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MARKERS OF BIOLOGICAL VARIATION OF BREAST CANCER

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Thesis submitted to the University of Glasgow

for the degree of Doctor of Medicine

January 1983

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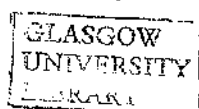
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Thesis

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Dedicated to

Eilish

Stuart and Robert

and also to my mother, Anne

With much love

## Preface

Our comprehension of the biological nature of breast cancer is obscure, despite generations of organised research. Clinical experience emphasises that this disease has a variable range of malignancy with different clinical manifestations and prognosis. One in fifteen women in the United Kingdom develops breast cancer and the majority of these die as a result of it. Primary treatment methods which are based upon anatomical or physical considerations, have been applied in a blanket fashion but have failed to achieve any improvement of mortality rates. A more specific primary treatment, appropriate to an individual situation and outlook could be provided if accurate markers of the range of malignancy in breast cancer were available. Attempts to define 'biological markers' within the primary growth which might shed some light upon its innate nature and possibly distinguish a favourable from an unfavourable type have met with limited success in recent years. The Nottingham-Tenovus Study of primary breast cancer, under the direction of Professor Roger Blamey has been at the forefront of these developments. Data from this centre have shown that oestrogen receptor status and histological grade of primary breast cancer are inter-related and each variable also bears some relationship to prognosis. However, studies of these 'intrinsic' prognostic variables remain at a preliminary stage. Many intrinsic variables have been identified, many are inter-related but their precise clinical applications have not been defined. It is known that many aspects of tumour behaviour have an influence upon prognosis but, to date we have been unable to isolate an ideal 'intrinsic marker' for any specific tumour characteristic.

This thesis describes a search for factors in the primary cancer which will identify specific tumour characteristics and allow accurate prediction of the likely clinical course in individual patients.

During a two year interval from August 1980, I was priveleged to hold the Tenovus Research Fellowship in Nottingham and I was involved in every aspect of investigation and management of patients which these studies concern. I personally carried out most mastectomies and lymph node biopsies and thus I harvested and distributed all specimens. Histological grading of primary cancers and examination of lymph node biopsies for metastatic tumour were carried out independently by Dr. C.W. Elston and Dr. Jane Johnson. Steroid receptors were measured in primary cancers by the Tenovus Institute for Cancer Research in Cardiff under the direction of Professor Keith Griffiths. As Tenovus Research Fellow I harvested, prepared and frequently transported tumour specimens in liquid nitrogen to Cardiff and carried out some receptor assays. I carried out all prostaglandin  $E_2$  radioimmunoassays in tumour explants, in the Department of Surgery, Queen's Medical Centre, Nottingham. I also personally carried out all cellularity counts in histological sections of cancers in which prostaglandins were measured.

Clinical follow up of patients after mastectomy continued in the Nottingham Post Mastectomy Clinic, which I conducted. I scrupulously documented all important 'events' in the 'Master Index' and later summarised the index to allow its transfer on to computer. I carried out a clinical examination and a full range of investigations on detection of recurrence in all patients. I

designed and used detailed proformas to allow (a) documentation of the precise dimensions and distribution of secondary disease before endocrine treatment, (b) clinical follow up with accurate documentation of any change in target metastases. These proformas are now complete on more than two hundred patients and make up the Nottingham Advanced Breast Cancer File.

I personally supervised treatment at the Advanced Breast Cancer Clinic and arranged external review for assessment of response to endocrine therapy. I carried out all statistical analyses although I received much valuable help from Dr. John Haybittle.

By these methods, intrinsic parameters in primary breast cancer were related to the clinical events which determine prognosis, in women in the Nottingham-Tenovus Study.

### Acknowledgements

It gives me the greatest pleasure to acknowledge the advice and encouragement which I received from Professor Roger Blamey, Professor of Surgical Science, University of Nottingham, during the execution of the studies of this thesis and during preparation of the manuscript. Professor Blamey initiated the Nottingham-Tenovus Study of operable breast cancer and it is entirely due to his foresight and energy that this Study has made such a profound contribution to our understanding of breast cancer.

I also wish to thank the following people who have contributed, directly or indirectly to the studies in this thesis.

Professor Keith Griffiths

Tenovus Institute for Cancer  
Research, Cardiff.

For providing facilities for  
steroid receptor analyses in tumour  
specimens, for advice and for  
encouragement.

Dr. C.W. Elston

Consultant Histopathologist, City  
Hospital, Nottingham.

For painstaking attention to  
pathological and histological  
specimens, for grading all tumours  
and for provision of the facilities  
for cellularity assessment.

Dr R.I. Nicholson

Tenovus Institute, Cardiff.

For supervising steroid receptor analyses of tumour specimens and for his enthusiasm in our many collaborative projects.

Dr Jane Johnson

Consultant Histopathologist, City Hospital, Nottingham.

For collaboration with Dr. Elston in morphological assessment of tumours and lymph nodes.

Dr. John Haybittle

Department of Medical Physics, New Addenbrooke's Hospital, Cambridge. For advice, regarding statistical analyses.

Dr. A.H. Morris

Consultant Radiologist, City Hospital, Nottingham.

For collaboration with myself in the examination of many difficult x-rays, in assessment of response to therapy.

Mr. J.M. Morrison

Consultant Surgeon, Selly Oak Hospital, Birmingham.

External assessor of response to endocrine therapy.

Dr. A. Howell

Consultant Medical Oncologist,  
Christie Hospital, Manchester.  
External assessor of response to  
endocrine therapy.

Dr. P. Holland

Medical Assistant, City Hospital,  
Nottingham.

For help in supervision of patients  
at the Advanced Breast Cancer  
Clinic.

Dr. S. Mann

Senior Registrar, Anaesthesia and  
Pain Specialist, City Hospital,  
Nottingham.

For making the life of many  
patients with advanced breast  
cancer more tolerable.

Julie Haynes

Technician, Department of Surgery,  
Nottingham.

For technical assistance on the  
prostaglandin project.

Veronica Lewis and  
Katherine Mullinger

Nursing Sisters, City Hospital,  
Nottingham

For their sympathetic approach to  
patients with breast cancer.

Mrs. Wendy Bartlam

For immaculate typing of this  
manuscript.

The work for this thesis was carried out while the author was Tenovus Surgical Research Fellow, in the University of Nottingham. The financial support of the Tenovus Institute was generous and is gratefully acknowledged.



# Markers of Biological Variation of Breast Cancer

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### Abbreviations

A number of abbreviations have been used throughout this treatise, whose meaning is given below:

ER	-	Oestrogen Receptor
PR or PgR	-	Progesterone Receptor
PgE <sub>2</sub>	-	Prostaglandin E <sub>2</sub>
fmol	-	femtomole
ng	-	nanogram

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Publications arising from this thesis

1. F.C. Campbell, R.W. Blamey, C.W. Elston, R.I. Nicholson, K. Griffiths, J.L. Haybittle. Oestrogen receptor status and sites of metastasis in breast cancer.  
British Journal of Cancer (1981) 44 : 456 - 459
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Extracta Gynaecologica (1982) 6 : 3 : 220 - 221
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Journal of Steroid Biochemistry (1981) 15 : 113 - 199

In Press

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In: Gynaecological Malignancy - Raven Press
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Submitted Paper

7. F.C. Campbell, Julie Haynes, D.F. Evans, R.W. Blamey, C.W. Elston, J.L. Haybittle, R.I. Nicholson, K. Griffiths. Prostaglandin  $E_2$  biosynthesis by tumour epithelial cells and oestrogen receptor.  
British Journal of Cancer

Published Abstracts

8. Histological differentiation and patterns of metastases from breast cancer.  
BASO December 1980  
Clinical Oncology (1981) 7 : 255
9. Oestrogen receptor status and sites of metastases in breast cancer  
SRS January 1981  
British Journal of Surgery (1981) 68 : 359
10. Oestrogen receptor status of primary breast cancer and response of metastases to endocrine therapy.  
BASO July 1981  
Clinical Oncology (1982) 8 : 85
11. Lymph node biopsy in breast cancer : The significance of 'missed' nodes.  
SRS July 1982  
British Journal of Surgery, November 1982
12. Prostaglandin E<sub>2</sub> synthesis by tumour epithelial cells and oestrogen receptor status of primary breast cancer  
SRS (Salzburg) September 1982  
Langenbeck 1982

## Chapter 1

### PRINCIPLES OF MODERN TREATMENT FOR BREAST CANCER

## Principles of Modern Treatment for Breast Cancer

The principles of treatment of any disorder ought to be formulated upon a true understanding of the primary pathological abnormality, if such treatment is to be successful. For example, in acute appendicitis the primary pathological abnormality is a localised inflammation within a useless organ, which may progress to cause severe, even fatal complications. Treatment principles incorporate an understanding of inflammatory processes and are directed towards early removal of the diseased organ. This treatment is highly successful and the mortality from appendicitis has fallen from 9.9 per 100,000 in 1939 to 0.4 per 100,000 in 1975 due mainly to improvements in diagnosis and treatment (Storer, 1979).

What of our understanding of breast cancer? Galen, a philosopher of ancient Greece, described breast cancer as a systemic disturbance due to an imbalance of natural humours, melancholia and black bile. These notions now seem laughable but have our comprehensions of the disease really improved since Galen's time? There has certainly been no decrease in mortality rate from breast cancer since records began and indeed, statistics show a disturbing upward trend (Registrar General's Statistical Review of England and Wales, 1973). Thus, it is vital that we reconsider our understanding of the disease and in this context it is essential to gain an historical overview of the scientific principles upon which present day treatment methods are based.

### Evolution of Surgical Therapy

Present day surgical management of carcinoma of the breast is based upon principles which were formulated approximately 100 years ago. Charles Moore, the surgeon in charge of the cancer wards at the Middlesex Hospital, first called attention to the patterns of local recurrence after limited excision of the tumour (the favoured surgical method of treatment at the time), in an article entitled "On the Influence of Inadequate Operations on the Theory of Cancer" (Moore, 1867). Moore demonstrated that local recurrences appeared most frequently adjacent to the old incision and he suggested that 'active microscopic elements' could be set free during the excision of the cancer. These 'active elements' could then lodge in the wound and their subsequent growth would cause the local recurrence. This view was contrary to the prevalent opinion of the time that a local recurrence represented a 'new cancer' and was a manifestation of constitutional susceptibility to the disease. Moore suggested that the whole breast be removed together with "adjacent unsound structures" including skin, pectoral muscles, and axillary nodes. Moore's ideas were accepted by Lister, in Glasgow and Mitchell Banks in Liverpool, but did not find general favour, possibly due to the limitations of operating time imposed by the relatively crude methods of anaesthesia and lack of resuscitative facilities at that period.

### The Radical Mastectomy

Towards the end of the nineteenth century, a resurgence of interest in these principles came about. William S. Halsted, an American surgeon devised and popularised the operation of radical mastectomy. Halsted based his operation partly on his own theories

of tumour biology and partly on prevalent ideas concerning mechanisms of dissemination. Halsted proposed two essential concepts which were central to the rationale of his operation. Firstly, he suggested that growing tumour remains localised at its site of origin for a period of time and thereafter, at some instant during their growth, tumour cells invade lymphatics and spread to regional lymph nodes in an orderly manner. In accordance with the ideas of other authorities (Stiles, 1892; Handley, 1904) Halsted considered that breast cancer disseminated primarily by lymphatic permeation and that initial entry of cancer cells into the bloodstream was unlikely. Secondly, in concurrence with the teaching of Virchow (Virchow, 1863) Halsted believed that lymph nodes provided an effective barrier to the passage of tumour cells and therefore, even when the regional nodes were clinically involved, the cancer was likely to be of a limited local-regional distribution.

In keeping with these concepts, therefore, an anatomical basis for cancer surgery was formulated. A cancer operation would of necessity consist of removal of the primary tumour together with the regional lymphatics and structures containing them, and lymph nodes by meticulous 'en bloc' dissection. The practical application of these principles to carcinoma of the breast culminated in the operation of the radical or 'Halsted' mastectomy which comprised removal of the entire breast, together with a wide circular expanse of overlying skin, the pectoral muscles and axillary contents, all in one piece (Halsted, 1894). At the same time, Meyer of New York described a similar operation, which he had conceived independently



of Halsted (Meyer, 1894). This 'radical' operation became the standard management for carcinoma of the breast both in the United Kingdom (Handley, 1906) and in America.

The widespread adoption of the procedure led to a most dramatic reduction of local and chest wall recurrences of the type which had been so well recorded by Moore. Halsted compared the results of his operation, with those of other authorities who practised less radical procedures and found that the incidence of local recurrence was reduced from 60 - 80% (Von Winiwarter, 1878) to 6% in his series of 50 patients (Halsted et al, 1895). Data relating to any improvement in survival however, were lacking. Indeed Greenough reported that the survival of patients treated by radical mastectomy was inferior to that of patients treated by lesser operations (Greenough et al, 1907). Lewis and Rienhoff (1932) later reported a 10 year survival of 12% in patients treated by the radical operation which compared with the 9% 10 year survival of patients treated with simple mastectomy alone, by Gross, some fifty years earlier (Gross, 1880).

#### 'Treatment Failure' with Radical Mastectomy

Local and distant recurrence, or death from the disease after the radical operation were considered to be a manifestation of 'treatment failure'. These 'failures' were thought to result from residual foci of cancer, left in situ after the mastectomy: local recurrences were thought to arise from tumour cells which had been left in the operative site (as initially described by Moore) as a result of 'poor surgical technique'. Distant metastases were considered to occur as a result of spread by lymphatics from any

nidus of cancer in lymph nodes which were not routinely removed. Sampson Handley thought that the anterior mediastinal and supraclavicular nodes were particularly likely to harbour clumps of cancer cells, and he wrote: "In such cases, it is probable, that not infrequently, permeation may smoulder along the lymphatics without giving rise to macroscopic nodules in its course. Thus, long periods of apparent immunity may be followed by recrudescence of the growth at some distant point." (Handley, 1922).

#### Extension of the radical principle

These ideas stimulated the search for some method of elimination of the "last involved lymph node" and the radical anatomical principle was 'extended' beyond the confines of the Halsted mastectomy. Surgical procedures became more expansive, and post operative radiation became a standard supplement to the mastectomy. Halsted extended his dissection to include removal of the supraclavicular lymph nodes, but later reverted to his original operation (Halsted, 1898). Handley administered a 'prophylactic' course of radiation to all of his patients after 1906 (Handley, 1922). In addition, Handley confirmed his own and others fears of residual cancer in lymph nodes which were 'beyond the knife' at radical mastectomy when he reported positive biopsies of internal mammary nodes. He subsequently advocated routine implantation of intercostal radium tubes and reported 77 cases treated in such a manner in 1927 (Handley, 1927). Twenty years later, R.S Handley, a senior surgeon at the Middlesex Hospital, and Sampson Handley's own son, recalled attention to the problem of residual tumour in internal mammary nodes (Handley and Thackray, 1949) when he

demonstrated positive biopsies in 31% of patients with inner quadrant tumours, and concluded that the classical radical mastectomy "was not radical at all and was likely to fail in its object in up to 25% of operable cases".

