit anti-guinea pig

gamma-globulin from Burroughs Wellcome.

The specificity of these assays has been considered in detail by Hunter and Bennie (1975) who showed that there was little interference by LH in the FSH assay (<0.035%) and FSH in the LH assay (0.54%). Similarly there was little interference by TSH, as shown by the fact that LH and FSH levels measured by these assays, were unchanged when TSH had changed markedly in vivo. However, some samples supposedly devoid of human gonadotrophins did yield slight responses in these assays due to matrix effects caused by substances of high molecular weight (Hunter et al, 1973).

The within-batch precision of assays, assessed from duplicate determinations was 11.8% for FSH and 15.4% for LH (n = 48 plasmas) whilst between assay CV's were 10.2% and 11.5% for FSH and LH respectively (n = 8 assays). The lowest detectable level of LH was 0.5 u/l and of FSH was 0.3 u/l.

The mean and range (±2SD) values for men with sperm counts of >60 x 106/ml were 5.50 (2.24-12.76) u/l for LH and 2.94 (1.29-6.70) u/l for FSH, (n = 111 for both hormones), (Hunter et al, 1974).

ii. Testosterone

Males and Females (Tenovus Institute, Cardiff)

The method used was a rapid procedure for the specific determination of testosterone as described by Joyce et al (1975). The assay entailed extraction of plasma (0.25 ml or 0.05 ml in men, 0.5 ml in

women) with 10 volumes of ethyl ether. The extract was then evaporated to dryness and a simple biphasic solvent partition between cyclohexane (4ml) and aqueous ethanol (2ml) was used to remove cross-reacting steroids (11-oxo-C19 steroids). Quantitation of testosterone was by saturation analysis (over the mass range 0.069 pmol-0.694 pmol) using an anti-serum raised in a rabbit against testosterone 11α-hemi-succinyl-bovine serum albumin at a dilution of 1:6000. Separation of free and bound steroid was achieved by using charcoal absorption.

The reproducibility of the assay was assessed from a series of duplicate measurements. At the levels encountered in females, S.D. was 1.35ng which it can be calculated is equivalent to approximately 0.10nmol/l, leading to a C.V. of 10% or less. Accuracy, established by the addition of known amounts of testosterone (0-0.63 pmol) to plasma from an ovariectomised, adrenalectomised female, ranged from 98.2-107.5%. The sensitivity of this assay was 0.076 nmol/l. The specificity of this method was confirmed by showing that, firstly, similar values were obtained using an alternative purification procedure, secondly less than 1% cross-reaction was observed with 11B-hydroxytestosterone and other steroids tested with the exception of dihydrotestosterone (11%), and thirdly, that levels were undetectable in the plasma from an adrenalectomised, ovariectomised subject.

iii. Oestradiol-17B

Males (Immunoassay Section, Dept. of Clinical Chemistry, Royal Infirmary, Edinburgh)

Females (Tenovus Institute, Cardiff)

Oestradiol-17B was measured by radioimmunoassay after extraction of plasma with diethyl ether, using a specific antibody generated against oestradiol-17B - 6-(O - carboxymethyl) oxime-bovine serum albumin. For samples from males, plasma (0.5 ml) was extracted with 5 ml diethyl ether by vortexing for 2 mins., and the ether extract was evaporated to dryness (Bolton and Rutherford, 1976). The residue was re-dissolved in 0.5 ml carbon tetrachloride and back-washed with 0.5 ml 50 mmol/l sodium hydroxide solution. An aliquot (0.2 ml) of the aqueous phase was used for radioimmunoassay. To 0.2 ml of standards (containing 2-100 pg oestradiol in 0.2 ml of NaOH) or samples 18.4 pmol (2.5 pg) [2,4,6,7^{3}H] oestradiol-17B was added a suspension of antibody coupled to a solid phase at a dilution sufficient to bind about 70% of the added tracer in a final volume of 0.5 ml. After overnight incubation, bound and free steroid were separated by dilution and centrifugation. The mass of oestradiol-17B present was determined from a semi-logarithmic plot of the standard curve. The precision of the assay was estimated from the values obtained for the same sample in 11 consecutive assays. The inter-assay CV was found to be 20%. From the values obtained from the same sample measured repeatedly in a single assay the intra-assay CV was 7.7%. Accuracy, established by measuring the recovery of 0.073-0.368 pmol of unlabelled steroid added perml plasma, was found to be 91.1 $\pm$ 7.0% (mean $\pm$ SD, n=11). The working

range of the assay was 7.5 - 370 pmol/l.

Specificity was achieved by using an antiserum selected with minimal cross-reactions with the potentially interfering oestrogens. The apparent concentration of oestradiol-17B was not significantly different when samples were assayed before and after chromatography on Sephadex LH-20 to remove interfering steroids, indicating that, in practice, the assay was highly specific.

Similarly, for samples from females, plasma (1 ml) was extracted and the dried residue from ether extraction was used for radioimmunoassay.

The assay was essentially as described above with the omission of the back extraction into alkaline carbon tetrachloride. The precision of the assay was assessed from replicate analysis of 0.2 ml samples for a plasma pool and was found to be 5.7% at a mean level of 1190 pmol/l. Even at lower levels, within-assay precision was less than 10%. The accuracy of this method, assessed from a series of quadruplicate determinations of the recovery of unlabelled oestradiol-17B from a pool of plasma was greater than 90% over the physiological range. The specificity of the method relies upon the use of the specific anti-E_2-17B-6-BSA and less than 1% cross reaction was observed with other steroids with the exception of oestriol (3%) and oestrone (7%). Sensitivity of the assay was 30 pmol/l.

iv. Prolactin

Males and Females (Tenovus Institute, Cardiff).

All the plasma samples were analysed using the method of Cole & Boyns (1973) modified by Cole et al (1976) and described more fully by Groom (1977). This employed a rabbit antiserum raised against an extract of amniotic fluid (antiserum R47) capable of binding human prolactin (HPr). Purified human pituitary prolactin used for labelling was provided by Dr. U J Lewis, and the unlabelled standard was 71/222 from NIBSC. All incubations were carried out at 4°C.

In brief, plasma (100 ul) was dispensed with 200 ul water and incubated with 200 ul first antibody (rabbit antiserum R47 final dilution 1:1200). A series of unlabelled standards (71/222) were similarly mixed with water and antibody. After overnight incubation at 4°C, 100 ul ^{125}I-labelled prolactin (human pituitary) was added and the incubation was continued overnight. On the third day, 200 ul of the second antibody (sheep antirabbit) was added and the tube was left till the following day. On Day 4, the precipitate was collected by centrifugation, the tubes were decanted and drained, and the precipitate was counted.

The precision within an assay, assessed from analysis of multiple plasma samples was less than 10%. All samples from one study were analysed within the same assay. The specificity was established by demonstrating parallelism between the displacements of radioactively labelled prolactin by samples of plasma and standards of human pituitary prolactin. Although some cross-reaction occurred with

human LH, FSH and TSH, this was eliminated by prior absorption of the antiserum with human chorionic gonadotrophin (HCG). Cross-reactions with human GH or human placental lactogen were insignificant. The sensitivity of the assay was approximately 20 mU/l.

v. Growth Hormone (Tenovus Institute, Cardiff)

GH was assayed by a method similar to that used for plasma LH, FSH and prolactin. This employed a rabbit antiserum obtained from Burroughs Wellcome Ltd., Beckenham, Kent (RD16, final dilution 1:300,000). Purified growth hormone for labelling (69/46) was donated by the National Institute for Medical Research, London while the unlabelled standard was the 1st IRP (international reference preparation) - human growth hormone (HGH) obtained from NIBSC.

In brief, plasma (100 ul) was dispensed with 200 ul water and incubated with 200 ul first antibody (RD16, final dilution 1:300,000). A series of unlabelled standards (1st IRP-HGH) were similarly mixed with water and antibody. After overnight incubation at 4°C, 100 ul ^{125}I-labelled GH (MRC 69/46) was added and the incubation was continued overnight. On the third day, 200 ul of the second antibody (sheep antirabbit) was added and the tube was left till the following day. On Day 4, the precipitate was collected by centrifugation, the tube was decanted and drained and the precipitate was counted.

The precision within an assay, assessed from analysis of multiple plasma samples, was less than 10%. All samples from one study were analysed within the same assay. Less than 1% cross-reaction was observed with highly purified preparations of LH, FSH, TSH and prolactin.

vi. TSH

(Tenovus Institute, Cardiff)

TSH was assayed using an antiserum (68/58) from the Medical Research Council (MRC) and the material for labelling (DE-32-3) was obtained from Dr. A Stockell Hartree. The unlabelled standard was MRC standard A.

In brief, plasma (100 ul) was dispensed with 200 ul water and incubated with 200 ul first antibody (68/58, final dilution 1:240,000). A series of unlabelled standards (MRC standard A) was similarly mixed with water and antibody. After overnight incubation at 4°C, 100 ul ^{125}I-labelled TSH (DE-32-3) was added and the incubation was continued overnight. On the third day, 200 ul of the second antibody (sheep antirabbit) was added and the tubes were left till the following day. All incubations were at 4°C. On Day 4, the precipitate was collected by centrifugation and the tube was decanted and drained and the precipitate was counted.

The precision within an assay, assessed from analysis of multiple plasma samples, was less than 10%. All samples from one study were analysed within the same assay. After absorption of the antisera

with HCG, less than 1% reaction was observed with highly purified preparations of LH, FSH, GH and prolactin.

vii. Cortisol

(Tenovus Institute, Cardiff)

Cortisol was determined by the method of Fahmy et al (1975). One hundred microlitre portions of plasma were diluted with 500 ul ethanol, mixed and centrifuged. The supernatant extract was decanted, dried under nitrogen and 100 ul antiserum (raised in rabbits against cortisol-3-(O-carboxymethyl) oximo-bovine serum albumen) was added to these tubes, and also to standard tubes containing 0.14-2.20 p mols unlabelled cortisol. After mixing and incubation for 30 min. at room temperature, [^{3}H] cortisol solution (100 ul containing 37,000 dpm) was added and incubation was carried out for 1 hour at 30^{o}C. After cooling in ice for 15 min., bound and free hormone were separated by the addition of 5 ml dextran-charcoal suspension, cooled on ice for 15 min. and centrifuged. An aliquot of the supernatant, bound fraction (0.5 ml) was used for counting.

The reproducibility of the assay was calculated from a series of duplicate estimations. The standard deviation was calculated for each of three different concentration ranges leading to intra-assay precisions at the mid-point for each range of 15.3% (0-165 nmol/l, n=10), 5.9% (165-550 nmol/l, n=31) and 3.5% (550-1102 nmol/l, n=31).

The inter-assay precision was calculated from values recorded from three individual plasma samples which were processed in five successive assays. The CV was 11.6% at a level of 380 nmol/l ± 44 S.D., 2.3% at 650 nmol/l ± 15 S.D. and 10% at 1350 nmol/l ± 143 S.D. The accuracy was assessed by determining the recovery of samples of increasing masses of cortisol added to plasma which had been obtained from a patient with Addison's disease and was known to be of low cortisol titre. The recovery of added standard varied from 94.4% to 110.4% over the range of concentrations produced (0-1322 nmol/l).

The assay, using anti cortisol-3-bovine-serum-albumin was highly specific. This anti-serum cross-reacted only at levels of 7.1% and 4% respectively with 11-deoxy-cortisol and corticosterone and none of the other naturally-occurring steroids which were examined cross-reacted at levels greater than 0.7%. The sensitivity of the assay (derived from estimates of the standard deviation at low concentrations) was 23 nmol/l.

3. Statistical Methods

Non-parametric statistical tests were used in most cases except for the larger groups of men where parametric methods were used.

In the male patients the distribution of plasma LH and FSH values were skewed both within individuals and for the distribution of patient means (Fig. 5). The skewness was reduced by using

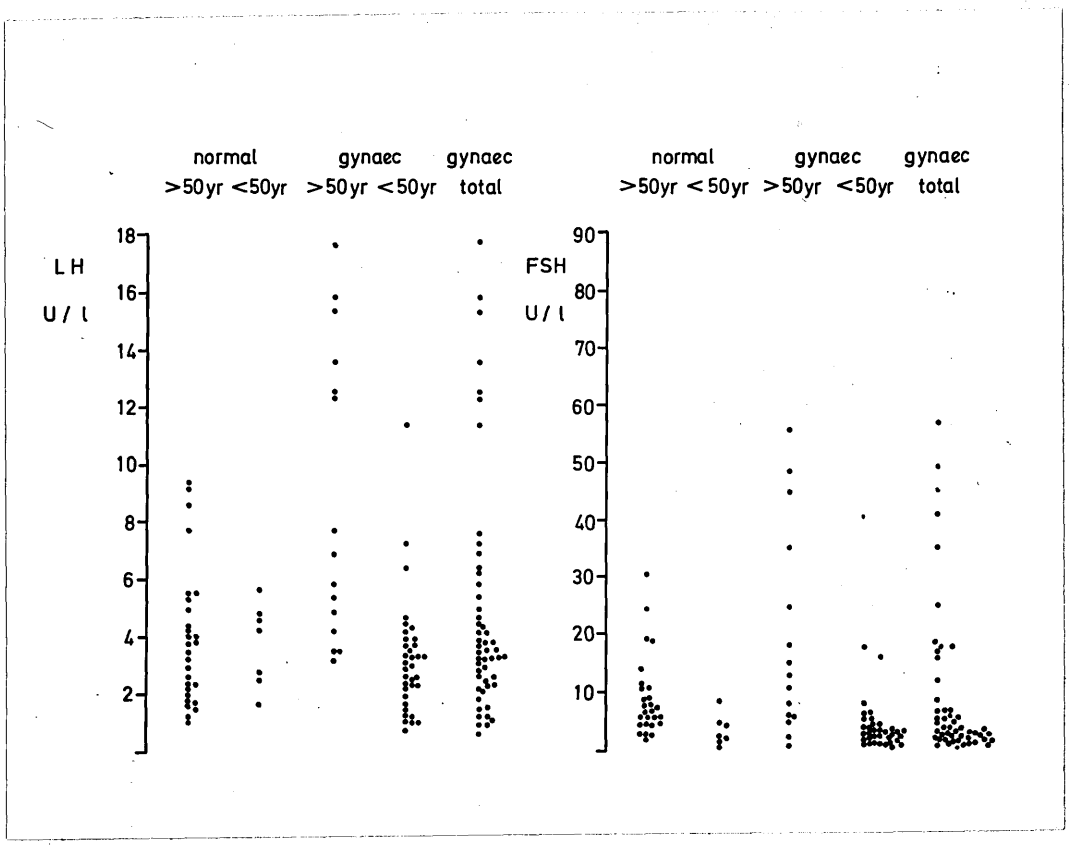


Fig. 5

Plasma LH and FSH concentrations in patients with gynaecomastia and normal controls - note the skewness.

logarithms (Fig. 6). Thus for analyses the natural logarithms of LH and FSH were used. Natural logarithms have the advantage that their standard deviation is approximately the numerical equivalent of the coefficient of variation of the sample value (Wide et al, 1973; Hunter et al 1974). In all other cases the raw values were used. The parametric methods used were t tests, Hotelling's T^2 test of which assume normal distribution.

To test whether the levels of the hormones tended to vary together, Kendal's rank correlation (τ) was used. The value τ can vary from +1 (when the values are completely correlated) to -1. All other cases give a value of between +1 and -1 (Kendal 1973).

Test for a pulsatile pattern of plasma LH and FSH

Several studies have shown that gonadotrophin levels particularly LH vary in a pulsatile pattern both in males and females (Nanken and Troen, 1971; Yen et al, 1972; Santen & Barden, 1973).

To examine for this possibility in the present date, a test by Moore and Wallis (1943) was used and which is as follows:-

For a given series of hormone levels, the total number of steps is the number of adjacent points with distinct hormone levels (ignore any where there is no measured change). These steps are then classed as upward or downward and the number of upward steps compared with that which we would expect in a random series. (Fig. 7).

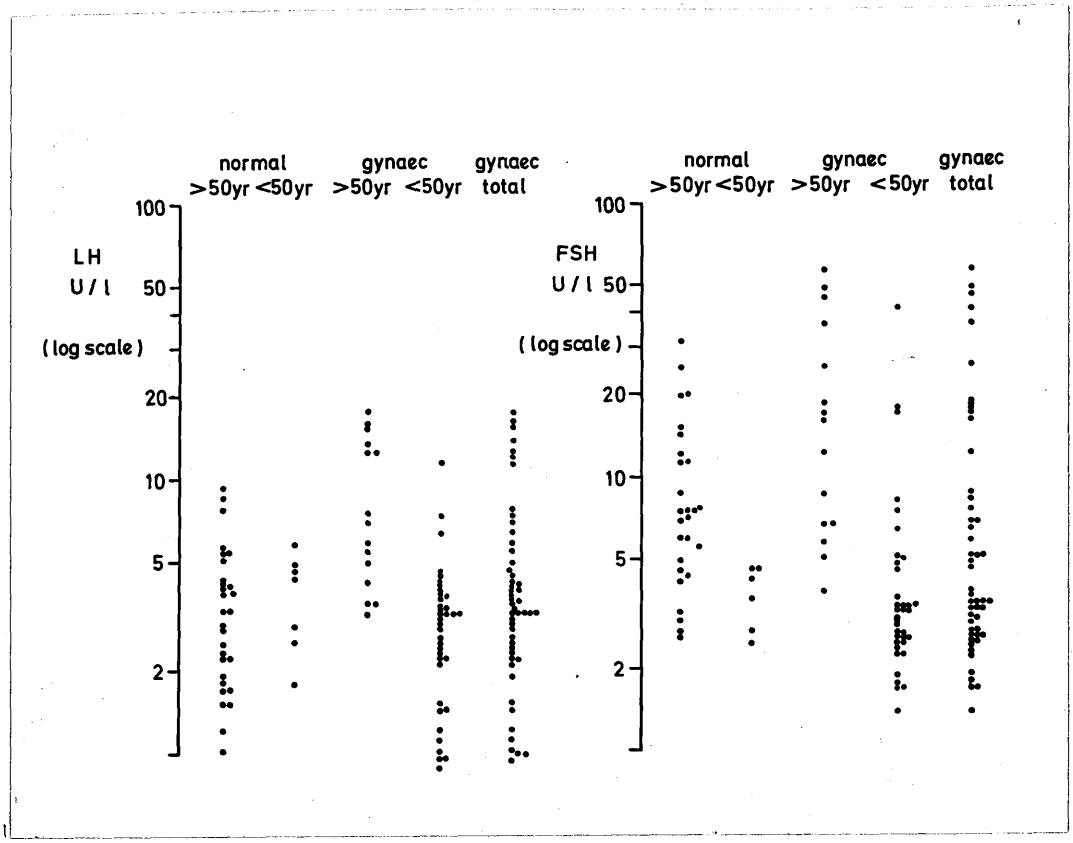
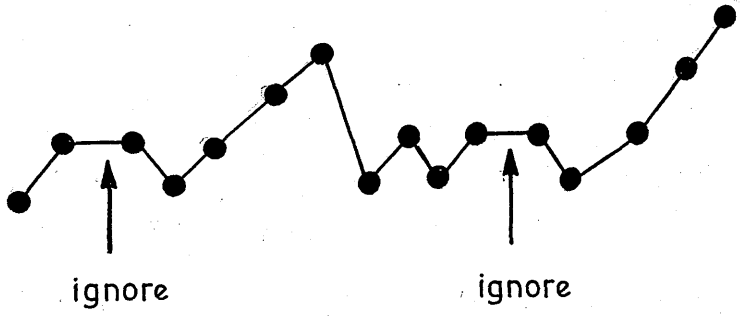


Fig. 6

Plasma LH and FSH concentrations in patients with gynaecomastia and normal controls expressed in logarithms - note the skewness is now reduced.



No. of distinct steps = 13

No. upward = 9

In calculating number of upward and downward steps ignore the steps where the levels remain the same.

The validity of this test is not affected by the variability of the assay, although its power may be. The only property of the assay which might affect it, is any drift effect, and quality control samples were included throughout the assay to check for this.

Fig. 7

The diagram is an example of the Moore and Wallis test.

Although this test will give a significant result for various other types of departure from randomness (e.g. a constant trend), a smaller number of upward steps than expected from a random series is at least a necessary condition for this type of pulsatile pattern. This test was used in both the male patients with gynaecomastia and the normal control subjects and in the women with breast cancer and their controls where samples were taken every 15 minutes for 8 hours.

In comparing the two groups of women a similar statistical test to the Moore and Wallis test was also used, namely Kendal's test which was based on a comparison of the observed number of turning points (rather than upward steps) to be expected if the sequence was random with the observed number. Kendal's rank correlation coefficient is represented by the Greek letter τ. Like in a correlation coefficient, it varies from +1 for complete positive correlation through 0 for no correlation to -1 for complete negative correlation (Swinscow, 1976). Both these tests should give a null result if there is a completely random series. Kendal's test is better for detecting cyclical variation while Moore and Wallis is better for pulsatile pattern.

In calculating number of upward and downward steps ignore the steps where the levels remain the same.

The validity of this test is not affected by the variability of the assay, although its power may be. The only property of the assay

which might affect it, is any drift effect, and quality control samples were included throughout the assay to check for this.

Test for 'Plasma Hormone Increments' in pituitary function tests

'Hormone increment' was the average of the hormone concentrations at specified times after injection in a stimulation test (TableI), minus the basal concentrations. Times were chosen after examining plots of all the patients' data so as to cover the time span over which any apparent response was evident.

Wilcoxon Signed Rank Test

Wilcoxon signed rank test is used on paired data and is the non-parametric test equivalent to the parametric 'paired t' test. The test is better than a paired t test when the numbers involved are small, while the t test is better if larger numbers are involved (Swinscow, 1976).

This test was used to compare the 'hormone increments' before and after pituitary ablations. Wilcoxon's signed rank test was also used to compare the values in the six women with breast cancer and their six matched controls as the numbers involved in this study were small.

Mann Whitney Test

The Mann Whitney test is a very similar test to the Wilcoxon two sample test. It is a non-parametric test equivalent to the parametric, unpaired t test (Swinscow, 1976). It was used to compare

TABLE I

Hormone	Time in Minutes
GH/insulin	60, 90, 120
LH/LHRH	30, 60
FSH/LHRH	30, 60
TSH/TRH	20, 30
Prolactin/TRH	10, 20, 30
Prolactin/Chlorpromazine	60, 90, 120

Times used to calculate increments of the various plasma hormone concentrations after releasing hormones.

the hormone values in the patients with breast cancer who did not have pituitary function tests both before and after pituitary ablation and the values were therefore unpaired.

Hotellings T^2 test

Hotellings T^2 test is a parametric test and is the equivalent of a simple t test when there is more than one measurement for each patient. It is used to compare simultaneously levels of two correlated hormones between two groups and the results are different from individual t tests as the measurements are correlated within each group. It was used to compare the levels of oestradiol-17B and testosterone levels in the older men with gynaecomastia with the older controls and also to compare the values of LH and FSH in the young men with gynaecomastic and younger controls and the older men with gynaecomastia and the older controls. Because changes in the levels of plsam LH and FSH or in testosterone and oestradiol-17B can be shown to be correlated in each group, Hotellings T^2 test was therefore used, which compared the difference between the means of the two groups of hormones simultaneously.

This test is illustrated in Fig 8. In diagram (a) group I and group II are each well separated on the LH and FSH plot, although LH and FSH individually would show considerable overlap. In diagram (b) there is no correlation within either group, and an ordinary t test would be adequate in this situation.

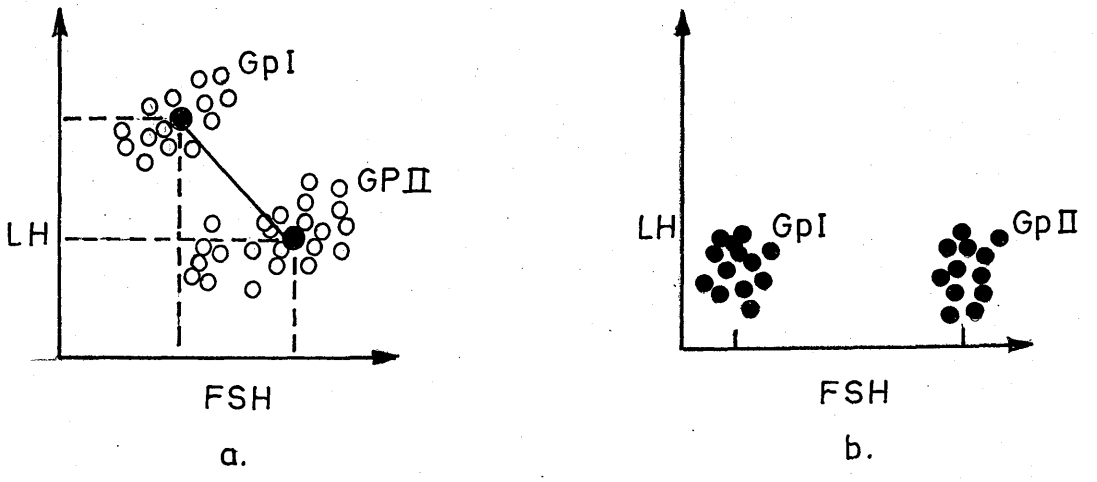


Fig. 8

Hotellings T^2 test - used to compare correlated plasma hormone levels.

a) In this example LH and FSH are correlated. Group I and Group II are each separated on the LH and FSH plot although individually LH and FSH would show considerable overlap.

b) In this example there is no correlation within either group and an ordinary t test would be adequate.

III

HORMONAL PROFILES IN BREAST DISEASE

Study 1 - Plasma Hormonal Profiles in Women with Breast Cancer

1. Literature review

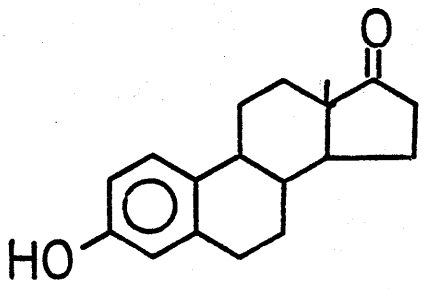
Considerable research has been performed in women with breast cancer to determine whether circulating levels of hormone are of prognostic value and to elucidate the effects of endocrine ablative and additive procedures on plasma hormone levels.

In the late 1950's and early 1960's the techniques then available for assays of hormones were mainly bioassays and chemicals methods and there were mainly, if not solely, applicable to urine. Twenty four hour urine samples did have an advantage in that they represented what was happening during a day, but it was difficult to collect good urine samples, and storage of assays was a problem. The recent development of precise assays by radio-immune methods has allowed the estimation of concentrations of hormones in circulating body fluids.

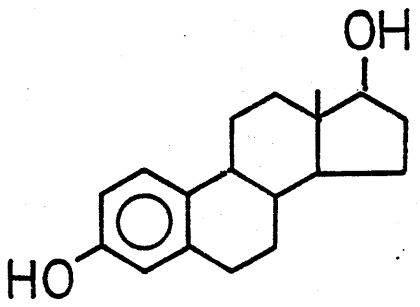
i. Oestrogens and breast cancer

In women, the two major oestrogens secreted from the ovary and adrenal are oestrone and oestradiol-17B, whose formulae are shown in Table II. In the pre-menopausal woman, the majority of the oestrogens is secreted from the ovary, while in the post-menopausal woman, most of the oestrone and oestradiol-17B is derived, not from a direct secretion from adrenal, which is small, but indirectly by their synthesis at peripheral sites (liver, muscle, fat) from androgens C-19 steroids Δ^4androstenedione and

TABLE II



1. Oestrone



2. Oestradiol 17β

Main oestrogens secreted in the human.

dehydroepiandrosterone.

Oestrogens are metabolised or conjugated prior to excretion primarily in the liver the principle ones being oestriol and oestrone sulphate. The conjugate oestrone sulphate is stongly bound to albumen in the plasma and is excreted slowly by the kidney. By contrast, oestriol, in the form of glucuronide is rapidly excreted. Oestrone sulphate thus is the major circulating oestrogen (Purdy, 1961); oestriol glucuronide probably the major urinary oestrogen (Brown, 1955).

Biologically, the most important naturally occurring oestrogen is oestradiol-17B but both oestrone and oestriol have biological activity. Oestrone sulphate acts only after hydrolysis, to yield oestrone, but the hydrolysing enzyme is present in many tissues. The carcinogenic potential of these three free oestrogens differs. Oestrone and oestradiol17B have repeatedly been shown to produce tumours in rats (Geschickler et al, 1942; Dunning et al, 1953; Mackenzie, 1955; Cutts et al, 1964); oestriol, which has not been as fully evaluated, has been found to be carcinogenic in mice (Rudali et al, 1975). It has also been reported that oestriol can stimulate macromolecular synthesis in human breast cancer cells to the same extent as oestrone and oestradiol-17B, can bind to oestrogen receptor and can stimulate protein synthesis (thymidine incorporation) of breast cancer in tissue culture (Lippman et al, 1977).

a) Urinary oestrogens

Oestrogens are present in the urine mainly in the form of glucuronides and sulphates but small quantities of free oestrogen are also present. In 1955-56, both Brown (1955) and Bauld (1956) developed chemical methods for the accurate chemical estimation of small amounts of oestrone, oestradiol-17B and oestriol in human urine. These were by hydrolysis of the urine extraction of the oestrogens by chromatography on alumino columns and determination of their concentrations by the Kober colour reaction.

Oestrogens in the intact female

Although these original methods were moderately precise and sensitive, no convincing association between urinary oestrogen levels and breast cancer could be demonstrated. Never the less, it was reported by Brown (1958) that postmenopausal women with breast cancer excreted higher amounts of oestriol than normal controls; Marmorston (1965) also reported abnormalities of oestrogen excretion in women with breast disease. In this study premenopausal women with breast cancer and benign breast disease excreted a significantly lower percentage of total oestrogen as oestrone and a significantly higher percentage of total oestrogen as oestriol than did normal, sick (emphysema) and well controls; postmenopausal women with breast cancer excreted significantly higher levels of oestriol than similar controls. These findings have not been confirmed. The results of early work on urinary oestrogens were conflicting and confusing (Irvine et al, 1961; Jull et al, 1963; Marmorston et al, 1965; Lemon et al, 1966; Schweppe et al, 1967;

Arguelles, 1973).

Oestrogens in endocrine ablated females

Several groups of workers measured urinary oestrogens (using Brown's method) after oophorectomy alone in premenopausal women and after oophorectomy and adrenalectomy in both pre and post menopausal women, and reported that while residual excretion of oestrogens in the urine might continue following these operations (Strong et al, 1956; Bulbrook and Greenwood, 1957; Birke et al, 1958) the presence of urinary oestrogen did not appear to jeopardise the clinical response to treatment. Continued excretion of oestrogens was also found after hypophysectomy (Greenwood and Bulbrook, 1957); there was no correlation between the oestrogen levels following this operation and the clinical course (Scowen, 1958). The source of oestrogens in patients whose ovaries, adrenals or pituitaries have been removed remains obscure. Dietary factors have been implicated.

However, the methods used for estimating urinary oestrogens were not sufficiently sensitive at low values, to measure precisely the amounts remaining in the urine in postoperative patients of this type, and it became generally accepted that the measurement of urinary oestrogens was of limited value in predicting response to endocrine ablation. These criticisms also apply to urinary oestrogens in intact women. In premenopausal women cyclical variation is known to occur and for meaningful assessment daily 24 hour collections throughout one or even two menstrual cycles are

required. In the postmenopausal woman, urinary oestrogen levels are very low and accurate chemical measurements are difficult.