#### The Extended Radical Mastectomy

Influenced by these findings, Urban (1956) and Sugarbaker (1964) both in America, extended the radical mastectomy to include a routine 'en bloc' dissection and removal of the internal mammary chain. Andreasson and Dahl Iversen in Denmark demonstrated tumour involvement of supraclavicular nodes in 33% of patients with positive axillary nodes (Andreasson and Dahl Iversen, 1949) and therefore they included a dissection of both the supraclavicular nodes and the internal mammary chain in their standard operation for breast cancer (Dahl Iversen and Tobiassen, 1963). Wangensteen, who was the most ambitious practitioner of the radical surgical principle advocated a 'super-radical' mastectomy which comprised removal of the breast and axillary contents; the internal mammary vessels and lymphatic chain; the upper and lower mediastinal and supraclavicular nodes all of which would be performed in two stages (Wangensteen, 1949).

As can be seen, these 'extended radical' mastectomies varied in scope and technique to an extent which made their comparative evaluation difficult. However, each of these procedures comprised a minimum of a radical mastectomy plus an 'en bloc' dissection of the internal mammary nodes with segments of overlying ribs and pleura and represented a formidable surgical assault. Haagensen reported that "the operation undoubtedly penalises the patient more than does

the classical radical mastectomy" (Haagensen, 1971). Shortly before they abandoned the procedure, Dahl Iversen and Toblassen (1969) described complications in 5% of their patients which were strictly referable to the 'extension' and not to the mastectomy component, viz. pleural perforations, rib necrosis, empyema and parasternal abscesses. Finally Wangensteen reported the prohibitive mortality of 12.5% after his two stage 'super-radical' procedure, although he was later able to reduce that to 3.6% by performing all steps in one stage (Wangensteen et al, 1956).

The first goal, in the management of any malignancy is the improvement of survival, particularly in those circumstances where the method of treatment itself carries such penalties. The most damning indictment of the extended mastectomies therefore, came from their failure to achieve that aim. Retrospective analyses by Gould, 1964; Cacaress, 1967 and Urban, 1971 all have failed to show any improvement of survival over that of the standard radical mastectomy. Furthermore a prospective trial comparing extended mastectomy with radical mastectomy (Lacour, 1976) did not show any survival benefit for the extended procedure. While it would appear that the extended procedures do convey some benefit, with regard to reduction of parasternal chest wall recurrences (Urban, 1971), local recurrences (Donegan, 1972) and prolongation of disease free interval (Urban, 1971), their prohibitive morbidity together with the lack of any survival benefit has led to their general abandonment.

### Radical mastectomy with supplementary radiotherapy

Other non surgical avenues have been explored for some means of eliminating residual local cancer after the standard 'Halsted' procedure. Radiotherapy, given pre or post operatively has been most popular in this respect. This modality of treatment in the early years of this century tended to be administered to selected patients for a variety of reasons, with variable doses and methods of administration, so that once again, there was a situation where any comparative assessment of results was difficult, if not impossible.

A number of non randomised retrospective studies were published often with conflicting conclusions. Greenough, in a retrospective review of 536 patients demonstrated a five year survival of 33% for patients treated by radical mastectomy alone and only 23% for those who received 'prophylactic' radiotherapy in addition to the operation and he recommended therefore that its routine use be discontinued (Greenough, 1929). Harrington, in a review of 3,381 patients failed to demonstrate any survival advantage in irradiated patients but details of radiation regimens were lacking (Harrington, 1935). However, McWhirter in Edinburgh, demonstrated a decreased rate of local recurrence and a greater five year survival, for both 'operable' and 'inoperable' cancer, in patients who received radiotherapy following the radical operation. These benefits were assessed against historical controls, treated by radical mastectomy alone, and were dependent on technique and dosage of radiotherapy (McWhirter, 1948).

It was not until the 'Manchester Trial' that accurate data became available (Paterson and Russell, 1959). In this prospective

study, patients with operable breast cancer were randomly allocated to receive a radical mastectomy either alone or followed by a supplementary course of radiotherapy. A total of 1,461 patients were entered into the trial between 1949 and 1956. This outstanding study became a 'milestone' in our approach to cancer therapy and had three important conclusions.

Firstly, there was no significant difference in survival despite the 'sterilising' effect which the additional DXRT would have had on internal mammary nodes. Secondly, 35% of the group who had radical mastectomy alone and then were 'watched' died without ever developing local recurrence and thus were spared the disadvantages of radiotherapy. Thirdly, the rates of local recurrence were markedly reduced in the irradiated group. However, these recurrences when they appeared were successfully treated by 'purposive' irradiation so that at death, the incidence of persistent local recurrence was approximately equal in both groups. Finally, Paterson and Russell concluded that "post operative radiotherapy as a routine procedure in all cases is associated with considerable disturbance and discomfort and occasional permanent morbidity. This seems unnecessary if it confers no increased overall protection and no benefit on the majority of patients".

A further prospective study in America, the National Surgical Adjuvant Breast Project (NSABP) clinical trial confirmed the Manchester conclusions, that local and regional recurrences were reduced by post-operative radiotherapy but that there was no survival advantage (Fisher et al, 1970). Haagensen concluded that "there was no justification whatever", for the use of 'prophylactic'

radiotherapy in addition to a radical mastectomy (Haagensen, 1971).

It seems, therefore, that extension of the radical mastectomy, either by more expansive surgery, or by the addition of radiotherapy, has not improved survival. By Halstedian principle, these more expansive techniques ought to have eliminated residual local regional cancer, the supposed focus from which disseminated metastases occurred, in a greater number of patients which likewise, ought to have been reflected in a greater cure rate. These unfulfilled expectations continue to challenge Halstedian principles. It is noteworthy, however, that most trials do report some benefit from these methods of 'augmented' radical surgery viz. there is general agreement that these delay the onset of local or regional recurrence with a consequent prolongation of disease free interval. Despite these benefits, however, the complications of these manoeuvres has led most surgeons to abandon their use and merely treat recurrent disease when it arises.

#### The shift to conservative surgery

The standard radical mastectomy itself, also carries significant complications e.g. sloughs, wound dehiscences and infections, deformity of the chest wall, arm oedema and limitation of shoulder movement (Crile, 1964) and clinicians in some quarters expressed concern at the widespread use of the procedure. Geoffrey Keynes, a surgeon at St. Bartholomew's Hospital, expressed "grave dissatisfaction" with the Halsted mastectomy in view of its "comparatively low cure rate and definite morbidity in the form of arm oedema and limitation of shoulder movement". Keynes considered the radical procedure to be a "hideous mutilation" and advocated a

combination of simple mastectomy plus radiotherapy as an alternative (Keynes, 1927). Ewing felt that the Halsted procedure was too radical for disease at either end of the spectrum of advancement, being performed on the one hand, for intraduct cancers, where the sacrifice of normal tissue was unlikely to be contributory to the prognosis, and on the other, for locally advanced "highly malignant" forms of the disease which were uniformly fatal whatever the treatment (Ewing, 1928). In a comprehensive review of patients treated by various combinations of surgery and radiotherapy for breast cancer, Adair documented the results of local operations such as simple mastectomy or lumpectomy plus radiotherapy and found to his surprise, that the survival rate was approximately the same as that of patients treated by the Halsted procedure (Adair, 1943).

Scientific objections to Halstedian principles were first raised by a London anatomist, J.H. Gray, in a masterly study of lymphatic anatomy of the breast. Using barium and thorotrast injections, Gray demonstrated normal lymphatics between the primary carcinoma and involved nodes, a finding which implied that carcinoma cells pass to the regional nodes as emboli, and not as a solid cord of cells occupying the whole lumen of the vessel has had been suggested by Handley and Halsted. Furthermore, Gray's studies failed to demonstrate lymphatic plexi in the deep fascia of the breast, and thus questioned the basis for the routine removal of the deep fascia and muscle in a radical mastectomy (Gray, 1936). Challengers of accepted principle were as popular then, as they are today. Sampson Handley wrote of Gray's findings,

"When on such flimsy evidence he denies the spread of breast



cancer by permeation of the deep fascia, one of the best established facts of pathology, it is necessary to state plainly that such ideas are a menace to the effective treatment of the disease, whether by surgery or irradiation." (Handley, 1937).

#### The "Modified" Radical Mastectomy

On the basis of Gray's findings, Patey and Dyson, devised a new "modified" radical mastectomy, which preserved the pectoralis major muscle and nerve, with the object of improving the cosmetic appearance and reducing operative blood loss. In 1948, they reported results of 46 cases, which they compared with those of 45 patients treated by radical mastectomy during the same interval. Patients were categorised according to lymph node involvement and were comparable in both groups. The Halsted procedure did not confer any advantage with respect to local recurrence or survival, over the new "modified" procedure (Patey and Dyson, 1948). This procedure remains popular today.

#### Simple mastectomy plus radiotherapy

McWhirter has long been regarded as a champion of the cause of limited surgery in the treatment of breast cancer, but it is noteworthy that his motives for his advocated combination of simple mastectomy and radiotherapy, were in fact radical in concept. McWhirter was impressed by the poor results, relating to both local recurrence rate and survival, of the radical operation and in 1935, he recommended to the surgeons of Edinburgh that post-operative radiotherapy be administered following the radical mastectomy. Analysis of these results six years later demonstrated a fall in the

rate of local recurrence, but little alteration of survival. In his review, however, McWhirter came across records of a number of patients who had been treated by a combination of radiotherapy and simple mastectomy and, being impressed by the favourable survival in this group, commenced an uncontrolled trial (incorporating this combination) in collusion with the Edinburgh surgeons. In 1948 McWhirter published results of a historical comparison of three different methods of treatment. The five year survival of all "operable" cases, treated by radical mastectomy alone, between 1930 - 1934 was 35.6% whereas, that for patients treated by radical mastectomy plus post-operative radiotherapy between 1935 - 1940 was 44%. The best results were achieved by simple mastectomy plus post-operative radiotherapy, between 1941 and 1945, in which the five year survival was 55.9%. Although not primarily motivated by a concern to reduce treatment morbidity, McWhirter was pleased to report an infrequent incidence of arm oedema following the less radical procedure (McWhirter, 1948).

Williams, in a comparative review of 1,044 cases, treated by local surgery, with or without radiotherapy or by radical local surgery also with or without radiation, found no difference in survival at five and 10 years. The simpler procedures carried the disadvantage of a higher rate of local recurrence but this, he felt was countered by a significantly greater incidence of other complications, notably arm oedema, after the radical procedures. Two randomised prospective trials have been conducted:

- 1) In Copenhagen, Kaae and Johanssen (1962) compared simple mastectomy plus radiotherapy with extended radical mastectomy and found no difference in survival or rate of recurrence

2) In England, Brinkley and Haybittle similarly failed to report any significant difference, in either survival or rate of local recurrence with the use of either simple mastectomy plus radiotherapy or radical mastectomy plus radiotherapy (Brinkley and Haybittle, 1966).

A study which progressed a step further, compared simple mastectomy with radical mastectomy with the proviso that radiotherapy was given to patients in both groups post-operatively only on confirmation of axillary metastases. Nothing in their preliminary data, concerning 230 patients suggested that a conservative approach was inferior to a radical one (Roberts et al, 1973). All of the above studies have really compared one form of radical treatment with another, in various combinations of surgery and radiotherapy and the similarity of results might have been expected.

#### Simple Mastectomy : Conservative Surgery versus Radicalism

Further studies truly compared simple conservative surgery with radicalism. Den Besten and Ziffren (1965) in a comparative review of simple mastectomy and radical mastectomy, failed to find any survival advantage at five years, for patients treated by the radical procedure, irrespective of axillary node involvement. Crile (1968) published similar findings. These reports, however, were based on relatively small series of patients. Any survival benefit of radical over simple surgery, is likely to be minimal at five years of follow-up (Baum, 1972) and therefore a relatively large number of patients, randomised to receive either radical or simple

therapy would be required to detect any such difference. For example, two thousand patients would be required to achieve a 90% chance of detecting a 7% difference at the statistical level of  $p < 0.05$  and if no difference existed, then a large series of patients would emphasise this finding with confidence (Baum, 1972).

Motivated by these concepts, a multicentre trial was initiated and co-ordinated at King's College Hospital, which compared the radical regimen favoured in the United Kingdom (simple mastectomy plus immediate post-operative radiotherapy to the operative site and the axillary, supraclavicular and internal mammary nodes) with a conservative regimen (simple mastectomy alone). Two thousand, two hundred and sixty-eight patients were randomised to one or other regimen between 1970 and 1975. At five years, there was no evidence that radiotherapy conferred benefit as regards survival or distant recurrence, in patients with Manchester Stage I or II disease (Fig. 1 : 1). The conservatively treated patients, however, did have a higher incidence of subsequent disease in the axilla and of local chest wall recurrences (Fig. 1 : 2). These complications however, were successfully controlled by additional treatment, in 70% of patients in whom they arose. These findings have also been confirmed at 10 years of follow up (Cancer Research Campaign Working Party, 1976, 1980).

In America, the National Surgical Adjuvant Breast Project (NSABP) working party initiated a similar multicentre trial in 1971 with three treatment options. Patients without nodal involvement were randomised to receive either (1) radical mastectomy alone or (2) simple mastectomy alone or (3) simple mastectomy plus post-

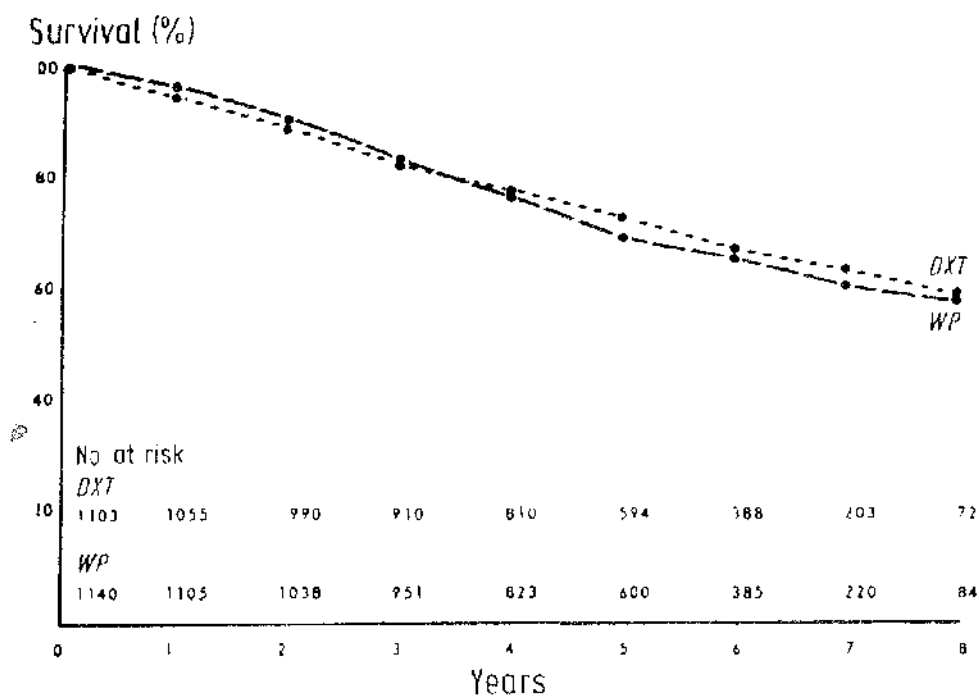


Fig. 1 : 1

Cancer Research Campaign Study (1980).

Survival in watch policy (simple mastectomy alone)  
and DXT (simple mastectomy with radiotherapy) groups.  
Differences not significant.