Conclusion

Despite the amount of work put into measuring urinary oestrogens, little has come out of it and it does not seem too productive to consider further studies of this type. At the same time urinary oestrogens did give some indication that one can not explain the behaviour of breast cancer to endocrine treatment by changes in urinary oestrogens.

b) Plasma Oestrogens

The introduction of radioimmuoassay methods in the late 1960's (Abraham, 1969) for the measurement of plasma oestrogens both in pre and postmenopausal women led to the reevaluation of the role of oestrogens in breast cancer. The two oestrogens, oestrone and oestradiol-17B in normal pre and postmenopausal women have been assayed by several workers (Baird, 1968; Abraham, 1969; Baird and Guevara, 1969; Korenman, 1969). Reported normal values are shown in Tables III and IV. Two very good studies in pre and postmenopausal women have been described by England et al (1974 a & b). These workers showed that the pattern of oestradiol-17B secretion throughout the menstrual cycle in the normal premenstrual woman was constant from decade to decade but that concentrations varied with age. The average concentrations for any given day of the cycle were reported to be higher in women in the 4th decade of life than either younger or older women. In normal postmenopausal women, oestradiol17B

TABLE III

SUBJECT	OESTRADIOL 17B			OESTRONE			AUTHOR
	Mean	±SE	Range	Mean	±SE	Range	
BOYS	83.8	-	-	72.7	-	-	Large & Anderson (1979)
	97.5	-	-	-	-	-	La Franchi (1975)
MEN	96.0	22	-	318	130	-	Baird & Guevara (1969)
	99.3	7.7	62.5-136	-	-	-	Chopra et al (1973)
	84.9	21.0 (±SD)	-	133	21.5 (±SD)	-	Hawkins et al (1974)
	154	-	86-206	125	-	58-180	Large & Anderson (1979)
	61	-	39-99	95	-	59-153	Pirke (1973)
	103	-	70-154 (95% CL)	181.5	-	88.9-363 (95% CL)	Zumoff et al (1982)
MEAN VALUE FOR MEN	99.7	-	-	170	-	-	

Normal values for men and boys. Results are the values found by several workers.

TABLE IV

SUBJECT	OESTRADIOL 17B			OESTRONE			AUTHOR
	Mean	±SE	Range	Mean	±SE	Range	
WOMEN POST-MENOPAUSAL	48.0	7.4	-	263	100	-	Baird & Guevara (1969)
	75.4	8.1	23-239	219	19.6	49-564	Bird et al (1981)
	21.2	1.2	-	-	-	-	England et al (1974)
	58.8	-	25.7-103	122	-	77.8-192	Vermeulen et Verdonck (1978)
MEAN VALUE	50.8	-	-	201	-	-	
PRE-MENOPAUSAL FOLLICULAR PHASE	107	25.8	-	148	15	-	Baird & Guevara (1969)
	371	88	106-537	-	-	-	Chopra et al (1973)
	132	18.5	-	-	-	-	England et al (1974)
MID CYCLE	1169	99	-	629	48	-	Baird & Guevara (1969)
LUTEAL PHASE	702	74	-	448	41	-	Baird & Guevara (1969)
	263.5	8.9	-	-	-	-	England et al (1974)

Normal values for pre and post-menopausal normal women. Results are the values found by several workers.

levels were very low, while oestrone levels were slightly higher. Day to day variation was slight. The concentrations of plasma oestradiol17B was oestrone in pre and postmenopausal women with early breast cancer or with advanced breast cancer have been compared with those in normal, healthy women by Wang and Swain (1974). No significant difference in plasma oestradiol-17B or oestrone concentrations was found in either group of patients, compared to controls; however, there was a trend in four, of higher plasma oestradiol-17B levels in premenopausal women with advanced breast cancer compared to the control group.

In 1974, England and his colleagues reported extension of their earlier work in normal females to women with breast cancer. They studied the plasma oestradiol-17B levels throughout one complete menstrual cycle in thirty-one pre-menopausal women with benign breast disease, and in ten pre-menopausal women with cancer of the breast - aged 40-49 years. They also studied twenty-five postmenopausal women with cancer of the breast, comparison of all groups was with those in thirty-two normal premenopausal and twenty-five normal postmenopausal females reported earlier. In the premenopausal women with breast cancer, the mean oestradiol-17B concentration was slightly but significantly greater than in the normal women. In the postmenopausal women, the concentration of oestradiol-17B was near the lower limit of the sensitivity of the assay in both normal and cancer groups. There was no difference between the values for the two groups. The authors suggested, that studies in postmenopausal women might be more rewarding were

oestrone to be assayed rather that oestradiol17B as the former is present at rather higher levels.

More recently it has been reported by Siiteri et al (1982) that the percentage of free oestradiol 17B is important. The percentage of free oestradiol 17B was estimated by these workers in a group of seventeen women with breast cancer and in controls matched for weight and menopausal status. The mean value of free oestradiol 17B in the women with breast cancer was significantly higher than in the control group but neither the body weight or serum hormone binding globulin (SHBG) was different. It was concluded that the elevation of free oestradiol 17B was due to other factors than a fall in SHBG, either due to competition by steroids or other substances for oestradiol 17B binding to SHBG or an abnormal steroid binding site.

Conclusion

There appears to be no concensus of opinion that either urinary or plasma oestradiol-17B concentrations are different between normal women and women with breast cancer.

ii Androgens and Breast Cancer

In the early 1960's a great deal of interest was also taken in the excretion of androgenic hormones in women with breast cancer. These estimations were carried out originally in urine but, as with other hormones, more sensitive radioimmunoassays were developed and determinations in plasma became possible.

(a) Urine

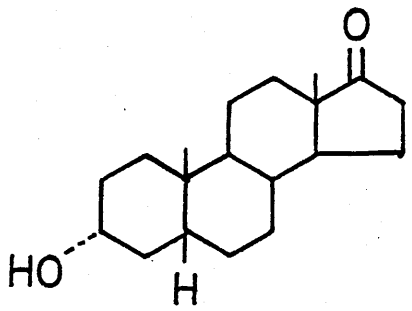
Quantitatively the main androgens excreted in female urine are the 11-deoxy-17-oxosteroids (previously termed 17-ketosteroids). These comprise aetiocholanolone, androsterone, dehydroepiandrosterone and their conjugates. Their chemical structure is shown in Table V.

Interest in androgen excretion in women with advanced breast cancer was aroused when Bulbrook et al (1960) showed that the pre-operative levels of aetiocholanolone, especially when combined as a function of the levels of hydroxycorticosteroids (17-OHCS) formed a 'discriminant function' which might predict the subsequent clinical response of the patient to adrenalectomy or hypophysectomy. The function was (80-80(17-OHCS mg/24 hours) + aetiocholanolone ug/24 hours). If this discriminant was positive, the likelihood of a clinical response to hormone manipulation was greater than if it had a negative value.

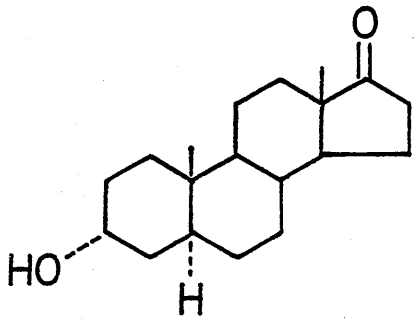
It was also suggested by Bulbrook et al, (1962), that the urinary levels of aetiocholanolone were lower in women with early breast cancer than normal controls but this was not confirmed by subsequent workers in a number of centres, e.g. Ahlquist et al (1968); Wade et al (1969); Cameron et al (1970); Miller et al (1975) who failed to find differences. It is relevant that studies of profiles of urinary androgen excretion in patients with lung disease and lung cancer have revealed differences similar to these described by Bulbrook in women with breast cancer, but reflect the degree of illness in patients with terminal disease (Marmorston, 1966; Rao,

TABLE V

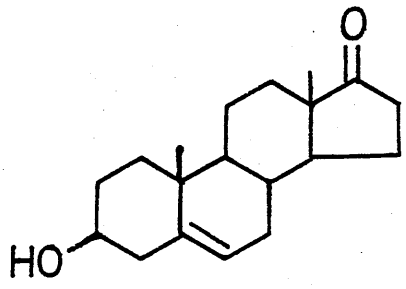
1. Aetiocholanolone



2. Androsterone



3. Dehydroepiandrosterone



The main 11-deoxy 17-oxosteroid androgens (unconjugated forms) excreted in female urine.

1970).

Estimations of urinary aetiocholanolone were also measured in four hundred and seventeen women, with either benign or malignant breast disease or with no breast disorder, the ages were between 20 and 70 years and the women came from South East Scotland and South Wales (Miller et al, 1975). No significant differences in urinary levels were detected between patients with breast disease, whether benign or malignant or control patients. It was also noted that the prognostic values of pre-operative aetiocholanolone measurements was limited in patients with early breast cancer. Low levels of aetiocholanolone were associated with post-menopausal patients, a group in which, the prognosis is generally poorer than that in premenopausal women.

In another study (Grattarola, 1973) urinary testosterone was measured in nineteen patients with breast cancer, clinically cured 5-15 years after mastectomy, and twenty-two breast cancer patients who had developed lung and/or bone metastases during the same period. All patients were between 50 and 65 years of age. Thirty-five postmenopausal women (age range 52-65 years) without breast cancer were taken as control subjects. Urinary testosterone was also measured in another group of postmenopausal women (age range 58 to 68) who had developed lung and/or bone metastases from other types of malignancies which had never been treated. It was found that the mean value of the urinary testosterone level of the normal control group did not differ significantly from that of the

clinically cured breast cancer patients or from that of the patients who developed lung or bone metastases from other malignancies. The urinary testosterone was found to be significantly above normal in the breast cancer patients, who developed recurrences 5-15 years after mastectomy for breast carcinoma. The conclusion was that increased andogenic activity was the hormonal factor in the development of breast cancer and that it can affect the course of this disease in the years following treatment of the primary tumour.

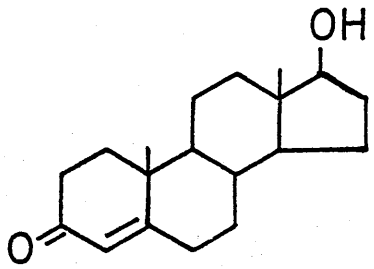
(b) Plasma Androgens

Whilst the urinary excretion of androgens in women with breast cancer has been studied fairly extensively, far less work has been done on the measurement of these hormones in the blood. The main androgens which have been found in plasma in females are dehydroepiandrosterone, androsterone and their sulphates, testosterone and $\Delta 4$ androstenedione. The chemical formulae for these substances are shown in Table VI. These androgens are the parent compounds of those which appear in the urine. Compounds found in the urine are derived by reduction and conjugation principally by the liver. The plasma compounds, being unmetabolised material, may represent a more direct estimation of androgen function.

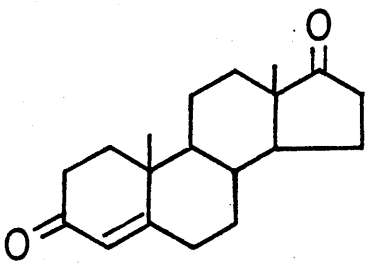
It has been reported that plasma levels of 17-oxosteroids are raised in women with breast cancer compared with controls (Benard et al, 1962).

TABLE VI

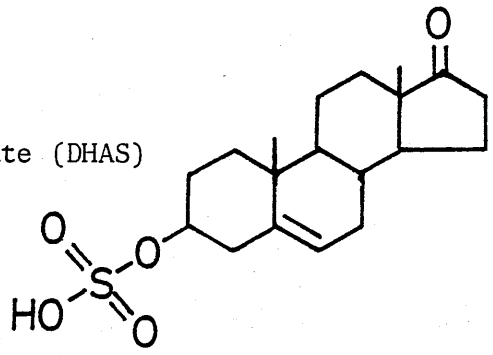
1. testosterone



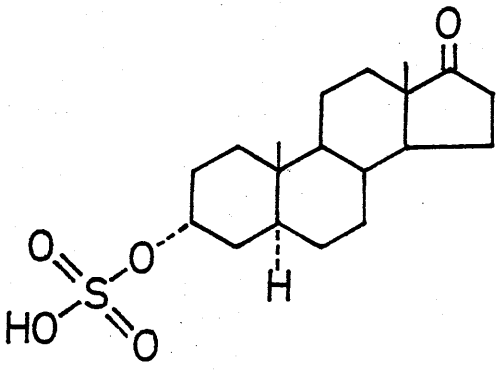
2. Δ 4 androstenedione



3. dehydroepiandrosterone sulphate (DHAS)



4. androsterone sulphate



The main plasma androgens found in normal women

A subsequent study, however, by Deshpande (1965) of normal women and patients with early and advanced breast cancer showed that levels of plasma 17-oxosteroids in cancer patients did not differ from those of the normal women. Plasma 17-OHCS level was normal in the early disease, but raised in advanced cases. In 1964, Deshpande and Bulbrooke showed that the total 17-oxosteroids in the plasma are mainly represented by dehydroepiandrosterone sulphate and androsterone sulphate and subsequent studies in breast cancer have concentrated on individual 17-oxosteroids.

It was reported that the concentrations of plasma dehydroepiandrosterone sulphate were lower in a group of patients with advanced breast disease than in a group of patients with benign breast disease (Brownsey et al, 1972). These findings agree with the work of Wang et al (1973) who reported that plasma dehydroepiandrosterone sulphate (DHAS) concentrations in women with early or advanced breast cancer was subnormal compared to the levels found in normal women. A further study was carried out by Wang in two hundred and eleven normal women, two hundred and two women with early breast cancer before and after mastectomy and ninety women with advanced breast cancer. Dehydroepiandrosterone sulphate sulphate and androsterone sulphate were significantly subnormal <u>after</u> mastectomy as they also were subnormal in women with advanced breast cancer. However, there was no difference in the mean levels of plasma dehydroepiandrosterone sulphate and androsterone sulphate in normal women compared with women with early breast cancer <u>before</u> mastectomy (Wang et al, 1974).

In the Guernsey population, studied by Bulbrook and his colleagues, Wang et al (1975) reported that these women with a family history of breast cancer had levels of plasma C-19 steroids lower than those who had no such association. The age adjusted geometric mean level of plasma dehydroepiandrosterone sulphate and androsterone sulphate were significantly lower in young sisters, (but not daughters) of women with breast cancer than in controls. The greatest difference was found in the younger age group.

It was reported by Thomas et al (1976) who used the Guernsey population as controls that plasma dehydroepiandrosterone levels in women with both early and advanced breast cancer were lower than in normal women. They were also lower in young women aged 20-29 with benign breast disease.

Studies have also been carried out to determine the concentrations of Δ 4 androstenedione in normal women and in women with early and advanced breast cancer (Wang et al, 1977). No significant difference was found between the mean levels of plasma Δ 4 androstenedione in normal women and those with early breast cancer assayed <u>before</u> mastectomy. However plasma Δ 4 androstenedione was subnormal in patients with early breast cancer <u>after</u> mastectomy and in patients with advanced breast cancer. In all groups, the plasma level of Δ 4 androstenedione was highly correlated with plasma levels of dehydroepiandrosterone sulphate. Simultaneous studies of plasma dehydroepiandrosterone sulphate and Δ 4 androstenedione levels in women with breast disease were also reported by Rose et al, 1977. These

included sixty-nine women with primary breast cancer (twenty-four before and forty-five one month after mastectomy) and forty-nine with advanced breast disease. The results were compared with similar estimations in a group of sixty-two healthy women. Plasma dehydroepiandrosterone sulphate levels were negatively correlated with age, and in all three breast cancer groups, significantly reduced levels were found, compared with age-comparable controls. No significant difference was found in plasma Δ 4 androstenedione levels between the cancer patients and controls. Contrary findings have also been reported. England et al (1981) found the mean levels of plasma dehydroepiandrosterone sulphate to be lower in ten normal women, compared with twenty-nine patients with breast disease (twenty benign disease and nine early cancers) but not significantly so. No significant difference in plasma dehydroepiandrosterone sulphate concentrations between postmenopausal patients with Stage 1 or 2 breast cancer and normal postmenopausal women were reported by Bird et al, 1981. These workers also found no significant differences in plasma levels of testosterone Δ5 androstene3B 17B diol, Δ4 androstenedione and dehydroepiandrosterone between these patients and the control group. It was, however, found that the mean plasma 5αdihydrotestosterone levels were lower for the patients with breast cancer. In another report (Zumoff et al, 1981) comparing eleven women with primary breast cancer (aged 31-78 years) and thirty-seven normal women, (aged 21-75 years) a marked and progressive decline of plasma dehydroepiandrosterone (DHA) and the dehydroepiandrosterone sulphate levels varied with age only in the normal women; in the cancer group the concentrations of both

steroids rose as age increased. Premenopausal patients with cancer had subnormal plasma dehydroepiandrosterone and dehydroepiandrosterone sulphate levels, postmenopausal patients had supranormal levels. When the ratio of dehydroepiandrosterone to androsterone was calculated it was found to be normal in the premenopausal patients but significantly elevated in the postmenopausal patients. The authors suggested that subnormal plasma adrenal androgen levels in the premenopausal patients was due to diminished production while the elevated plasma levels in the postmenopausal patients was due to slowed metabolic removal.

In summary, plasma dehydroepiandrosterone and plasma dehydroepiandrosterone sulphate tend to be decreased in breast cancer within a race but there are contrary findings. Menstrual status may affect the concentrations. The report that C-19 steroids such as dehydroepiandrosterone sulphate inhibits breast cancer in mice lends possible clinical relevance.

Sebaceous glands are stimulated by endogenous androgen with testosterone, $\Delta 4$ androstenedione and dehydroepiandrosterone as the likely agents (Pochi et al 1963). There is some biological evidence that patients with breast cancer have increased androgenicity and various studies have suggested that there is increased sebum excretion in patients with breast cancer (Krant et al, 1968; Burton et al, 1970; Wang et al, 1972). These findings suggested the possibility that postmenopausal women with advancing breast cancer were, in general, in an androgen stimulated internal

environment and, as a group, were different in this regard from healthy postmenopausal women. However, Wang et al (1972) did not find that high amounts of plasma oxosteroids were associated with the higher level of sebum production in women with breast cancer.

Work also has been carried out in relation to plasma testosterone Wang et al (1966). He compared the levels of plasma testosterone in fourteen patients with benign breast disease, in twenty-four patients with early breast cancer and nineteen normal women. No difference in the plasma testosterone levels was found between the three groups. In 1974, Horn and Gordon also studied plasma testosterone levels in thirteen postmenopausal women. No difference in the plasma testosterone levels was found between these two groups. Jones et al (1977) measured plasma testosterone levels in forty-four women with breast cancer and those in sixty-five women with benign breast disease. Again no difference was found in the plasma testosterone levels between the two groups.

iii Progesterone and Breast Cancer

Progesterone is mainly secreted by the corpus luteum. It is a hormone whose relationship to breast cancer has only recently received attention.

Wang and others (1974) measured plasma progesterone levels in the luteal phase in three hundred and forty-one control premenopausal women, twenty-five patients with early and twelve with advanced breast cancer. Assay was by competitive protein binding. The

plasma progesterone levels in the group of patients with early breast cancer was similar to those in the control group. In the study all subjects with progesterone levels greater than 3 ng/ml of plasma were considered to have ovulated. There was no evidence of an increased incidence of ovulatory failure in patients with early cancer. There was however, a significant increase in the incidence of ovulatory failure in the advanced cases. The mean plasma progesterone level of the patients with advanced disease who had ovulated was not significantly lower than both the control group and the patients with early breast cancer. Four obese normal women with irregular menstrual cycles for six cycles were studied by Sherman and Korenman (1974) who found that progesterone levels during the luteal phase were far below those observed in ten women with normal cycles. These workers suggested that altered hormonal environment of the mammary gland consequent to persistent oestrogenic stimulation, in the absence of an adequate progestational phase, i.e. "luteal phase deficiency", could provide a hormonal setting in the breast favourable to the development of carcinoma.

Plasma levels of progesterone in ten postmenopausal women with early breast cancer were compared with the levels in a control group of fifteen postmenopausal women with early cancer at other sites by Smethurst et al (1975). The mean plasma progesterone was significantly raised in the breast cancer patients compared to the control group.

Plasma progesterone levels were compared in fifty-three normal British (high risk group) and fifty-four Japanese (low risk group) (premenopausal, menopausal and postmenopausal) women - no significant differences between the two groups were found (Bulbrook et al 1976). In the following year Malarkey et al (1977) also reported that the serum progesterone levels, determined pre-operatively in sixteen women with benign breast disease and seventeen patients with breast cancer were similar to those found in twenty-five age- and weightmatched control women. These patients and controls were in the luteal phase of the cycle or were postmenopausal.

iv Gonadotrophins and Breast Cancer

Direct attempts to correlate measurements of gonadotrophins and pituitary function with the development of breast tumours or with the clinical course of the disease have been relatively unsuccessful. This was probably due to the lack of accurate methods for measuring the pituitary hormones in the plasma until recently. Even allowing for this, very few associations have been demonstrated between pituitary function and tumour growth or response to therapy.

Many of the early estimations or urinary gonadotrophins used bioassay techniques. Compared with later radioimmunoassay methods these methods were relatively insensitive and therefore much of the early work may have to be reassessed. In 1958, Loraine used bioassay to measure gonadotrophins in post-menopausal patients with

breast cancer. Urine was collected for eight days, but the variability between samples in some patients represented a twofold difference in the gonadotrophin levels. Despite this he noted that patients who failed to respond to stilboestrol therapy, had a significantly higher mean urinary gonadotrophin excretion than the patients who obtained a remission. Urinary gonadotrophins were later estimated in forty-one patients with advanced breast cancer prior to adrenalectomy or hypophysectomy (Hayward et al, 1961). The difference between the levels in responsive and unresponsive patients was not significant, but high gonadotrophin levels tended to be present in those who subsequently responded. The evidence for alterations in urinary gonadotrophin excretion in breast cancer patients and on the correlation of measurements with response to hormone therapy are few and conflicting. With the development of a radioimmonoassay for plasma LH and FSH by Midgley (1966;1967) more accurate studies could be performed, and considerable basic knowledge on the secretion of LH and FSH has become available. This is regulated by complex interactions between releasing hormones from the hypothalamus and ovarian hormones (Schalley et al, 1971). Episodic pulsatile patterns of gonadotrophins have been shown to occur in pre- and postmenopausal women (Yen et al, 1972). A periodicity of 1-2 hours was seen during the early follicular phase and mid-cycle surge while a four hourly periodicity was seen in the mid and late luteal phase. The frequency of pulses of LH and FSH in the postmenopausal subjects was the same as in the follicular and mid-cycle women - every 1-2 hours.

Little, however, is known about the relationship, if any, of plasma gonadotrophins to breast cancer. A study in which plasma LH and FSH levels in post-menopausal women with early breast cancer, advanced breast cancer and normal controls was carried out and it was found that the values in the cancer patients did not differ from the controls (Wang et al 1976). Malarkey and his colleagues (1977) measured serially gonadotrophins in the serum over a twenty-four hour period in sixteen women with benign breast disease, seventeen women with breast cancer and twenty-five age- and weight-matched controls; no differences were detected. Others have reported similar findings.

No significant difference in the plasma LH or FSH levels in ninety-eight postmenopausal women with early breast cancer compared with the levels in thirty-four postmenopausal, control women were detected by Bird et al (1981). Only a marginal difference in plasma gonadotrophin values between normal British and Japanese women, who have different risks of developing breast cancer was reported by Kumaoka et al (1976). In the premenopausal group, plasma LH in Japanese women was marginally higher than in British subjects while in the postmenopausal group plasma LH was significantly higher in British women.

In summary, plasma gonadotrophin concentrations seem to be within normal limits in patients with breast cancer. A complicating factor in women with advanced breast cancer is the report that in postmenopausal women, illness may depress LH and FSH levels and

obscure the true result (Warren et al, 1977).

v. Prolactin and Breast Cancer

Although some hormones and drugs can act directly on the pituitary to influence prolactin secretion, prolactin in contrast to other pituitary hormones is largely under control of an inhibitory factor (PIF) from the hypothalamus. Talwalker et al (1963) found that the hypothalamus contained a factor which inhibited synthesis and release of prolactin by the rat anterior pituitary in vitro: this factor was not any of the recognised hormones in the hypothalamus and it was subsequently termed PIF.

The measurement of human prolactin was delayed by difficulties in distinguishing prolaction activity from that of growth hormone. Purified growth hormone had been found to have considerable prolactin activity, and there had been doubts whether in man the two hormones were separate entities. However, evidence of the existence of two distinct hormones was forthcoming and human prolactin was isolated and purified (Lewis, 1971). These advnaces led to the development of homologous radioimmunoassay of the hormone (Hwang et al, 1971).

As early as 1971, Turkington et al, raised the question regarding the importance of prolactin in the growth of human breast cancer. Their work indicated that evidence of regression of advanced disease could occur despite increase in prolactin levels. He and his colleagues had measured serum prolactin by a bioassay method in

eleven patients who had undergone pituitary stalk section for treatment of metastatic mammary carcinoma. In eight out of the eleven, there was remission of the disease but five of these eight patients had elevated prolactin levels. Thus increased prolactin levels in these patients did not prevent regression of the disease. Evidence is also available from epidemiological studies that early pregnancies, which would involve an earlier but considerable increase in prolactin secretion (Hwang et al, 1971) reduce lifelong risk of breast cancer (MacMahon, 1973).

Comparisons of serum prolactin in normal women and women with breast cancer have not provided evidence of an important relationship. Such evidence if conflicting. For example, Murray and his colleagues (1972) compared plasma prolactin levels in twenty-four women with metastatic breast cancer and twenty-three postmenopausal patients hospitalised for other reasons. The prolactin levels of the cancer group were statistically higher than the controls. In contrast Wilson et al (1974) compared plasma prolactin levels in forty-nine patients with breast cancer against thirty-nine hospital controls and found no difference in the prolactin levels between the two groups. A similar finding was also reported by Kwa et al (1974) who compared one hundred and fifteen patients with breast cancer with one hundred and fifteen matched controls and found no difference in the plasma prolactin levels in the two groups. A further study was carried out by Franks et al (1974) who measured the plasma prolactin levels in one hundred and thirteen patients with a breast lump prior to surgery. They found no difference in

the prolactin levels between patients with breast cancer and those with benign breast lumps. Racial comparisons have also been carried out. In a study by Kumaoka and his colleagues (1976) no differences were found between the plasma levels of prolactin in normal British women (high risk breast cancer) and comparable Japanese women (low risk breast cancer), irrespective of whether they were adolescent, premenopausal, menopausal, postmenopausal. However, women with a family history of breast cancer were reported to have an increased prolactin level in the evening (Kwa et al, 1976). Nocturnal estimates of plasma prolactin were performed by Malarkey et al (1977) in twenty-five control women, sixteen women with benign breast disease and twenty-three women with breast cancer before breast surgery, who showed that the nocturnal plasma prolactin concentration was significantly decreased in twelve of the postmenopausal breast cancer patients but significantly increased in five premenopausal women with breast cancer, as compared to the control women. Plasma prolactin levels throughout the mentrual cycle were studied by Cole et al (1977) in eleven patients who had undergone mastectomy for primary breast cancer and in thirty-two normal women at various stages of the menstrual cycle. The cancer patients had higher plasma prolactin levels, but this was believed to be an acute effect. There was little difference in prolactin levels between patients with primary cancer who had had a mastectomy more than three months previously, and normal women.

Increased nocturnal plasma prolactin levels were also reported by Tarquini et al (1978) in nulliparous premenopausal normal women (at

high risk) in comparison with parous premenopausal women. In addition, five of seven nulliparous premenopausal women with breast cancer, had elevated nocturnal prolactin levels compared to five of thirteen parous women, also with breast cancer. It was concluded that premenopausal nulliparous women with benign or malignant breast disease generally exhibited a significant peak of plasma prolactin in the early evening but that this was rarely seen in parous patients.

In summary, the results of estimates of plasma prolactin in patients with breast cancer are variable and depend greatly on the form of the study carried out. In the majority of studies plasma prolactin has been shown to be within normal limits. It would seem apparent that a single sample is inadequate for assessing the role (if any) of prolactin in breast cancer and that further work should involve multiple sampling.

vi. The Thyroid Hormone and Breast Cancer

In 1896 Beatson described the use of thyroid extract as an adjuvant to oophorectomy in the treatment of advanced breast cancer. Its use since then has been only occasional and its value never proven. It is not known whether thyroid hormones have a role in the pathogenesis of breast cancer. It was past belief that there was prevalence of goitre or evidence of other thyroid disease in patients with breast cancer. Thus (Bogardus and Finley 1961) reported that forty-two out of seventy-nine patients with breast cancer had an abnormality of the thyroid gland on physical

examination, while in another report a high incidence of breast cancer was found in ninety-two female patients with thyroid cancer (Chalstrey and Benjamin, 1966).

It has been reported that iodine deficiency enhances the response of rat breast tissues to sex hormone injections. Oestrogen and testosterone administration to iodine deficient hypothyroid rats results in lesions which mimic human cystic disease of the breast and suggests another physiologic factor which may act to modify breast sex steroid interaction (Eskin et al, 1967). Further more rates of morbiditiy and mortality due to breast cancer are reported to be higher in areas of iodine inadequacy than in regions where iodine is available (Eskin, 1970). Studies have also shown that abnormal breast tissue (dysplasia or neoplasia) has increased radio iodine uptake compared to histologically normal breast tissue from the same patient (Eskin et al, 1974).

The effect of an intravenous injection of thyrotropin releasing hormone (TRH) on plasma TSH was measured in fifty women with early and fifty women with advanced cancer of the breast (Mittra and Hayward, 1974). The results were compared with those obtained in a group of fifty age-matched women in hospital awaiting surgery for a variety of reasons. The results suggested that the mean basal level of TSH in both cancer groups was significantly higher than in the controls and that more cancer patients had TSH levels above the normal range. After TRH stimulation, the mean response to TSH was significantly higher in the breast cancer groups than in normal

women. Approximately three times as many cancer patients as controls had an exaggerated response of TSH to TRH indicating a relative primary thyroid deficiency. From these results it was suggested that breast cancer patients as a group had a level of thyroid function which was lower than that found in control women in hospital with conditions unrelated to breast.

A hypothesis was put forward that a suboptimal level of circulating thyroid hormones may abnormally sensitise mammary epithelial cells to prolactin stimulation, thus leading to eventual neoplasia. It was shown by Rose and his colleagues (1978) that plasma TSH was negatively correlated with plasma T_3 in early breast cancer and that reductions in plasma T_3 might explain increased plasma TSH levels. T_3 has been shown to be the important hormone in the regulation of pituitary TSH release (Belchetz et al 1978). Rose suggested that these considerations might support the view that patients with early breast cancer with elevated plasma TSH and reduced T_3 levels had a degree of impaired thyroid function and suggests that this abnormality might preceed the disease and could be a factor in brest cancer risk. Later MacFarlane et al (1980) however studied a large number of consecutive patients with breast cancer, a group of women with benign breast disease, and a group of normal women by plasma TSH, T_3 and T_4 levels. No increase in the incidence of thyroid dysfunction was found in patients with breast cancer compared to patients with benign breast disease or controls. Comparisons of plasma TSH in British and Japanese women were reported by Kumaoka et al (1976). Plasma TSH was higher in the British women but not

significantly so.

Thyroid hormones are known to affect enzyme systems concerned with oestrogen and androgen metabolism. Fishman et al (1962) reported that, when levels of thyroid hormones are raised there is a fall in the conversion of oestradiol-17B to oestriol while the conversion to 2 methoxyoestrone is increased. The metabolism of testosterone to androsterone is also reported to be stimulated by thyroid hormones.

The androsterone-aetiocholanolone excretion rate was worked out by Schorted and his colleagues in 1966 who found that this ws increased in 76% of patients with myxoedema and decreased in 59.1% of patients with thyrotoxicosis. It is known that in patients with thyrotoxicosis and after administration of thyroid hormone, there is a rise in the level of sex hormone binding globulin (SHBG) (Ruder et al 1971). Because of the different affinities of this binding protein for testosterone and oestradiol-17B, a rise in its level results in a greater fall in the plasma level of unbound testosterone, than of unbound oestradiol-17B. SHBG is an important determinant of the ratio of unbound testosterone and oestradiol-17B. Increased SHBG will have a feminizing influence, and decreased SHGB levels a masculinizing influence. In thyrotoxicosis, SHBG increases considerably with resulting changes in oestradiol-17B and testosterone metabolism (Burke and Anderson, 1972).

In summary plasma TSH, T_3 and T_4 seem to be within normal limits in patients with breast cancer. The importance of thyroid hormone and

these various factors in the development of breast cancer still remain known.

vii. Growth Hormone and Breast Cancer

Patients with breast cancer frequently have abnormalities in carbohydrate metabolism. Thus it was reported by Glicksman et al (1956) that 35% of patients with breast cancer had a diabetic-type glucose tolerance curve in contrast to only 10% of subjects with benign breast disese. An accurate method for the estimation of GH by a radioimmunoassay technique became available in 1962 (Hunter and Greenwood) and with its availability, more precise measurements could be made in patients with breast cancer. However, plasma growth hormone levels are influenced by many psychological factors e.g. stress, exercise, fasting (Greenwood and Landon, 1966), and measurements can only be meaningful under strictly controlled conditions.

A standard stimulus provided the most appropriate condition for studying growth hormone and Greenwood et al (1968) used the insulin hypoglycaemia test. Growth hormone measurements have therefore been used in breast cancer mainly either after a glucose load or after insulin hypoglycaemia.

Stimulation of growth hormone levels has been used mainly as a test of pituitary function (see Study 3) and studies to determine differences between women with breast cancer and normal women are few. In one study 24 hour growth hormone levels in sixteen women

with benign disease, seventeen with breast cancer and twenty-five age- and weight-matched controls were measured by Malarkey et al, 1977. The serum GH concentrations and the pattern of GH secretions were similar in all the groups evaluated.

Defects in studies

There appears to be no consensus of opinion that there is a difference of either urinary or plasma oestrogens between women with breast cancer and normal women. More consistent results were reported on C-19 steroids, both in urine and plasma. However the abnormality of urinary 11-deoxy-17-oxo steroids reported in patients with advanced breast cancer may be a non-specific effect of illness. So also may the reduced concentration of plasma dehydoepiandrosterone sulphate be due to secondary effects. The results of measuring C-19 steroids in the plasma of patients with early breast cancer are more equivocal. Part of the confusion may be caused by poor matching of breast cancer patients and control women. The need to study well-matched groups is obvious. The type of plasma sampling, for the various hormones, used also seems to vary from study to study. The types of control women used have also been variable. Sometimes, single blood sampling has been carried out and sometimes multiple samples over long periods of time. Although progesterone, growth hormone, thyroid stimulating hormone, prolactin and gonadotrophins all appear to be within normal limits, the need for multiple sampling over a period of time is required if one is to take into account the cyclical variation of these hormones. This is particularly important in the case of

gonadotrophins and prolactin.

Plasma Hormone Levels in Women with Breast Cancer

2. Objective of Study

a) The object of this present study was to study precisely a small group of very well matched breast cancer patients and controls - not only matched for age, but for parity and years past the menopause.

b) To use a precise multiple plasma sampling regime, and a well recognised radioimmunoassay (see method section). Sequential samples of blood were taken at 15 minute intervals for 8 hours from individual patients with breast cancer and the levels of plasma LH, FSH, oestradiol-17B, testosterone, prolactin and cortisol were compared with the levels in control postmenopausal women carefully matched for age, parity and last menstrual period.

3. Methods in Patient Sampling

Postmenopausal women were specifically chosen for the study because it was felt a more accurate and detailed comparison could possibly be made between the women with breast cancer and the control women only if the age, parity and years past the menopause were accurate. It seemed that a truly accurate hormonal status could not be achieved in premenopausal women due to the variability of the menstrual cycle in some women with variability of the day of ovulation and also the possibility of early pregnancy in the control pre-menopausal women. The study therefore was carried out only on

postmenopausal women.

a) Women with Breast Cancer

All patients studied had presented with histologically proven cancer, diagnosed initially either at the Breast Clinic of the Department of Clinic Surgery, or at the Combined Breast Clinic in the Radiotherapy Department of the Royal Infirmary, Edinburgh. The patients were classified as having either primary or advanced disease. Primary disease was considered to be present when detectable tumour was limited to one breast and its lymph nodes with no evidence of local or distant metastatic disease. This, therefore, was amenable to surgery which involved removing the breast and examining the lymph glands in the axillary tail later. If these glands proved histologically to contain tumour then the patient was treated with radiotherapy six weeks after mastectomy. Advanced breast disease existed when tumour cells had spread beyond the breast and axilla to a distant site. The histology of this was proven by biopsy in as many cases as possible. This disease was out of the scope of local surgery and required treatment either by local radiotherapy to the primary lesion or systemic therapy either by hormone manipulation or chemotherapy. The patients were further subdivided according to menstrual status as follows:-

1. Premenopausal : patients menstruating regularly
2. Menopausal : patients with irregular periods or within 5 years of the last menstrual period.

3. Postmenpausal : patients beyond 5 years of their last menstruation.

A detailed clinical history was taken from all new patients on their arrival at either of the above clinics. This included noting the duration of disease, previous treatment and time which had elapsed since that treatment.

In an effort to detect invasion of the other breast or skeletal metastases, all patients were given standardised mammography and thermography of both breasts and a full skeletal survey of x-rays. All patients wherever possible, had pathological confirmation of their disease either at the time of mastectomy or by biopsy of assessible tumour in advanced disease.

b) Control Women

The controls were all normal women attending the Regional Blood Transfusion Centre as blood donors. All were volunteers with no previous history of breast disease, thyroid or any other endocrine disorders. None were taking drugs.

Design of Study

Six patients with early or advanced breast cancer were studied and paired with six control women matched for age, parity and last menstrual period. The details of the two groups are shown in table VII.

TABLE VII

	Controls		
Control	Age	Years since Menopause	Parity
1	69	21	1+0
2	62	15	0+0
3	55	9	0+0
4	55	12	2+0
5	73	21	0+0
6	61	10	2+0

	Cancer Patients		
Cancer Patient	Age	Years since Menopause	Parity
1	73	22	3+0
2	65	17	0+0
3	53	7	0+0
4	56	9	5+0
5	76	25	0+0
6	56	10	2+0

Details of patients with breast cancer and their matched controls

The cancer group consisted of two patients with early breast cancer, two with advanced local disease and two with disseminated disease. Four patients were tested as hospital in-patients, and two attended as out-patients. The control women were all out-patients. The blood samples were taken as described for multiple blood samplings, every 15 minutes for 8 hours, the plasma was assayed for LH,FSH, oestradiol-17B, testosterone, prolactin and hydrocortisone (cortisol).

The mean concentration of each hormone for the six patients during the 15 minute period was calculated and compared graphically with the mean of the six controls.

4. Results of Study

The results are shown in Fig. 9.

In the graphs, means were calculated for a period of time by the clock. Some points on the graph at either end did therefore represent the means of five or even four patients. For this calculation, only samples of those 15 minute periods by the clock for which there were both test and control samples were included. Results are shown in table VIII.

The mean concentrations of oestradiol-17B, LH, FSH, and cortisol did not differ between the six patients with breast cancer and their matched controls. However, prolactin and testosterone did differ.

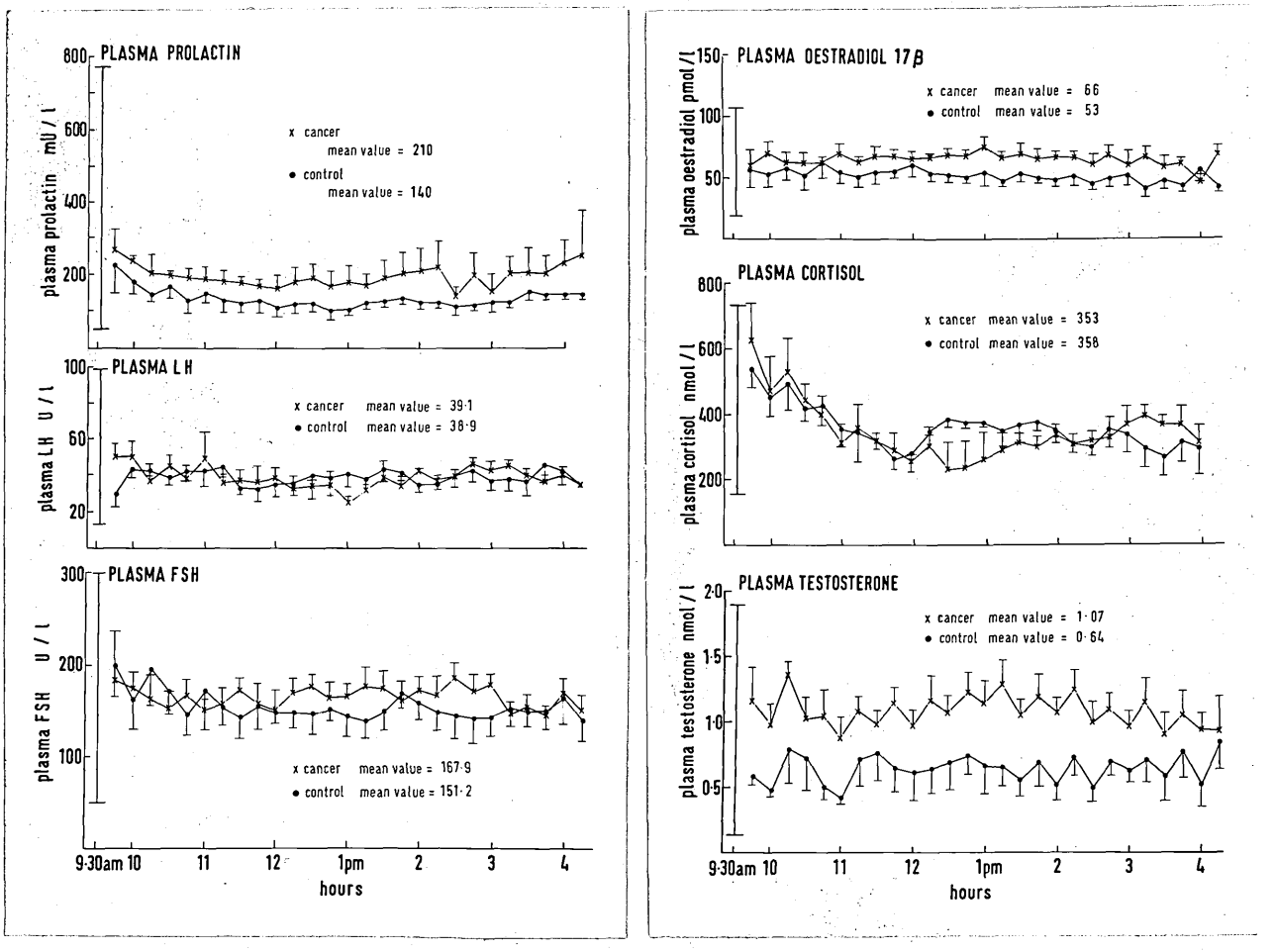


Fig. 9

Plasma hormone concentrations in patients with breast cancer and in controls.

TABLE VIII

PLASMA HORMONES

	L.H. U/L		F.S.H. U/L		TESTOSTERONE n mol/L		OESTRADIOL 17/3 p mol/L		PROLACTIN m U/L		CORTISOL n mol/L	
	cancer	control	cancer	control	cancer	control	cancer	control	cancer	control	cancer	control
1	28.8	26.2	120.5	154.5	1.52	1.36	83.8	69.5	90	90	424	358
2	42.2	34.9	174.0	193.9	0.60	0.53	60.7	44.5	160	180	319	328
3	53.9	35.0	219.2	84.9	0.97	0.60	51.8	65.4	180	100	375	333
4	35.3	42.5	170.5	135.5	0.75	0.44	54.4	50.0	400	140	369	369
5	28.3	41.4	147.1	185.9	1.33	0.36	77.2	36.4	220	150	361	380
6	45.9	53.4	176.2	152.5	1.26	0.52	66.2	51.8	200	120	264	377
mean difference	0.17		16.72		0.44		12.7		78		5.5	
p (Wilcoxon's test)	N S		N S		< 0.05		< 0.1		N S		N S	

N S : not statistically significant $p > 0.1$

Comparison of sample means for each patient compared with those of each time matched control.

The difference in prolactin concentrations was sharply accentuated by the levels in one patient (number 4 in table VII) who was subsequently found to have been taking Thyroxine 0.1 mg./day and spironolactone plus hydroflumethiazide 25 mg three times a day, before the test; accordingly the prolactin concentration for this patient was excluded and the date from the group were re-calculated. This is shown in Fig. 10. In table VIII the p. value for prolactin has been calculated without patient No. 4.

A significant difference (at the 5% level) was observed in the plasma testosterone concentrations which were higher in every woman with breast cancer than in the matched control. Five patients had also higher plasma oestradiol-17B levels, but this only reached significance at the 10% level. Both plasma testosterone and oestradiol-17B levels however, were within the normal range for post-menopausal women although the cancer group had higher values within this normal range.

A statistical analysis of the sequential samples in both patients and controls was carried out to determine if there was a detectable fluctuating pattern in hormone concentration during the eight hour period. These values were obtained from each woman separately and the amalgamated results are presented. In a relatively short series of observations, the results of statistical tests from an individual woman are not likely to demonstrate significant differences from randomness values unless the pattern is obvious. However, an overall test of randomness within the group of subjects may be based

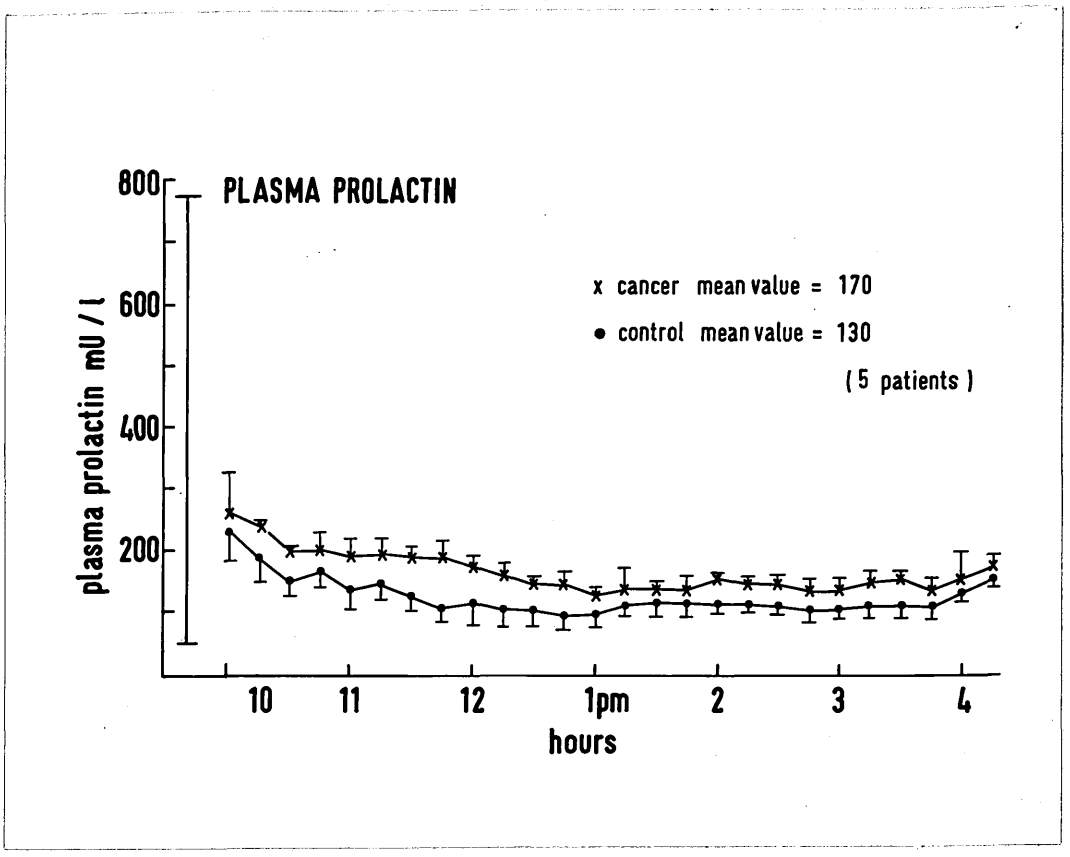


Fig. 10

Plasma prolactin concentrations in five patients with breast cancer and in five control women.

on the sum of the number of turning points. These amalgamated results are presented separately for patients with breast cancer and their controls as well as for all subjects studied. (Table IX). LH showed no significant pattern while the results for prolactin, FSH and cortisol showed fewer turning points than would be expected by random variation indicating changes in these variables during the observation period of eight hours. Cortisol concentrations showed a pronounced fall 2 hours or more after starting the test, usually followed later by a rise. The pattern for prolactin was not consistent for all women and generally showed only undulations from sample to sample. Oestradiol-17B and testosterone levels both showed significantly more turning points than would be expected by random fluctuations.

A Moore-Wallis test was also used and showed that the pattern was significantly different from random in prolactin and oestradiol-17B but in neither of these cases did examination of the plots show any evidence of a pulsatile pattern. (Table X). If anything, there was a morning fall in prolactin and the oestradiol-17B fall was in the evening. The results of this test also showed that neither LH or FSH showed a pulsatile pattern or trend in either the patients with breast cancer or the control women.

5. Discussion

Plasma testosterone

The mean value found in normal postmenopausal women was 0.63 nmol/l - range 0.17-1.91 nmol/l and it is in keeping with those reported by

TABLE IX

	CANCER				CONTROLS				TOTAL			
	T	E(T)	Var(T)	C†	T	E(T)	Var(T)	C†	T	E(T)	Var(T)	C†
LH	111	107.3	28.8	0.6	71	76.0	20.5	-1.0	182	183.3	42.5	0.1
Pr	74	98.0	26.3	-4.6***	63	70.0	18.9	-1.5	137	168.0	45.2	-4.5***
E_2	121	114.7	30.8	1.1	96	86.0	23.1	2.0*	217	201.7	53.9	2.0**
T	150	119.3	32.0	5.3***	95	87.3	23.5	1.5	245	206.7	55.5	5.1***
FSH	114	118.7	31.8	-0.7	79	90.0	24.2	-2.1*	193	208.7	56.0	-2.0*
Cortisol	79	117.3	31.5	-6.7***	58	76.7	20.7	-4.0***	137	194.0	52.2	-7.8***

$C† = \frac{[T-E(T)]-\frac{1}{2}}{\sqrt{var(T)}}$

E(T) = expected number of turning points if the sequence was random

* = $p < 0.05$
** = $p < 0.01$
*** = $p < 0.001$

T = observed number of turning points

Pattern of sequentialsampling in patients and controls and significance of differences (Kendall's Test).

TABLE X

Moores Wallis Test

	Cancers			
	n (up)	Exp (ups)	var (t)	Z
LH	80	83	14.8	-0.78
Pr	64	78	13.9	*-3.75
E_2	84	86	15.3	-0.71
FSH	92	91	16.2	0.25

	Controls			
	n (up)	Exp (ups)	var (t)	Z
LH	59	59	0	0
Pr	48	54	10.0	-1.89†
E_2	59	66.5	12.1	-2.16 sig 5% level
FSH	64	69.5	12.6	-1.55

* significantly fewer upwards than downwards trend

† not quite significant at the 5% level, but in the same direction as above

Pattern of sequential sampling in patients and controls and significance of differences - using the Moore-Wallis Test

This table compares the number of ups on the graph with the number of expected ups (Figure p 71)

other workers (Wang et al, 1975; Chakravarti et al, 1976). Plasma testosterone levels showed significantly more turning points over the sampling time, than would be expected by random fluctuations. There was, however, no definite pulsatile pattern, either in the women with breast cancer or in the control women. At no time did these fluctuations vary outwith the normal range. A zig-zag serial pattern of change was present which might be explained either due to technical reasons or due to biological reasons. The number of samples which could be included in a single run of testosterone assay was limited, and for this reason alternate samples from all individuals were taken for each run. Alternatively, these fluctuations of plasma testosterone levels agree with the findings of Rosenfield and Helke (1974) who, in a study of premenopausal women, reported small random fluctuations in testosterone levels superimposed upon a diurnal rhythm, which was greater than could be accounted for by the imprecision of the method. In another study (Tyler et al, 1975) plasma testosterone was measured at 4 hourly intervals for 24 hours in nine premenopausal women and the results showed considerable individual variation in both concentrations and the pattern of the serial samples.

Although the plasma testosterone levels in breast cancer patients were within normal limits, each of the six women with breast cancer had significantly higher plasma testosterone values than her matched control at each time. Others have also reported that plasma testosterone levels are within normal limits in patients with breast cancer (Horn and Gordon, 1974; Wang et al, 1975; Jones et al,

1977; Malarkey et al, 1977). No differences, however, were observed in these previous studies between the women with early or late breast cancer and normal controls. The failure of these workers to produce similar findings to this present study may be explained by the non-use of sequential plasma sampling by some and the use of group comparison rather than matched pairs. As C19 steroid hormones have been shown to vary with the number of years past the menopause (Chakravarti et al, 1976) it is important that patients should be matched at least for age and years past the menopause. The significance of the high normal circulating plasma testosterone levels shown in this study must remain in doubt until larger numbers have been studied. It is impossible to assess the real importance of testosterone in breast cancer until different risk groups are compared and to determine whether small differences in plasma testosterone could cause the disease in patients already predisposed, or whether these differences are just casually related to the disease.

It may be important that testosterone can be metabolised by human breast cancer to form oestradiol-17B. The possibility exists that higher testosterone levels might result in higher oestrogen concentrations within the tumour (Miller and Forrest, 1974).

A certain amount of biological evidence exists that women with breast cancer are more androgenic than normal women. Some workers have suggested that ovarian interstitial tissue is a gland of internal secretion concerned with the formation of androgens (Rice

and Savard, 1966). Evidence for this is supported by a study from Grattarola (1973) who found the excretion level of testosterone to be significantly increased in patients with endometrial hyperplasia and in patients with breast cancer who had an atypical endometrial pattern. Breast cancer patients with anovulation and endometrial hyperplasia also presented with higher urinary testosterone levels than the women with endometrial hyperplasia but no breast cancer. In a later report, Grattarola and his colleagues found increased urinary testosterone values in patients with advanced breast cancer with metastases present, but not in patients who were clinically cured. They concluded that increased androgenic activity was the hormonal factor involved in the development of breast cancer (Grattarola, 1975). Other workers have reported that women with breast cancer in Britain secrete sebum at a higher rate that normal women (Krant et al, 1968; Burton et al, 1970). As sebum secretion may be increased by androgens, this would indirectly add support to the possibility of raised plasma testosterone levels in women with breast cancer.

There is, however, evidence that <u>low</u> excretion of plasma dehydroepiandrosterone sulphate (DHAS) and dehydroepiandrosterone (DHA) and urinary aetiocholanolone may be associated with an increased risk and the presence of breast cancer (Bulbrook et al, 1967a). On the other hand, there is also evidence of a converse effect. Young Japanese women who have a <u>low</u> risk of breast cancer have been reported to have lower urinary excretion of androgen metabolites than their Western counterparts (Bulbrook et al, 1967b). It may be

important to note that plasma DHAS and DHA are almost exclusively adrenal in origin, whereas an increase in testosterone excretion is more likely to be associated with ovarian abnormality.

Since this study, further studies on multiple plasma testosterone concentrations have been reported.

The mean 24 hour serum testosterone levels (by radioimmunoassay) was measured in sixteen women with benign breast disease, seventeen patients with breast cancer and in twenty-five age- and weight-matched control women. Mean 24 hour testosterone levels were found to be significantly elevated in women with breast cancer in the luteal phase of their cycles but were normal in postmenopausal breast cancer women (Malarkey et al, 1977), while in another report (Adami et al, 1979) significantly higher mean plasma testosterone levels were found to be present in one hundred and twenty-two postmenopausal patients with newly diagnosed breast cancer as compared to the values in one hundred and twenty-two age-matched control women.

A further study by Jones et al (1981) reported that plasma testosterone levels (total and apparent free) were measured in one hundred and forty-seven women - age range between 14 and 90 years (mean 53 years). Forty-five of these women were normal controls and the remainder were classified into groups, those with histologically confirmed benign (twenty) or malignant breast tumours (seventy-one) and those with non-metastatic cancer at other sites

(eleven). In the malignant breast group forty-three were classified as early and twenty-eight as advanced breast disease. The women were then further categorized as in the present study into menopausal status and years past the menopause. No significant difference was found in the plasma testosterone levels between any of the clinical groups. It would seem that although there may be some abnormality in plasma androgens in breast cancer, particularly in patients with advanced disease, the levels of the plasma androgens, such as testosterone, compared and expressed as a group are apparently variable depending on the format of the study.

Oestradiol-17B

The mean value for plasma oestradiol-17B concentrations in the normal postmenopausal women was 53 pmol/l - range 15-110 pmol/l. This is in agreement with other workers (Baird and Guevara, 1969; Vermeulen and Verdonck, 1978; Bird et al, 1981). With regard to variation throughout the day, plasma oestradiol-17B concentration in both groups of women studied, was found to have more turning points than would be expected by random fluctuations. Graphically, the plots showed no evidence of a pulsatile pattern, but the levels of plasma oestradiol-17B tended to fall in the evenings. These findings agree with a study by Alford et al (1973) who found that plasma oestradiol-17B levels showed wide fluctuations throughout the day in two premenopausal women, a consistent pulsatile pattern not being observed.

Plasma oestradiol-17B levels in postmenopausal women with breast cancer were higher, though within normal limits, than in the control women. A significant difference was not proved. These findings are in agreement with previous reports of normal values in patients with breast cancer (Wang and Swain 1974; England et al, 1974; Malarkey et al, 1977).

Since the work was completed, it has also been reported by Adams et al (1979) that plasma oestrone (also $\Delta 4$ androstenedione and testosterone) were significantly higher in postmenopausal patients with newly-diagnosed breast cancer compared to the levels found in age matched normal women. In the control group of women, plasma oestrone levels were shown to be significantly correlated with plasma $\Delta 4$ androstenedione and testosterone, but in the cancer group plasma oestrone was only slightly correlated to testosterone and was not correlated to $\Delta 4$ androstenedione. These workers concluded that an incresed availability of $\Delta 4$ androstenedione and testosterone led to increased androgenic stimulation, decreased availability of sex-hormones binding globulin and an increased plasma oestrone level. There is also a report by Drafta et al (1980) that levels of plasma oestradiol-17B were subnormal in premenopausal patients with breast cancer in the late cycle, as compared to control women, while in postmenopausal patients, plasma oestradiol-17B was significantly higher in the breast cancer group. Bird and his colleagues (1981), however, found no significant difference in plasma oestradiol-17B or oestrone levels between postmenopausal patients with breast cancer (Stage 1 and 2) and

normal postmenopausal women.

In another report, Moore and his colleagues (1982) studied total and non-protein-bound-oestradiol-17B in pre and postmenopausal patients with Stage 2 breast cancer and in normal control women. They reported that in premenopausal women with breast cancer, total serum oestradiol-17B levels were normal, but non-protein-bound-oestradiol-17B levels were raised. In the postmenopausal group with breast cancer, both total and non-protein-bound-oestradiol-17B levels were significantly elevated. In these patients, the sex-hormone-binding globulin (SHBG) was lower and there was a highly significant correlation between non-protein-bound-oestradiol-17B and SHBG, but, for a given SHBG binding capacity, the cancer patients had more non-protein-bound-oestradiol-17B than controls. These workers suggested that the breast in women with breast cancer may be exposed to elevated levels of biologically active oestradiol-17B (ie. unbound).

Prolactin

The mean plasma prolactin value for the control women was 140 mu/l range 50-780 mu/l, which is in agreement with the findings of other workers (Kumaoka et al, 1976; Malarkey et al, 1977). No pulsatile pattern of plasma prolactin was found - only undulations in both groups of women over the test period. The prolactin concentrations were found to be lowest in the morning which agrees with previous work in which prolactin was reported to be lowest at 12 noon (Vanhaelst et al, 1973; Parker et al, 1975). The variation in

plasma prolactin levels did not correspond with the plasma cortisol levels which were highest in the morning and became lower in the afternoon. Therefore, it is unlikely that prolactin levels were increased by the stress of venepuncture. L'Hermite et al (1972) have also shown that only rarely does venepuncture cause blood release of prolactin.

No differences were found in plasma prolactin levels between patients with breast cancer and the matched control women. Other workers have also failed to show differences in prolactin levels (Franks et al, 1974; Kwa et al, 1974; Wilson et al, 1974). Malarkey and his colleagues (1977) reported nocturnal levels of prolactin to be diminished in twelve postmenopausal women with breast cancer compared to the levels in control women. The daytime and mean prolactin levels and patterns of secretion, however, were not different between the two groups. More recently, it has been reported that significantly higher plasma prolactin values (on a single morning sample) are present in postmenopausal women with early breast cancer than in postmenopausal control women (Bird et al, 1981).

Plasma Cortisol

The plasma cortisol values were within normal limits, throughout the test period (Control mean 358 nmol/l - range 193-716 nmol/l). Levels were highest at the beginning of the test period and then declined, reaching a steady state after about two hours. This suggests that the patients may have been under stress at the

beginning of the test period, the cortisol level returning to normal after two hours. More likely, the higher values at the start of the test and the falling levels throughout the morning were due to normal diurnal variation (Hamanaka et al, 1970). Only part of this variation was observed in the present study as there were no observations between 6-8 a.m. or at 12 midnight.

The report by Malarkey et al (1977) or an elevated plasma cortisol at 4 pm in breast cancer patients was not substantiated by this study.

There was no difference in the mean levels between the cancer and control patient.

Plasma Gonadotrophins

The mean value of plasma LH (38.9 u/l range 15-100 u/l) and plasma FSH (151.2 u/l range 50-300 u/l) described in this study for control women agrees with that quoted by other workers for normal post-menopausal women (Yen et al, 1972; Kumaoka et al, 1976; Wang et al, 1976; Malarkey et al, 1977).

Plasma FSH levels in both groups showed small changes during the observation period. These probably represent random fluctuation in levels detected between samples, there being no consistent evidence for a pulsatile pattern of plasma FSH. Plasma LH levels showed no fluctutations at all.

Episodic, pulsatile pattern of plasma gonadotrophins have been reported in normal women (Midgely et al, 1971; Yen et al, 1972; Wide et al, 1973). In one study, samples were taken hourly from seven premenopausal women and it was reported that plasma LH concentrations and possibly FSH were maintained as a series of peaks of variable magnitude (Midgely et al, 1971). Later Yen and his colleagues (1972) reported changes in plasma LH and FSH levels which they described as minute to minute fluctuations resembling a pulsatile pattern, with a periodicity of 1-2 hours. However, only three women were studied and only two were postmenopausal and both of these were early postmenopausal. In another study (Wide et al, 1973) the changes in plasma LH and FSH levels were described as episodic. Seventeen women were studied but only seven were postmenopausal, and the evidence for a pulsatile pattern in these postmenopausal women is poor.

In this present study plasma FSH did show some fluctuation, while plasma LH levels did not fluctuate, which agree with the graphical changes displayed by Wide and his colleagues, whose women were also older than those described by Midgely et al (1971) and Yen et al (1972). It could be that with increasing age and declining values of plasma LH, the pulsatile pattern is lost.

The inability to detect differences in plasma LH and FSH levels between patients with breast cancer and the control women agrees with previous work by Wang et al (1976) and Malarkey et al (1977).

As neither plasma LH nor FSH levels had a pulsatile pattern of secretion either in normal postmenopausal women or in postmenopausal women with breast cancer, it would seem unnecessary to assay more than one blood sample for gonadotrophins. If, however, multiple sampling had been omitted in this study, the small but significant difference in plasma testosterone between women with breast cancer and well matched controls would have passed unnoticed on one single sample which would have been within normal limits. A compromise of four half-hourly samples over two hours, as will be suggested for the regime for the men with gynaecomastic, is probably suitable for the women as well.