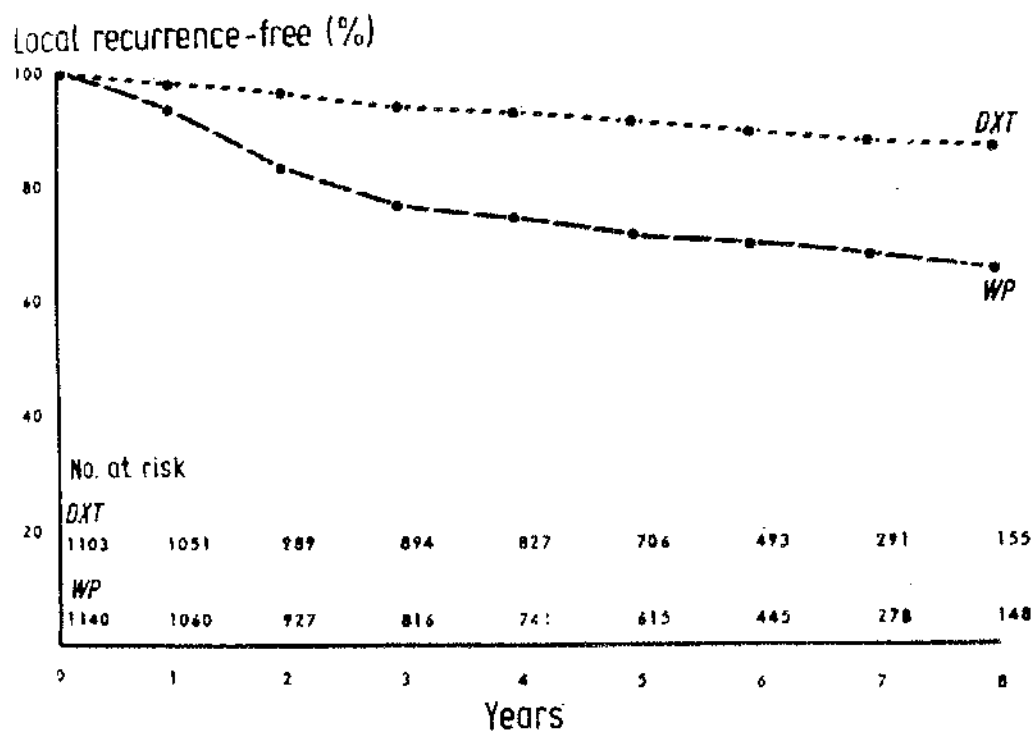


Fig. 1 : 2 Cancer Research Campaign Study (1980).  
 Local recurrence free in watch policy (simple mastectomy alone) and DXT (simple mastectomy with radiotherapy) groups.  $p < 0.001$  by Logrank.

operative radiotherapy to the axillary, internal mammary and supraclavicular nodes. By 1977, 980 patients had been treated and followed up for a mean interval of 36 months when results were reported. There was no significant difference in disease free interval or in cumulative survival between the three treatment options although the incidence of local and regional recurrences was marginally higher in the group treated by simple mastectomy alone (Fisher, 1977a).

These studies undoubtedly demonstrate that radical local treatment, whether by surgery alone or in combination with radiotherapy, does not carry any survival advantage over that of simple mastectomy alone. In particular, it would seem that prophylactic ablation of regional lymph nodes irrespective of whether they are involved by tumour, has no contribution to achievement of cure. Indeed, Fisher concluded that, "Tertiary metastases from neglected nodes is either minimal or is inconsequential in the overall course of the disease" (Fisher, 1977a).

#### Routine post-operative radiotherapy and local recurrence

The above studies demonstrate that post-operative chest wall irradiation is unlikely to convey any survival advantage but that this measure is likely to reduce the incidence or delay the onset of local recurrences. The question arises of whether its routine use is justified solely on these grounds. This question, even today, remains controversial (Leading Article B.M.J. 1981).

The observed rate of local recurrence at five years after simple mastectomy is between 15% and 30% (Friedlander, 1981) and routine irradiation administered immediately after surgery would prevent the development of this complication in approximately 60% patients (Chu, 1976). However, as was first emphasised by Paterson and Russell, a more conservative policy of delaying radiation treatment until recurrences appear, is successful in controlling this complication in approximately 70% of those in whom it arises (Paterson and Russell, 1958; Chu, 1976; Cancer Research Campaign, 1976). Therefore the incidence of 'resistant' local recurrences, that is having either made their appearance following prophylactic radiation or persisted despite 'purposive' irradiation is likely to be similar at five years of follow up (Friedlander, 1981).

Routine post-operative radiotherapy carries significant side effects such as rib necrosis, pneumonitis, skin ulceration, myelopathies, etc. which occur in 7 - 24% of treated patients (Chu, 1955; Meyer, 1978; Polansky, 1980). A conservative policy, therefore of delaying treatment until local metastases appear would spare 70 - 85% patients these possible complications without any palliative loss (Friedlander, 1981). The clinician's choice of treatment for early breast cancer must be based upon his personal evaluation of these relative pros and cons but for many surgeons the morbidity attendant upon routine 'prophylactic' radiation and also the lack of any survival advantage or indeed of any palliative gain, would argue strongly against its continued use.



### Local excision with radiotherapy

The final step in the progressive shift to conservative local treatment ends with this procedure. Preservation of breast tissue with a superior cosmetic end result are the aims of this method, which is no less 'radical' in principle than a simple mastectomy.

Mustakallio was the first advocate of this method and in a series of 127 patients whose primary tumour was "no bigger than a hen's egg", treated by lumpectomy and irradiation, he reported a five year survival of 84% (Mustakallio, 1954). Other uncontrolled series followed. In a review, Porritt (1964) found a five year survival of 65% in 74 women treated by lumpectomy and irradiation and only 50% in 109 women treated by radical mastectomy. Levene et al (1977) reported a five year rate of local control of 100% in 64 women with stage I and II breast cancer treated by this method. All patients with stage I disease and 62% with stage II disease survived five years. Calle et al (1978) reported similar results with this method. Five and 10 year survival rates of 120 women (whose primary was less than 3.0 cms diameter) were 85% and 75% respectively. In Calle's series only 16 women (12%) required secondary surgery for local recurrence. Two prospective randomised trials have been completed.

1) Atkins et al (1972) randomly allocated 387 women aged over 50 years with operable breast cancer to receive either -

a) radical mastectomy plus post-operative radiotherapy (2,500 - 2,700 rads to axilla supraclavicular fossa and to internal mammary chain) plus 3 doses of Thiotepa

or

b) wide local excision of the lesion (extended tylectomy) plus post-operative radiotherapy (2,500 - 2,700 rads to the axilla, supraclavicular fossa and internal mammary chain plus 3,500 - 3,800 rads to the breast) plus 3 doses of Thiotepa.

In patients with stage I and II disease, a higher rate of local recurrence was seen in the extended tylectomy group. However, the majority of these "local" recurrences appeared in axillary nodes, treated by radiotherapy doses which have been considered inadequate (Fisher, 1977b). No survival difference was noted in all patients (Fig. 1 : 3) or in those with stage I lesions (Fig. 1 : 4), but in stage II cases radical mastectomy gave a significantly better survival at 10 years, although not at five years (Fig. 1 : 4). It is noteworthy that this trial has been criticised on the grounds of inadequate radiotherapy, given not only to the axilla, but also to the breast (Orthovoltage irradiation was used) (Veronesi et al, 1981).

2) Veronesi and colleagues (1981) randomised 701 patients with tumours smaller than 2.0 cms with no palpable nodes to receive either

a) A Halsted radical mastectomy

or

b) A 'quadrantectomy' with axillary dissection and radiotherapy to the ipsilateral residual breast tissue. No difference in disease free interval or survival was seen between the two groups at seven years follow up (Figs. 1 : 5, 1 : 6).

Although this study concerned patients with 'clinical' stage I breast cancer, approximately 25% patients in each group had

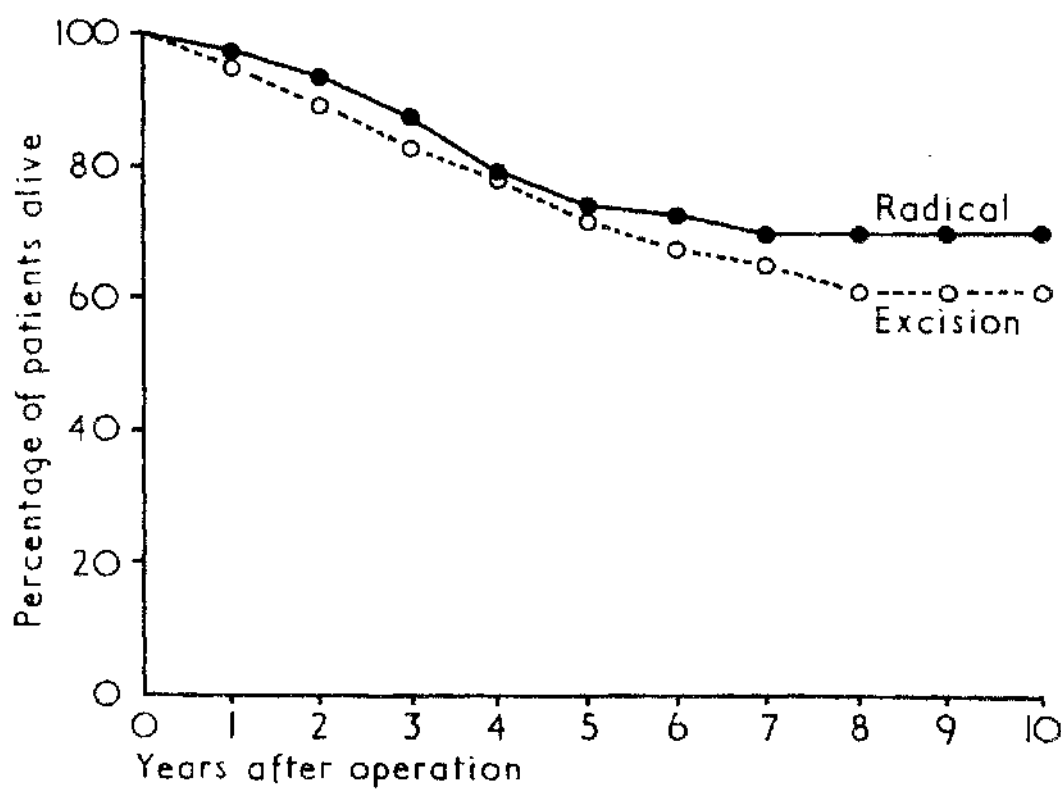


Fig. 1 : 3      Hedley Atkin's Study (1972)  
Radical mastectomy versus tumour excision with  
radiotherapy : All cases.  
Survival differences not significant.

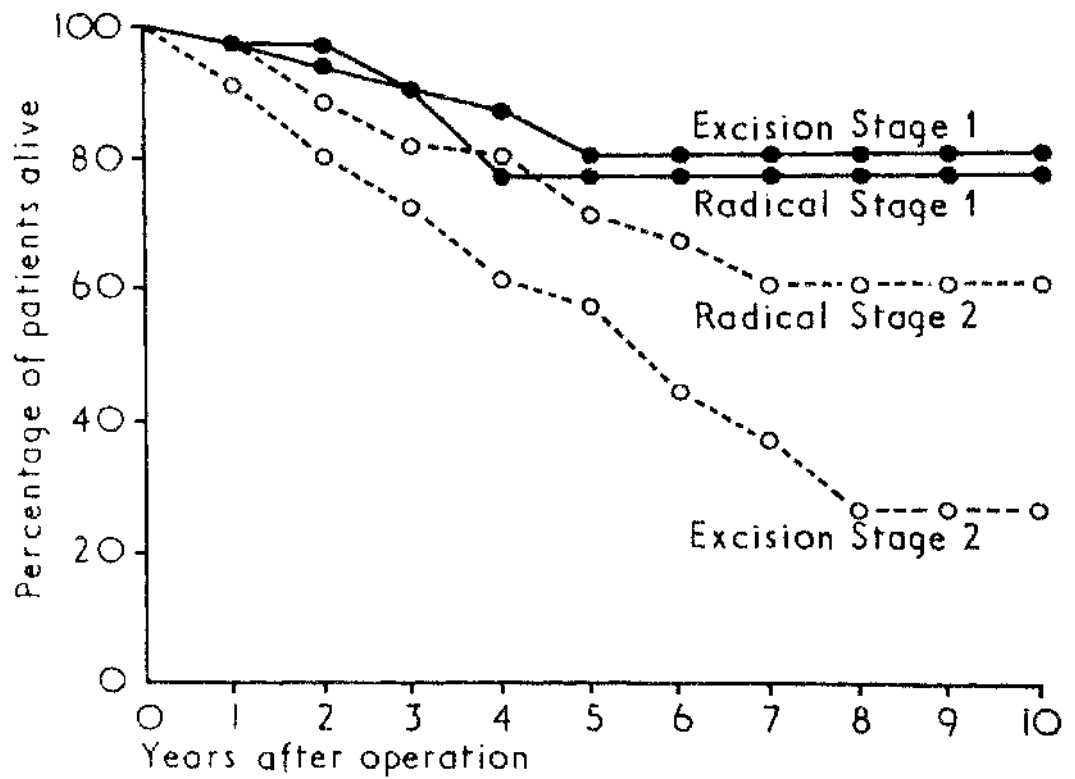


Fig. 1 : 4 Hedley Atkin's Study (1972)  
 Radical mastectomy versus excision with radiotherapy.  
 Survival according to clinical stage.  
 Stage I : No significant difference  
 Stage II :  $p < 0.05$  by Logrank.

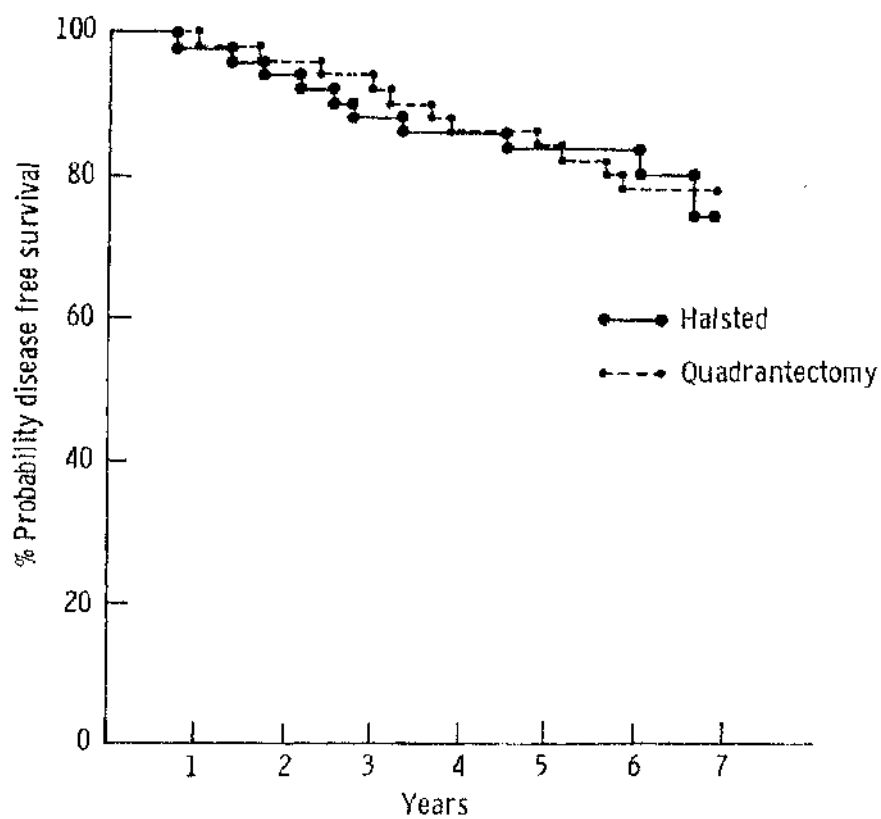


Fig. 1 : 5 Veronesi Trial. Radical mastectomy versus quadrantectomy, axillary dissection and radiotherapy. Disease free interval: no significant difference.

(Veronesi et al., 1981)

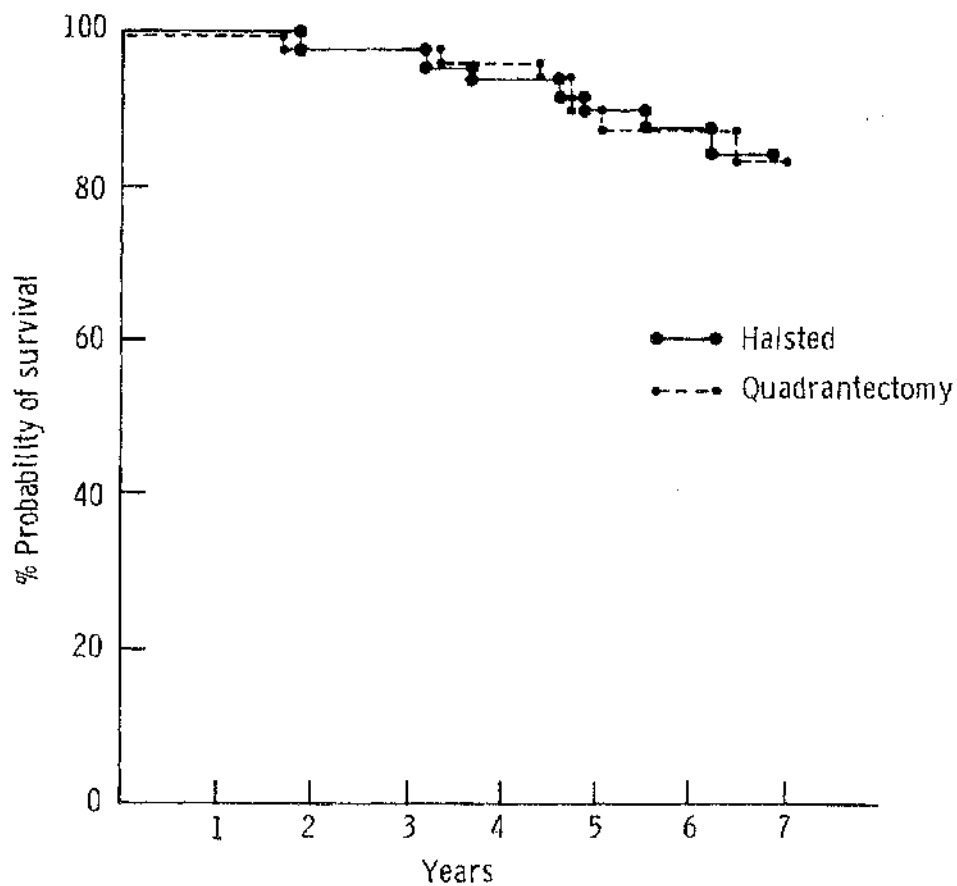


Fig. 1 : 6 Veronesi Trial. Radical mastectomy versus quadrantectomy, axillary dissection and radiotherapy. Survival: no significant difference.

(Veronesi et al, 1981)

pathologically defined axillary node metastases. When these patients were considered separately, no survival difference was found, a finding rather at variance with the Atkins study (Fig. 1 : 7)

Thus in patients with early breast cancer, without clinical evidence of involved nodes, lumpectomy and irradiation appears to be as effective as a radical mastectomy. No conclusion can as yet be drawn for clinical stage II disease. In America, the NSABP is currently evaluating 'segmental' mastectomy (wide local excision) both alone and in combination with radiotherapy against simple mastectomy in patients with clinical stage I and II disease. We await their results with interest. It is therefore rather early to draw valid conclusions about lumpectomy and radiotherapy. Its case for all operable disease is unproven.

#### The place of local therapy in breast cancer

Breast cancer appears to be a systemic disease in the majority of patients at the time of presentation. As MacDonald pointed out approximately 30 years ago, "the fact that uniform cure is not obtained by radical surgery in cancers which by morphological evidence are still localised to their site of origin, indicates frequent dissemination, early in the infiltrative period" (MacDonald, 1951). When it is considered that breast cancers as small as 1.0 centimetre in diameter have already progressed through approximately 30 doublings (Spratt, 1977) then the principle of 'early' dissemination becomes less surprising. Circulating cancer cells have frequently been reported associated with 'early' breast cancers (Roberts et al, 1958).

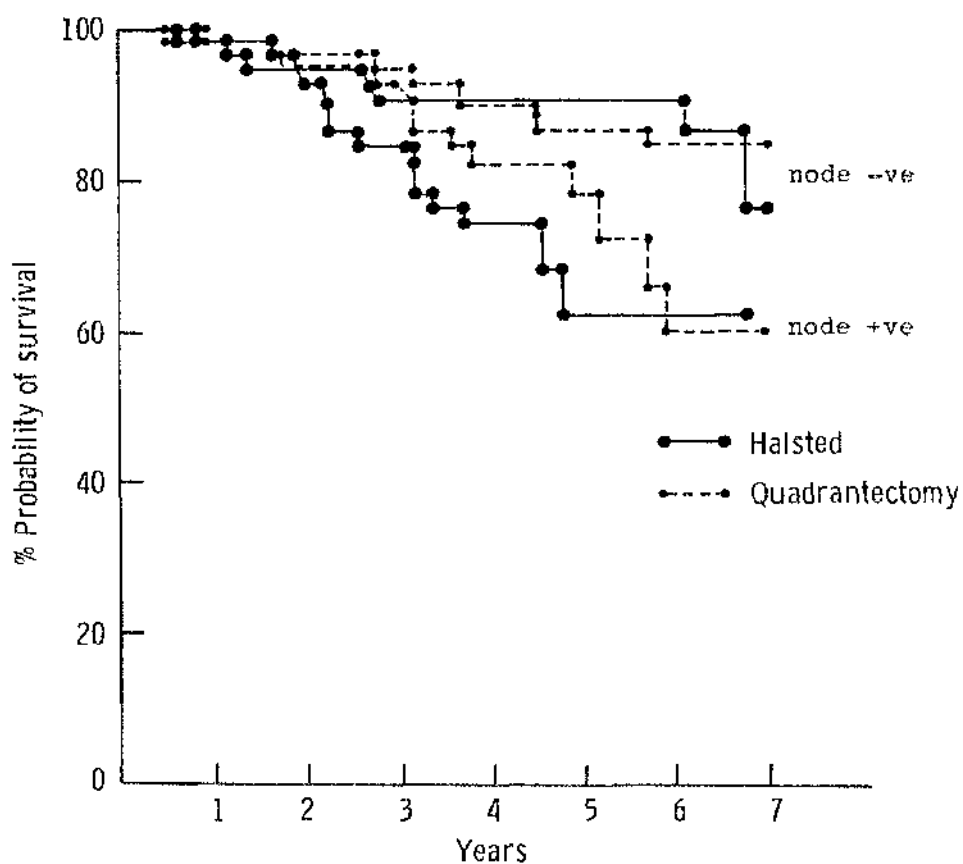


Fig. 1 : 7 Veronesi Trial. Radical mastectomy versus quadrantectomy, axillary dissection and radiotherapy. Survival by node status.

Node negative group - no significant difference

Node positive group - no significant difference



What place does local therapy have in a disease of this nature?

Several important points of dogma have emerged from the aforementioned trials in breast cancer. Firstly, local therapy will prevent local advancement of the disease from a small circumspect lesion to a large fungating mass, which oozes blood and protein to the detriment of the patient. Secondly, local therapy will not only remove the lesion but will delay or prevent local recurrences. As first emphasised by Moore in 1867, the likelihood of local recurrence is inversely related to the extent of local treatment: the incidence is lowest after an extended radical mastectomy (Lacour, 1976) but unacceptably high after simple excision alone (Halsted, 1896). However, as local treatment becomes more aggressive, the incidence of complications rises until the paradoxical point is reached, where the morbidity due to treatment exceeds that due to the complication which it is designed to prevent (Devitt and Beattie, 1964). Today, the standard local treatment for operable breast cancer in the United Kingdom is a simple mastectomy which achieves adequate local control with a low incidence of complications (Cancer Research Campaign Working Party, 1980). It is uncertain at the present time whether lesser procedures may be as satisfactory.

Thirdly, we know that 20 - 25% patients with clinical stage I or II breast cancer will have a normal expectation of life after effective local treatment (Brinkley and Haybittle, 1968; Adair et al, 1974). This concept has been described as a "practical cure" by Adair et al (1974). How has local treatment achieved this end? In the small number of cases (whom we cannot identify) where cancer is

truly confined to the breast, then cure by mastectomy alone is both logical and feasible. However, when spread outside the breast has occurred, then the concept of 'cure' by mastectomy alone is more difficult to comprehend. In Brinkley and Haybittle's series, one quarter of the patients alive at 25 years after mastectomy had involvement of axillary nodes at the time of their operation and many of these received no additional therapy. How can these patients have been cured by a mastectomy alone? Fisher has proposed the hypothesis that removal of the bulk of tumour by a mastectomy leaves behind a small "critical tumour load" which may be satisfactorily eradicated by the host's natural immunological defences (Fisher, 1977b). There is no direct evidence which supports this hypothesis, however and others have demonstrated contrary effects, that the growth rate and growth fraction of residual cancer cells increase after removal of the primary lesion (De Wys, 1972), so that immune defences would appear to have little inhibitory effect.

One important factor which has received little attention is the natural variation of behaviour or growth rate of these cancers. Relatively few patients with breast cancer refuse mastectomy, but nonetheless, 30 year survivors who show no evidence of distant metastases have been reported within this group (Baum, 1980). To reconsider Brinkley and Haybittle's data (1975): the death rate in the 'cured' mastectomy patients was identical to that of an age matched population without breast cancer at 20 years of follow up, but the death rate from breast cancer in the former was sixteen times greater. These findings suggest that a form of natural

selection, due perhaps to the cancer's slow growth rate or limited metastasising potential, may be partly responsible for the apparent 'cures' observed after surgical treatment.

#### Principles of "Adjuvant" systemic therapy for breast cancer

The limitations of local therapy for carcinoma of the breast have become apparent over the past few decades. No matter what local treatment is given, metastatic relapse is the major cause of treatment failure and ultimate demise. The concept of breast cancer which is systemically disseminated at the time of presentation (in the majority of patients) has become accepted and it would seem logical that some form of systemic therapy be incorporated into the initial management. Chemotherapy and hormonal therapy are the two most popular modalities which have been used in this respect and numerous trials have been carried out.

#### Adjuvant chemotherapy

This method is the more recent of the two, and it has been enthusiastically accepted in America, despite the caution of the initial investigators. The principles and results of this method will be considered.

#### The agents

It is rather ironic that research on chemical weapons of war has led to the modern era of chemotherapy for malignant disease. Systemic analysis of the nitrogen mustards began in 1942 and at the outset considerable toxicity was described particularly in tissues with renewable cell populations, eg lymphoid tissues, bone marrow and the epithelium of the gastrointestinal tract (Gillman, 1963).

The ideal cytotoxic drug which will control the growth of cancer cells or destroy them completely without serious damage to the host, has not yet been found. Common features of present day chemotherapeutic agents are their disparate origin, their marginal anti-tumour selectivity and their narrow therapeutic index i.e. a narrow margin between an effective dose and a lethal dose (Dowling et al, 1970). These agents are considered to be most active against rapidly proliferating cells, but their precise mechanism of action (with few exceptions) is obscure (Calman et al, 1980) and they must be regarded as non specific cell toxins.

Nevertheless, these compounds have revolutionised the treatment of some cancers eg. Choriocarcinoma - where permanent regression can be anticipated in approximately 80% patients after Methotrexate therapy with or without the use of other agents (Li, 1956). Carcinoma of the breast is only moderately sensitive to these drugs which have consequently had a disappointing effect upon advanced stages of that disease. Certainly the results of early trials using combinations of these agents were encouraging: Cooper (1969) claimed complete responses in 88% patients with advanced breast cancer treated by Cyclophosphamide, Methotrexate, 5-Fluorouracil, Vincristine and Prednisone. However the promise of these preliminary findings have been largely unfulfilled and total (partial and complete) response rates of 40 - 60% with a median duration of remission of approximately seven months are more frequent findings (Kardinal, 1979). It is noteworthy that rather less than 5% patients who receive chemotherapy for recurrent breast carcinoma survive five years (Fisher, 1977b).

## Toxicity

Cytotoxic agents have short and long term side-effects. Our knowledge of the former is greater, and certainly, short term complications are of greater importance to the patient with advanced disease.

Chemotherapeutic agents are most active against rapidly dividing cells, both in a target cancer and those which comprise normal tissues, eg. Gastrointestinal tract, epithelium, skin, hair follicles, bone marrow (Kardinal, 1979). The external manifestations of these actions are the complications of nausea, vomiting, stomatitis, diarrhoea, haemorrhagic cystitis, alopecia and marrow depression, all of which are easily recognisable. In addition, most cytotoxic drugs in general and alkylating agents in particular have some action against resting cells (Dowling et al, 1970). Their action against stable cells of the host are more difficult to quantify, but have certainly been documented, eg. Vincristine Neuropathy, Adriamycin Cardiomyopathy. While criteria of assessment of response of a cancer to chemotherapy are relatively stringent (Hayward et al, 1977), methods of evaluation of damage to normal tissues are rather less precise. Often the only standard measurement which is taken to give an indication of the degree of cellular toxicity is a white cell count.

Long term side effects are of concern in patients who are clinically well and receive these agents as 'adjuvant' therapy. Chromosomal breakage may occur (Schien and Winokur, 1975) and the leukemic potential of these agents has been shown in patients who have received them as an adjuvant (Seiber and Adamson, 1975; Rizzo

et al, 1981). The consequences of immunosuppression 'early' in the course of the disease are unknown. In view of the limited effectiveness of these agents in advanced disease and their known toxicity, what is their place in treating undetectable disease at the time of mastectomy?

#### Principles of adjuvant chemotherapy

Two ideas underline the use of cytotoxic agents as 'adjuvant' therapy.

Firstly, the efficacy of chemotherapy is dose dependent and therapy in tolerable doses is more likely to be effective against small numbers of residual tumour cells (Hill and Price, 1977). Therefore, the administration of this treatment immediately after mastectomy when only 'small' deposits of cancer remains in the form of micrometastases is likely to increase the cure rate (Bonadonna et al, 1978). Evidence for this contention is provided from in vivo experimentation upon animal models. Using C5781 mice, in which mammary adenocarcinomata had been transplanted, Shapiro and Fugman (1957) demonstrated a 57% "cure" rate by the concomitant use of surgery and chemotherapy, whereas either modality, given alone, failed to achieve any "cures". ("Cure" was defined as survival for a period of 30 days without evidence of cancer recurrence.)

Secondly, the sensitivity of tumour cells to a specific drug is a function of the proliferating state of the tumour and surgical removal of the primary cancer possibly causes an increase in the rate of cellular replication of metastases. This second principle has been shown only in the animal model (Simpson-Herren and

Griswold, 1970; De Wys, 1972). Thus rapidly cycling micrometastases ought to be more susceptible to cytotoxic agents (Fisher and Wolmark, 1977).