Since this study was completed, there have been other reports of multiple plasma sampling throughout 24 hour and 8 hour time periods. In 1977, Malarkey et al reported studies in which 24 hour serum oestradiol-17B levels were measured in sixteen patients with benign breast disease, seventeen patients with breast cancer and twenty-five age-matched and weight-matched control women. Samples were taken every four hours and a radioimmunoassay was used. These workers found that the serum concentrations and patterns of plasma oestradiol-17B secretion were similar in all three groups.

Further multiple plasma studies on plasma prolactin have been reported. Using a multiple sampling regime, Kwa et al (1978) found that nulliparous and obese postmenopausal normal women (at risk of developing breast cancer) had raised early evening plasma prolactin levels compared to a similar group without such risk factors. The

same group of workers also reported that evening plasma prolactin levels in nulliparous premenopausal women with benign and malignant breast disease were increased compared to similar parous women.

Plasma prolactin was estimated at four hourly intervals throughout a 24 hour span from healthy women, women with benign breast disease and women with carcinoma of the breast by Tarquini et al (1980). These showed considerable variations in prolactin levels throughout a day and also differences in circadian mesor of prolactin among the groups were significant. This underlined the importance of multivariable and multifrequency investigations in breast cancer. An increase in 24-hour mean luteal phase plasma prolactin level was reported in thirteen young females whose mothers had breast cancer. These females were also found to have a partial resistance of prolactin to dopamine suppression (Levin and Malarkey, 1981).

Conclusion

In this study, plasma LH, FSH, testosterone, oestradiol-17B, prolactin and cortisol concentrations were all within normal limits in these patients with breast cancer, but, within the normal range, the plasma testosterone concentrations in each cancer patient were significantly higher than in each matched control.

Study 2 - Effect of Endocrine Treatment : Relationship of Hormonal Changes

A. Hypophysectomy - 90 Yttrium implantation and pituitary function tests

The pituitary stalk has two elements. One is vascular and is concerned with the control of the anterior part of the anterior lobe. The other is neural and connects with the posterior lobe in continuity with the hypothalamus.

The secretion of hormones produced in the anterior lobe is controlled by neurohormones secreted by the cells of the hypothalamus and carried down the pituitary stalk in the vessels of the vascular connections. This system is called the hypophyseal portal system. The active endocrine part of the anterior lobe consists of epithelial secretory cells. The human pituitary is a confederation of five largely independent functioning units each represented by a specific cell type synthesizing and releasing one or two pituitary hormones. The older classification of pituitary cells into acidophile, basophile and chromophobe types is inadequate to explain the independent secretion of six hormones. Progress has been made by the application of histochemical immunofluorescent and electron microscopic techniques (Pelletier et al, 1978). As a result of these studies, the concept has been strengthened that each major hormone is secreted by a distinct cell type except both gonadotrophins which are secreted from a single

cell. Pituitary cells are classified on the basis of the hormone secreted. The hormones secreted are growth hormone (GH), adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), gonadotrophins, follicular stimulating hormone (FSH) and luteinizing hormone (LH) and prolactin (HPr). Unlike the majority of anterior pituitary hormones which are under hypothalamic stimulatory control by releasing factors, prolactin is under tonic and clonic inhibitory control. This control is exercised by the median eminence of the hypothalamus. The neuroinhibitor in the case of prolactin (HPr) has been termed prolactin inhibiting factor (PIF). The existence of a prolactin releasing factor (PRF) has also been postulated. The normal physiological level of prolactin is maintained by short (pituitary-hypothalamic-axis) and long (systemic-hypothalamic-pituitary) feedback mechanisms. Proof that prolactin activity is due to escape from a hypothalmic inhibitory factor comes from observations that the addition of a hypothalamic extract inhibits the secretion of prolactin into the culture media (Pasteels, 1963). The presence of an inhibiting factor has also been demonstrated in other studies in which hypersecretion of prolactin has been observed in experimental animals after pituitary stalk section, following the creation of specific median eminence lesions or by transplantation of the pituitary gland to other sites (Meites and Nicoll, 1966). In man, pituitary stalk section leads to an increase in the concentration of prolactin in the plasma (Newsome et al 1971).

There is no direct evidence of the existence of a factor which stimulates prolactin secretion in mammals but such a factor probably plays a part in the maintenance of normal prolactin levels by means of the short (internal) feedback control. Although PRF has not been isolated, injections of pure thyrotropin releasing hormone (TRH) in humans causes an elevation of plasma prolactin levels and of thyroid stimulating hormone (TSH).

In the early 1950s only the estimation of urinary gonadotrophins was available for the direct assessment of pituitary function. Studies of pituitary function in patients with breast cancer was therefore limited to this particular group of hormones. The measure of gonadotrophin function most frequently used was the increase in mouse uterine weight, following the injection of an extract of urine which estimated both FSH and LH (Klinefelter et al 1953). The development of a method using adsorption on to kaolin with elution followed by acetone precipitation (Loraine & Brown, 1956) allowed large volumes of low-titre urine to be extracted, which gave consistent and reliable results. The estimation of urinary gonadotrophins was used as a direct measure of pituitary function following attempted ablation of the pituitary gland. In eleven out of twelve patients studied at the Mayo Clinic, gonadotrophin secretion fell to zero within three to ten days of surgical hypophysectomy (Blackburn et al, 1956). Paradoxically, Lipsett & Pearson (1956) recorded significant gonadotrophin excretion in two patients after surgical removal of the pituitary, despite subsequent autopsy evidence that this was complete. However, the method of

assay used (Klinefelter et al, 1953) lacked precision.

During the 1960's the development of radioimmune-assays allowed a method to become available for the estimation of growth hormone (GH) in the blood. Tests evolved in which the release of growth hormone was stimulated by hypoglycaemia indirectly by the injection of insulin; following which plasma levels of GH were sequentially measured. (Greenwood, Landon & Stamp, 1966). These tests were used to estimate the completeness of destruction of the pituitary e.g. following yttrium implantation. In one such study, reported by Stewart et al (1971), histological assessment of destruction of the pituitary gland after yttrium implantation was correlated with the results of the insulin test. It was reported that an absent GH response to hypoglycaemia was associated with an almost complete destruction of the pituitary gland. In a further study the growth hormone response to insulin induced hypoglycaemia after yttrium implantation was compared with that after transethimoidal hypophysectomy. There was no difference between these two procedures in terms of the degree of pituitary ablation achieved, although persistent small but significant fasting levels of GH which did not respond to the hypoglycaemic stimulus was more common after yttrium 90 implantation. It was suggested that this probably arose from pituitary remnants divorced from hypothalmic control (Roberts et al, 1973).

By the 1970's as a result of the further development of precise immunoassay techniques it became possible to estimate most of the

hormones in the plasma. This included luteinizing hormone (LH), follicular stimulating hormone (FSH), thyroid stimulating hormone (TSH) and prolactin (HPr). More recently the hypothalamic releasing hormones - luteinizing releasing hormone and follicle stimulating releasing hormone (LH/FSH - RH) and thyrotropic releasing hormone (TRH) have been synthesised and are available for clinical use. Direct stimulation of the pituitary with subsequent estimation of circulating trophic hormones (LH,TSH, FSH) can now be usedto assess pituitary reserve. Plasma concentrations of LH and FSH thereby increase if functioning gonadotrophic cells are present in the pituitary. Similarly TRH administration raises the plasma levels of TSH and also prolactin (Bowers et al, 1971) while chlorpromazine is also known to raise plasma prolactin concentrations (Turkington, 1972,b). Thyroid stimulating hormone (TSH) secretion after the administation of synthetic TRH has been extensively studied both in normal people and in patients with hypothalamic-pituitary disease (Fleischer et al, 1970; Hall et al, 1972). Similarly LH and FSH response to synthetic LH/FSH-RH have been reported in normal people (Besser et al, 1972). These releasing hormones have been found to be specific and secretion of other pituitary hormones was unchanged after TRH and LH/FSH-RH administration (Fleischer et al, 1970). Initially for the assessment of pituitary function each provocative test was used separately involving the patient in a considerable number of tests. A single test, similar to that described by Harsoulis et al, 1973, has been developed and evaluated, this with the objective of causing ill patients with breast cancer as little upset as possible.

Results of these tests have been correlated with other estimates of pituitary function and also with the clinical response to removal or destruction of the pituitary gland.

2. Methods

Hypoglycaemia could be worrying in the pituitary ablated patient and the growth hormone test that was used in the present study was the insulin infusion test of Carter, Dozois & Kirkpatrick (1972). For stimulation of LH, FSH and TSH secretion the releasing hormones LHRH and TRH were used. For prolactin stimulation tests using TRH and chlorpromazine were carried out. TRH has a direct action on the pituitary prolactin secretion, (Lister et al, 1974) while chlorpromazie acts indirectly on the pituitary by the inhibition of release of prolactin inhibiting factor. This is brought about by blocking dopamine uptake into storage granules in the hypothalamic nuclei (Friesen et al, 1972; Leblanc and Yen, 1976)

The patients with breast cancer were assessed for clinical response to Y90 six months after treatment, according to the criteria suggested by the British Breast Group (1974). Patients were grouped as responders, non-responders and those with an equivocal response.

Thirteen patients with breast cancer were investigated, nine patients before and one month after Y90 implantation, and four post-operatively only. In addition three patients with diabetic retinopathy were studied. Two of these had tests before and again

one month after; only one post-operatively. The pituitary function tests used were:

a) LHRH test

b) LHRH plus TRH test

c) Insulin infusion test

d) LHRH plus TRH plus insulin infusion test

e) Chlorpromazine test.

Test Procedure

The pattern of test undergone by the patients are shown in Table XI. Most patients on day one had LHRH alone (Table XI), on day two LHRH plus TRH followed afterwards by the insulin test and on day three the chlorpromazine test. Other patients (6,7 and 8) had a combination of LHRH plus TRH plus insulin on day one and chlorpromazine on day two.

2. Pituitary Function Tests

a) LHRH Test

The patient was fasted overnight and kept in bed the morning of the test. An 18G. teflon cannula was inserted into an arm vein and a basal blood sample was withdrawn. A second basal sample was taken after 10 minutes and 100 ug. LHRH injected through the cannula. Blood was withdrawn at 10,20,30 and 60 minutes, centrifuged and the plasma (serum) deep frozen and subsequently assayed in batches for LH and FSH as described in the Method Section.

TABLE XI

Patient Number		LHRH (a)	LHRH TRH (b)	Insulin (c)	LHRH TRH Insulin (d)	Chlorproma-zine (e)
	1	OX	OX	OX	-	OX
	2	OX	OX	OX	-	OX
	3	OX	OX	OX	-	OX
	4	OX	OX	OX	-	OX
	5	OX	OX	OX	-	OX
	6	-	-	-	OX	OX
Breast	7	-	-	-	OX	OX
	8	-	-	-	OX	OX
Cancer	9	O	OX	OX	-	OX
	10	X	X	X	-	X
	11	X	X	X	-	X
	12	X	X	X	-	X
	13	X	X	-	-	X
Diabetic	1	-	OX	OX	-	OX
Retinopathy	2	-	OX	-	-	OX
	3	-	X	X	-	X

O - Test done pre y-90

X - Test done post y-90

Tests of pituitary function carried out

b) LHRH plus TRH Test

The patient was fasted overnight and kept in bed the morning of the test. An 18G. teflon cannula was inserted into an arm vein and a basal blood sample was withdrawn. Following a second basal sample 100 ug. LHRH plus 200 ug. TRH was injected through the cannula. Blood was then withdrawn at 10, 20, 30 and 60 minutes and deep frozen and subsequently assayed in batches for LH, FSH, TRH and prolactin.

c) Insulin Infusion Test

After the completion of the LHRH plus TRH test, a 21G. butterfly needle was inserted into a vein in the dorsum of the hand. Blood was withdrawn from a cannula in the other arm. Following two basal samples at 15 minute intervals, infusion of insulin was commenced at a constant rate to deliver 0.04 u/kg/hour. Blood was withdrawn at 30, 60, 90 and 120 minutes for GH and blood glucose.

d) LHRH plus TRH plus Insulin Test

Patients were again fasted and as before, a cannula was inserted into one arm. Two basal samples of blood were taken. A butterfly needle 21G. was inserted into a vein on the dorsum of the other hand and through this 100 ug. LHRH and 200 ug. TRH were injected. An insulin infusion using a dose of 0.04 u. insulin/kg. body weight/hour was immediately commenced through this needle and blood was withdrawn from the cannula in the other arm at 10, 20, 30, 40, 60, 90 and 120 minutes. The basal samples were used for the measurement of LH, FSH, prolactin, TSH, GH and blood glucose; those

at 10, 20, 30, 40 and 60 minutes for LH, FSH, TSH and prolactin, and those at 30, 60, 90 and 120 minutes for GH and blood glucose.

e) Chlorpromazine Test

Patients were fasted and as before, a cannula was inserted into an arm vein through which basal samples were taken. Chlorpromazine (50mg.) was injected intramuscularly into the buttock and blood was withdrawn at 30, 60, 90 and 120 minutes and assayed for prolactin.

3. Results

Measurement of Pituitary Function

I. Before Implantation of the Pituitary

The typical response of plasma hormone levels to the stimulation used in a patient with breast cancer tested before and one month after yttrium implant is shown in Fig. 11. It can be seen that the peak circulating concentration of the various hormones occurs at different times after injection of the stimulating agent. For each hormone the increment in hormone concentration on stimulation has been calculated by subtracting the basal value from the observed concentration. For each test the average of these increments at specified times after injection was calculated. Specified times were those when the hormone concentrations were greatest. These times are listed in Table I.

The median and range of these increments for each test is given in Table XII. The majority of patients showed a large hormonal increment to the appropriate releasing hormone.

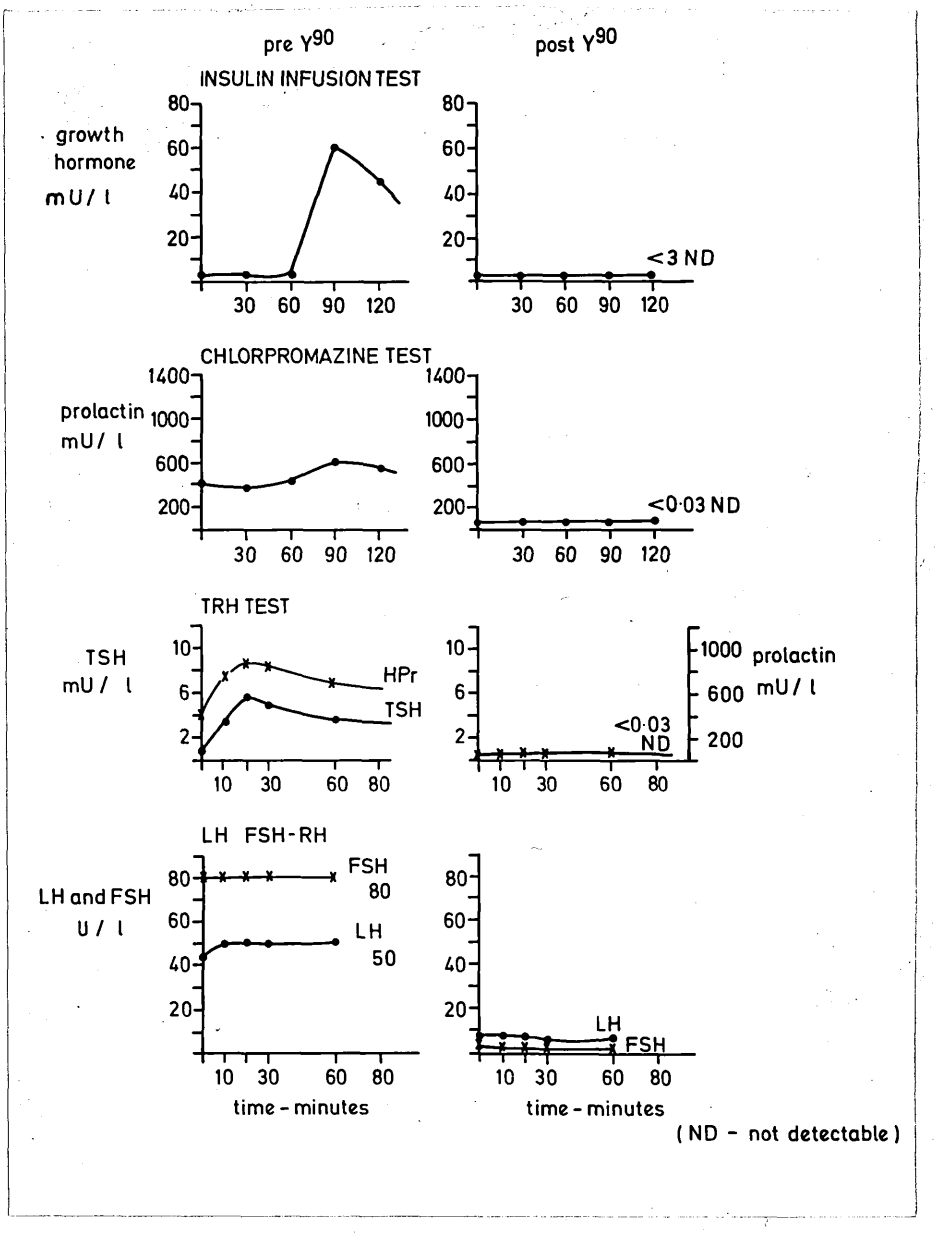


Fig. 11

Measurement of pituitary function in a patient aged 70 years before and after yttrium implant.

TABLE XII

Before γ^{90} Implantation

Test	No of patients	Median of hormonal increments	Range of hormonal increments
GH/insulin	10	25 mU/1	7 - 93 mU/1
LH/LHRH	6	15 U/1	-1 - 73 U/1
LH/LHRH + TRH	10	32 U/1	3.5 - 67.5 U/1
FSH/LHRH	6	20 U/1	3.5 - 95 U/1
FSH/LHRH + TRH	10	26 U/1	4.5 - 83.5 U/1
Prolactin/LHRH + TRH	11	880 mU/1	70 - 1560 mU/1
Prolactin/Chlorpromazine	11	550 mU/1	-350 - 840 mU/1
TSH/TRH	11	10 mU/1	0 - 20.5 mU/1

Median and range of increments of hormone concentration in patients before γ^{90} implantation.

The exceptions were one patient (No. 1 in response group), Table XIII, who showed very little response to LHRH in either test and low prolactin response to chlorpromazine. Another two patients had negative prolactin response to chlorpromazine (patients No. 2 and 4 in the non-response group), and a further two a low TSH response to TRH (patients No. 3 and 5 in the non-response group).

The mean incremental hormone levels for each test are plotted against time in Figure 12. All patients undergoing the tests before yttrium 90 implantation are included. For this purpose the results for the simultaneous LHRH plus TRH plus insulin test have been combined with the results in those patients in whom LHRH plus TRH and insulin were given on separate days.

Comparison of Tests of Pituitary Function before yttrium 90 implantation

(1) LHRH alone and LHRH plus TRH

Five patients underwent both stimulatory tests before Y90 implant. Levels of plasma LH and FSH increments in hormone concentrations to the two tests were compared using Wilcoxn's signed rank test. There was no significant difference between the mean increments in concentrations of LH and FSH for LHRH alone or in combination with TRH. Also the two tests produced a very similar ordering of the five patients with respect to the increments of the concentration of hormones in Spearman's $p/=0.9$ just significant at 5% level.

TABLE XIII

Pre-Implant

Patients		GH mU/1	LH U/1	FSH U/1	Hpr (TRH) mU/1	Hpr (Chlor) mU/1	TSH mU/1
Responders	1	7.7	3.5	4.5	990	50	20.5
	2	-	-	-	-	-	-
	3	-	-	-	-	-	-
	4	33	35.5	31.5	420	130	11.5
Equivocal	1	-	-	-	-	-	-
	2	16.7	19.5	19.0	1560	61	5
Non-Responders	1	16.7	66	83.5	910	550	8.5
	2	7.0	32	27.5	380	-350	15
	3	37.0	67.5	59.0	1130	690	0
	4	15.0	-	38.0	610	50	7
	5	39.0	36.5	25	880	170	1.5
	6	60.0	-	-	580	770	5
	7	-	-	-	-	-	-

Increments in plasma hormone concentration in individual patients with advanced breast cancer before ^{90}Yttrium implantation

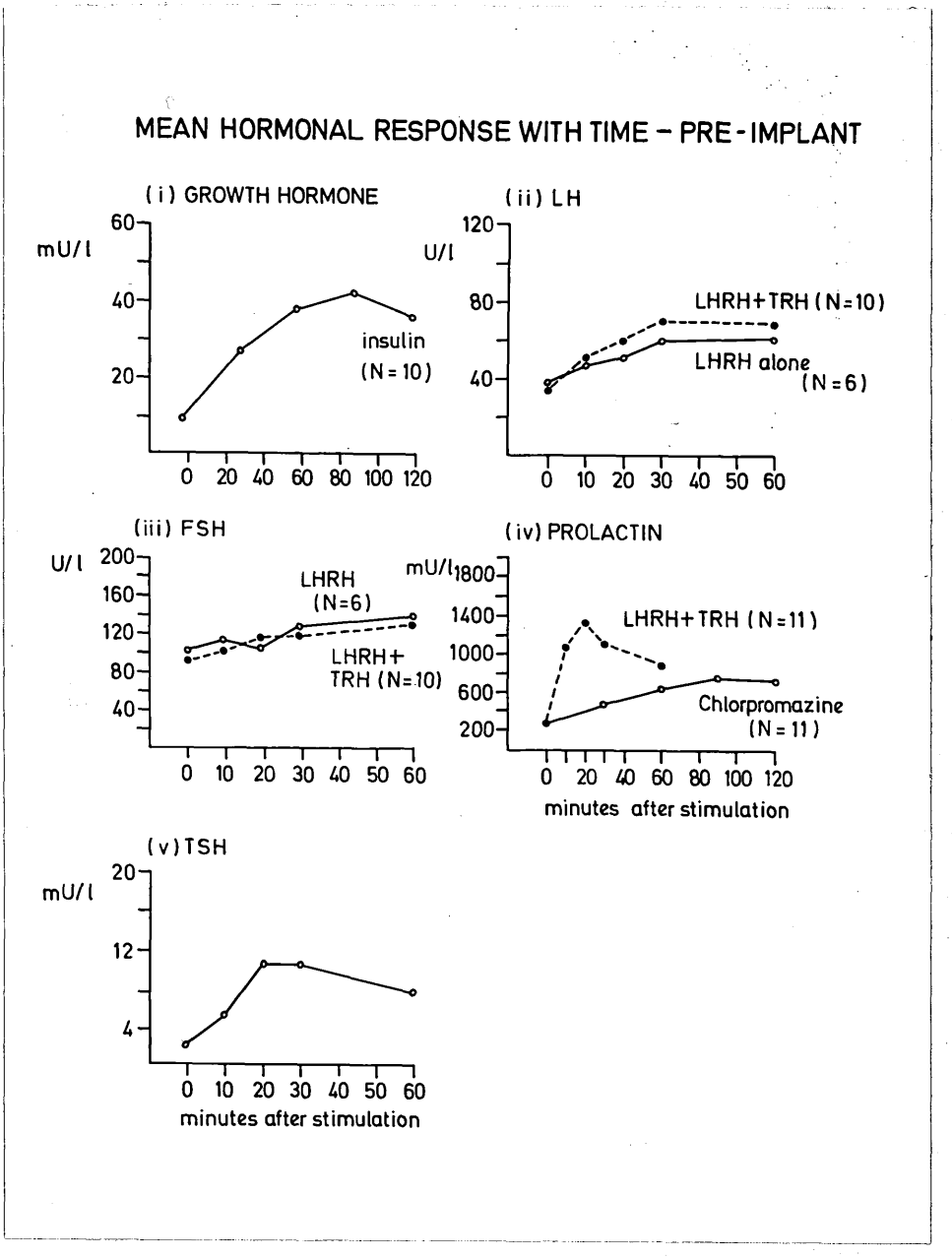


Fig. 12

Mean plasma hormone concentration response with time pre-implant for GH, LH, FSH, prolactin and TSH.

(2) Prolactin/TRH and prolactin/chlorpromazine

Eleven patients underwent both of these tests of prolactin stimulation. The prolactin response was greater when stimulated by TRH than by chlorpromazine (sig. at 5% level using Wilcoxon's signed rank test). Results expressed as medians are shown in Table XII. Also the two tests produced a quite different ordering of the eleven patients, i.e. (Spearman's $\rho = 0.28$ which does not differ significantly from zero at the 5% levels). Therefore these two different tests of prolactin stimulation are not correlated. The time course was different too with an early peak of plasma prolactin concentration at about 20 minutes after TRH and a late peak at about 90 minutes after chlorpromazine.

II. After pituitary implantation

In Table XIV are given the median and range of hormonal increments for all patients who were tested before and one month after yttrium 90 implant of the pituitary.

There is an obvious and significant decrease in pituitary function in patients tested after implant compared with those tested before ($p<0.01$ on the Mann Whitney rank sum test for increments of all hormones). (See Methods page). Some patients showed a complete absence of pituitary function on all tests, whereas others still gave a response to one or more stimulatory factors. The results for the individual patients can be seen in Fig. 11 and Table XV.

TABLE XIV

After Y^{90} Implantation

Test	No of patients	Median of hormonal increments	Range of hormonal increments
GH/insulin	14	0 mU/1	-2 - 13 mU/1
LH/LHRH	9	2 U/1	-0.5 - 4 U/1
LH/LHRH + TRH	16	1 U/1	-2 - 9.5 U/1
FSH/LHRH	9	0.5 U/1	0 - 13.5 U/1
FSH/LHRH + TRH	16	1 U/1	0 - 13 U/1
Prolactin/LHRH + TRH	16	100 mU/1	0 - 890 mU/1
Prolactin/Chlorpromazine	16	0 mU/1	-50 - 190 mU/1
TSH/TRH	14	1.5 mU/1	0 - 5.5 mU/1

. Median and range of increments of hormone concentration in patients after ^{90}Yttrium implantation

TABLE XV

Post-Implant

Patients		GH mU/1	LH U/1	FSH U/1	Hpr (TRH) mU/1	Hpr (Chlor mU/1	TSH mU/1
Responders	1	0	1	1	170	0	2
	2	0	-2	0	0	0	1
	3	-1.67	1	1	50	-50	1.5
	4	0	-2	0	0	0	0
Equivocal	1	-	2.5	0	150	-10	1.5
	2	0	0.5	0	0	0	0
Non-Responders	1	-2	0.5	12.5	350	130	5.5
	2	0	-0.5	0	0	0	0
	3	3.3	31	13	890	150	0
	4	0	1.5	0.5	10	-20	3.5
	5	13	9.5	10	200	80	1.5
	6	0	-0.5	0	0	0	0
	7	11	5	1.5	150	-40	1.5

Increments in plasma hormone concentrations in individual patients with advanced breast cancer after ^{90}Yttrium implant.

Comparison of tests of Pituitary Function after Y90 Implant

(1) LHRH alone and LHRH with TRH

Nine patients underwent both of these tests of gonadotrophin release after Y90 implant. No significant difference could be detected between the increments of either LH or FSH to each of the two forms of stimulation. The two tests produced a very similar ordering of the patients with respect to LH and FSH increments (Spearman's ρ=0.7 (LH) and 0.8 (FSH), both significantly greater than zero at the 5% level).

(2) Prolactin/TRH and prolactin/chlorpromazine

Sixteen patients underwent tests of both type after implant. As in the tests before implantation the prolactin increment after TRH was the greater (sig. at 1% level on Wilcoxon's signed rank test). Results are shown in Table XIV. Patients with a low response to TRH tended, however, to have a low response to chlorpromazine, although the reverse was not true (Spearman's ρ=0.5 significant at 5% level).

Pituitary Function and Clinical Response

Of the thirteen breast cancer patients, four had objective regression of disease at six months, seven had no response and in two the clinical response was equivocal (Table XV). An example of a patient with a clinical response after hypophysectomy is shown in Fig. 13. The results of the pituitary function tests after the implant in patients grouped by clinical response are shown in Fig. 14. The prolactin response is shown in Table XVI.

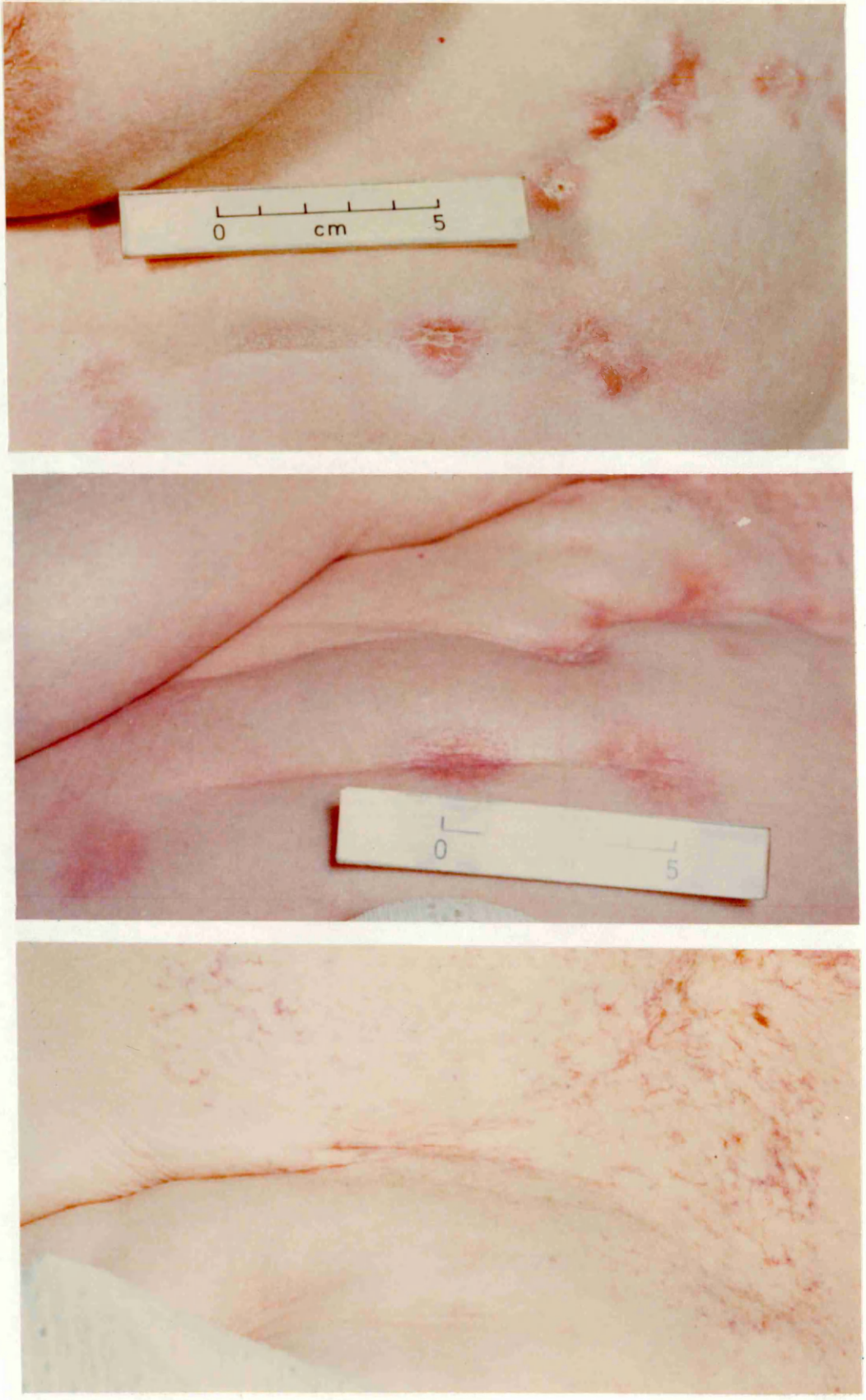


Fig. 13

Photographs of skin lesions of a patient with advanced breast cancer before, 6 months and 1 year after yttrium implant.