To take the first principle, it must be remembered that cytotoxic agents are non specific and the dose-response effect of this treatment on micrometastases may be rather diluted by its affinity for normal host cells. On the second principle, the evidence that micrometastases are more susceptible to cytotoxic agents following removal of the primary is controversial and indeed there is experimental work (again on animal models) which suggests the opposite, that micrometastases are more susceptible before removal of the primary (Van de Velde et al, 1977) or that micrometastases are less susceptible than the primary tumour itself (Van Putten et al, 1979).

Indeed, it is a little surprising that principles elaborated from experiments performed on induced cancers which comprise a particularly homogenous strain of cancer cell (Currie, 1979), or transplanted cancers studied in different species and under a unique set of conditions, have been applied so readily to the human disorder.

#### Adjuvant chemotherapy: Results of treatment ~ The Trials

This brief review shall consider only the larger clinical trials of this therapeutic modality. The initial rationale for administration of adjuvant chemotherapy was the desire to eradicate circulating cancer cells, dislodged during a mastectomy.

In 1958, the NSABP initiated a prospective randomised double blind study of thiotepa vs placebo. Eight hundred and twenty-six women were entered until 1961. Treatment was given at the time of surgery and for two days thereafter. Only premenopausal patients with  $> 4$  positive nodes derived any benefit and the recurrence rate in this group was approximately 40% less than in controls. At five years of follow up differences in rate of recurrence were not significant, but a significant survival benefit ( $p < 0.05$ ) was apparent in premenopausal patients with  $> 4$  positive nodes who received adjuvant treatment (Fisher et al, 1968). These initial results appeared to support the principle of adjuvant chemotherapy and a conceptual change of emphasis occurred at this time from the destruction of circulating cancer cells, to the need to destroy occult micrometastases.

In 1972, a randomised trial of adjuvant Melphalan (L-PAM) vs placebo was initiated by the NSABP in patients considered to be at risk of recurrence ie. those with positive axillary nodes. Treatment was given for five days, at six week intervals over two years. Once again only premenopausal node positive patients showed any benefit in that significantly fewer recurrences were seen at 18 months. However at 30 months, differences were not significant (Fisher, 1975). A British study has failed to find any benefit with L-PAM in either pre or postmenopausal women (George et al, 1981).

Combination chemotherapy appears superior to single agents in the treatment of advanced disease (Taylor, 1974, 1976) and, based upon this concept, the Tumour Institute of Milan initiated their randomised prospective trial of CMF (Cyclophosphamide, methotrexate



and 5-fluorouracil) versus placebo, in patients with involved lymph nodes. Treatment was started two to four weeks following mastectomy and continued for 12 monthly cycles. Observed toxicity was much greater than encountered with single agents. Three hundred and eighty-six patients were entered and have since been followed up for five years (Rossi, 1981). At 12 months of follow up, a highly significant decrease in recurrence was noted in both pre and postmenopausal patients treated with CMF. The greatest improvement was observed in patient with > 4 nodes involved (40.7% controls had recurred vs 8.8% CMF) (Bonadonna et al, 1976). By 36 months of follow up, the benefit in premenopausal patients was still striking, but differences in postmenopausal patients were no longer significant. Patients with 4 involved nodes once again derived greatest benefit (Rossi et al, 1981). At five years of follow up, a significant survival advantage ( $p < 0.05$ ) was observed in the treated group. The recurrence free interval was significantly prolonged in patients with 1-3 nodes, but not in those with > 4 nodes. Similarly, significant differences were observed between treated and control groups in premenopausal subjects, but not in postmenopausal patients (Rossi, 1981). These findings which are of immense interest raise certain important questions:

1. Are the benefits of treatment due to true eradication of micrometastases or to short term inhibition of tumour growth?

The beneficial effects of chemotherapy were transient in certain patient subgroups:

a) Postmenopausal patients (Milan Study)

A significant decrease in recurrence rate was observed at 12

months of follow up in treated postmenopausal patients, but not at 36 months or five years. Bonadonna and Valagussa now suggest that the apparent lack of benefit in this group was erroneous and was a result of the lower dosages of chemotherapy which were administered, in an attempt to reduce toxicity. In a retrospective review, the authors found that those postmenopausal women who received full doses of treatment did in fact have a significantly greater five year survival ( $p < 0.05$ ) than untreated controls (Bonadonna and Valagussa, 1981).

This argument is not convincing. The numbers of patients concerned in this retrospective analysis were small: only 20 postmenopausal women had received a full course of therapy of whom only nine had been followed up for five years. Furthermore, disparities could have occurred in other undefined variables, which conceivably could have contributed to the observed difference.

These implications then, concerning drug dosage are inconclusive and it is conceivable that the observed advantage of therapy in postmenopausal patients could have been due to a temporary growth inhibition.

#### b) Patients with 4 involved axillary nodes

Relapse free interval was prolonged for only a temporary interval among premenopausal women in this category in the Thiotepa study and also in all such patients in the Milan project.

It might be argued that patients with significant nodal involvement could have a large residual tumour burden which is beyond extirpation by such measures, that palliation would be the best that could be achieved and that cures would only be possible in

those with 'minimal residual cancer'. This argument is almost impossible to test. Patients with 'minimal disease' following mastectomy eg. node negative patients, have a high survival rate (about 80% at five years) and certain authorities would feel that exposure of such patients to the hazards of therapy is not justified (leading article Lancet, 1981; Carter, 1981). A very large number of patients would have to be treated and followed for a prolonged interval, before any significant advantage could be demonstrated. To date, therefore, adjuvant chemotherapy has had the effect of simply delaying recurrence in certain patient subgroups. Further follow up is required to ascertain whether a true eradication of micrometastases has been achieved in others.

## 2. Does adjuvant chemotherapy convey a true survival advantage?

This question follows in logical sequence to the last and will be the critical test of the principles and rationale of adjuvant treatment. We must be concerned only with a persistent survival advantage and not simply with a short term benefit in treated women, when compared with untreated controls. Such a short term survival advantage was demonstrated for adjuvant Thiotepa, in premenopausal women with > 4 nodes in the NSABP study (Fisher, 1968). The crucial question, which was unanswered by the NSABP study, is whether treatment at the time of recurrence will equalise the life intervals in each group. The recent data from Milan has attempted to resolve this matter (Rossi et al, 1981). Secondary systemic therapy was given at recurrence to both study arms, but a small proportion of patients in each arm did not receive it. Thus, secondary treatment

was given to 91.3% of controls and 93.9% of the adjuvant group, on detection of recurrence. Various treatment modalities were given to variable proportions of the patients but the majority (approximately 60%) in each group received secondary chemotherapy alone for their recurrences. Control patients received CMF and the adjuvant group were given Adriamycin and Vincristine at relapse. All analyses pertaining to secondary treatment were confined to these patients. The rate and duration of response to this secondary chemotherapy were comparable in both study arms and the authors concluded on this basis, that the overall survival advantage of the adjuvant patients was due to adjuvant CMF and that secondary therapy at recurrence, had little influence.

A closer analysis of the Milan data raises doubts about these conclusions. When the survival of the same groups of women, who in both arms received secondary chemotherapy for recurrence was considered, it was shown that the control group fared better. Median survival from the time of mastectomy was 55 months for controls but only 50 months for the adjuvant group (Table 1 : I, Rossi et al, 1981). Thus, when that proportion of the patients who, in both study arms were followed from mastectomy and given similar systemic therapy at relapse (chemotherapy) were considered separately it became clear that the adjuvant patients fared worse. With that observation, it becomes difficult to account for the favourable survival advantage of adjuvant patients in the study considered as a whole. It is possible that survival differences in favour of the adjuvant group as a whole could be entirely due to differences in the minority of women in both study arms who received

Table 1 : IMedian survival in patients with relapse (months)

	<u>From first relapse</u>	<u>From radical mastectomy</u>
Controls	38	55
CMF Group	30	50

Reproduced from Table IV Rossi et al, 1981

no systemic therapy at relapse. In this minority, we would expect the adjuvant group to have a better outlook, since they received systemic treatment at mastectomy, controls received none and treatment ought to be better than no treatment irrespective of when it is given.

The numbers of patients who received salvage therapy at relapse were not revealed in this study and the important question of whether adjuvant chemotherapy conveys a true survival advantage must be regarded as being unanswered. Preliminary data does not appear promising.

### 3. Is the toxicity justifiable?

It is impossible to make an objective judgment on this question without a conclusive answer to the last. Early toxicity is considerable (Table 1 : II). Palmer et al (1980) reported side effects which were sufficiently severe to interfere with lifestyle in 79% patients receiving a multiple drug regimen (Chlorambucil, Methotrexate, Fluorouracil, Vincristine and Adriamycin). Twenty-nine percent volunteered that the treatment "could never be gone through again".

Late complications require further patient follow up for full evaluation. In particular, Rossi et al (1981) reported an equal incidence of second cancers between treated and control groups and no cases of acute leukaemia. One of the treated patients, however, has already developed a fatal acute leukaemia which was considered to be due to adjuvant therapy (Rizzo et al, 1981).

Table 1 : IIToxic manifestations of CMF

Leukopenia	%
2,500 - 3,999	67
< 2,500	4
Thrombocytopenia	
75,000 - 129,000	57
< 75,000	14
Alopecia	55
Cystitis	28
Amenorrhoea	54
Oral mucositis	18
Conjunctivitis	25
No toxicity	4

Reproduced from Table III (Bonadonna et al, 1976)

## Hormonal Therapy as Adjuvant

### The mechanism of oestrogen action and oestrogen deprivation

The response of target tissues to oestrogen is related to the presence of cytoplasmic receptor proteins which bind oestradiol as it enters the cell with selective high affinity (Jensen and De Sombre, 1973). The binding of the hormone to the receptor forms a complex which migrates to the nucleus where it reacts with specific acceptor sites in the chromatin. Here, the nuclear oestrogen receptor complex initiates a series of events on the DNA template which lead to production of macromolecular components essential for continued cell maintenance and function and also to DNA replication and cell division (Griffiths and Nicholson, 1981). See Fig. 1 : 8) Thus, any process involving suppression of oestrogen production or oestrogen blockade at cellular level would lead to tumour regression. The actions of oestrogen are therefore specific to cells which possess the receptor and processes of oestrogen deprivation are similarly specific. As a consequence, therapeutic side effects are less severe than with non specific cytotoxic agents. Thus good quality remissions, which are often prolonged may be induced in approximately 30% patients with advanced breast cancer (Stoll, 1969).

### Principles of hormonal therapy as 'adjuvant'

An experimental basis for the use of adjuvant hormonal therapy has been elaborated from observations on the rat mammary model. It has been shown that a proportion of induced rat mammary tumours can be extinguished completely by anti-oestrogen therapy (De Sombre and



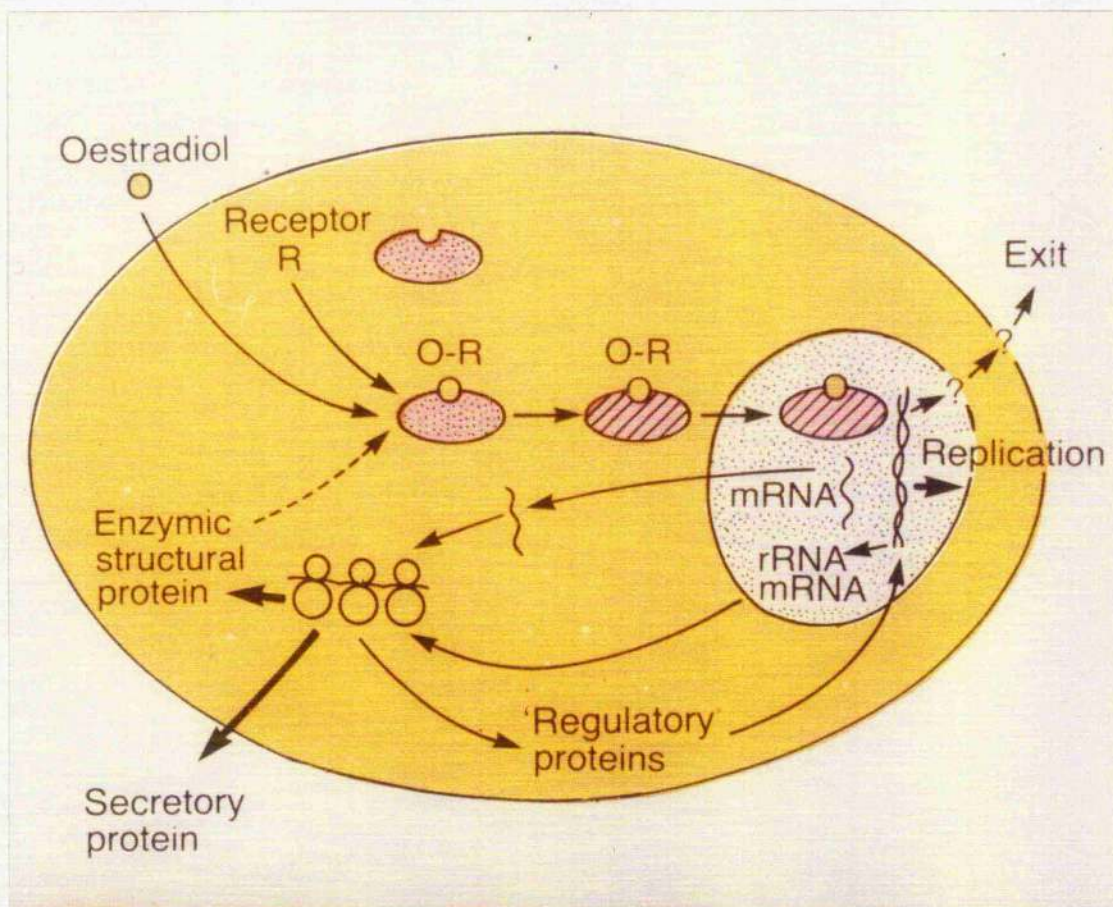


Fig. 1 : 8

Diagrammatic representation of oestrogen effects within the target cell: oestradiol binds to cytoplasmic receptor and the complex becomes translocated to the nucleus where it alters the DNA template. Messenger (MRNA) and ribosomal RNA then enhance the production of protein components essential for cell maintenance and function.

(Griffiths and Nicholson, 1981)

Arbogast, 1974). Tumours which are early in the course of their development are most likely to have the most complete response to oophorectomy or anti-oestrogen therapy (Griswold and Green, 1970; De Sombre and Arbogast, 1974) which suggests a "dose-response" principle, similar to that of adjuvant chemotherapy. However, the criticisms already mentioned of principles elaborated from animal models also apply in this context. Nevertheless, it would seem that hormonal treatment, with its relative lack of side effects would comprise the ideal modality to be used as an 'adjuvant'. Various trials have been conducted and will be reviewed briefly.

#### Adjuvant hormonal therapy - The trials

The first trial to incorporate hormonal therapy as an "adjuvant" measure, was conducted by C.W Taylor in Boston between 1935 and 1939. Taylor had previously shown the therapeutic value of a "radiation menopause" in premenopausal patients at recurrence. This treatment, interestingly, was particularly useful for bony metastases. He then treated 47 premenopausal patients by routine "radiation castration" at mastectomy until 1939, but was unable to demonstrate any difference in the incidence of recurrence between treated patients and a historical control series of 50 premenopausal women and concluded that prophylactic castration was not advantageous (Taylor, 1939).

Kennedy et al (1964) in a review, reported a prolongation of disease free interval in a group of women who received 'adjuvant' oophorectomy, but oophorectomy at the time of recurrence in the untreated group equalised matters so that the net effect upon survival was the same.

Of particular interest are the results of five randomised prospective trials. Nevinney randomised one hundred and forty-three patients to oophorectomy within one month of mastectomy or to be delayed until recurrence. Patients receiving the adjuvant oophorectomy had a decreased rate of recurrence (32% vs 46%) and a slight survival benefit, but differences were not statistically significant (Nevinney et al, 1969). The NSABP project evaluated prophylactic oophorectomy in a multicentre trial which involved 154 patients and 82 controls followed up for 36 months. Patients who were at high risk of recurrence, viz. > 3 involved axillary nodes derived a transient benefit and the investigators concluded that results were disappointing and that no further studies of this modality were warranted (Ravdin et al, 1970). Nissen-Meyer (1965) reported a favourable prolongation of disease free interval and survival in postmenopausal women treated by adjuvant oophorectomy and a small dose of prednisolone, but this finding was not confirmed by Meakin in Toronto who reported no benefit in postmenopausal women. Meakin's study had more than two arms, and some patients received prednisolone in addition to a 'radiation menopause'. However, 67 patients received a prophylactic x-ray castration only and these patients enjoyed a prolonged recurrence free interval and survival, when compared to 70 controls but again differences failed to achieve statistical significance (Meakin et al, 1979).

All four of these studies may be justly criticised on the grounds of small numbers and short intervals of follow up. The most complete study of 'adjuvant' castration was conducted in Manchester and began in 1948. In this study patients were randomised to have

either a radiation induced artificial menopause, shortly after mastectomy, or no treatment until the development of recurrence (Cole, 1964). Twenty percent of the patients in this study had locally advanced disease, but fortunately, these were analysed separately and we shall consider only the 596 women with operable breast cancer. A radiation menopause significantly delayed the onset of distant metastases and this was reflected in an improved survival at five years, compared to the control group, which approached significance ( $p = 0.07$ ). At ten years of follow up, however, there was no survival difference (Cole, 1968) possibly due to the equalising effect of therapeutic castration at the time of recurrence.

Therefore, in spite of five randomised trials it is still not known whether adjuvant oophorectomy is of benefit to patients with primary breast cancer. The results indicate a delay in relapse for some patients, but there may be no survival advantage of 'early' prophylactic treatment over treatment delayed until the time of recurrence.

## Current Therapy of Early Breast Cancer

### Success and Failure

#### Local treatment

Surgical removal of the breast bearing a tumour is the mainstay of therapy for early breast carcinoma (Nemoto et al, 1980). Whether or not supported by other local measures, this treatment prevents local advancement of the cancer and is associated with a prolonged survival in 20 -25% of women (Brinkley and Haybittle, 1968; Adair, 1974) although the variable malignity of the neoplasm may have some influence upon the apparent "cure" rate. While successful in preventing ulceration, fungation etc. this treatment clearly fails in its prime objective of cure, in the majority of women. The principles of local treatment methods, wrongly based upon an orderly sequence of events in the progression of breast cancer have failed to embrace either the systemic nature of the disease or its variable biology and with the benefit of hindsight the failures of local therapy can come as no surprise.

#### Adjuvant systemic therapy

Adjuvant therapy of various means has consistently prolonged disease free interval (Cole, 1968; Fisher et al, 1976; Bonadonna et al, 1976) although cytotoxic regimens achieve this end at the cost of considerable side effects.

A physical principle governs the use of adjuvant therapy in breast cancer. Curable cancer is thought to differ from incurable cancer only by virtue of its bulk. Cure is theoretically related to the concept of a "critical tumour load" and is thought likely to

occur only in that nebulous interval when the residual "critical load" of cancer is amenable to extirpation by non specific cytotoxic or other agents (Skipper, 1971; Schabel, 1975). This principle was elaborated from animal models (Shapiro and Fugman, 1957; De Wys, 1972) but the gross variation of tumour behaviour in humans (Bloom, 1968; Devitt, 1971) casts doubt on the reliability of concepts derived from observations on homogeneous strains of cancer cells in the experimental animal.

Nevertheless, in the general conception of breast cancer, the physical principle of adjuvant therapy has largely superseded the anatomical principle of radical surgery. Neither take note of the variable nature of breast cancer. Other analogies can be drawn. Failures of radical surgery have been attributed to an inadequate extent of resection (Sugarbaker, 1964; Wangensteen et al, 1956) and failures of adjuvant chemotherapy to inadequate doses (Bonadonna and Valagussa, 1981). Doses are currently being increased and treatment time extended. Both radical surgery and adjuvant therapy do convey some benefits:- a reduced rate of local recurrence and a prolonged disease free interval, respectively. As with radical surgery the surgeon must weigh the benefits of adjuvant therapy against its morbidity. The main objective of adjuvant therapy is cure, by the eradication of micrometastases (Schabel, 1975) which ought to be reflected in a true survival advantage. This objective is still unproven, but as discussed above preliminary data from certain trials are discouraging (Cole, 1968; Rossi et al, 1981).

## Chapter 2

### BIOLOGICAL VARIATION OF BREAST CANCER

### Biological Variation of Breast Cancer

When compared to the weight of effort and expertise which have been invested in the study of other aspects of breast cancer, this important subject has received relatively little attention. Eggers, De Cholnoky and Jessup (1941) in the attempt to explain the relatively favourable outlook for patients in their series who presented with breast cancer after a two year delay, drew attention to the variable clinical progress of the disease and emphasised that a range of malignancy existed. MacDonald, in an elegant article published ten years later, made the suggestion that "variations in biological behaviour generally may be of determinative importance in respect to the possibility of therapeutic control of an individual neoplasm" and thus formulated his theory of 'Biological Predeterminism'. This author cited cases of bulky, locally advanced tumours of the breast, still apparently confined to their site of origin after a duration of 10 years as examples of 'biological predetermination' at work and made a plea that treatment methods be designed to take account of the "complex biological nature of cancer" (MacDonald, 1951). A similar theme was explored by Park and Lees in the same year, who emphasised that the 'biological phenomena' of breast cancer, in particular the metastasising potential, direction of spread and rate of growth were very variable but that these factors were likely to have a greater influence upon survival than surgical therapy (Park and Lees, 1951).

Other authors have related certain patterns of the clinical course of the disease, to survival and a number of clinically relevant observations have emerged, viz.



1. A prolonged recurrence free interval tends to be associated with a prolonged overall survival (Shimkin et al, 1954; Cutler, Asire and Taylor, 1969).
2. The anatomical site of secondary metastasis is an important prognostic factor: patients with bone metastases have a better outlook than those with predominantly visceral secondaries (Shimkin et al, 1954; Devitt, 1971).
3. A response to systemic therapy at relapse, particularly to hormonal therapy, conveys a survival advantage (Taylor, 1962). These three aspects of clinical behaviour are recognised determinants of prognosis and appear to be interrelated. It has been shown that responders to endocrine therapy have a longer disease free interval than non responders and patients with bony secondaries are three times as likely to respond to this modality than those with visceral metastases (Taylor, 1962). Thus, it is conceivable that the intrinsic biological nature of the cancer determines behavioural patterns, that patients with favourable tumours have a long disease free interval, develop metastases at favourable secondary sites and have a good rate of response to secondary endocrine therapy.

The prognostic significance of the physical extent of cancer, as reflected in the clinical stage, has long been recognised (Shimkin, 1954). Devitt has suggested that the clinical course in individual patients can be predicted accurately if both clinical stage and clinical observations pertaining to growth behaviour, viz. recurrence free interval and anatomical sites of secondary metastases, are taken into account (Devitt, 1976). Thus, it would

appear that the intrinsic nature of the neoplasm has an important influence upon clinical disease patterns and its prognostic significance may be separate from that of conventional staging methods.

#### Markers of biological variation in breast cancer

Certain 'intrinsic factors' have been identified in primary breast cancers and have been related to various natural phenomena which occur during the mastectomy to death interval of patients with the disease. In particular these markers have been related to prognosis, both to disease free interval and survival. Such markers have been identified by diverse methods, often by painstaking and logical process, often by chance and their number becomes exhaustive. For the sake of clarity, therefore, I have categorised them in the following arbitrary manner (Table 2 : I) and shall offer a brief review of their reported clinical value.

Table 2 : I

Markers of Biological Variation of Breast Cancer

1. Morphological Features
  - a) Tumour differentiation
  - b) Tumour type
  - c) Other factors
    - 'Invasiveness' - Parenchymal,
    - lymphatic, vascular,
    - tumour necrosis
  
2. Cellular Products
  - i) Steroid receptors
  - ii) Prostaglandins
  - iii) Other - casein, lactalbumin
    - tumour associated antigens
  
3. Physical Properties    ~    Tumour Cell Kinetics

## 1. Morphological Features

### Histological Differentiation

Even before comprehensive histological classifications of breast cancer type were developed, a relationship had been noted between tumour differentiation and prognosis. Dennis (1891) noted that "the tumours which show structures departing but slightly from the normal correspond with the group of case histories that are favourable ..... tumours which show a great departure from the normal structure correspond to unfavourable case histories ..... the more typical the structure the better the prognosis, the more embryonic the structure, the greater the liability of recurrence".

Von Hanseemann (1893) estimated the degree of "anaplasia" by the loss of glandular arrangement of the cell, and the number of mitoses and concluded that the greater the degree of anaplasia, the greater the tendency to form metastases. Greenough (1925) was the first to segregate breast cancer into three grades of malignancy on the basis of the degree of tubule formation, variation in size and shape of the nuclei and the number of mitotic figures. This author related these categories to prognosis in 73 women and noted that 68% of those with Grade I (well differentiated) cancers were alive at five years whereas none of those with Grade III (poorly differentiated) tumours survived for that interval. Scarff and Patey (1928) and Haagensen (1933) used similar criteria for grading and arrived at similar conclusions. Twenty years later, Bloom (1950) renewed interest in this method and used a scheme of summary scores for each of the three epithelial elements identified by Greenough (tubule formation, pleomorphism and mitoses) to designate three grades of

malignancy: (well differentiated - Grade I, moderately differentiated - Grade II and poorly differentiated - Grade III). Bloom and Richardson (1957) graded the breast cancers of 1,544 who had presented to the Middlesex Hospital, between 1936 and 1949 for whom five, ten and fifteen year survival rates were available, and found a strong correlation with survival (Table 2 : II).

Similar findings were reported by Tough et al (1969) and by Elston et al (1980) in a study of 205 patients from Nottingham.

Grading has been criticised on the grounds that its assessment is subjective. Champion et al (1971) reported variance of opinion between two observers in 18% cases. Nonetheless, grading by either observer offered useful prognostic information. In Bloom and Richardson's series (1957) each observer cross checked the others grades independently and agreement was reached in over 90% cases. Similarly, Fisher et al (1980) experienced no more than 10% inter-observer variation within a single institution. A further criticism of grading is that variation in the histological appearance occurs in different parts of the same tumour (Willis, 1967). However, Bloom (1950) reported that different sections from the same tumour have a comparable grade of malignancy and a total individual pattern can be recognised. Tough et al (1969) found that grading was impossible due to variation within the same tumour, in only 1% cases.

Histological grading has been recognised as an important prognostic discriminant by the World Health Organisation (Scarff and Torloni, 1968), but it has not yet achieved the widespread acceptance that it deserves.

Table 2 : IIRelationship between tumour differentiation and prognosis

<u>Tumour Grade</u>	<u>Patients (%)</u>	<u>Survival (%)</u>		
		<u>5 years</u>	<u>10 years</u>	<u>15 years</u>
I	26	75	53	31
II	45	47	27	18
III	29	32	19	10

(Bloom and Richardson, 1957)

### Histological Tumour Type

The differences of clinical course associated with in situ and invasive breast cancers demonstrate the optimum prognostic yield from histological tumour typing (Rosen et al, 1980). However, in situ carcinomas ought to be regarded as a different entity, and histological typing is less valuable for invasive cancers. While papillary, colloid and tubular carcinomas are associated with a better clinical course than average (McDivitt et al, 1968), these are rare, each accounting for less than 1% of the total of invasive breast cancers (Fisher et al, 1975). It is of interest that tubular and colloid cancers tend to be histological Grade I (Bloom, 1957; Fisher et al, 1975). Medullary cancers are frequently considered to have a favourable outlook but in fact they comprise a heterogeneous group. That subgroup which is identified by well defined margins and a marked lymphoid infiltrate have a good prognosis but the remainder, termed atypical medullary carcinomas by Fisher, have a less favourable prognosis than average (Fisher et al, 1975). By far the majority of breast cancers (approximately 80%) are ductal. Fisher separated this group into pure ductal (50%) and ductal with other features (30%), yet found no prognostic difference between the two (Fisher et al, 1975).

### Other Histological Features

It is an attractive assumption that the proliferative potential and invasive activity of breast cancers can be directly visualised in fixed sections. Certain authors have attempted to relate various histological appearances to aggressive forms of tumour behaviour and ultimately to prognosis.

### Infiltrative tumour borders

Lane and associates (1961) held that the degree of infiltration at the tumour border was of prognostic significance and demonstrated an impressive 10 year survival of 80% for tumours with a well delineated margin (n = 40) compared to only 38% for those with an infiltrative margin (n = 158). Patient groups were relatively small in this study and other workers have failed to confirm these findings (Hamlin, 1968; Silverberg et al, 1971).

### Lymphatic invasion

Fisher reported lymphatic invasion within the tumour itself in one third of cases, but felt that this feature was possibly present in a further 23% cases. This feature was frequently present in poorly differentiated tumours and was associated with short term treatment failure, ie. a short recurrence free interval (Fisher et al, 1975).

### Vascular invasion

Weigand et al (1980) stained sections of 155 cancers for elastic tissue and found blood vessel invasion in 60 (38%). Patients with cancers in this category had a short subsequent disease free interval. However, Fisher outlined the difficulties of identifying blood vessel invasion in that elastin staining was an unreliable means of distinguishing blood vessels from mammary ducts in paraffin sections since the latter also contain a proportion of elastic tissue. By reliance on classical criteria, of fibrin thrombus or endothelium on the surface of any mass of cancer cells within a lumen, as evidence of vascular invasion, Fisher found an incidence of only 4.7% of this abnormality and was unable to demonstrate any prognostic significance (Fisher et al, 1975).



### Tumour necrosis

This feature is regarded as a classical marker of malignant transformation (Willis, 1967) and confers a small unfavourable effect upon prognosis in invasive cancers. Tumour necrosis is present in some degree in two thirds of all cases of breast cancer but the more marked degrees are associated with the most malignant histological grade (Fisher et al, 1975). It has no prognostic significance, however, in intraduct cancers.

### Conclusions

From a review of the literature pertaining to histomorphic prognostic factors it is apparent that all of the factors have some value if used in isolation.

Histological typing will identify certain rather rare cancers associated with a good prognosis but does not permit any stratification of the vast majority of cancers which fall into a single pathological and prognostic category (ductal carcinomas). The "other" factors have some prognostic significance but their identification is subjective and results are variable.

Histological grading, on the other hand appears to be a simple and reproducible system of prognostic stratification. This method would also detect those cancers whose histological type was associated with a good prognosis (colloid and tubular cancers tend to be histological grade I) and also those with "other" unfavourable features (lymphatic invasion and tumour necrosis tend to occur with poor histological grades), associated with a poor prognosis.

Thus it seems possible that grading may obviate the need for other histological markers in this context.