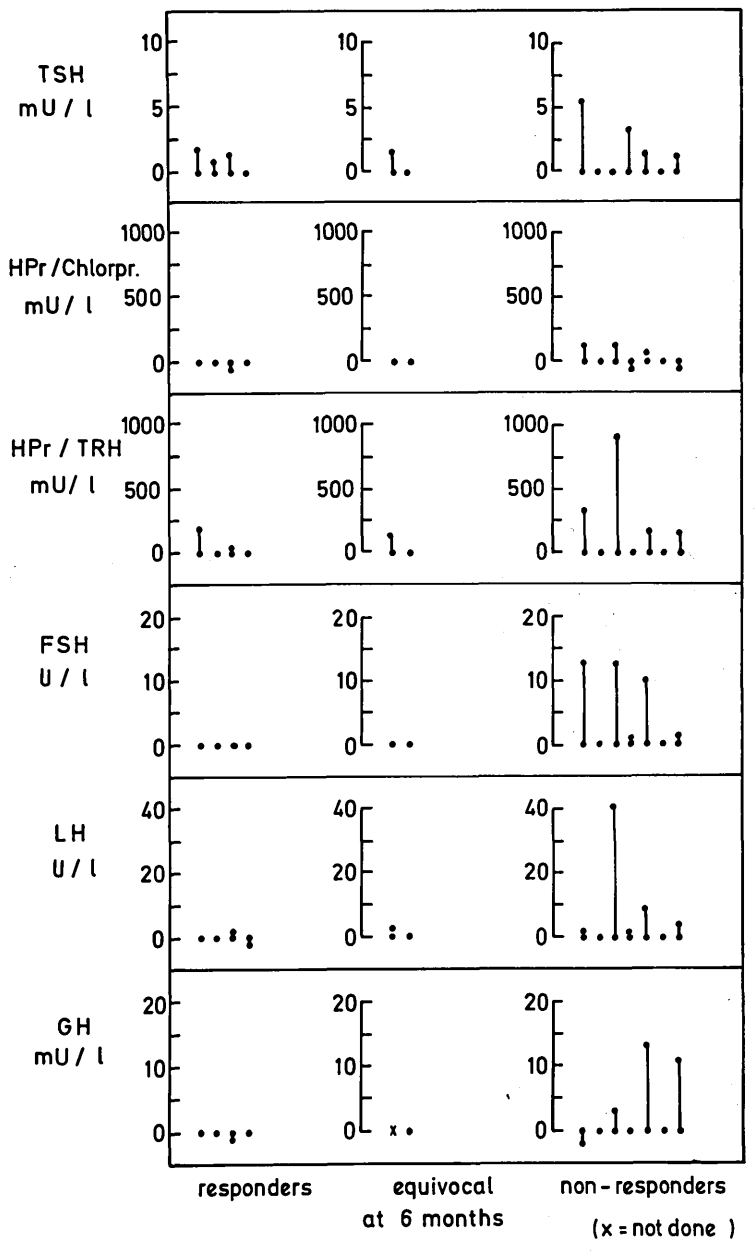


Fig. 14

Increment of plasma hormone concentration after yttrium implant with the clinical response at six months.

TABLE XVI

Pre-Implant

Stimulant	Plasma Prolactin mU/l	
	Median	Range
TRH	610	70 - 1560
Chlorpromazine	170	-350 - 840

Post-Implant

Stimulant	Plasma Prolactin mU/l	
	Median	Range
TRH	95	0 - 890
Chlorpromazine	0	-50 - 190

Mean plasma prolactin concentrations after stimulation with TRH or Chlorpromazine before and after ^{90}Yttrium implantation

Since the administration of TRH with LHRH did not have significantly different effects on LH and FSH compared with LHRH alone, the results of tests in different patients can be combined. When both tests have been performed in one patient an average rate has been calculated.

These six patients with an objective or equivocal response showed no evidence of pituitary function other than a small increment of prolactin when stimulated by TRH (Responders No. 1 and 3 and equivocal responder No. 1 who also had a small TSH increment). (Fig. 14 and Table XV.

By contrast, only two of the seven patients with no evidence of clinical response (non-responders No. 2 and 6) had no hormone increments and appeared to have had a complete ablation. Four of the patients, Nos. 3, 5 and 7, had considerable increments of all hormones, while patient No. 4 had very low increments of LH and TSH (Fig. 14 and Table XV).

Because of the small numbers of responders (maximum 4) and the limited total number of patients, none of the hormone increments could be shown to be significantly different between the non responders and the responders plus equivocal responders. (Wilcoxon's rank sum test : not significant). However, the results quoted above suggest that significantly greater hormone increments would be found in the non-responders if these tests were repeated on a larger series of patients.

4. Discussion

Basal hormone levels after pituitary ablation are often difficult to detect even with the most sensitive assay, yet they cannot necessarily be regarded as zero. As residual activity may be important in continued tumour growth, it is essential to use provocative stimulation tests.

Degree of pituitary ablation

Assessing the degree of ablation after Yttrium implantation involves measuring the degree of destruction of the pituitary cells. It is notable that their resistance to destruction following Yttrium 90 may vary. Histological studies of pituitaries after implantation reveal that surviving pituitary tissue is usually limited to a subscapular rim supplied by the capsular vessels. (Forrest et al, 1959) This variable resistance by some cells may explain why some and not other plasma hormones are still present after implantation.

It is necessary to cause as little upset as possible to a patient already ill with advanced breast cancer. For this reason the various combinations of releasing hormones used in pituitary function tests were evaluated to allow the simplest combination affording the most information about pituitary destruction to be used. It was found that the addition of TRH to LHRH given as one injection did not affect the plasma, LH or FSH response to LHRH alone, either before or after Yttrium implant. The numbers were small and the conclusions therefore were tentative but the results were consistent with those of Harsoulis and his colleagues (1973).

A test of pituitary function using LHRH stimulation combined with an insulin infusion test for growth hormone was useful. Since TRH could be given in combination with LHRH with no extra discomfort to the patient, and the addition of TRH stimulated the levels of prolactin secretion, the recommended test of pituitary function was LHRH plus TRH plus insulin both before and after Yttrium implant. A comparison was made between the prolactin response to chlorpromazine and TRH stimulation. There was a significant difference in plasma prolactin responses, this being significantly greater after TRH administration than after stimulation with chlorpromazine both before and after Yttrium implant. Chlorpromazine tended to produce little prolactin response and this apparent suppression of pituitary function was often inconsistent with the result of the other stimulation tests including TRH for prolactin secretion. The results of the chlorpromazine test was therefore ignored in favour of the TRH test for prolactin secretion; TRH would appear to be a better stimulant of prolactin than chlorpromazine.

The definition of a complete ablation, that following none of the stimulation tests was there any evidence of increments of any plasma hormones. Incomplete ablation was assumed when the majority if not all the tests showed a significant rise in any hormone to any stimulation test.

When the patients with advanced breast cancer were grouped by clinical response into those with objective regression of disease at

six months, those with an equivocal response and those with no response to Yttrium implantation, a relationship between the degree of pituitary destruction and clinical response was observed. An absence of measurable pituitary function did not guarantee a clinical response but all patients with a response or an equivocal response had minimal pituitary function. Gonadotrophins and growth hormones were completely suppressed in these patients with a response or an equivocal response, but some of these patients did show a small prolactin and TSH response stimulation by TRH. These results seem to indicate that if a hypophysectomy was incomplete a clinical response was less likely to occur due to residual pituitary tissue, continuing to secrete hormones. Conversely a complete ablation did not guarantee clinical response.

These conclusions were not in agreement with views expressed by other workers. (Peck and Olson, 1963; Stoll, 1969; Bates et al, 1976; Fairney et al, 1976; Wang et al, 1979) who did not find any relationship between residual pituitary function and clinical response to hypophysectomy. It has been suggested that two factors are required for a clinical response (Hawkins et al, 1980) - the pituitary destruction must be complete and the breast tissue must be hormone sensitive i.e. the tumour must at least be oestrogen receptor positive. These other workers removed the pituitary surgically and so the general level of destruction may have been more complete than by Yttrium implant. The receptor status of the groups of patients may also have been different.

The results of this present study indicate the need for testing pituitary function in patients with advanced breast cancer who have been treated by Yttrium implantation. If, at one month after implantation, residual pituitary tissue is evident from the tests, particularly LH, FSH and growth hormone secretion, then it seems reasonable to assume that pituitary ablation is incomplete and a clinical response will be unlikely to occur. A change of treatment then to another regime would therefore be advisable and time-saving.

5. Conclusion

1. The most suitable combination of pituitary function test to use in a patient with advanced breast cancer is a combination of LHRH, TRH and insulin by infusion.

2. TRH is a better stimulant of prolactin secretion than chlorpromazine particularly after Yttrium implantation.

3. The results of these tests suggest that when the ablation of the pituitary, as measured by residual hormones on stimulation, is incomplete a clinical response is unlikely to occur, while a clinical response is more likely if the pituitary destruction is complete though not guaranteed.

B. Chemotherapy

The extent to which chemotherapy exerts its action by inhibition of the endocrine glands is uncertain.

In 1976 Bonnadonna and his colleagues studied the effects of cyclophosphamide, methotrexate and 5-fluorouracil (CMF) used as adjuvant

treatment to radical mastectomy. They found that half the premenopausal patients with breast cancer being treated by this regime developed amenorrhoea, an effect produced mainly by the cyclophosphamide, and it was suggested that when amenorrhoea did occur in these patients the CMF treatment was more likely to be successful and the patient more likely to have a clinical response. It was suggested by Rubens et al (1977) that the probable action of chemotherapy used prophylatically in early breast cancer was on the ovary, the effect being similar to that of carrying out a prophylactic oophorectomy. Another group of workers studied the effect of either L-phenylalanine mustard (L.PAM) or cyclophosphamide, methotrexate, 5-florouracil and vincristine (CMFV) on pituitary and steroid hormones in pre and postmenopausal patients with breast cancer (Rose and Davis, 1977). They suggested that a fall in plasma oestrogens (oestradiol-17B and oestrone) was accompanied by a rise in plasma LH and FSH, as would be found naturally at the menopause. In the postmenopausal woman, only plasma $\Delta 4$ androstenedione levels fell; the other hormones were unchanged. These results suggested ovarian suppression by a direct effect on the ovaries in the premenopausal group and could also be due to suppression of the ovary in the postmenopausal group since after the menopause this organ secretes some testosterone and $\Delta 4$ androstenedione. The same workers studied ovarian and adrenal function in premenopausal breast cancer patients before and at intervals during adjuvant therapy with cyclophosphamide, methotrexate and 5-fluorouracil (CMF), CMF plus prednisone (CMFP) or CMFP plus tamoxifen (Rose and Davis 1980). Amenorrhoea developed

within 10 months in thirty-four of the thirty-eight patients. In the patients receiving CMF there was a reduction in plasma total oestrogens and an increase in plasma LH and FSH, again indicating that these drugs suppress ovarian function. These authors found that the age of the patient was inversely correlated with the time to the onset of the drug-induced amenorrhoea, reflecting perhaps an increasing ovarian sensitivity to cytotoxic agents as a woman approaches her natural menopause.

After adjuvant chemotherapy plasma $\Delta 4$ androstenedione levels in the premenopausal women who developed amenorrhoea fell to levels similar to those of untreated postmenopausal patients. There was also a small but significant fall in plasma $\Delta 4$ androstenedione concentrations in postmenopausal patients. However, the plasma dehydroepiandrosterone sulphate (DHAS) concentrations were unaffected by adjuvant chemotherapy in either group of patients. These observations suggested that the altered plasma $\Delta 4$ androstenedione concentrations after adjuvant chemotherapy were due to the loss of ovarian secretion whereas the adrenal source of both $\Delta 4$ androstenedione and DHAS remained intact. On the basis of plasma LH and oestradiol-17B levels (Schulz et al, 1979) suggested that combination chemotherapy led to alteration in the hypothalamic-pituitary-ovarian axis in premenopausal and perimenopausal women but not in postmenopausal women who continued to have physiologically high levels of LH and FSH and low plasma prolactin and 17B oestradiol levels under cytotoxic treatment. The effect of chemotherapy was on the ovary and the hypothalamic and

pituitary functions were resistant to the influence of combination chemotherapy; the effects on the ovary were more likely to be involved in premenopausal and perimenopausal women than in postmenopausal women, perimenopausal women being more likely to develop amenorrhoea than premenopausal women. In women who continued to menstruate during adjuvant chemotherapy, Sherman et al (1979) found no major alterations in plasma hormone secretions (LH, FSH, oestradiol-17B, progesterone and prolactin). Despite these recent reports it was thought at the time this study was originally set up that the effects of chemotherapeutic drugs on ovarian hormone levels could be secondary to the effects at the pituitary level, and so this study has investigated the effect of chemotherapy on pituitary function.

2. Methods of Study

Fifteen women with progressive advanced breast cancer were included in the study. Following an initial trial of additional hormone therapy, they were randomly allocated into three groups, one of which was treated by yttrium 90 implantation of the pituitary alone; one by chemotherapy alone and the third by yttrium 90 implantation plus chemotherapy. All patients had received no therapy one month prior to their inclusion in the study.

Chemotherapy was by the cyclophosphamide, methotrexate, 5-fluorouracil (CMF) regime in which two doses of 5-fluorouracil (700 mg/m^2) and methotrexate (60 mg/m^2) were given intravenously on days 1 and 8 of a 28 day cycle and cyclophosphamide 1100mg/m^2/day was

given orally for days 1 - 4 inclusively.

Pituitary function studies were carried out during the week prior to the operation or the commencement of chemotherapy and were repeated exactly one month later. In the case of those patients treated with combined modalities, chemotherapy was started one day following yttrium 90 implantation. The pituitary function test used was the combination of LHRH plus TRH plus insulin as described in the preceding section and the hormones measured were plasma prolactin, GH, FSH and LH.

The increments of plasma hormones were calculated by subtracting the basal levels from the average of those at specified times after injection. The times used for estimating the average peak response level as previously defined; 60, 90 and 120 minutes for growth hormone, 30 and 60 minutes for LH and FSH and 10, 20 and 30 minutes for prolactin.

3. Results

The basal levels and increments in hormone concentrations have been plotted for each patient before and after treatment, in Figs. 15 and 16.

The number of patients in each group is small so that real changes in increments of hormone concentrations may not be significant.

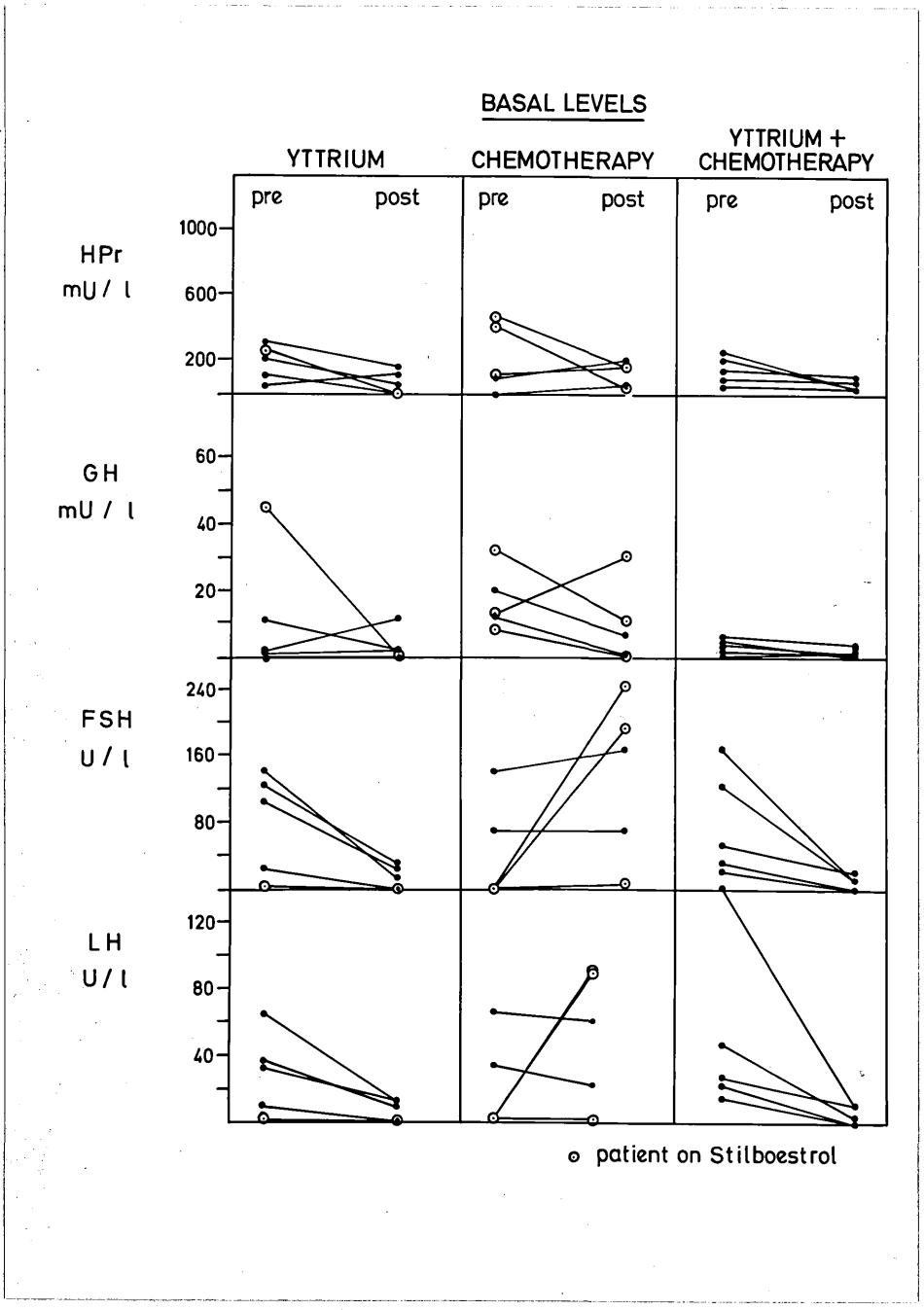


Fig. 15

Basal hormone levels pre and post ^{90}yttrium implantation, chemotherapy and ^{90}yttrium implantation and chemotherapy.

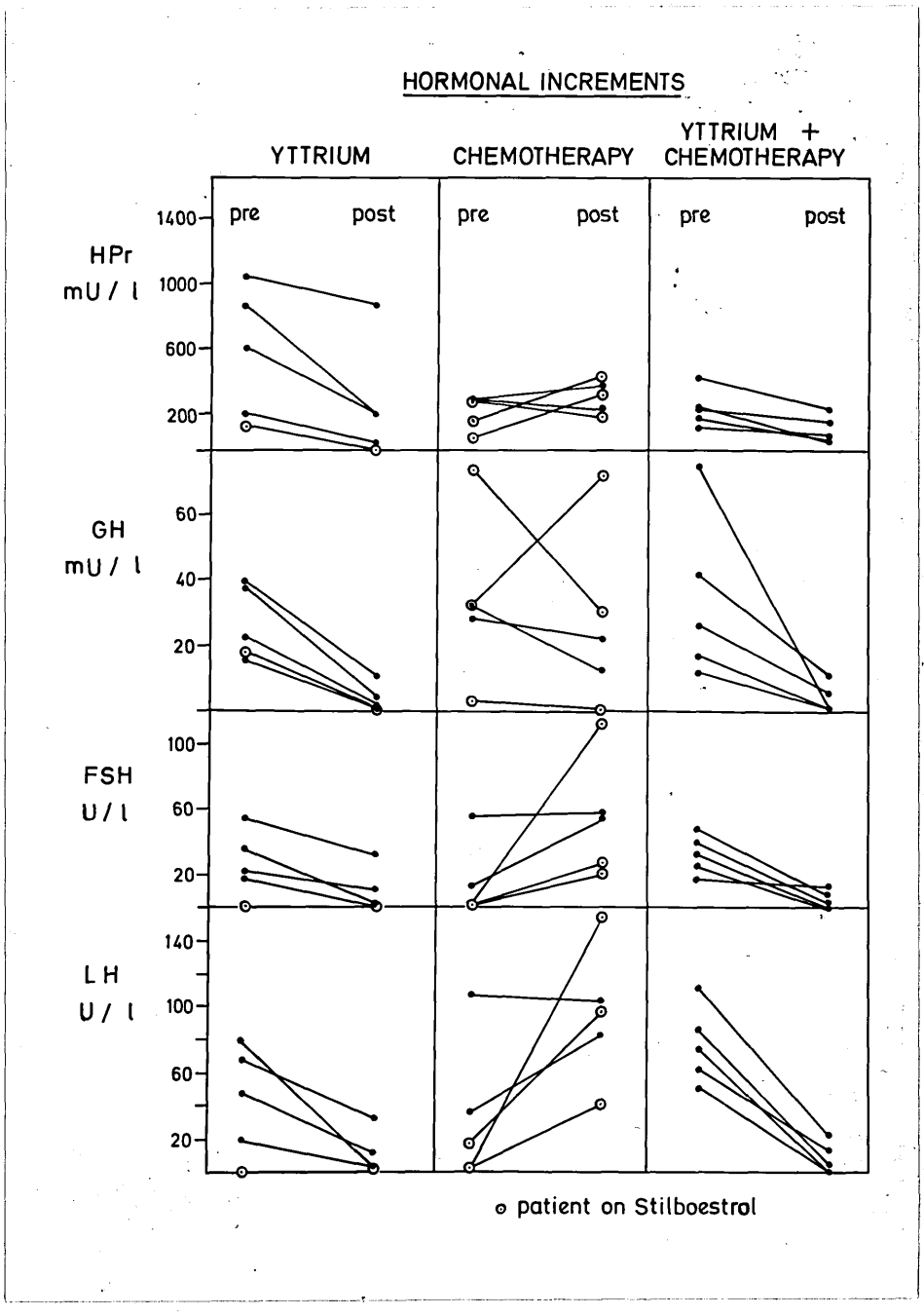


Fig. 16

Increments in hormone concentrations in response to LHRH, TRH and insulin pre and post ^{90}yttrium implantation, chemotherapy and ^{90}yttrium implantation and chemotherapy.

Basal levels of plasma LH and FSH were significantly lowered ($P<0.05$ Wilcoxon's signed rank test) after yttrium implantation with or without chemotherapy. Although the average basal levels of plasma prolactin and growth hormone levels were lower after these treatments, this barely reached statistical significance ($P>0.05<0.1$ Wilcoxon's signed rank test).

Patients who had an yttrium implant (either with or without chemotherapy) had significantly lowered increments for all hormones ($p<0.05$ Wilcoxon's signed rank test). No such reduction of hormone increments could be detected in those patients who received chemotherapy.

Three of the five patients on chemotherapy alone and one who had an yttrium implant, had taken stilboestrol in the month prior to chemotherapy, and this is likely to account for the suppression of LH and FSH basal levels and the reduced increments after stimulation prior to commencing chemotherapy or the performance of yttrium 90 implantation. The post-treatment values of all patients on chemotherapy, however, were not different from the pre-treatment values for all patients who were not on drug treatment. Nor did the addition of chemotherapy to yttrium implant cause any further suppression of the increments of hormonal concentrations (Wilcoxon's Rank Sum Test:NS)

4. Discussion

The present studies of the effects of multiple chemotherapy on pituitary function, using a combined stimulation test of LHRH, TRH and insulin, showed that chemotherapy (CMF) alone did not produce an effect on pituitary function. Furthermore, the administration of chemotherapy to patients treated by implantation of Y90 into the pituitary did not produce any changes in pituitary function additional to those observed by Y90 implantation alone. It should, however, be remembered that the number of patients in each group was small and it is just possible that the administration of stilboestrol prior to the test may have compromised the results. However there ws no evidence that this was so.

Although the present studies were carried out in postmenopausal women with advanced disease, some support for our conclusions can be derived from the work of Samaan et al (1978) who studied fifty-five premenopausal women with early breast cancer, of which thirty-eight became amenorrhoeic; eleven of these amenorrhoeic women were compared with eleven of the women who did not develop amenorrhoea. Basal plasma LH, FSH, oestradiol-17B and prolactin were measured before and after LHRH and TRH administration. The results showed that the amenorrhoea was a result of primary ovarian failure.

On the basis of previous studies of hormone levels, the following tentative conclusions can be drawn. In the premenopausal woman, it seems likely that chemotherapy has two sites of action: 1. Directly on the cell division in the tumour. 2. On the ovary. In

the postmenopausal woman, however, there may be possibly three sites of action: 1. The tumour. 2. The ovary. 3. The adrenal.

The reason for including the action on the ovary as a possible site in the postmenopausal woman is because a fall in plasma $\Delta 4$ androstenedione concentration could be due either to action on the adrenal or the ovary. The pituitary seems not to be involved except perhaps when prednisone (strictly a hormone rather than a cytotoxic drug) acts via the pituitary to alter adrenal function. These hypotheses can also be examined in the light of knowledge concerning the oestrogen receptor and response to chemotherapy. It is well established that oestrogen receptors relate to (1) a response to endocrine therapy (Croton et al, 1981) and (2) to prognosis (Knight et al, 1977; Cooke et al, 1980). However, the relationship to chemotherapy is less clear.

If in the premenopausal women chemotherapy acts by suppressing hormones, then one would expect mainly receptor-positive patients to benefit, leading to approximately 33% response rate. This is not so. Firstly, in advanced disease, chemotherapy is effective in approximately 55-60% of patients (Taylor et al, 1976; Helman et al, 1982). Secondly, despite varying evidence it would appear on balance that neither oestrogen receptor-positive nor oestrogen receptor-negative tumours respond preferentially to chemotherapy (Hawkins, Roberts and Forrest, 1980). This argues for the effects of chemotherapy being at least partially not related to ovarian suppression. Despite this, since patients with oestrogen receptor-

negative tumours are unlikely to benefit from adjuvant endocrine treatment, it may well be that patients with early disease, of poor prognosis, with oestrogen receptor-negative tumour, should still be selected for adjuvant chemotherapy in the absence of any suitable alternative (Croton et al, 1981).

5. Conclusion

A 28-day cycle of chemotherapy in patients with advanced breast disease does not produce an effect on pituitary function, nor does its addition to the yttrium 90 implantation procedure produce any additional effect over and above that seen for yttrium 90 implantation alone. It is concluded that these chemotherapeutic agents, therefore, probably act directly on the tumour, though the possibility of some ovarian action cannot be excluded.

C. Treatment with tamoxifen or stilboestrol

Stilboestrol had been used in the treatment of advanced breast cancer since the 1930's, while tamoxifen has been introduced more recently. The latter, ICI 46, 474 - the trans-isomer of L-(pB dimethylamino ethoxy phenyl 1.2 diphenyl but-1-ene) is a non-steroidal antioestrogen which has emerged as a hightly effective non-toxic drug for the treatment of women with advanced breast cancer. Early clinical studies indicated that benefit from tamoxifen therapy could occur in 30-40% of women with advanced breast cancer (Cole et al, 1972; Ward, 1973)

A remission rate of 38% in postmenopausal women with advanced breast cancer has been reported by Lipton et al (1982). A similar response rate to tamoxifen of 30% was found by Smith et al (1982) in a controlled randomised trial of women with advanced breast cancer who were given either tamoxifen or aminoglutethimide. In another series of one hundred and thirteen consecutive selected patients with Stage IV breast cancer, tamoxifen induced an objective remission in 50% lasting an average period of sixteen months. Tumour regression occurrred in visceral, osseous and soft tissue sites with equal frequencies. Patients with oestrogen receptor-positive tumours had a significantly higher remission rate (63%) than did those in whom oestrogen receptor measurements were not done (44%). Women who were more than ten years postmenopausal had a significantly higher remission rate 58% than did those who were less than ten years postmenopausal (41%) (Pearson et al, 1982).

More recently, and since the time this study now reported used tamoxifen, two controlled trials between tamoxifen and stilboestrol were initiated - one at the Mayo Clinic and the other in Edinburgh. Both studies showed that the two drugs were equal in efficacy, but since tamoxifen was less toxic it appeared to be the preferred agent (Stewart et al, 1980; Ingle et al, 1981).

Tamoxifen appears to compete with oestradiol for its cytoplasmic receptor, the oestrogen receptor (Furr et al, 1979). The precise mechanism by which the anti oestrogens antagonize oestradiol is not clear, but they appear to produce a receptor complex that it not

able to interact properly with the genome specific for the receptor-oestrogen complex (Edwards et al, 1979).

Patients with tumours which contained oestrogen receptors and those patients who had responded to previous hormonal manipulation tended to respond to tamoxifen (60% and 69% respectively) (Kiang et al, 1977). On the other hand, patients with receptor negative tumours or with a history of failure of response to previous hormonal treatments did not respond to tamoxifen therapy. It has also been reported from a review of two hundred and thirty five patients that 54% of these patients responded to tamoxifen when oestrogen receptors were present and only 14% responded when oestrogen receptors were not present. (Hawkins et al, 1980). Since this study was started, it has been reported that tamoxifen had no effect on plasma oestradiol-17B, testosterone or prolactin levels, but it reduced the concentration of plasma FSH (Golder et al, 1976; Willis et al, 1977; Wilking et al, 1982; Coombs et al, 1982).

The aim of this present study was to examine (a) the effect of therapy on circulating plasma hormone levels, and (b) if the clinical response was related to changes in plasma hormone levels.

2. Methods of Study

Thirty-five postmenopausal women with advanced breast cancer were studied within the Edinburgh trial. None had been given any previous hormone or other systemic therapy for the treatment of their disease.

Studies were carried out at the Combined Breast Clinic of the University Department of Clinical Surgery and the Department of Radiotherapy at the Royal Infirmary of Edinburgh. This clinic is specifically concerned with the conduct of therapeutic trials in patients with advanced cancer of the breast.

Following documentation of the state of the disease, patients were entered into a double blind crossover trial in which they received identical tablets of either tamoxifen (10mg.) or stilboestrol (5 mg.), one tablet being taken by mouth thrice daily. Prior to allocation, the patients were stratified according to the distribution of their recurrent or metastatic disease, the performance of a previous mastectomy and the recurrence-free interval.

Some patients, because of cardiac or respiratory problems, were considered to be unsuitable for treatment by stilboestrol. They were excluded from the trial but given elective tamoxifen therapy in the same dose as in the trial. As their pattern of hormonal changes did not differ from that of the patients included in the trial, they have been included with the patients given tamoxifen in the trial. Patients attended the clinic at monthly intervals when their response to therapy was assessed.

Documentation of the response was by the criteria advised by the British Breast Group (1974) and patients were classified as undergoing objective regression, an equivocal or intermediate

response, or no response at three and six months.

If no response occurred or when a good response had ended, administration of the drug was stopped for one month before the patient was started on a second agent. Some patients, therefore, received both drugs sequentially.

Before starting a drug, 10 ml. of blood was withdrawn from an arm vein. The blood was heparinised, centrifuged and stored as described in General Methods Section. Similar samples were taken at the same time of day at one, three and six months after starting therapy. The plasmas were sent in as batch to the laboratories and assayed for FSH, oestradiol-17B, testosterone and prolactin. The number of treatments on each drug is shown in Table XVII. Conduct of the trial allowed opportunity to study the effect of the drugs on plasma hormone levels in all patients receiving the treatment as first or second drugs; but analysis of the relationship of these hormonal changes to clinical response was restricted to those patients receiving the drug on their <u>first</u> treatment.

3. <u>Results</u>

Tables XVIII and XIX give the results of the concentrations of plasma hormones in those patients who received tamoxifen or stilboestrol. The median increase or decrease is shown for each hormone at one, three and six months after commencement of treatment with the significance of these changes.

TABLE XVII

	1st Drug No. Patients	2nd Drug No. Patients	Total No. 1st and 2nd Drug
Stilboesterol	11	5	16
Tamoxifen	15	3	18
Elective Tamoxifen	9	-	9

Treatment on Stilboesterol or Tamoxifen. Total number of treatments on each drug, given to 35 patients. Some patients had both drugs sequentially.