## 2. Cellular Products

### Steroid Receptors

1) Oestrogen Receptor (ER): It is interesting to trace the history of studies of hormone dependence of breast cancer to the discovery of oestrogen receptors.

### Human studies

Schinzinger in 1889 is credited with the first suggestion that there might be a relationship between the ovaries and human breast cancer, although he did not put his ideas into practice. George Thomas Beatson conclusively demonstrated the therapeutic value of surgical oophorectomy, in premenopausal women with advanced breast cancer (Beatson, 1896). Sir Stanley Boyd collected together the case reports of 54 women who had undergone oophorectomy for a cancer of the breast and reported a benefit in 19 (35%) - a figure which has remained relatively constant in most reports ever since (Boyd, 1900). Lett (1905) reported a series of 99 patients and documented a temporary improvement in about one third. As techniques of radiotherapy progressed, ovarian irradiation was introduced and achieved response rates in advanced disease, similar to that of surgical oophorectomy (De Courmelles, 1926). Atkins performed a subtotal adrenalectomy on six patients between 1947 and 1948 and noted some measure of improvement in two of the women (Atkins, 1966). When cortisone replacements became generally available, Huggins and Bergenstal (1952) were able to carry out adrenalectomy with greater safety, and with good therapeutic effect. Luft and Olivercrona (1953) published the first account of successful remission of the disease following transfrontal hypophysectomy.

However, despite the more radical measures to eliminate oestrogen synthesis, response rates did not improve. Stoll (1969) and Hayward (1970) were unable to demonstrate any correlation between quantitative changes in oestrogen synthesis following an ablative procedure and the degree of clinical response.

Paradoxically, additive hormones have been shown to be therapeutic in advanced breast cancer. Haddow et al (1944) reported temporary retardation of tumour growth in 10 of 22 women, with advanced cancer treated by triphenylethylene, and in 5 of 14 women treated by Stilboestriol. In the same year, results of treatment of 100 cases of advanced breast cancer by Stilboestriol were reported by Ellis et al (1944). Seventeen of 52 patients over the age of 58 years had shown improvement, with spectacular success in some cases. No patient under the age of 58 years had shown spectacular improvement, although some benefit was noted in about one fifth. With further experience Stilboestriol became the drug of choice in the treatment of advanced cancer in postmenopausal women.

#### Animal studies

Leo Loeb demonstrated that an early oophorectomy (before three months of age) in a strain of mice with a naturally high incidence of breast cancer, caused a fall in the rate of cancer development (Loeb, 1919). Laccassagne published a report describing the development of breast cancer in male mice injected with oestrone benzoate (Laccassagne, 1932). Shimkin and Wyman (1945) reported a reduced incidence of murine breast cancer following a bilateral adrenalectomy with oophorectomy.

## Prediction of response of advanced breast cancer to endocrine therapy

With the recognition of some relationship between hormones, breast cancer and therapeutic regression, here came a search for methods of predicting response, which centred upon two areas.

### a) The hormonal 'milieu'

The studies of R.D. Bulbrook centred upon the hormonal environment, in individual patients as a means of predicting response. Bulbrook et al (1960) measured androgen and corticosteroid metabolites in the urine of patients with breast cancer. A discriminant function was devised from the ratio of the metabolites: a high titre of aetiocholanone and a low titre of 17 Hydroxy corticosteroids (positive discriminant) was associated with a good response to ablation. Hayward and Bulbrook (1968) later went on to relate this discriminant to incidence and prognosis of breast cancer, and those with a negative discriminant had a reportedly worse prognosis.

The specificity of this test was called into question however, by Durant and Miller (1973) who reported a reduced excretion of androgen in patients with disorders other than breast cancer viz. hepatic disorders, advanced non mammary cancers etc., the implication being that the discriminant function merely represented a non specific reaction to stress.

### b) Biological characteristics of the target tumour

A great deal of effort has been expended in the investigation of the hormonal responsiveness of the cancer itself. Folca and his colleagues (1961) advanced our knowledge with an elegant study which

concerned 10 patients. The investigators demonstrated that a proportion of breast cancers (four of the ten) had the ability to trap labelled oestrogen (tritiated hexoestriol) and that the patients with these cancers had a good response to endocrine therapy. Ling, Cowen and Inman (1965) demonstrated selective trapping of tritiated oestradiol by hormone responsive DMBA induced mammary cancers, in Sprague Dawley rats. Toft and Gorski advanced our knowledge further when they isolated a macromolecular component from rat uteri which had the characteristics of a specific oestrogen receptor. In this experiment, the investigators injected tritiated oestradiol intraperitoneally, in experimental rats and demonstrated selective binding to a macromolecular component in the uterus. No uptake was detected in hormone independent tissue (serum or intestine). Because of the sedimentation rate of this molecule, its specificity in binding and its sensitivity to proteolytic enzymes, the authors postulated that the receptor molecule was a large protein (Toft and Gorski, 1966). Korenman and Dukes (1970) are credited with the first demonstration of the presence of oestrogen receptors in human breast cancer. Using a method of sedimentation analysis, similar to that employed by Toft and Gorski, the former investigators demonstrated in 15 patients with breast cancer, that certain of the tumours had high concentrations of specific oestrogen binding protein when compared to the quantities found in fat or in the uninvolved gland. Further studies confirmed this preliminary data. Feherty et al (1971) demonstrated the presence of oestrogen receptors in 37 of 53 breast cancer specimens (70%) with higher concentrations in postmenopausal patients. Witliffe et al (1971)

was able to demonstrate the presence of receptors in only 37% breast cancers (29 of 75) but confirmed the tendency for higher concentrations to occur in lesions of postmenopausal patients.

Jensen et al (1973) first demonstrated the clinical value of receptors in human breast cancer by correlating the presence of oestrogen receptor with a high rate of response to endocrine therapy. These findings were confirmed by numerous investigators, whose data were summarised by McGuire et al (1975): the presence of oestrogen receptor in target cancer is associated with a high rate of response to hormonal measures (50 - 60%) irrespective of the type of endocrine treatment but more importantly very few patients (< 10%) with receptor negative cancers respond (McGuire et al, 1975).

#### Oestradiol - receptor interaction at cellular level

The receptor for oestrogen is a large protein molecule present in cell cytoplasm. Oestradiol passes into the cell by simple diffusion and binds to the cytoplasmic receptor, which undergoes a structural change before becoming transferred to specific acceptor sites in nuclear chromatin. Once bound, the nuclear complex initiates and integrates series of transcriptional events on the DNA template leading to production of other macromolecular components, many of which are essential for cell maintenance and function. One of these macromolecular 'end products' is the progesterone receptor (Fig. 1 : 8) (Griffiths and Nicholson, 1981).

#### Oestrogen receptors and prognosis

Terenius et al (1975) first correlated ER with prognosis, when they reported that the interval between recurrence and death, in a

small series of patients was shorter in those with ER negative breast cancers, but surprisingly this group of women had a longer disease free interval than those with ER positive cancers (Terenius et al 1975). In a series of 41 patients, Singhakowinta et al (1975) reported a favourable survival advantage and a longer recurrence free interval for patients with ER positive tumours although the differences did not reach statistical significance. A similar trend was noted by McGuire et al (1975) but contrary findings were reported by Leclercq et al (1973) and Gorlich and Heise (1975). Walt et al (1976) reported a mean survival from mastectomy to death of 40.5 months for ER positive cancers and 27.2 months for ER negative cases.

Patient numbers were small in all of the above studies. In addition receptor assays were performed largely on biopsies of metastatic tumour and an element of selection must have been involved, in that patients whose metastases were inaccessible for biopsy would have been excluded from the analysis. Knight et al (1977) are usually credited with the first large study to relate ER in the primary cancer to prognosis. In a series of 145 women in whom ER estimations had been performed upon the primary tumour, the authors reported a favourable prolongation of recurrence free interval in patients with ER positive primaries although differences only reached significance in that group with four or more involved lymph nodes. In this study, however, patients with positive lymph nodes had received a variety of adjuvant regimens and this treatment was given to a proportionately greater number of patients with ER positive primary cancers, which almost certainly biased results.

The first study relating ER status of the primary cancer to recurrence free interval in patients who remained untreated after mastectomy came from Nottingham. In an analysis of the data concerning the first 300 patients in the study, at 18 months median follow up, Maynard et al (1978a) reported a significant prolongation of the interval to a major recurrence in patients with an ER positive primary cancer, when compared to patients with ER negative tumours ( $p < 0.05$ ). ER status was unrelated to the degree of lymph node involvement, but its effect in terms of differences in recurrence free interval became more marked in those with involved nodes ( $p < 0.025$  for patients with axillary node involvement and  $p < 0.001$  for apical or internal mammary node metastases). Rich et al (1978) demonstrated a similar relationship of ER in the primary cancer to recurrence free interval, but no statistical evaluation of significance was given.

A parallel study to the Nottingham series has been conducted from Liverpool since 1975, in which tumours were harvested, stored and assayed for oestrogen receptors in a similar manner. The findings of that study were similar to Nottingham data. In an analysis of data relating ER in the primary cancer to disease free interval in 286 women who remained untreated after mastectomy, Cooke et al (1979) reported a significant prolongation of disease free interval for ER positive tumours ( $p < 0.001$ ). The authors also noted that ER status was independent of lymph node involvement, but patients who were free of nodal metastases with ER negative tumours had the same high rate of recurrence as all women with positive axillary nodes.



In a previous report from Nottingham, Bishop et al (1979), noted a survival advantage for postmenopausal women with ER positive primaries ( $p < 0.025$ ). Rather similar results were reported by Hahnel et al (1979) who in a study of 335 patients found significant differences in both recurrence free interval ( $p < 0.05$ ) and survival ( $p < 0.01$ ) in favour of patients with ER positive primaries. This author also noted that the effect of ER on recurrence free interval was maximal in the first two years after surgery but gradually dissipated thereafter, although the survival difference persisted. A similar observation was made by Furmanski et al (1980) who noted that differences in recurrence rates were most marked between ER positive and negative groups up to 30 months after surgery, but the rates converged thereafter until at 40 months no difference existed. Blamey et al (1980), from this centre, reported no difference in recurrence free interval between ER positive and negative primaries in 250 women who had been followed for a minimum of 30 months, whereas a previous report at 18 months median follow up had noted a difference which was significant at the 95% level of confidence (Maynard et al, 1978a). A significant survival difference persisted, however. Samaan et al (1981) reported a difference in disease free interval between ER positive and negative groups only in premenopausal women ( $p < 0.05$ ) but a survival advantage was evident for all women in the series with ER positive primaries ( $p < 0.05$ ). Croton et al (1981) of the Liverpool group reported a significant survival advantage ( $p < 0.001$ ) for all women with ER positive primaries.

Hormone dependency of breast cancer may be distributed along a continuous gradient and thus qualitative assessment of receptor

status by some arbitrarily defined value may be misleading (Paridaens et al, 1980). In a recent report, Godolphin and colleagues (1981) reported a correlation between ER concentration in the primary cancer and disease free interval. The association with survival was even stronger: a linear trend of increasing survival was noted through variation of ER concentration from  $< 1$  to  $> 260$  fmoles/mg cytosol protein. The authors noted that ER and TNM stage (TNM was assessed by clinical measurements retrospectively) were independent and that ER concentration was as strongly associated with survival as stage.

All studies are in agreement that there is no relationship between ER activity and measures of clinical stage such as tumour size (Rosen et al, 1975; Millis, 1980; Cooke, 1982) or lymph node involvement (Maynard et al, 1978; Millis, 1980). Thus ER is independent of tumour bulk and the prognostic interaction of both factors is additive (Maynard et al, 1978; Blamey et al, 1980; Cooke et al, 1982).

#### Oestrogen receptors and tumour morphology

Oestrogen receptor status of breast cancer has been related to various tumour types and histological features. It has been shown that lobular carcinomas tend to be ER positive (Rosen et al, 1975; Eusebi et al, 1978) as do mucinous, tubular and papillary cancers (Meyer et al, 1978; Fisher et al, 1980). Nuclear pleomorphism is associated with low ER levels (Fisher et al, 1980).

Of greater interest is the relationship of ER to histological differentiation. The majority of studies would support such a

relationship but the strength of that association is controversial. It is noteworthy that no correlation was found in two relatively small studies by Rosen et al (1975) or by Rich et al (1977) in series of 120 and 50 patients respectively. Several studies have found a tendency for poorly differentiated tumours to be ER negative, but this trend failed to reach statistical significance (Maas et al, 1975; Pich et al, 1978; Cooke et al, 1982). Two publications from Nottingham have emphasised that a highly significant relationship exists between the two parameters, that well differentiated (grade I) cancers tend to be ER positive, both in postmenopausal women and in all women in the study (Maynard et al, 1978b; Elston et al, 1980). Others have since published findings in agreement (Fisher et al, 1980; Millis, 1980; McCarty et al, 1980). In a study of 207 tumours, Thoresen et al (1981) noted a highly significant relationship between the two qualitative parameters, but questioned the biological significance of the relationship on the grounds that the measured ER values in the receptor positive group, were similar in each histological grade. Thoresen's study may be criticised on two counts, however. Firstly, in any test of correlation of quantitative variables, all values of the variables should be considered. The investigators in this study excluded the cases (approximately 40% of the total) with a value of zero. Secondly, statistical analysis was erroneous: the distribution of ER values was extremely skewed but simple parametric tests, which are inappropriate for this type of data, were used. Data provided was too scanty for independent statistical analysis and this study must be regarded as being inconclusive.

### b) Progesterone receptors

Like oestrogen receptors, progesterone receptors (PgR) were identified and investigated as a means of predicting response to endocrine therapy in patients with advanced breast cancer.

When a hormone dependent cell undergoes malignant transformation it might retain part or all of its regulatory mechanisms by which the endocrine system influences its activity. Thus a successful endocrine effect and in particular, a successful application of endocrine therapy might be dependent upon a complete regulatory pathway. As outlined previously, oestrogen receptor binds oestradiol to initiate a sequence of events which in oestrogen target tissues culminates in protein synthesis. Thus ER constitutes a marker of only the first step of a hormone regulatory pathway. However, progesterone receptors are one of the measurable end products of hormonal action in oestrogen sensitive tumours (Fig. 1 : 8) and ought to be a better marker of the integrity of regulatory pathways. Based upon these hypotheses Horwitz et al (1975) postulated that progesterone receptors ought to be an ideal marker of endocrine sensitivity of breast cancer. Preliminary results of some studies relating progesterone receptors to response of advanced breast cancer to hormonal therapy lend support to this hypothesis (Brooks et al, 1980; Osborne et al, 1980) but others differ (Manni et al, 1980).

In metastatic tumour, a strong relationship has been demonstrated between oestrogen and progesterone receptors in breast cancer: approximately two thirds of ER positive tumours also contain progesterone receptors (Horwitz et al, 1975; Brooks et al, 1980).

In addition, the likelihood of a tumour being PgR positive increases with the measured value of ER (Osborne et al, 1980; Skinner et al, 1980). As with ER, no correlation has been observed between PgR and tumour bulk, ie. tumour size or lymph node involvement (Millis, 1980), but PgR has been significantly correlated with tumour grade: PgR negative tumours tend to be poorly differentiated (King, 1980; Pichon et al, 1980). The precise relationship of progesterone receptors to other variables in the primary cancer remains uncertain.