TABLE XVIII

	FSH U/1	Oestradiol-17B pmol/1	Testosterone nmol/1	Prolactin mU/1
Pretreatment				
n	27	26	25	26
median	137	452	1.39	200
range	55to+261	0to+603	0to+2.08	0to+450
1 Month				
n	24	24	24	25
median	-25	3.7	0	0.00
range	-72to+74	-426to+408	-1.25to+1.32	-420to+600
3 Months				
n	20	20	20	19
median	-33	37	0	-20
range	-188to+54	-441to+261	-1.18to+1.53	-370to+340
6 Months				
n	14	14	14	13
median	-33	3.7	0	-70
range	-178to+24	-99 to +198	-1.08to1.46	-300to+120
Significance WSRT*	Significant decrease at 1/12, 3/12&6/12	NS	NS	NS

WSRT - Wilcoxon signed rank test

n - number of patients

Changes in median plasma hormone concentration values and ranges on treatment with Tamoxifen as 1st or 2nd drug. Changes all refer to pretreatment values.

TABLE XIX

	FSH U/1	Oestradiol-17B pmol/1	Testosterone nmol/1	Prolactin mU/1
Pretreatment				
n	16	16	16	16
median	106	110	1.39	120
range	1to+208	14to+202	0.24to+3.23	70to+960
1 Month				
n	16	16	16	16
median	-105	3.7	0	300
range	-203to+1	-99to+673	-1.60to+2.22	-440to+580
3 Months				
n	8	8	8	8
median	-104	-18.4	0.03	310
range	-203to+2	-48to+44	-0.97to+0.80	-620to+780
6 Months				
n	3	3	3	3
median	-120	0	-0.24	130
range	-69to-145	0to+99	-0.28to+0.49	-570to+310
Significance WSRT*	Significant decrease at 1/12, 3/12&6/12	NS	NS	Significant increase at 1/12

* WSRT - Wilcoxon signed rank test

n - number of patients

Changes in median plasma hormone concentrations and ranges on treatment with stilboestrol as 1st or 2nd drug. Changes all refer to pretreatment values.

For clinical reasons, some of these patients were withdrawn from the hormone therapy before the six month period, and collection and analysis of blood samples was incomplete in others. The results at three and six months of drug therapy are therefore based on smaller numbers than those at one month, so at a later time, evidence may then be insufficient for even a considerable change in hormone level to be statistically significant. Patients compared at three and six months also over-represent those who responded to treatment, and this should be borne in mind when interpreting Tables XVIII and XIX.

Plasma prolactin level rose in eleven out of twenty-five patients treated with tamoxifen at one month, and in six out of nineteen patients at three months. In contrast, levels of plasma FSH fell in nineteen out of twenty-four patients at one month, and seventeen out of twenty patients at three months. Administration of tamoxifen therefore significantly suppressed the median circulating levels of FSH in the plasma at one, three and six months after administration. Tamoxifen did not change the plasma concentration of oestradiol-17β or testosterone. Stilboestrol was more effective than tamoxifen in depressing plasma FSH concentrations. Levels of FSH fell in fifteen out of sixteen patients at one month and seven out of eight at three months. No patient taking stilboestrol had plasma FSH levels more than 3 u/l above the limits of detection of the assay when on treatment. In addition, stilboestrol significantly increased plasma prolactin concentration (fifteen out of sixteen patients at one month, and seven out of eight patients at three months) but had no effect on plasma oestradiol-17B or

testosterone levels.

To relate these hormonal changes with clinical response to therapy, patients with a definite clinical response at three months and six months were selected and compared with those with no response at either time. All those patients with a short-lived or equivocal response, and those who were not fully assessed due to intolerance or other causes were excluded. The numbers of definite responders and non-responders to stilboestrol was insufficient for meaningful comparison to be made. Results for tamoxifen are shown in Table XX. No relationship was detected between the changes in hormone levels and the clinical response to treatment.

4. Discussion

The effect of tamoxifen and stilboestrol on basal plasma hormone levels has been studied.

The effect of stilboestrol

Stilboestrol (diethylstilboestrol) failed to have an effect on the plasma levels of testosterone or oestradiol 17B and these findings are in agreement with those of previous workers (Mahajan et al, 1978). However as was reported by Franchimont and his colleagues (1971), stilboestrol reduced the plasma FSH level. Plasma gonadotrophins are known to rise naturally at the menopause due to the fall in plasma oestrogen levels. Therefore the administration of oestrogen might be expected to cause a fall in plasma gonadotrophins due to the inhibition of the pituitary feedback mechanism. There

TABLE XX

Drug - Tamoxifen

Hormones		Responders			Non Responders			
		Median change	Ranges	n	Median change	Ranges	n	
FSH U/1	1 mth	-26	(-63→74)	6	-15	(71→26)	9	Responders vs non-responders on Rank Sum Test = Not Significant
	3 mths	-38	(-188→451)	6	-18	(-97→23)	7	
Oestradiol 17B pmol/1	1 mth	0	(-48→154)	6	-14.7	(-412→408)	9	
	3 mths	18.4	(-99→147)	6	7.4	(-441→261)	7	
Testosterone nmol/1	1 mth	-0.16	(-1.18→0.03)	6	0.14	(1.25→0.94)	9	
	3 mths	-0.17	(-1.18→0.03)	6	0	(-0.90→1.53)	7	
Prolactin mU/1	1 mth	90	(-110→580)	6	0.00	(-420→150)	9	
	3 mths	90	(-360→230)	6	-10	(-370→340)	7	

n = number of patients

Median changes with ranges in plasma hormone concentrations for responders and non-responders to Tamoxifen.

is ample evidence that this is so in experimental animals. It has been reported by Piacsek and Meites (1966) that oestrogen given for twenty-one days depressed hypothalamic luteinizing releasing hormones (LHRH) content in ovariectomised rats. The pituitaries of the ovariectomised rats showed a four-fold rise in LH concentration whereas oestrogen by injection prevented this increase. Their results also suggested that oophorectomy stimulates the release of hypothalamic LHRH more than its synthesis while systemic injections of oestrogen can inhibit both release and synthesis of LHRH. Oestrogen can act directly on the pituitary to stimulate LH release.

Yen and his colleagues (1974) in a study of five hypogonadal women, (three of whom were postmenopausal, one ovariectomised and one had gonadal dysgenesis) found oestrogens exert a direct negative feed-back action on pituitary gonadotrophins. A rapid increase in circulating oestradiol-17B concentration induced a marked diminution in pituitary responsiveness to LHRH.

In our studies stilboestrol raised the concentration of plasma prolactin. This has been reported by other workers. L'Hermite et al (1974) showed that prolactin secretion was increased in women with breast cancer treated with oestrogen. In animal studies in which oestrogen was given to rats, it has been shown that the oestrogens decrease hypothalamic prolactin inhibiting factor content and increase the release of prolactin (Meites 1972). There is a certain amount of evidence that oestrogens have a similar action in humans with prolactin.

The effect of tamoxifen

Tamoxifen was found in this present study to have no effect on plasma oestradiol-17B, testosterone or prolactin levels but it reduced the concentration of plasma FSH, though to a lesser extent than stilboestrol. These findings are in agreement with previous reports (Golder et al, 1976; Willis et al, 1977; Coombes et al, 1982; Wilking et al, 1982). The mode of action of tamoxifen must either be a direct effect on hormone synthesis or indirectly via oestrogen receptor. The mechanism of action of tamoxifen was originally studied mainly in animals. In rats, tamoxifen has been reported as a weak and atypical oestrogen with anti-oestrogen properties (it inhibits the effect of the response to exogenous oestrogen of the vaginal epithelium (cornification) and uterus (weight increase), and has little pituitary inhibitory activity. In mice, it is more oestrogenic when given in large doses than in rats (Harper and Walpole, 1967), but so far there is no convincing evidence that this is so in man. Therefore the mechanism by which FSH is suppressed by tamoxifen is still unknown.

Tamoxifen presumably behaves in man similarly to animals. It can be inferred from the work of Klopper and Hall (1971) that it has an effect on the hypothalamus in the human. In this respect it resembles its chemical relative, clomiphene - another substituted triphenyl ethylene compound which has been used to induce ovulation. Clomiphene has been shown in rats to exhibit a marked depression in the uptake of tritiated oestradiol-17B by the anterior hypophysis and a small depression of oestrogen uptake by the anterior

hypothalamus (Kato et al, 1968). This depression of uptake of (^{3}H) oestrogen indicates binding of the clomiphene with oestrogen receptor proteins as in other tissues. There is reason to believe that tamoxifen has a complex mode of action, but it appears to operate primarily through the oestrogen receptor system to inhibit the biological effect of oestrogen in the tumour cell (Nicholson, 1979). It competes with oestradiol at the cytoplasmic receptor sites. Tamoxifen receptor complexes are formed and translocated into the nucleus where it remains for prolonged periods and interferes with the regeneration of cytosol oestrogen receptors (Jordan et al, 1975; Nicholson et al, 1976; Clark et al, 1977).

In our studies both stilboestrol and tamoxifen lowered plasma FSH concentrations both in patients who responded to these agents and in those who did not. This is in agreement with other reports (Willis et al, 1977; Coombes et al, 1982; Wilking et al, 1982) but is in disagreement with the results of the study reported by Golder et al, 1976 who found that plasma FSH levels in those responding returned to pre-treatment levels in the second and third month of treatment. By contrast those in the non-responding group remained lower than in the pre-treatment value. A significant difference between plasma FSH levels in responders and non-responders was found during the second and third month of treatment by these workers.

The failure of patients to respond to tamoxifen and stilboestrol is thus not due to failure of the drugs to reduce plasma FSH levels. Changes in circulating hormones may only be indirectly involved.

5. Conclusion

Tamoxifen decreases plasma FSH levels in post-menopausal women with breast cancer at one, three and six months, but has no effect on prolactin levels. Stilboestrol has a more profound effect in suppressing FSH levels at one, three and six months and also increased prolactin levels. Neither drug affects plasma testosterone or 17B-oestradiol levels. These effects were present in patients with and without a clinical response to the drug. The effects of tamoxifen and stilboestrol may relate to the presence of oestrogen receptors and not necessarily to changes in plasma hormone levels.

Gynaecomastia

The breast in the male remains rudimentary, but in certain circumstances there is abnormal breast hyperplasia which is termed gynaecomastia.

1. Literature review

Aetiology of Gynaecomastia

The first recorded clinical description of gynaecomastia was that of Paulus Aeginate - the last of the Byzantine physicians who lived at the end of the 6th century AD. He wrote "As at the season of puberty, the breasts of females swell up. so in like manner those of males also swell to a certain extent, but in the most part they subside again. In some cases, however, having acquired a beginning they go on increasing owing to the formation of fat below."

During and after the Renaissance, gynaecomastia received the passing mention afforded to a medical curiosity. An interesting case of gynaecomastia with secretion from the nipples was described by Charleton in 1669. Later in that century, Diemerbroeck (1694) used knowledge of the occurrence of gynaecomastia in males as an argument against the view that lactation in females was due to the expression of menstruous blood, retained during pregnancy and the puerperium. Little appears to have been written on the subject of gynaecomastia for the next one hundred and fifty years. However, in 1848, Von Basedow described a man suffering from a severe degree of

thyrotoxicosis who showed gynaecomastia. In the first two volumes of the Annals of Surgery, three papers reporting cases of gynaecomastia were published - one of the patients then described was a boy whose mother had acromegaly. At about that time gynaecomastia was attributed to diseases, injury or atrophy of the testes or its excision (Williams 1894). It was noted that testicular atrophy was also seen in some cases of cirrhosis of the liver (Corda 1925). In 1926, Sylvestrini drew attention to an observation he had made in 1904 that gynaecomastia was sometimes seen in patients with cirrhosis of the liver - the triad of cirrhosis, testicular atrophy and gynaecomastia is sometimes referred to as the Sylvestrini-Corda syndrome. It was believed by Erdheim (1928) that in most cases, gynaecomastia was the primary condition, though in some cases the lesion was obviously secondary to its condition. A case of bilateral pubertal gynaecomastia was described by Richardson (1943) who suggested that the gynaecomastia, was due either to excessive production of oestrogens by the testes at puberty or due to an abnormally vigorous response on the part of the breasts to normal endocrine stimulation.

It is now recognised that there are many causes associated with gynaecomastia - a suggested classification as described by Hall (1959) is given in Table XXI.

TABLE XXI

THE CAUSES OF GYNAECOMASTIA (Hall, 1959)

1. Gynaecomastia associated with physiological states.
    (a) Neonatal gynaecomastia.
    (b) Essential gynaecomastia.
    (c) Involutional gynaecomastia.
2. Gynaecomastia due to underlying disease.
    A. Diseases of the endocrine glands.
        I. Diseases of the testes.
            (a) Prepubertal testicular failure.
            (b) Klinefelter's syndrome.
            (c) Leprosy.
            (d) Mumps.
            (e) Orchitis of unknown aetiology.
            (f) Tumours: Teratoma.
                Chorionepithelioma.
                Seminoma.
                Interstitial cell tumour.
                Sertoli cell tumour.
            (g) Radiation.
            (h) Undescended testes.
            (i) Castration.
            (j) Trauma.
            (k) Unilateral testicular lesions: Varicocoele;
                atrophy of unknown aetiology.

II. Diseases of the adrenal cortex.

(a) Tumour.

(b) Hyperplasia.

(c) Stress.

III. Diseases of the tyroid gland.

(a) Hyperthyroidism.

(b) Hypothyroidism.

IV. Diseases of the pituitary gland.

(a) Acromegaly.

(b) Chromophobe adenoma.

(c) Hypopituitarism.

V. Disorders of sex.

(a) Hermaphroditism: True

False.

(b) Transvestism.

VI. Miscellaneous.

(a) Following prostatectomy.

(b) Albright's syndrome.

(c) Diabetes mellitus.

(d) Cushing's syndrome.

B. Diseases of the liver.

I. Cirrhosis.

II. Hepatitis.

III. Carcinoma.

IV. Haemochromatosis.

C. Diseases of the alimentary canal.

Ulcerative colitis.

D. Malnutrition and renutrition.

E. Diseases of the central nervous system.

I. Traumatic paraplegia.

II. Friedreich's ataxia.

III. Syringomyelia.

IV. Dystrophia myotonica.

F. Pulmonary disease.

I. Bronchogenic carcinoma.

II. Tuberculosis.

III. Bronchiectasis.

IV. Empyema.

G. Drugs.

Oestrogens.

Androgens.

Chorionic gonadotrophin.

Adrenocortical hormones.

Digitalis.

Radioactive iodine.

Amphetamine.

CLASSIFICATION (amended)

From the clinical experience in the study later described it is now recognised that there are many causes of gynaecomastia. A simple classification is suggested as follows:

I Physiological (associated with physiological states)

1. neonatal gynaecomastia
2. pubertal gynaecomastia
3. gynaecomastia of 'old age'

II Due to:

a) Diseases of the testis:- eg.

1. tumours
2. Klinefelters syndrome
3. castration
4. undescended testes
5. unilateral testicular lesions - varicocoele

b) Diseases of the liver:- eg.

1. cirrhosis

c) Diseases of the pulmonary system:- eg.

1. bronchiectasis
2. bronchogenic carcinoma

d) Diseases of the endocrine system:- eg.

1. pituitary tumours
2. adrenal tumours
3. diabetes mellitus
4. thyrotoxicosis

e) Malnutrition and renutrition:-

refeeding prisoners of war

III Pharmacological

Gynaecomastia due to the following drugs:-

oestrogens, reserpine, mariuhana, Salbutamol, spironolactone, cimetidine, Paroven, androgens, alcohol.

1. Clinical Appearance of Gynaecomastia

At the time of puberty, 70% of boys show a palpable button-like nodule in the chest wall. The altered mammary gland appears as a mass or a nodule under the nipple. Occasionally it is attached to the nipple, but it is not adherent to other structures. The mass may become fairly large and appear as a more or less globular body (Karsner 1946). It is usually firm, smooth in outline and may be tender. (A typical picture is shown in Fig. 17 of a 21 year old man who had had gynaecomastia since the age of 14). During the process of growth which can go on for months, or even years, the enlargement is usually gradual, although there can be periods of interruption or accelerated growth. After it reaches a certain size, growth ceases. There are variable degrees of enlargement and projection of the nipple with widening of the areolar ; sometimes there is also increased pigmentation and prominence of the glands of Montgomery. Unilateral gynaecomastia is more frequent but bilateral involvement

Fig. 17

A photograph of a 21 year old man with right sided gynaecomastia of 7 years' duration.

can occur. Menville (1933) reported bilateral disease in 12.8% of eighty-eight cases, Geschickler (1941) found it bilateral in 20% of one hundred and eight cases, while Karsner (1946) found bilateral involvement in only 4.6% of two hundred and seventy-four cases. Such estimates may not be accurate as slight, or even moderate enlargement of a second breast may not be noticed by the patient or examiner. Mammographically, gynaecomastia is more often bilateral, even when clinically appears to be unilateral.

It is evident that gynaecomastia arising in early life may be a continuation or augmentation of the pubescent node, but when arising in later life, after the node can be assumed to have disappeared, the aetiology is presumably different.

2. Pathology of Gynaecomastia

Gynaecomastia breast tissue histologically and in its gross appearance closely resembles the mammary tissue of girls at the time of puberty (Goodman 1937). The microscopic appearance is a picture of growth of mammary ducts and of periductal stroma; lobule formation is absent. Karsner (1946) pointed out that the enlargement of the breast in gynaecomastia is chiefly due to a proliferation of connective tissue, and that adipose tissue, although present, does not contribute much to the size of the breast. Epithelial cell change also occurs. Ducts which are normally lined by one or at the most two cells, can in gynaecomastia become 5 - 6 cells thick. Budding of the ducts is sometimes seen either as narrow branching structures or as spherical projections

from the duct or its branched prolongations. Typical histology of gynaecomastia is shown in Fig. 18 (a,b and c).

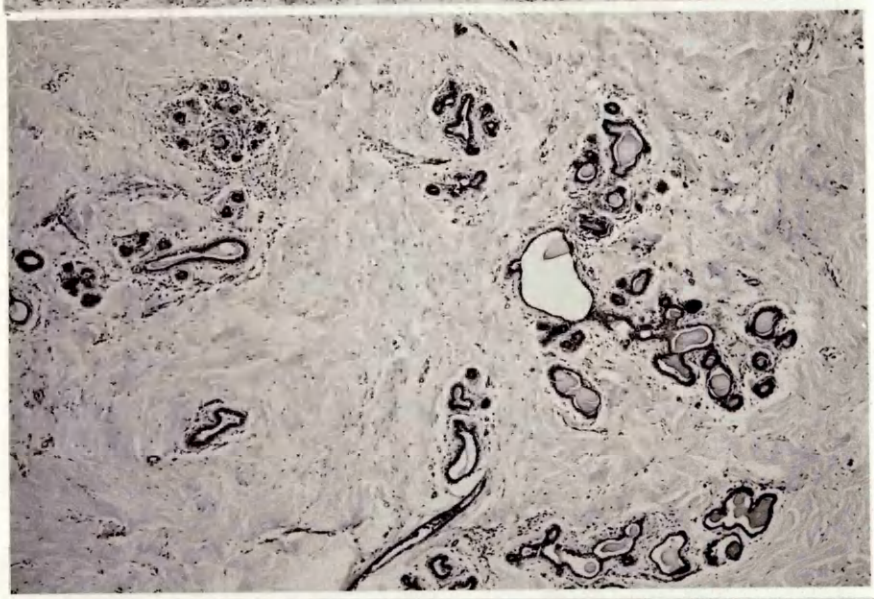
It was demonstrated by Karnauchow (1954) in twenty patients with gynaecomastia that the myo-epithelial cells of the breast in gynaecomastia also show proliferation. These cells lie within the basement membrane of the lactiferous ducts and occasionally proliferate to such an extent that they push their way into the lumen of the duct. He also found that myo-epithelial cells may proliferate alone or with the ordinary ductal epithelium to form benign, intraductal papillomatous growths. When regression with ductal atrophy occurred in gynaecomastia, the myo-epithelial cells persisted after the ordinary epithelial cells had vanished and remained as bizarre cell groupings in dense fibrous tissue. Alveoli such as are seen in the female breast do not appear in gynaecomastia, with one exception; small acini do develop in neonatal gynaecomastia, and may produce "witches milk". Although there are few reports of this, neonatal gynaecomastia in general resembles miniature lactating glands in microscopic appearance (Dietrich 1927).

3. Hormone levels in men with gynaecomastia

A hormone is a substance produced by an endocrine gland and secreted directly into the blood stream where it circulates in extremely low concentrations to have an effect at a distant target organ. In the past decade considerable progress has been achieved in the development of techniques for the assays of plasma hormones with the

a -

b -



c -

X 38

Fig. 18

Typical histology of gynaecomastia. Three different photomicrographs from the same breast.

sensitivity and specificity necessary for the measurement of normal levels of most blood-borne hormones.

As gynaecomastia is most frequently associated with either puberty (at which time there is great hormonal change), diseases of the endocrine glands, or hormone administration, it is natural to suppose that the underlying cause of breast disorder is hormonal imbalance. Even in gynaecomastia associated with other aetiological factors such as liver disease, malnutrition or drug administration the effect may be via a secondary action on hormone metabolism and excretion.

In animals at all stages of development, among the many factors that control mammary growth, hormones play a leading role (Lyons et al 1958). It has been shown that breast development in foetal male and female mice is different (Raynaud 1971). The mammary growth patterns in both male and female foetuses up to the 14th day are similar. After this, sex differences in breast development occur. In the male mouse foetus, there is no nipple formation and in the adult male mouse, the mammary gland has no connection to the skin and no nipples. However, early gonadectomy (by localised irradiation of the mouse foetus by X-rays at 13 days) prevents the destruction of the primary duct in the foetal male mouse and leaves the glands connected to the skin in the adult and the newborns, regardless of their genetic sex, have mammary glands similar to those mammary glands in females (Raynaud 1971). The effects of castration of male foetuses demonstrate that it is the secretion of

the foetal testis which is responsible for the male pattern of development of the mammary gland. In the early stage of mammary rudiment development, growth is slow and is uninfluenced by pituitary hormones but is affected by ovarian or testicular hormones. Later growth is rapid and is affected by pituitary hormones but resists ovarian or testicular hormones (Raynaud 1971; Ceriani 1974).

Male rats feminised prenatally by an anti-androgen develop nipples, teat ducts, a complete gland system, main milk ducts and mammary glands. It seems likely that the androgens of the male foetus inhibit the development of the mammary glands and that in the male rat foetuses, the anti-androgen prevents endogenous testosterone from producing the incomplete organogensis with athelia and without adnexa that normally occurs in the male mamma (Neuman et al 1966 a,b). Hormones are also involved in post-pubertal growth. It has been shown in rats and mice, in which hypophysectomy, adrenalectomy and oophorectomy has been carried out, that no single hormone has been found capable of inducing complete ductal mammogenesis and at least five hormones play an important part in mammogenesis and lactation. LH and FSH stimulate the ovary to produce oestrogen, while corticoids (under the influence of ACTH) are required for inducing ductal growth. Prolactin and progesterone are necessary as well, for full lobulo-alveolar development (Lyons 1958; Nandi 1958).

No similar studies are available in humans. It seems likely that hormones must play a part in male breast development, and that men may have the potential for full mammary gland development. Research has therefore been focussed on determining individual hormone values in patients with gynaecomastia.

i) Oestrogens and the male breast

The changes produced by oestrogens in man are almost identical with those found in gynaecomastia due to other causes. As early as 1940, Dunn reported gynaecomastia occurring in men taking 5 mg. stilboestrol orally daily for two months, while Fitzsimmon (1944) reported twenty-eight cases of gynaecomastia out of thirty-eight men who were employed in the manufacture of stilboestrol. In 1945, Moore et al, described the biopsy findings of the enlarged breasts of men on stilboestrol for carcinoma of the prostrate. However, no hormone measurements were carried out at that time as satisfactory assays for plasma hormones were not available.

The most important circulating oestrogens in the male are oestradiol-17B and oestrone. The formulae for these compounds are shown in Table I. These circulating plasma oestrogens in the male arise from testicular secretion, adrenal secretation and peripheral conversion of androgens to oestrogens (Van Thiel et al 1975). Males are, in fact, capable of greater peripheral aromatization of androgens to oestrogens than menstruating females and the conversion of $\Delta 4$ androstenedione to oestrone and of testosterone to oestradiol-17B is a highly significant source of oestrogens in man (Longscope

et al 1969). In normal men, approximately 80% of the oestrogens are derived from peripheral metabolism of circulating androgens (Kirschner 1972). It has been suggested that in the adult, peripheral aromatization of C_{19} steroids takes place partially in the liver (Smuk and Schwers 1977). Similarly, changes in rates of catabolism of oestrogens (eg. in liver disease) might indirectly lead to changes in androgen secretion rate and therefore influence oestrogen production.

A study of the urinary excretion of oestriol, oestrone and oestradiol-17B in pubertal boys with and without gynaecomastia concluded that gynaecomastia of puberty was not associated with an increased concentration of urinary oestrogens (Jull et al 1964). A similar study was carried out at the same time in older men. In this group, the results of analysis of urinary oestrogen levels in the gynaecomastia group were variable but there was no conclusive evidence of increased concentrations of urinary oestrogen in the group as a whole.

Normal oestrone levels in patients with gynaecomastia were also reported by Hall (1959), though he found high urinary oestrogen values in patients with thyrotoxicosis - a condition often associated with gynaecomastia; Larsson and Sundborn (1963) confirmed the existence of elevated urinary oestrogens in patients with thyrotoxicosis and gynaecomastia; others have reported raised oestrogen in the urine of gynaecomatic males. For example, Okano et al (1963) found urinary oestrogens increased in five patients with

gynaecomastia, while in a large series of patients with gynaecomastia, Rupp et al (1951) found thirteen out of twenty-eight patients with gynaecomastia to have increased urinary oestrogen levels.

Therefore, studies of urinary oestrogen levels in gynaecomastia have yielded inconsistent results with normal oestrogen values in some studies - and elevated values in other.

More recently investigators have turned to study the oestrogens circulating in blood. One of the first studies of serum oestrogens in gynaecomastia was reported by Goldfine (1971) who described a single case of a 14-year old boy who developed spectacular gynaecomastia in association with pubescent growth and rapid sex maturation. Amongst several hormones which were abnormal the plasma oestradiol-17B concentration was elevated.

Plasma oestradiol-17B levels were also studied in sixteen boys with pubertal gynaecomastia, by La Franchi et al (1975). They found that six of the boys had elevated oestradiol-17B levels. However, Bidlingmaier et al (1973) found that in twenty-nine patients with pubertal gynaecomastia the plasma oestradiol-17B and oestrone levels did not differ from normal, though some individual values were slightly above the normal range. In a report by Korenman (1969), using a radioligand binding assay (employing rabbit uterine receptors as binding protein) unconjugated oestradiol-17B was measured in normal men, in seven men with severe cirrhosis in the

presence or absence of gynaecomastia and in gynaecomastia of non-cirrhotic aetiology. He found that the levels of circulating oestrogens were elevated in cirrhosis and, also, gynaecomastia per se was associated with increased mean plasma oestradiol-17B level.

A moderately raised plasma oestrogen concentration in one case of gynaecomastia which had developed after mild infective hepatitis was found by Anderson et al (1972). It was suggested that increased sex hormone binding globulin (SHBG) also associated with liver disease, may have caused the rise in oestradiol-17B which is bound by the sex hormone binding protein. Elevated SHBG levels had also been found in thyrotoxicosis, a condition associated with gynaecomastia (Crepy 1967; Chopra and Tulchinsky 1974). In most cases, the gynaecomastia appeared during hyperthyroidism and receded when the patient became euthyroid. It was proposed that alterations in balance between circulating oestrogen and unbound androgen might play a significant role in the genesis of gynaecomastia (Chopra and Tulchinsky 1974). It has been demonstrated that thyroid hormone alters the metabolism of oestradiol-16-14^{C}-in vivo in human subjects, resulting in decreased conversion of oestradiol-17B to oestriol and increased conversion to 2-methoxyoestrone (Fishman et al 1962).

In another study of gynaecomastia and cirrhosis, Chopra et al (1973) measured oestradiol-17B concentrations by radioimmunoassay in a group of thirteen patients with chronic hepatic cirrhosis, six of whom had gynaecomastia. The serum oestradiol-17B concentration was

elevated in eight of the thirteen patients. (Only one of these eight had gynaecomastia), and the mean value was significantly higher than that of normal men. The percentage of oestradiol-17B that was dialyzable (percentage unbound oestradiol) in the sera of men with cirrhosis averaged 20% higher than that in the sera from normal men. The concentration of unbound oestradiol-17B in serum (Percentage unbound x total oestrogen concentration) was elevated in eleven of the thirteen patients. However, these differences in serum concentration of the total and unbound oestradiol-17B between patients with and without gynaecomastia were not statistically significant. It was concluded that in some of cirrhotic patients, the tissues might be perfused with supra normal concentrations of unbound oestradiol-17B. These workers also found low levels of serum testosterone (which is described later) and found the oestradiol-17B/testosterone (E_2/T) ratio in the patients with gynaecomastia was higher, although not significantly so. They concluded that both oestrogens and androgens may be involved in the development of gynaecomastia.

Plasma oestrone levels were measured by radioimmunoassay in fifty men with chronic alcoholism and varying degrees of alcoholic liver disease, seven of whom had gynaecomastia (Van Thiel et al 1975). It was found that the plasma oestrone levels in these seven men with gynaecomastia was significantly higher than that found in those who did not have gynaecomastia. They concluded that chronically alcoholic men had an absolute increase in plasma oestrone and this may provide an explanation for the development of signs of

feminization, eg. gynaecomastia and spider angiomata, which are common in alcoholism.

In summary studies of plasma oestrogens suggest that these may be raised or normal in gynaecomastia. The SHBG concentrations are elevated and there is often an elevated oestradiol-17B/testosterone ratio.

ii) Androgens and Gynaecomastia

In gynaecomastia most of the emphasis on hormonal abnormalities has been placed on the oestrogens, but in many studies androgens have also been measured. The results of these studies are summarised below.

The most active and abundant naturally occurring androgens in the male are 5 α dihydrotestosterone, testosterone, $\Delta 4$ androstenedione and dehydroepiandrosterone sulphate. Testosterone and 5 α dihydrotestosterone are strongly bound to plasma globulins while $\Delta 4$ androstenedione and dehydroepiandrosterone sulphate are weakly bound. All except 5 α dihydrotestosterone can be converted to oestrogens in the male.

5 α dihydrotestosterone, testosterone and $\Delta 4$ androstenedione are produced mainly by the testes, but some testosterone and $\Delta 4$ androstenedione are also produced by the adrenals while dehydroepiandrosterone sulphate is almost exclusively andrenal-derived, ie. dehydroepiandrosterone is produced by the adrenal and converted

peripherally to dehydroepiandrosterone sulphate.

It has been reported that the plasma testosterone levels in a 14-year old boy with gynaecomastia and pubescent growth with rapid sexual maturation, were consistently above the range observed in normal men and other pubertal boys (Goldfine et al 1971). These authors concluded that the gynaecomastia in this case was the result of elevated levels of plasma testosterone. This hyperleydigism, as it is called, was explained on the basis of a failure of the negative hypothalamic pituitary feedback system to respond appropriately to circulating testosterone and oestradiol-17B.

Plasma testosterone and oestradiol-17B concentrations were measured by La Franchi et al (1975) in sixteen pubertal patients with gynaecomastia. Eleven of the sixteen had elevated oestradiol-17B/testosterone ratios. These authors concluded that these increased ratios played a causative role in most cases of pubertal gynaecomastia.

24-hour profiles of plasma oestrogens and androgens levels in males at puberty (eleven) with gynaecomastia and eight without gynaecomastia were studied (Large and Anderson 1979) who found that there was no difference between the 24 hour plasma testosterone and $\Delta 4$ androstenedione profiles of pubertal boys and those boys with gynaecomastia. The major findings in male puberty was that levels of plasma oestradiol-17B and to a lesser extent, of oestrone were high relative to testosterone levels for prolonged period of the

afternoon and evening (when testosterone levels were lowest). A finding (seen only in the boys with gynaecomastia) was that of elevated and markedly fluctuating levels of plasma oestradiol-17B. There was an absolute increase in the area under the 24 hour oestradiol-17B. These authors concluded that normal men require sustained adult circulating testosterone levels to prevent their oestrogens from stimulating breast development.

Earlier, Anderson (1972) had measured plasma oestradiol-17B and testosterone concentrations in a patient with mild jaundice attributed to infective hepatitis. The patient subsequently developed gynaecomastia and Anderson found plasma testosterone levels to be high with oestradiol-17B values high or normal, though the concentration of unbound testosterone was low. He suggested that an increase of hepatic serum hormone binding globulin (SHBG) production following the attack of jaundice resulted in a fall in unbound testosterone. In turn this would stimulate LH release to the plasma and, consequently, increased Leydig cell activity, resulting in androgen production until the free testosterone levels (monitored by the hypothalamus) returned to normal.

Plasma testosterone levels were measured by radioimmnoassay in thirteen patients with cirrhosis, six of whom had gynaecomastia (Chopra et al 1973). It was found that the serum testosterone levels were below the normal range in nine out of the thirteen men with cirrhosis and that the mean value was significantly lower than that found in normal men of comparable age. The percentage of

serum testosterone which was dialyzable (ie. unbound fraction) in cirrhotic men was similar to that in normal men, but the concentration of unbound testosterone (total testosterone concentration x dialyzable fraction) was below normal in twelve out of thirteen patients. The serum concentrations of total and unbound testosterone in the six patients with gynaecomastia were not significantly different, however, from those patients with cirrhosis without gynaecomastia. The authors concluded that it was not the oestrogens or androgens on their own which were the main abnormality, but it was a ratio of the two which was important. Thus, the mean oestradiol-17B/testosterone ratio in patients with cirrhosis was significantly higher than that in the normal men and was also further elevated in the presence of gynaecomastia, although this difference was not statistically different. They concluded that the lack of a clear relationship between an elevated oestradiol-17B/testosterone ratio and the occurrence of gynaecomastia in cirrhotic patients suggests that the development of gynaecomastia in cirrhosis may be related to factors in addition to oestrogen androgen imbalance, but these are obscure.

Plasma testosterone levels were also measured in fifteen patients with thyrotoxicosis, four of whom had gynaecomastia (Chopra et al 1974). The mean serum testosterone level was significantly raised in those patients with thyrotoxicosis as compared to the mean value for normal men, but the oestradiol-17B/testosterone ration did not differ from that in normal men. However, the mean ratio of unbound oestradiol-17B to unbound testosterone in the hyperthyroid men was

significantly higher than in normal men. The values of serum testosterone, dihydrotestosterone and unbound testosterone in the presence of gynaecomastia did not differ from those without gynaecomastia. Chopra and his colleagues proposed that it was the alteration in the balance between circulating unbound oestrogen and unbound androgen which might play a significant role in the genesis of gynaecomastia, particularly in hyperthyroidism.

More recently, Aiman et al (1980) reported studies of oestradiol-17B and testosterone secretion rates in three cases of gynaecomastia after testicular atrophy due to mumps orchitis. At the time of study, the ages of the men varied from 63-68 years. The mean plasma production rate of testosterone was 20% of the value found in normal elderly men without gynaecomastia, while the mean production rates of oestradiol-17B and oestrone were comparable to those of normal young men. Plasma gonadotrophins were elevated due to decreased testosterone production. Conclusions were that the capacity of Leydig cells to secrete testosterone was impaired after mumps, but the capacity to form oestrogen was not similarly impaired, since most oestrogen is formed in extraglandular sites. Therefore, there was a reduction in the ratio of testosterone to oestrogen production rates and gynaecomastia resulted.

In summary, plasma androgen concentrations in gynaecomastia are variable while the plasma oestradiol-17B/testosterone ratio is frequently elevated and the balance between circulating unbound oestrogen and unbound androgen is important.

iii) Gonadotrophins and gynaecomastia

The above considerations lead to review of the findings related to gonadotrophin concentrations in gynaecomastia. Immunoassay estimation of gonadotrophins only became available in the early 1970's and so the levels of gonadotrophins have been measured in a small series of patients with gynaecomastia and the results of gonadotrophin estimates in the condition are conflicting.

Normal values for plasma LH and FSH, using a radioimmunoassay, were reported by Goldfine (1971) in a 14-year old boy who developed spectacular gynaecomastia associated with pubescent growth and rapid sexual maturation. Lee (1973) studied a large number of normal children and some with endocrine abnormalities. It was found that the plasma LH and FSH concentrations, using a double antibody radioimmunoassay technique, were within normal limits in six boys with gynaecomastia. Normal concentrations of plasma LH and FSH again using radioimmunoassay, were reported by La Franchi et al (1975) in sixteen boys with pubertal gynaecomastia.

In a report of men with gynaecomastia associated with chronic haemodialysis, the plasma LH and FSH levels, - measured by radioimmunoassay - were studied in eight patients on haemodialysis without gynaecomastia and compared with seven men with gynaecomastia (Swerdloff et al 1970). The presence of gynaecomastia was not associated with elevated levels of plasma oestradiol-17B, testosterone or FSH but all patients with gynaecomastia had increased plasma LH concentrations.

In another group of five men with gynaecomastia, which had developed during haemodialysis for chronic renal failure plasma LH and FSH were measured using a radioimmmonassay. All patients had elevated plasma LH levels and four also had elevated plasma FHS levels (Sawin et al 1973). Whilst the cause of gynaecomastia was not clear these workers thought it was more likely to be related to elevation of plasma LH or plasma FSH or both, than to be due to increased plasma oestradiol-17B or oestradiol-17B/testosterone ratio.

In another study, however, the plasma LH and FSH concentrations, using a radioimmunoassay. were reported to be low in one patient with gynaecomastia of two and a half years duration (Smith, 1971).

In another case of gynaecomastia following infective hepatitis Anderson (1972) found that the plasma LH levels were persistently higher, or at the upper limit of normal for an adult male. He thought the elevated levels were associated with a low plasma testosterone level which stimulated the LH production.

In a study by Chopra (1973) it was found that the serum concentration of LH was elevated in seven out of thirteen patients with cirrhosis, but serum FSH levels were normal. The method of estimation was by radioimmunoassay. However, the serum LH levels in the six patients with gynaecomastia and cirrhosis were not statistically different from the plasma LH levels in seven patients who did not have gynaecomastia.

From these conflicting results, the role of gonadotrophins in intitiating gynaecomastia must still be in doubt, although in some groups with particular aetiology, for example, gynaecomastia commomly associated with renal dialysis, plasma LH and FSH appear to be consistently elevated.

iv) Prolactin and gynaecomastia

In a recent comprehensive study, Large et al (1980) carried out 24-hour profiles of circulating prolactin levels in eight boys with delayed puberty, eleven boys with gynaecomastia and two normal adult men. The mean 24-hour plasma prolaction levels in the four boys with delayed puberty and in ten boys with gynaecomastia exceeded the mean levels for the two adult men. Boys with gynaecomastia had higher 24-hour mean levels of plasma prolactin, higher day time levels and higher sleep associated levels than did control subjects. The levels were not related to the degree of duration of gynaecomastia, but 24-hour mean plasma levels of prolactin and oestradiol-17B were positively correlated. The authors believed that oestrogen androgen imbalance during the day time was the major cause of gynaecomastia, with hyper-prolactinaemia sometimes occurring, as a response to relative hyperoestrogenaemia.

Using a bioassay, Turkington (1972) examined the plasma prolactin levels in twenty-nine adult patients with gynaecomastia associated with a variety of clinical conditions. The results showed that the prolactin levels were normal in all the patients. These results failed to provide evidence that prolactin was aetiologically related

to the development of gynaecomastia.

In another study plasma prolactin levels were measured by an immunoassay in nineteen patients undergoing chronic haemodialysis (Nagel et al, 1973). Of these nineteen patients, eleven had gynaecomastia. The plasma prolactin concentration was elevated in seven of the patients, but these elevations did not correlate with the presence of gynaecomastia:plasma prolactin concentration was increased in four of the eight patients without gynaecomastia and only in three of the eleven with gynaecomastia.

In contrast, Van Thiel and others (1975) measured the plasma prolactin levels by radioimmunoassay in fifty men with chronic alcoholism and varying degrees of alcoholic liver disease. Seven patients had gynaecomastia and these seven had plasma prolactin levels significantly greater than the values for men without gynaecomastia.

In summary, plasma prolactin concentrations have been found to be normal or elevated in gynaecomastia. The elevation may be a secondary phenomenon to increased oestrogen levels.

Results from all these studies suggest that in gynaecomastia plasma hormone results are variable. The plasma oestradiol-17B/testosterone ratio is frequently elevated. The plasma gonadotrophins may be elevated or normal and plasma prolactin may also be elevated or normal.

It must be emphasised that in many of these reported studies the number of patients investigated were few. One of the reasons for results being variable may be the varied aetiology of gynaecomastia and it may well be that more meaningful interpretation of these studies may be obtained by categorising the results into different aetiologies.

While some conditions associated with gynaecomastia are not directly connected with an endocrine abnormality, hormones are probably associated with most types of gynaecomastia. Most conditions associated with gynaecomastia are diseases of endocrine organs, or with the administration of steroid hormones, eg. oestrogens or drugs which may resemble or release endogenous hormones. The occurrence of gynaecomastia at birth, puberty and old age in many otherwise healthy subjects is probably related to some hormone imbalance at these times.

With the availability of radioimmunoassays, hormone analysis can now be done on small amounts of blood taken sequentially and the concentrations of several hormones can be studied simultaneously. This is the basis for the present study of plasma hormones in gynaecomastia.

2. Methods of Study

PATIENTS

Between 1972 and 1975, fifty-five patients with gynaecomastia were referred for an opinion at the breast clinic and these were all

studied in a similar way. Four patients were not included in the plasma studies, as three of these patients did not have clinical or mammographis evidence of breast tissue and one further man had Klinefelter's syndrome and was excluded. None of the patients studied were suspected of having hormone-secreting tumours.

For each patient a standard questionnaire was completed which documented the age of onset of the breast swelling, length of history of the condition, the breast involved, the presence of breast pain or secretion, shaving habits, drug history, number of children, sexual function and occupation.

Clinical examination included careful palpation of the testes, the abdomen and both breasts. Presence of acne was also sought.

In younger boys a button-like nodule was usually easily detected and there was normally no confusion with adipose tissue. This was not the case in older men in whom the distinction was more difficult.

All patients aged twenty years and over had mammography carried out. Only when this confirmed the presence of breast tissue was a patient considered to have gynaecomastia. A mammogram typical of gynaecomastia is shown in Fig. 19. All men over twenty years also had X-rays of chest, skull and abdomen to exclude tumours of lung, brain and adrenals. Younger men did not have these X-rays routinely so as to avoid radiation.

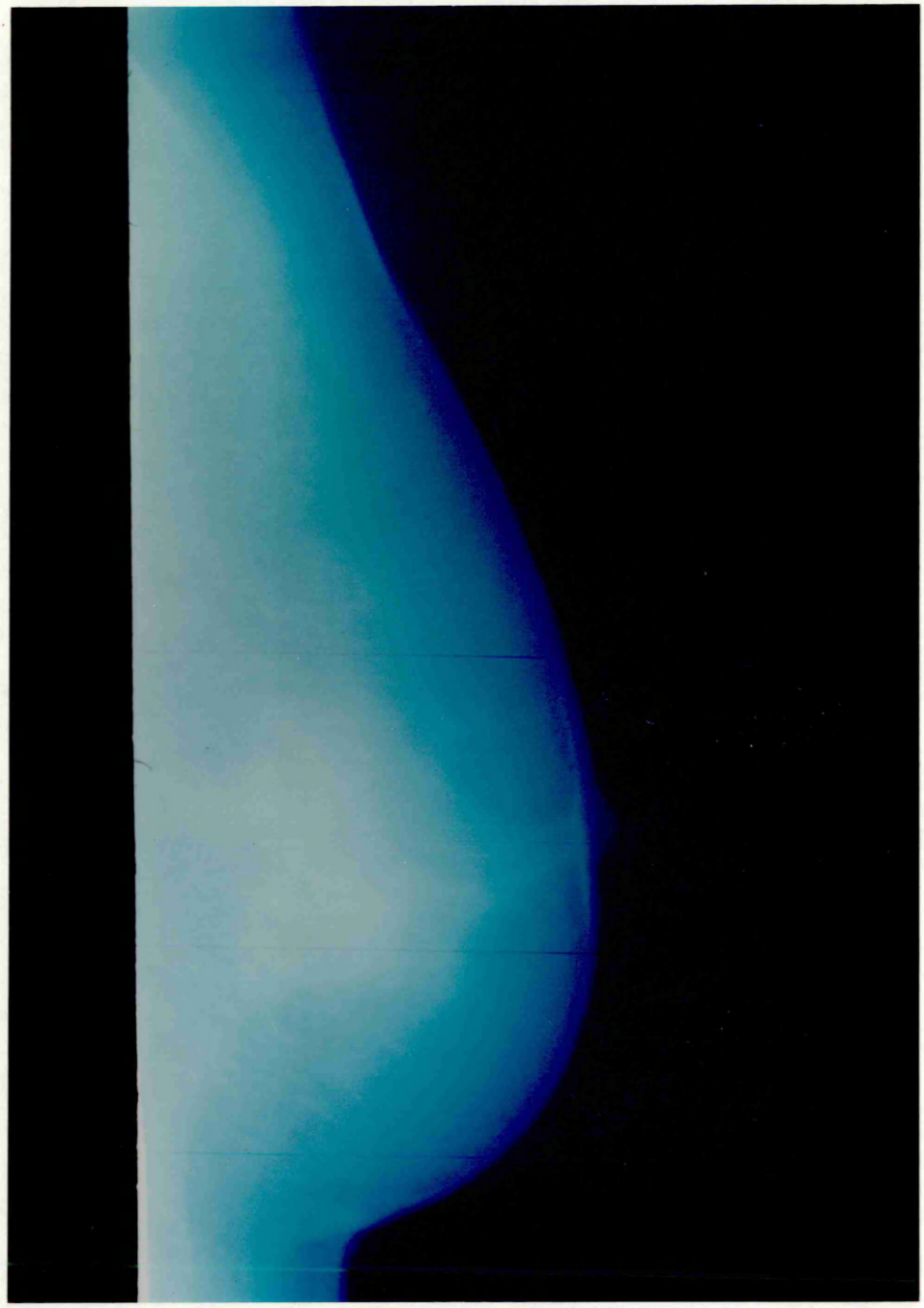


Fig. 19

Mammogram of a patient with gynaecomastia.

Laboratory investigations were performed in all patients. These included measurement of haemoglobin, protein-bound iodine or plasma thyroxine to exclude hyperthyroidism or hypothyroidism, blood sugar concentration, (random sample) - to exclude diabetes mellitus, plasma cortisol concentration and liver function tests including measurement of serum bilirubin, SGOT, SGPT, lactic dehydrogenase and plasma proteins (to exclude liver disease) to delineate factors in gynaecomastia.

Age

The median age of the total group was 26 years - range 14-78 years - and in most cases the aetiology of the gynaecomastia was unknown (see later). The group was arbitrarily divided into two - young and old, 50 years of age being taken to be the dividing age (50 years and above being included in the older group). In the young group, thirty-six men were studied, median age 25 years, twenty-five of whom were at or near puberty, the remainder being over 20 years of age. There were fifteen men over 50 years, median age 65 years - range 54-78 years.

Controls

These comprised twenty-eight normal older men who had no known history of endocrine disease and were not taking drugs of any kind. They were all ex-patients from a surgical ward who had had minor surgery more than one year prior to the study.

The young controls were seven normal men from the Immunoassay Section Hormone Laboratory. All were healthy men with no history of endocrine disease and none were taking drugs. The median range of ages are shown in Tables XXII and XXIII.

Results

(a) General and histological appearance

In the group of fifty-one patients, fourteen had right sided gynaecomastia, twenty-four had left sided and thirteen men had bilateral disease. Gynaecomastia was of longer duration in patients with bilateral disease (an average of 4 years 3 months) as compared with unilateral gynaecomastia (1 year 8 months).

Two typical cases are shown in Figures 20 and 21 and the course summarised in the legends. Figure 20 shows the typical external appearance of gynaecomastia as seen in a 25 year old man who had had gynaecomastia for two months. Figure 21 is of a 14 year old boy who three years previously had had breast tissue removed - the old scar is seen. The swelling had recurred two months prior to this picture. In patient Number 1 the swelling subsided and no operation was necessary, but patient Number 2 had a mastectomy because of breast discomfort.

Thirty-one patients had breast tissue removed by surgery under general anaesthesia. This was sent for routine pathology and in all cases, gynaecomastia was confirmed histologically on H and E paraffin section. Figure 22 is a photomicrograph of breast tissue

TABLE XXII

	Gynaecomastia under 50 years n = 36		Controls under 50 years n = 7			
	Mean	± SD or range	Mean	± SD or range	t	p
	Median Age		Median Age			
	25	14 - 46	28	22 - 47		
Log LH LH	1.62 5.04	±0.62 2.7-9.4	1.97 7.20	±0.04 6.9-7.5	1.63	NS
Log FSH FSH	1.29 3.63	±0.5 2.2-6.0	1.20 3.32	±0.63 1.8-6.2	0.42	NS
Oestradiol 17B	92.0	±30.9	98.3	±27.6	-	-
Testosterone	22.0	±8.9	-	10.4-35 NR	-	-
Prolactin	120	±60	-	0-600 NR	-	-

LH - expressed in U/l
FSH - expressed in U/l
Oestradiol 17b - expressed in pmol/l
Testosterone - expressed in nmol/l
Prolactin - expressed in mU/l
NR = normal range
NS = not significant

Mean plasma concentrations ± SD or range for each hormone from a single blood sample in younger men (under 50 years) with or without gynaecomastia.

TABLE XXIII

	Gynaecomastia 50 years and over n = 15		Controls 50 years and over n = 28			
	Mean	± SD or range	Mean	± SD or range	t	p
	Median Age		Median Age			
	65	54 - 78	70	61 - 86		
Log LH LH	2.69 14.80	±0.61 8.0-27.1	1.84 6.31	±0.58 3.5-11.2	4.61	<0.001
Log FSH FSH	2.62 13.74	±0.85 6.2-32.1	2.03 7.61	±0.64 4.0-14.4	2.61	<0.02
Oestradiol 17B	143.1	±43.4	111.9	±63.7	4.20	<0.001
Testosterone	22.7	±10.1	21.6	±7.3	0.33	NS
Prolactin	140	±20	-	0-600 NR	-	-

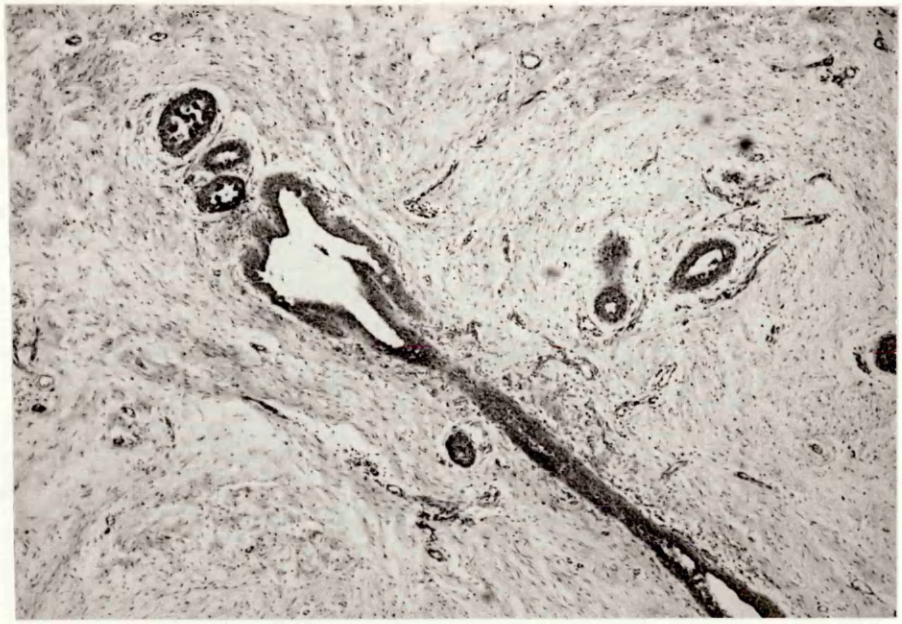
LH - expressed in U/1
FSH - expressed in U/1
Oestradiol 17B - expressed in pmol/1
Testosterone - expressed in nmol/1
Prolactin expressed in mU/1
NR = normal range
NS = not significant

Mean plasma concentrations ± SD or range for each hormone from a single blood sample in older men (50 years and over) with and without gynaecomastia.

Fig. 20

Patient No. 1

A photograph of a 25 year old man with right sided gynaecomastia of 2 months' duration.



X 38

Fig. 22

Photomicrograph of breast tissue removed from patient in Fig. 21.

removed at surgery from patient Number 2. This shows the typical picture of gynaecomastia with elongated dilated ducts associated with stromal hyperplasia.

Past History

Associated disease was found in ten patients with gynaecomastia and in nine patients there was a history of treatment with drugs. The details are detailed in Table XXIV.

Routine Screening Test Results

Liver function tests, including the measurement of serum bilirubin, SGPT, SGOT and alkaline phosphatase level and protein bound iodine or plasma thyroxine were normal in all patients. No case of diabetes mellitus was found. None of the patients were anaemic and none had abnormal changes in diurnal plasma cortisol levels. All X-rays of chest, skull and abdomen were normal.

Plasma Hormones in Single Samples

The first twenty-seven from the total of fifty-one men had only one single sample of blood taken at 9.30 am for estimation of plasma LH, FSH, oestradiol-17B, testosterone and prolactin. The following twenty-four consecutive patients who were not selected in any different way from the first twenty-seven patients, had multiple blood samples taken, including one at 9.30 am. The latter patients with multiple blood samples form the basis of the studies described in more detail below.

TABLE XXIV

Associated Conditions	Number of Patients
Seminoma of Testis	2
Tuberculosis of Testis	1
Hypospadias	1
Dystrophica Myotonica	1
Bilateral undescended Testes	1
History of alcohol ingestion	1
Congestive cardiac failure	3
History of drug intake	9

Clinical conditions associated with the patients who had gynaecomastia.

Single Samples

To allow comparison of plasma hormone concentrations on all the total group of fifty-one men, the value for the 9.30 am sample has been used. The results are presented in Table XXII and XXIII along with similar samples in thirty-five normal men. These have been divided into two groups - younger (<50 years) and older (50 years or more). In the younger men no significant change was detected in any plasma hormone concentration between men with gynaecomastia and normals. In the older men with gynaecomastia the gonadotrophin and oestradiol-17B concentrations were elevated as compared with the corresponding control group. This elevation was highly significant.

Multiple Samples

Study 1: Plasma hormone concentrations over 8 hour sampling period in men with gynaecomastia and control subjects

Six men with gynaecomastia were studied whose ages and clinical background are shown in Table XXV. The control subjects were seven healthy normal male volunteers from the Regional Hormone Laboratory (now Immunoassay Section, Clinical Chemistry Department, Royal Infirmary) aged 22-47 years and two normal older men with no history of endocrine disorders or drug history (aged 62-64 years). In this group of fifteen men, intensive sampling was carried out to determine the pattern of change with time.

TABLE XXV

Subject	Age in years	Possible Aetiology of Gynaecomastia
1	16	Pubertal
2	37	Seminoma testis treated by orchidectomy and radiotherapy 1 year before gynaecomastia
3	55	Drugs: Bendrofluazide and Digoxin for 6 months before gynaecomastia
4	70	Drugs: Salbutamol and Prednisolone for 3 months, 2 years before gynaecomastia
5	71	Unknown
6	73	Bilateral undescended testes

Age and clinical details of the six patients with gynaecomastia in Study 1.

Method of Sampling

Both patients and control subjects had their blood withdrawn as described in the General Methods Section every 15 minuts for 8 hours starting between 9.30 and 10 am. Plasma was prepared as described previously and the levels of plasma LH, FSH, oestradiol-17B, testosterone and prolactin were measured.

Results - Study 1

The plasma hormone levels showed considerable variation over the 8 hour period for all the hormones studied in six patients with gynaecomastia and nine control men. Plasma FSH had the least variation with coefficients of variation for each patient around 15%. Plasma LH and oestradiol-17B had coefficients of variation around 25% over the 8 hour period.

To seek pulsatile patterns of hormone secretion a test described by Moore and Wallis (1943) was used. This consisted of counting the number of upward steps in the hormone levels in the patients who were studied with gynaecomastia and normal subjects and comparing this with the number of upward steps expected in a completely random series (see Method Section). Pulsatile secretion should result in a fewer upward steps, at the pulses, followed by a larger number of downward steps during the decay phase. This analysis was carried out in all six patients and nine controls.

Only the plasma levels of LH in the young controls showed a significantly raised number of upward steps compared with a random series, and plots of the plasma LH levels with time confirmed the existence of a pulsatile pattern in all subjects in this group with pulses at intervals ranging from 30 minutes to two hours. This pattern of LH was not observed in any other group.

The plots of FSH and oestradiol-17B levels with time for individiual patients and controls were also examined by this statistical test and these did not suggest any common pattern of variation for any group. An example of these patterns is given in Figure 23. This includes one of the young men with gynaecomastia as contrast to one of the young controls, and an old man with gynaecomastia as compared with a normal old man. This seems to illustrate the existence of a pulsatile pattern of LH in the normal young man, but not in the other groups.

To test whether any of these hormones measured varied one with another, rank correlations (Kendall's) were compared for the sets of hormones measured at 15 minute intervals for each patient. Pooled tests for each group showed only one correlation that was signficant at the 5% level, namely a value of 0.37 for the correlation between LH and FSH, in the young controls (Table XXVI).

Conclusion

This study of sequential sampling indicated that there was no obvious pulsatile variation in the levels of the hormones studied

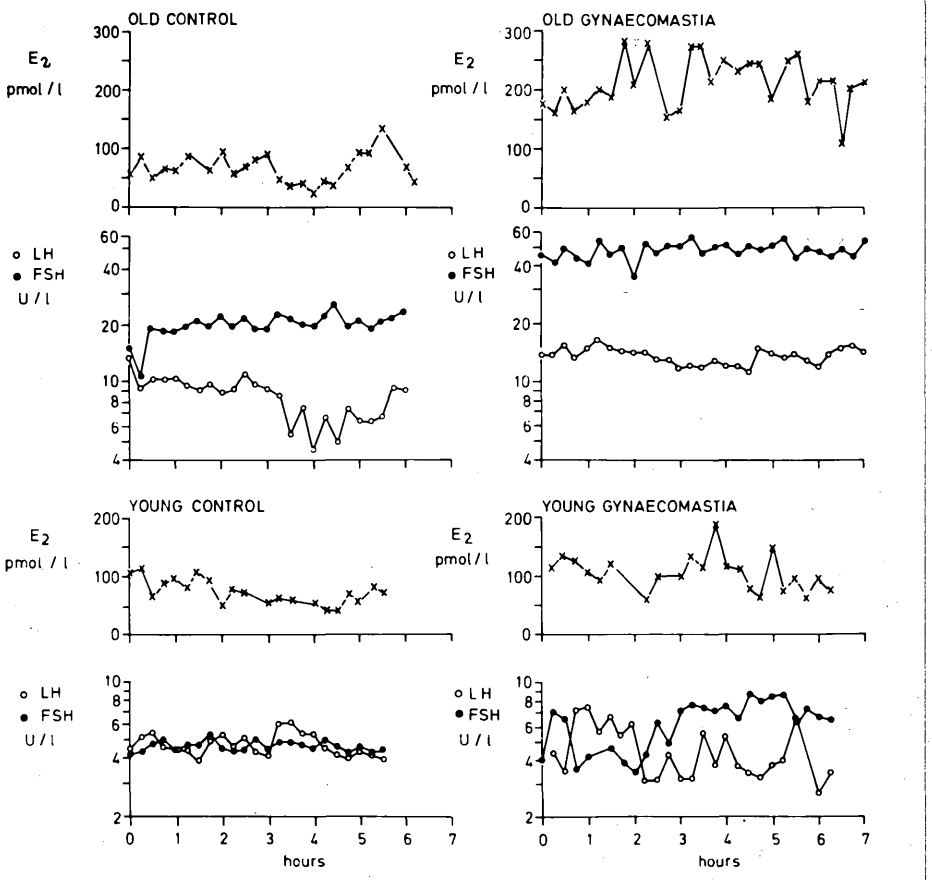


Fig. 23

Example of plasma LH and FSH and oestradiol 17β profiles from 2 patients with gynaecomastia and 2 controls one from each age group.

TABLE XXVI

	Young Gynaecomastia	Controls	Gynaecomastia	Controls
LH - FSH	0.03	0.37*	0.04	-0.10
E_2 - LH	0.01	0.07	0.09	-.
E_2 - FSH	0.04	0.10	0.16	-

*$p<0.01$ on a combined test i.e. that correlation is zero for all the subjects.

Pooled correlation for each group of patients with gynaecomastia and control subjects for daily variations in hormone levels i.e. average rank correlation (Kendall's τ).

(other than LH in normal young men) over an 8 hour period. For this reason it was considered justifiable to restrict sampling to a 2 hour period, during which, 4 half-hour samples were taken and to use this method for studying a larger series of patients.

STUDY 2

Comparison of mean plasma hormone concentrations of 4 half hourly samples in patients with gynaecomastia and normal males

A total of twenty-four male patients were studied, including the six subjects investigated in Study 1 and a further eighteen men. Of these twenty-four patients, eight had right sided gynaecomastia, twelve left sided and four bilateral gynaecomastia. The apparent duration of gynaecomastia was one year or less in eighteen out of twenty-four patients and more than two years in only three patients, two of whom were in the bilateral group.

Seventeen patients under the age of 50 years were classified as "young men" and formed a group with a median age 21 years (range 15-46). The remaining seven had a median age of 68 years (range 55-71); Table XXVII. Four of the seventeen young men with gynaecomastia were married with children. The remainder were unmarried. All, except two had testes which were normal in size and consistency. These two men had a history of seminoma of testis treated by orchidectomy and radiotherapy before the development of

TABLE XXVII

	Numbers	Median age in years	Range in years
Young Controls	7	28	22 - 47
Young Gynaecomastias	17	21	15 - 46
Old Controls	28	70	61 - 86
Old Gynaecomastias	7	68	55 - 71

Study 2

Numbers studied and median age of patients with gynaecomastia and control subjects who had multiple plasma hormone sampling.

gynaecomastia.

Buccal smears in all the younger patients were negative for the Barr antibody. Klinefelter's syndrome was therefore excluded.

Two patients had received drugs (one man had had a short course of stilboestrol seventeen years before the onset of gynaecomastia and also reserpine five years before; the other patient had been smoking marihuana for one year until one month prior to onset of gynaecomastia).

Five of the seven older men with gynaecomastia were married with children, one married with no children and one was unmarried. In this group, one patient had congestive cardiac failure, one an undescended testis of long standing and three others were on drugs. One had been taking salbutamol and prednisolone, one bendrofluazide and digoxin and the third man spironolactone, methyl dopa and Distalgesic (all drugs were continued till one day prior to the studies). The various clinical conditions and drugs are shown in Tables XXVIII and XXIX.

Controls comprised seven normal young and twenty eight older men. The young controls were seven normal men from the Immunoassay Section, Clinical Chemistry. All were healthy men with no history of endocrine disease and none were taking drugs. The older controls comprised of twenty-eight normal older men (described previously) who had no known history of endocrine disease and were

TABLE XXVIII

Associated Condition	No. under 50	No. over 50
Seminoma Testis	2	0
Undescended Testis	0	1
Cardiac Failure	0	1
History of drugs	2	3
Unknown aetiology	13	2

Clinical conditions associated with 24 patients with gynaecomastia who had plasma hormone studies.

TABLE XXIX

Number of Patients	Drugs Involved	Age
1	salbutamol, prednisolone	70
1	bendrofluazide, digoxin	55
1	reserpine, stilboestrol	46
1	spironolactone, methyl dopa, "Distalgesic"	62
1	marihuana	20

Pharmacological agents taken by this group of 24 patients with gynaecomastia who had intensive plasma studies.

not taking drugs of any kind. They were all ex-patients from a surgical ward who had had minor surgery more than one year prior to the study. The median range of ages are shown in Table XXVII.

Method of Sampling

All men had blood withdrawn at 30 minute intervals for two hours in the morning. The appropriate samples from the subjects in Study 1 were also included. The procedure for obtaining blood and the method of assay for plasma LH, FSH, oestradiol-17B, testosterone and prolactin were as described in the General Method Section.

Results - Study 2

The hormone levels between different groups of patients were compared using the mean concentrations from the four half-hourly morning samples. The median starting time of sampling for the patients with gynaecomastia with four half-hourly samples was 10 am. (range 9 am - 11.25 am). For those with the longer series of 15 minute samples, (Study 1 patients) the equivalent samples were used to represent each patient in order to ensure that valid comparisons were made. Since serum levels of gonadotrophins have been shown to have a log-normal distribution (Wide et al 1973; Hunter et al 1974), logarithms of LH and FSH values were used in the statistical analysis. However, the distributions of the arithmetic values for testosterone and oestradiol-17B appeared to be symmetrical and log-transformation was not used. To allow for correlations between various hormone levels, multi-variate statistical tests (Hotellings T^2 test) were used instead of individual t tests for each hormone

(described previously in Statitistical Methods Section).

Mean Plasma Hormone Levels using four half hourly samples - Expression of results

The results are expressed in three ways.

1. Scatterplots of the mean values of plasma LH, FSH for each patient for the two groups of patients and their controls are produced in Figures 24 and 25, and for oestradiol-17B tests in Figure 26. The two patients with seminomas had markedly increased plasma levels of LH and FSH compared with the rest of the young patients with gynaecomastia (Fig. 24), and were therefore excluded from all further analysis of plasma hormone levels. Therefore the results in Table XXX include only twenty-two men (fifteen under 50 years). Those patients with a drug history in either the young or older group did not have levels different from the other patients with gynaecomastia in their respective age groups and so were included.

2. In Table XXX the mean values of the four hormones for each group of patients are shown. The means of LH and FSH are geometric means. For testosterone and oestradiol-17B arithmetic mean values and standard deviations are given.

3. The correlation coefficients between the mean hormone levels for pairs of hormones in each group of patients and controls have been calculated and are given in Table XXXI.

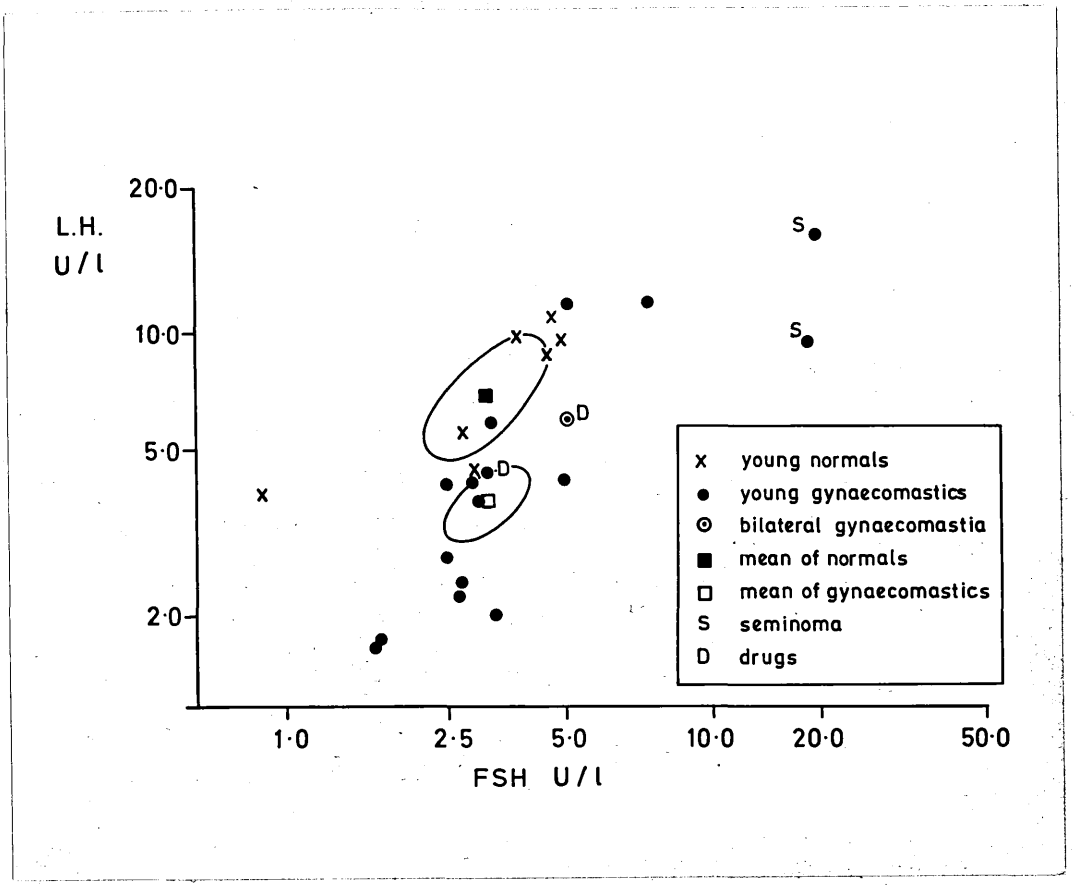


Fig. 24

Plasma LH and FSH concentrations for all patients and controls under 50 years. Ellipses show 95% confidence regions for the estimated means-controls (upper ellipse) and patients with gynaecomastia (lower ellipse). The two seminoma patients are excluded from the calculations of the mean for the gynaecomastic group. Note log. scales for both LH and FSH.

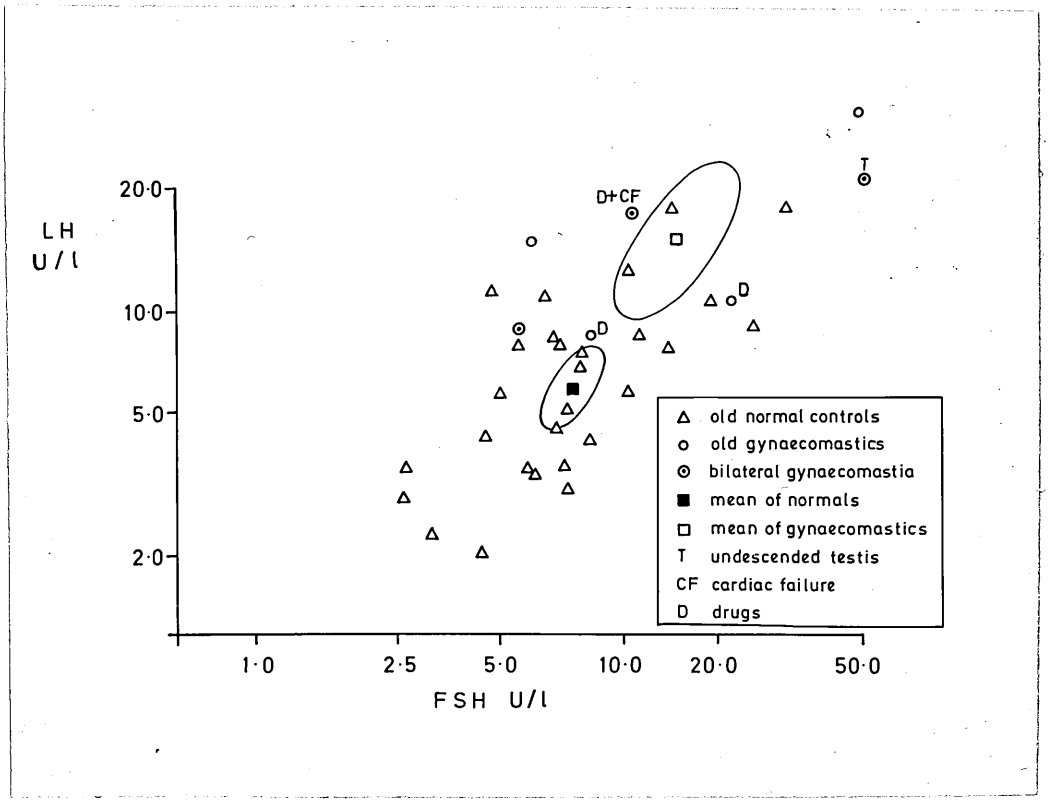


Fig. 25

Plasma LH and FSH concentrations for all patients with gynaecomastia and controls over 50 years. Upper ellipse shows 95% confidence region for the estimated mean of patients with gynaecomastia. Lower ellipse gives 95% confidence region for the controls. Note log. scale on both axis.

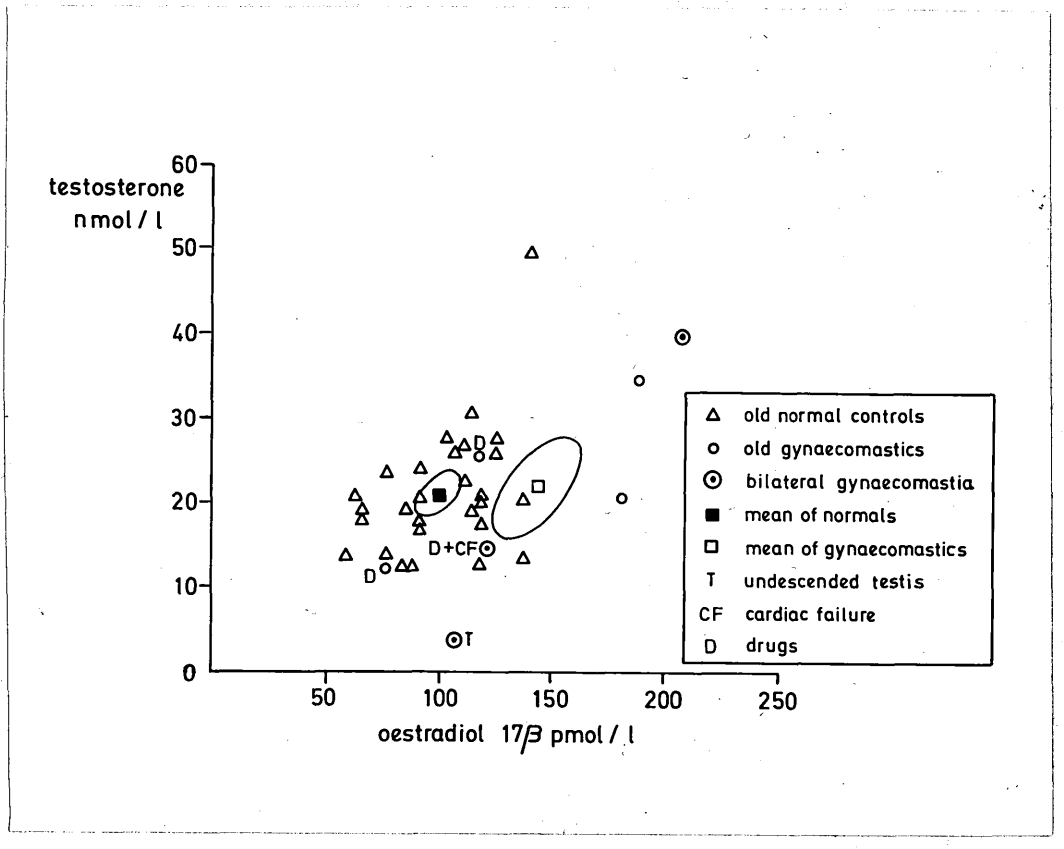


Fig. 26

Plasma oestradiol 17β and testosterone concentrations for all patients with gynaecomastia over 50 years. Smaller ellipse gives 95% confidence region for the estimated mean of the controls; larger ellipse the 95% confidence region for the estimated mean of the patients with gynaecomastia.

TABLE XXX

		Gynaecomastia under 50	Controls under 50	Gynaecomastia over 50	Controls over 50
N		15	7	7	28
LH u/l	Mean* ±1 sd	3.82 2.32-6.30	6.98 4.97-9.78	14.54 8.98-23.40	5.98 3.62-9.37
FSH u/l	Mean* ±1 sd	3.12 2.36-4.13	3.00 2.38-3.78	15.07 5.96-38.30	7.58 3.84-14.97
E_2 pmol/l	Mean sd	96 (31)	101 (26)	142 (48)	101 (24)
T nmol/l	Mean sd	23.0 (9.4)	† †	21.9 (12.7)	21.5 (7.4)

* For LH and FSH these means are calculated from the logarithms of the raw data, and are thus the geometrical means of the LH and FSH values; the range gives the raw values equivalent of ±1 standard deviation of the log values, other means are arithmetic.

\- No testosterone values are available for normal young controls.

Mean plasma hormone concentrations from four morning samples, in patients with gynaecomastia and in control subjects.

TABLE XXXI

	Gynaecomastia under 50	Controls under 50	Gynaecomastia over 50	Controls over 50
N	15	7	7	28
LH/FSH	0.81*	0.87*	0.72*	0.67*
LH/E_2	0.25	-0.71*	0.17	-0.10
LH/Test	0.15	†	-0.14	0.29
FSH/E_2	0.18	-0.82*	-0.35	-0.14
FSH/Test	†	†	-0.37	0.25
E_2/Test	0.58*	†	0.81*	0.43*

* $p < 0.05$ that correlation is zero

† No testosterone values available on young controls

Note: LH and FSH values are on a log. scale

Correlations between the mean plasma hormone concentrations in men with and without gynaecomastia.

In considering these results, change in absolute levels of gonadotrophins will be considered first; oestrogen and testosterone second and thirdly correlations between them.

(i) Plasma LH and FSH Mean Levels

In Fig. 24 the distribution of LH and FSH for the controls and patients with gynaecomastia under 50 years is shown. The ellipses give the 95% confidence intervals on a one-dimensional plot, and their elongation is a consequence of the correlation between the two hormones. The separation of the ellipses indicates a significant difference between the mean values of the two groups and the appropriate test of significance confirms this (Hotellings's T^2 test $p<0.01$). The young patients with gynaecomastia had lower plasma LH values than the controls, but similar plasma FSH values and thus exhibit a lower LH/FSH ratio.

The equivalent plot for the older groups is shown in Figure 25. Again the patients with gynaecomastia differ from the controls but now the pattern is different. The men with gynaecomastia have raised values of both LH and FSH and the ratio LH/FSH is similar in the two groups (Hotellings's T^2 $p<0.01$). The mean levels of LH and FSH given in Table XXX reflect these changes. However there is also evidence that the plasma FSH levels but not the LH levels are raised in the normal older controls compared with the younger controls (p value <0.01 student's t test). The scatter of FSH but not LH is also greater for the older men ($p<0.01$ variance ratio (F) test).

(ii) Plasma Oestradiol-17B and testosterone mean levels

Figure 26 shows the scatter of testosterone and oestradiol-17B values for the two older groups. Here there was a significant difference in the group means ($p<0.01$), the patients with gynaecomastia having raised plasma oestradiol-17B but similar testosterone levels, ie. a raised E_2/T ratio, as compared with old controls. This is also shown by the mean value in Table XXX. From the standard deviations in Table XXX it can be seen that the scatter of both oestradiol-17B and testosterone is greater for this group with gynaecomastia than for the controls ($p<0.01$ on a likelihood ratio test).

Due to technical reasons, no plasma testosterone levels were available for the young controls. However, plasma oestradiol-17B levels for the young men with gynaecomastia were not significantly different from those for the young controls ($t=0.64$, not sig).

Plasma Prolactin - mean levels

No values for plasma prolactin concentrations were obtained from controls. The values for the two groups of patients with gynaecomastia are given in Table XXXII. The known normal range for plasma prolactin values in males is 0-600 mU/l and both groups are within this normal range. It would seem therefore that plasma prolactin levels are within normal limits in this group of patients with gynaecomastia.

TABLE XXXII

Plasma Prolactin mU/l

	N	Mean	Variance
Gynaecomastia < 50	28	155	6
Gynaecomastia > 50	7	198	25

Mean plasma prolactin concentrations in patients with gynaecomastia.

(iii) Correlations of hormones

The correlation coefficient between hormones in Table XXXI indicate that a significant correlation existed between LH and FSH values in all groups, and also between oestradiol-17B and testosterone in older men. However, with the exception of plasma FSH and oestradiol-17B in the young controls, the levels of steroids within a group were not related to these of gonadotrophins.

4. Discussion

Gynaecomastia is a disease of many different aetiologies, as described in the earlier suggested classification. Many varied conditions e.g. adrenal tumours, cirrhosis of the liver and drugs are associated with breast enlargement in men. There may be common reasons for gynaecomastia, including abnormal target organ sensitivity and or a disordered hormonal profile. There is, however, still the possibility that certain forms of gynaecomastia may not be associated with hormone abnormalities. Nevertheless, gynaecomastia associated with a physiological aetiology, e.g. gynaecomastia in puberty or in old age is most likely to be associated with hormonal disturbances; it is at these stages in the male life at which the biggest changes in hormones occur. The aim of this study was to ascertain whether circulating levels of hormones in men with gynaecomastia differed from those in normal men. Before it was possible to determine if hormonal abnormality existed in patients with gynaecomastia, it was necessary to have reliable reference values for hormone concentrations in normal men without gynaecomastia. There were potentially problems in

determining base line data because of reported pulsatile fluctuations in hormone secretion. Furthermore, it was not a simple matter of measuring one hormone, as it was important to investigate the relationship and ratios of various hormones.

The present study has confirmed that there was a pulsatile pattern of plasma LH in normal young men (Nanken and Troen 1971, Santen and Barden 1973) but no evidence of this was found in normal older men or in older men with gynaecomastia, or indeed in the group of young men with gynaecomastia. It could be that as the patient grows older and the levels of LH and FSH rise (Rubens et al 1974), the pulsatile pattern is lost. Possibly both raised plasma gonadotrophin levels and absence of pulsatility of LH are consequences of testicular dysfunction. Such subnormal gonadal function might occur in old age but has yet to be consistently proven in young men with gynaecomastia.

Because of the known pulsatile variation of plasma LH, a suitable sampling procedure was required. The two hour sampling period used in this study reduced the considerable within-day variation seen in all the hormones. Its success was gauged by calculation of the coefficient of variation of the means of all possible sets of 4 half-hourly samples taken from our 8 hour series. In all cases the coefficient of variation was reduced by a factor of about 2, indicating that samples taken over a 2 hour period had a range of values similar to those found in the 8 hour series.

Based on these mean levels, the younger group of patients with gynaecomastia showed evidence of abnormality of plasma gonadotrophin levels with decreased LH/FSH ratio, while their oestradiol-17B levels did not differ significantly from control values.

The older men with gynaecomastia were found to have two-fold higher values of both plasma LH and FSH compared to normal old men with a similar LH/FSH ratio, and elevated oestradiol-17B levels. Plasma testosterone values were within normal limits.

It appears that plasma LH/FSH ratio is reduced in young men with gynaecomastia. Therefore, it could be supposed that a lowered plasma LH concentration is not stimulating the Leydig cells to produce an adequate plasma androgen level, the negative feedback mechanism in the pituitary is stimulated, and more FSH is produced by the sertoli cells to increase plasma oestrogen concentrations. The mechanism of the anti-developmental effect of androgens on the breast has not been fully elucidated, but it could be a lower androgen value allows unopposed oestrogen to stimulate breast development.

The findings of raised plasma gonadotrophin levels in normal older men agree with the findings of previous workers (Schalch et al, 1968; Rubens et al, 1974; Ryan and Faiman, 1968). Thus the mean plasma FSH level was 2.5 fold higher in our twenty-eight older controls (median age 70) as compared with eight young controls (median age 28) and the values more widely spread about their group

mean; no elevation was observed for LH levels. These changes appear to represent a response of the pituitary to decreasing testicular function characterised by a falling testosterone and rising oestradiol-17B and oestrone levels (Burger et al, 1974; Rubens et al, 1974). In the present work too, plasma oestradiol-17B concentration was raised but testosterone was unchanged.

In the younger patients with gynaecomastia the oestradiol-17B levels did not differ significantly from those in controls of the same age. Although no control values were available, the plasma testosterone values in young patients with gynaecomastia did not differ from either the older controls or the patients over 50 years with gynaecomastia.

Of four previous reports on plasma oestradiol-17B levels described in the literature, there were two studies (each on a single patient) one of which described similar findings (Smith, 1971), while the other reported an elevated oestradiol-17B level. (Goldfine et al, 1971). A more intensive study on sixteen pubertal boys, reported elevated plasma oestradiol-17B levels in six out of sixteen patients and a raised oestradiol-17B/testosterone (E_2/T) ratio in eleven (La Franchi et al, 1975). Large and Anderson (1978, 1979) found that oestradiol-17B levels rose with advancing puberty, the highest values were found in two boys with gynaecomastia and in a boy who later developed gynaecomastia. They also found a marked variation in the E_2/T ratio throughout 24 hours, and suggested that the exposure of the male breast at puberty to significant levels of

oestrogens unopposed by androgens during the daytime, when plasma testosterone levels were lowest, might account for transient gynaecomastia at puberty.

In the older patients with gynaecomastia studied here, the oestradiol-17B levels were elevated while the plasma testosterone values were within normal limits. The E_2/T ratio was therefore raised in these older men with gynaecomastia, in agreement with the finding of Chopra et al (1973) who found an elevated E_2/T ratio in thirteen older men with cirrhosis (mean age 46 years) compared to the ratio in normal men, and an even higher ration in men with cirrhosis who also had gynaecomastia. This study reported results in older men with gynaecomastia in a similar age group to our group of men.

In the absence of any significant evidence for gonadotrophins directly acting on breast tissue and the fact that oestrogens are known to stimulate breast growth (Dunn, 1940; Fitzsimmons, 1944), it may be that gynaecomastia is not due to an abnormality of LH/FSH ratios but that these ratios lead to an increase in oestrogens in the presence of normal androgen levels and therefore an increased E_2/T ratio. A rise in plasma oestrogens without a concomitant rise in androgens may be the primary cause of breast tissue growth. Thus the abnormality in circulating gonadotrophins and oestradiol-17B may ultimately be united in a single hypothesis.

The mechanism whereby raised plasma gonadotrophin levels in the older men with gynaecomastia are associated with oestrogen/androgen imbalance still remains to be elucidated. However, if the elevation of plasma LH and or FSH are a result of primary gonadal dysfunction due to old age, then it is possible that in association with a plasma LH stimulation of the Leydig cells to maintain a normal level of androgen production, there is also a concomitant rise of FSH levels which would in turn stimulate the synthesis of oestrogen from testosterone by the Sertoli cells.

Oestradiol-17B can be shown to be synthesised by Sertoli cells from the testes maintained in cell culture. Addition of FSH caused a slight but significant increase in oestradiol-17B synthesis while in the presence of testosterone, FSH produced a 12-fold increase in oestradiol-17B synthesis. The conclusion was that not only do Sertoli cells synthesis oestradiol-17B from testosterone, but this conversion is markedly stimulated by FSH when added with testosterone (Dorrington and Armstrong, 1975). There are, however, other possible pathways to oestrogen/androgen imbalance which include changed capacity for metabolism of steroids by breast tissue or changes in adrenal function.

The mechanism whereby disturbed oestrogen/androgen balance in male breast tissue, leads to gynaecomastia is also unknown. Oestrogen receptors are probably present in both normal male and female breast since the tissues are oestrogen-sensitive, but by present methods the level of receptor activity is barely detectable (Leclerq et al,

1975). The anti-oestrogenic effects of androgens in breast cancer may be mediated via the oestrogen receptor (Davies et al, 1977; Nicholson et al, 1978; Zava and McGuire, 1978; Adams et al, 1981). At low concentrations androgens may bind only to the androgen receptor but at very high concentrations they can also bind to the oestrogen receptor and cause translocation of the oestrogen receptor to the cell nucleus where the androgen receptor complex is inactive; this process may be involved in tumour regression. It may be that equally in normal men, normal concentrations of androgens are involved in suppressing breast growth by this kind of mechanism whilst in gynaecomastia, lowered oestrogen/androgen ratio leads to a greater translocation by oestrogen rather than androgen. Alternatively, it may be that androgens inhibit oestrogen action in the normal male by a different mechanism eg. by affecting nuclear type II receptor sites in a manner similar to that described for the effects of progesterone and dexamethasone (Clark, Upchurch and Markaveich, 1981). Thus it is possible that in the breast tissue from pubertal and aging males the elevation of plasma oestradiol-17B levels and fluctuations in the E_2/T ratio might permit expression rather than suppression of oestrogen-induced stromal hypertrophy.

It has been shown (in vitro) that gynaecomastic breast tissue lacks the enzyme 3 β-hydroxysteroid dehydrogenase; (3 β HSD) (Miller et al, 1974). In the testis this enzyme is influenced by both steroid and pituitary hormones (Hafiez et al, 1972). If 3 β-hydroxysteroid dehydrogenase in breast tissue is also influenced by hormones, an imbalance of circulating hormones could diminish the enzymic

activity in gynaecomastic breast leading to diminished production of testosterone or other 3 B-hydroxy androgens and diminished suppression of breast growth.

Changes in pituitary hormones other than gonadotrophins e.g. prolactin, may also be associated with the aetiology of gynaecomastia. However, in the present limited study, plasma prolactin values were within normal limits for both young and older men with gynaecomastia. This is in agreement with a previous study by Turkington (1972) who, using a bioassay technique, found normal plasma prolactin levels in twenty-nine men with gynaecomastia. Nagel and his colleagues (1973) however reported variable prolactin levels in patients with gynaecomastia on a chronic dialysis programme, while Van Thiel et al,(1975) found increased plasma prolactin concentrations in chronic alcoholics particularly those with gynaecomastia. Large et al (1980) reported higher mean 24-hour plasma prolactin levels in boys with gynaecomastia than controls. Thus despite our negative findings, a role for prolactin in the aetiology of gynaecomastia cannot be dismissed. Nevertheless, changes in plasma prolactin could be secondary to those in oestrogen and/or gonadotrophin secretion.

Whilst as a group, patients with gynaecomastia may have abnormal levels of hormones, many individuals with gynaecomastia appear to have normal values. In order that these normal levels may give rise to gynaecomastia it is likely that a second factor ie. abnormal sensitivity of breast tissue to endocrine stimulation, is present in

these cases. Certainly some degree of differential sensitivity to hormones must exist between each breast. Both breasts are exposed to the same circulating hormone levels and one would suppose that if these were the only causative factors, then gynaecomastia would always be present initially as bilateral disease.

5. Conclusion

In summary, both alteration in hormone levels, (especially in the oestrogen/androgen ratio) and in tissue sensitivity appear to be involved in the aetiology of gynaecomastia. In the normal situation, the steroids from the testis are under control of the pituitary-hypothalamic system by a negative feedback mechanism. If in old age, or other predisposing conditions, the testicular activity diminishes the negative feedback mechanism will be diminished also, permitting an enhanced release of FSH with or without LH to the plasma in an attempt to maintain a normal plasma testosterone level. This may lead to an over-stimulation of the Sertoli cells (independent of the Leydig cells) which will produce more oestradiol-17B, resulting in a raised oestrogen/androgen ratio and a greater stimulation at the oestrogen receptor sites. However not all people have raised oestrogen/androgen ratios, which could be due to, for example inherited or acquired differential sensitivity (a) to feedback in the hypothalamus, or (b) of the Sertoli and Leydig cells in the testis. Furthermore, not all people with raised oestrogen/androgen ratios develop gynaecomastia and this may be due to variations in the sensitivity in the breast tissue to hormones; the reasons for these variations are also as yet unknown.

5. Conclusion

1. In young men with gynaecomastia, the concentration of plasma LH and consequently the LH/FSH ratio was lower than that in the control subjects.

2. In older men with gynaecomastia, both plasma LH and FSH concentrations were higher than those in normal controls, but the LH/FSH ratio was similar in the two groups.

3. In older men with gynaecomastia, plasma oestradiol-17B levels and the oestradiol-17B/testosterone ratio were higher than those in the controls.

4. Older controls had higher plasma FSH levels than younger controls.

5. A pulsatile pattern of plasma LH was present only in the young controls.

IV

CONCLUSION

IV. CONCLUSION

There can be little doubt that hormones influence breast development both in males and females. The aim of this study was to examine in more detail plasma hormone levels in women with breast cancer and in men with gynaecomastia.

The conclusions of the studies of the women with breast cancer are summarised below.

In the intensive sampling regime of plasma hormones over a six to eight hour period, plasma LH, FSH, testosterone, oestradiol-17B, prolactin and cortisol levels were all within normal limits in six postmenopausal patients with breast cancer, when compared with six patients without the disease, matched for age, parity and menstrual status.

Within the normal range, plasma testosterone levels were significantly higher in each breast cancer patient, compared with her control.

Plasma oestradiol-17B levels were also higher in five out of six women with breast cancer but not significantly so.

No pulsatile pattern of plasma LH levels was detected in any woman whether with or without breast cancer.

In both groups of women there was a fall of plasma cortisol concentration two hours after the start of the tests.

Plasma FSH, prolactin, testosterone and oestradiol-17B levels were measured in postmenopausal women with advanced breast cancer, before and on treatment with stilboestrol or tamoxifen. Tamoxifen treatment significantly decreased plasma FSH levels at one, three and six months but had no effect on plasma prolactin concentrations, or any of the other hormones measured.

Stilboestrol treatment had a more profound effect in suppressing plasma FSH levels at one, three and six months but also significantly increased the levels of plasma prolactin.

These effects on plasma hormone levels were present in all patients and therefore were not related to clinical response to the drug.

When specific pituitary releasing hormones and drugs were used to devise an optimum test of pituitary function, it was found that TRH was a better stimulant of plasma prolactin concentrations than chlorpromazine.

LHRH increased plasma LH and FSH levels; the addition of TRH to LHRH did not change the response of LH and FSH.

It was concluded that the best test of pituitary function in a woman with breast cancer about to undergo yttrium implantation of the pituitary was a combination of LHRH, TRH and insulin by infusion.

This test was used in women with advanced breast cancer as a test of pituitary reserve before and one month after yttrium implantation.

Of the patients who had residual pituitary hormones on stimulation after implantation, none of these had a clinical response. In contrast, those women with minimal residual hormones on stimulation (and therefore presumably with the nearer complete pituitary destruction), were more likely to have a clinical response, although this was not guaranteed.

This stimulation test was also used to test the effect of quadruple chemotherapy on pituitary function. When three groups of women with breast cancer were treated by either yttrium implantation alone, chemotherapy alone or a combination of both and tested for pituitary reserve before and one month after treatment, chemotherapy was found to have no effect on pituitary function.

<u>The conclusion of the studies of the men with gynaecomastia</u>

In a group of young men (under 50 years) with gynaecomastia, plasma LH concentrations were lower than those in controls of a similar age without the disease. As plasma FSH levels were similar in both groups, young patients with gynaecomastia exhibited a lower LH/FSH

ratio.

Older men with gynaecomastia (over 50 years) had higher values of both plasma LH and FSH than controls of a similar age without the disease, but the LH/FSH ratio was similar in the two groups.

The pulsatile pattern of plasma LH was present only in the young controls, being absent in the young and old men with gynaecomastia.

The pulsatile pattern of plasma LH was also absent in the old control group and this group of men had higher plasma FSH levels than the younger control group.

Plasma oestradiol-17B levels were elevated in the older men with gynaecomastia, as compared with normal, age-matched controls.

Plasma testosterone levels were similar in the two older groups, ie old men with gynaecomastia and older controls. Thus, the oestradiol-17B testosterone ratio was raised in the older men with gynaecomastia in comparison to older controls.

V

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