Studies relating PgR to prognosis remain at a preliminary stage. Skinner et al (1980) demonstrated a favourable trend of prolongation of recurrence free interval for patients whose tumours contained both receptors (ER+/PgR+) but differences from other categories were not significant. Pichon et al (1980), in a study of 105 patients with all stages of breast cancer found a favourable association between the presence of PgR in the primary cancer and prognosis: the incidence of all metastases except local recurrences were 3.6 times less frequent in the PgR positive than in PgR negative tumours ( $p = 0.02$ ). Furthermore, the likelihood of metastatic spread was inversely related to the measured concentration of progesterone receptor ( $p < 0.01$ ). However, the number of patients with Stage III cancer was proportionately greater in the PgR negative group and this is very likely to have biased results. Further data is necessary to validate this important point.

## Cellular products (ii)      Prostaglandins

Since the discovery of prostaglandins in extracts of human seminal plasma and sheep vesicular glands (Von Euler, 1936), a remarkable list of biological activities have been attributed to this family of closely related compounds. Their role in cancer is perhaps more controversial than any other aspect of oncology. In this brief review two aspects of their activity will be considered:

- 1) Bone resorption and hypercalcaemia
- 2) Tumour growth and metastases.

### Prostaglandins, Bone Resorption and Hypercalcaemia

In 1970, Klein and Raisz demonstrated that prostaglandins caused osteolysis with the liberation of labelled calcium ( $^{45}\text{Ca}$ ) when added to organ culture of embryonic mouse calvaria. Several experimental tumours which produce large amounts of prostaglandins, have been shown to cause bone lysis in vitro (Tashjian et al, 1972). Santoro, Jaffe and Simmons (1977) demonstrated that long term intraperitoneal administration of prostaglandin  $\text{E}_2$  caused an accumulation of osteoclasts at the trabecular surface, a fall in the percentage of trabecular bone and a significant loss of total bone calcium in mice. Tashjian (1978) demonstrated that the transplantable mouse fibrosarcoma HSDN<sub>1</sub> and the VX<sub>2</sub> carcinoma in rabbits produce large quantities of prostaglandin E and that this factor is responsible for the hypercalcaemia in the animals bearing these tumours. Hypercalcaemia could be prevented by treating the animals with the prostaglandin synthetase inhibitor, Indomethacin. Powles et al (1973) demonstrated that the prostaglandin inhibitor

Aspirin prevented the development of bone metastases in rats given an intraperitoneal injection of Walker fibrosarcoma cells, but interestingly Aspirin therapy did not prevent the development of metastases at other sites.

#### Human Studies

Using an in vitro culture system of human primary breast cancer explants, with radiolabelled bone fragments, Powles et al (1976) reported that tumours with the greatest in vitro osteolytic activity were most likely to develop bone metastases, but with a longer follow up, refuted this claim (Dady et al, 1981). It has been shown that breast cancers have a greater capacity to synthesise prostaglandins than non-malignant breast tissue (Bennett et al, 1975). This latter investigator also demonstrated that primary breast cancers which produced the greatest amounts of "prostaglandin like material" were most likely to be associated with a positive bone scan at the time of mastectomy (Bennett et al, 1975, 1977). Bennett's studies may be criticised on two counts, however. Firstly, his method of bioassay is an insensitive and non specific measure of prostaglandins and cannot identify any given type (Olley and Coceani, 1980). Hence, Bennett's use of the term "prostaglandin like material". Secondly, bone secondaries were identified only by bone scanning, which itself can be misleading (Bishop et al, 1979). The incidence of positive scans (23%) was rather higher than anticipated in this series and the possibility of false positives exist.

In conclusion, there is a great deal of impressive evidence that E series prostaglandins are potent bone resorbing agents, but

their role in the pathogenesis of bone metastases from human breast cancer is unclear.

## 2. Tumour growth and metastases

The role of prostaglandin  $E_2$  in this highly complex area is controversial and there are two diametrically opposed views, viz.

### a) Prostaglandin $E_2$ inhibits tumour cell growth

There is some impressive evidence for this contention shown by in vitro experiments in which endogenous and exogenous prostaglandin  $E_2$  has inhibited growth of a number of cell lines including neuroblastoma (Prasad, 1972), mouse melanoma (Santoro et al, 1976, 1977) and friend erythroleukemia (Santoro and Jaffe, 1979). The effects of  $PgE_2$  may be partly reversed by the administration of prostaglandin inhibitor Indomethacin, which results in stimulation of tumour cell growth (Santoro et al, 1976). Similar effects have been shown with various tumours in vivo. The administration of synthetic analogues of  $PgE_2$  to mice inoculated with melanoma caused a delay in the establishment and a reduced size of metastases, compared with controls. Survival was prolonged by  $PgE_2$  (Santoro et al, 1977).

Kibbey, Bronn and Minton (1978) showed in a small experiment that the concentration of prostaglandin E and the activity of prostaglandin synthetase is lower in rat mammary tumours which metastasise than in other types which do not. Powles et al (1973) noted that although the administration of prostaglandin inhibitor to rats with Walker fibrosarcoma was beneficial in reducing bone secondaries, it was associated with a non significant increase in the weight of soft tissue secondaries.



There is no data relating to any growth inhibitory effect of  $\text{PgE}_2$  in humans.

b) Prostaglandin  $\text{E}_2$  stimulates tumour growth and potentiates metastases

In contrast with the data quoted above, there is an equally impressive mass of studies showing that inhibition of prostaglandin synthesis by Indomethacin or similar agents limits the growth of tumour in vivo (Humes, Cupo and Strausser, 1974; Plescia, Smith and Griswicz, 1975) and reduces the rate of metastases and prolongs the animal's survival (Lynch et al, 1978; Bennett et al, 1979).

Various explanations have been offered for these discrepancies:

Claesson et al (1980) emphasised the different effects of Indomethacin at different concentrations viz. the growth of fibroblasts was inhibited at low concentration, but the opposite effect occurred at higher concentrations and the latter effect could be reversed by the addition of prostaglandin  $\text{E}_2$ . Others have suggested that the effect of Indomethacin upon tumour weight may arise simply from its anti inflammatory action, due to a reduction of local oedema (Santoro et al, 1977). However, it is also possible that other, as yet unidentified factors have a significant influence upon tumour growth, which in different experimental systems, could bias results.

Of greater interest is the relationship of prostaglandin  $\text{E}_2$  synthesis to tumour growth in human breast cancer, but data in this area is scanty. Bennett et al (1979) reported that patients whose tumours produced high amounts of "prostaglandin like material" tended to die soonest after mastectomy, but factors such as patient

age and tumour stage were not taken into account and patient numbers were small in this study. Indeed the median age (60 years) of the patients who died within three years of mastectomy was higher than that of the survivors (54 years) and one could postulate that prostaglandin production is simply a function of age, and that older patients had higher mortality rates. Rolland et al (1980) related high prostaglandin production to histological features indicative of a poor prognosis in 105 women with breast cancer, but no data relating to actual prognosis was given.

In summary, data concerning the role of prostaglandins in the regulation of tumour growth and development of metastases is contradictory in the experimental animal studies, and is inconclusive in human breast cancer.

#### Cellular products      iii) Other factors

Casein and  $\alpha$ -lactalbumin can be identified by fluorescent or immunoperoxidase techniques in approximately 30 - 50% of breast cancers but preliminary studies of the relationship of these factors to prognosis have been negative (Walker, 1979) or inconclusive (Fortt et al, 1979). Carcinoembryonic antigen can be identified by the immunoperoxidase technique and its presence is related to differentiation: well differentiated tumours tend to stain positively for CEA while poorly differentiated tumours tend to lack this antigen (Walker, 1980). All of these parameters are at a preliminary stage of investigation.

#### Tumour cell kinetics

Observers have attempted to measure tumour growth rate in patients by serial measurements of recurrences in skin (Philippe and

Le Gal, 1968) or of radiographs of lung secondaries (Spratt, 1977). Despite the wide range of doubling times, these measures of tumour growth rate have been related to prognosis (Philippe and Le Gal, 1968) but would clearly be unhelpful in the majority of patients. The influence of treatment which is given upon the detection of recurrences, on the doubling times would be impossible to evaluate.

Other workers have measured cell division by measurement of the uptake of  $^3\text{H}$ -Thymidine after incubation of breast cancer tissue with this isotope. An inverse relationship between high thymidine labelling (Rapid cell division), and oestrogen receptor status has been observed (Silvestrim et al, 1979; Cooke et al, 1982) but Thymidine labelling itself has not been related to prognosis.

#### Intrinsic biological factors - Commentary

Attempts to define 'biological markers' within a primary breast cancer, which might shed some light upon its variable nature have continued sporadically over the past half century and, as seen from the preceding review have met with some success. Each of the parameters previously mentioned would have some value if used in isolation but some are clearly more useful than others. The weight of evidence strongly demonstrates the prognostic value of steroid receptor assays and histological grading and it is these, with which this thesis will be primarily concerned.

If we anticipate that 'intrinsic factors' are related to prognosis, then we might also expect that they would be related to other prognostic variables, such as tumour bulk, but associations such as these have not been adequately investigated. Bloom (1950) reported a tentative relationship between histological grade and

tumour size, but this finding remains unconfirmed. Before any attempt is made to relate intrinsic factors to prognosis it must be demonstrated that they are independent of the prognostic influence of tumour bulk or alternatively if a relationship does exist, then a correction must be introduced. Pichon et al (1980) failed to do this in their study of the prognostic influence of progesterone receptors in breast cancer. There was an uneven distribution of stage III breast cancers between the PgR positive and negative groups and the findings of that study must be considered inconclusive.

Studies of intrinsic variables in breast cancer remain in their infancy. For example, when we measure multiple factors, we may simply be measuring the same aspect of malignancy by disparate methods, eg. by morphological or biochemical methods. Strong relationships exist between certain parameters, eg. oestrogen receptor status and histological grade and we might anticipate that there will also be overlap of the prognostic yield. The exact inter-relationships of newer factors, such as quantitative oestrogen receptors, progesterone receptors, and grade are uncertain. It has been suggested that all measures of intrinsic tumour malignancy (eg. histological differentiation, ER status and quantitative value, PgR status etc.) tend to express themselves at the same prognostic level (Fisher et al, 1975). However, there is no data to support this view. There has been no study which has carried out a comparative assessment of the predictive value of these parameters in any clinical situation.

Metastasis is an essential phenomenon of malignancy and certain factors related to this event govern the prognosis for the patient. For example the rapidity of onset of metastases (disease free interval), the predominant secondary sites of involvement and their response to systemic therapy are the most important clinical criteria which determine outlook. It is likely that the phenomenon of metastasis is governed by certain extraneous influences, and is not simply a haphazard occurrence. Evidence for this contention comes from observations of different metastatic patterns of different malignant neoplasms. The adult melanoma is a highly malignant lesion which disseminates widely, yet the juvenile melanoma, a lesion with a very similar histological appearance is virtually always cured by excision. Other examples are the predilection for bone of breast cancers and the predilection for liver of bowel neoplasms. Certain studies have related intrinsic parameters of breast cancer to the time of occurrence of metastases (disease free interval) yet few have been concerned with patterns of metastatic spread, or with predilection for any specific secondary sites. Biochemical pathways which might be involved have not been elucidated.

Many studies have reported a relationship of intrinsic factors, mainly steroid receptor status to response of metastases to endocrine therapy. However, almost all studies have related receptor assays performed upon secondary tumour to response, and data relating steroid receptors in the primary cancer to response is scant (De Sombre and Jensen, 1980). This point is important because it is only a minority of patients who have metastases which are accessible for biopsy.

Survival is the ultimate yardstick against which prognostic factors are evaluated. There has been no study which has carried out a comparative evaluation of intrinsic factors as predictors of survival.

### The present project

The essence of the present project is the investigation of whether the innate malignancy of primary breast cancer might be identified at the time of mastectomy by measures of steroid receptors (both receptor status and quantitative level) and histological grade. Secondly, this project will investigate means of using any such information to clinical advantage. In view of the gaps in our knowledge, concerning intrinsic factors, which were highlighted in the preceding section, studies have been conducted in the following fashion:

- 1) The prognostic influence of tumour bulk (both primary tumour size and the degree of lymph node involvement) will be considered. The Nottingham methods of measurement of tumour bulk will be critically analysed and results evaluated against disease free interval and survival.
- 2) Since it has been suggested that prognostic overlap occurs with these factors, the exact inter-relationship between oestrogen and progesterone receptors, both status and quantitative level and histological grade will be investigated.
- 3) Intrinsic factors will be related to tumour bulk and if necessary a corrective factor applied.

Thereafter a comparative assessment of the predictive value of all intrinsic factors will be carried out in a variety of clinical situations. Specifically, steroid receptors and histological grade will be related to:

- 4) Disease free interval. The interval to all types of recurrence viz. local, regional and distant will be considered.

- 5) Patterns of metastases. Intrinsic factors will be related to secondary sites of recurrence. The prognostic significance of specific sites of involvement in the Nottingham-Tenovus series will be considered.
- 6) Biological mechanisms of metastases. Prostaglandin  $E_2$  is a potent osteolytic agent and possibly promotes bone secondaries. Biosynthesis of prostaglandin  $E_2$  will be related to intrinsic variables in the primary cancer.
- 7) Response of metastases to endocrine therapy. The objective assessment of a response to therapy will be critically considered and response rates of the Nottingham series assessed. Prediction of response by means of intrinsic factors in primary breast cancer will be evaluated.
- 8) Survival. All intrinsic variables will be considered against survival.

Ideally, studies such as those proposed should involve wholly untreated patients, but such circumstances would be unethical. However, the clinical course of patients who remain untreated following mastectomy is likely to be similar to that of wholly untreated women (Baum, 1976). All patients with which this thesis is concerned, are in this category.

The studies of this thesis have further implications which will not be investigated in detail. An improvement of the mortality rate from breast cancer may require the development of adequate measures of prevention or more effective systemic agents. It is possible, however, that available therapeutic resources, tailored to the most appropriate patients by the use of such markers may achieve some improvement. For example, a fairly constant proportion of patients



with secondary breast cancer respond to endocrine therapy (20 - 30%). It is possible that a similar proportion of patients with undetectable micrometastases will respond to any 'adjuvant' endocrine treatment. In an unselected group the benefits to the 'responders' would be concealed by the poor results of the larger numbers of patients who would be treatment failures. Stratification of patients therefore into groups for whom certain treatment modalities are appropriate, might be beneficial. Indeed, treatment principles based upon a knowledge of the systemic distribution of breast cancer and also of the innate biological nature of the disease at the time of presentation may offer some hope for the future.

## Chapter 3

### THE NOTTINGHAM-TENOVUS STUDY

### The Nottingham-Tenovus Study

The Nottingham-Tenovus study of breast cancer is a broad based research project which embraces the overall spectrum of disease, from the preclinical phase by breast screening to the treatment of the terminal illness. This thesis therefore represents a small part of the total project, being primarily concerned with intrinsic biological factors in the primary cancer, which have been evaluated in variable numbers of patients.

#### Patients

The Nottingham-Tenovus primary breast cancer series includes all patients with breast cancer who have presented to one surgeon, Professor Roger W. Blamey since 1973, with the following exclusions:

- i Patients aged over 70 years.
- ii Patients with locally advanced cancers which are judged to be inoperable.
- iii Patients with distant metastases at presentation.
- iv Patients with a second primary cancer of another organ.
- v Patients who refuse follow up.

To date, the series comprises 800 women of whom the first 550 have been followed for a minimum interval of three years. Studies upon this 550 largely form the basis of the present project.

#### Diagnosis and Surgical Treatment

A 'Trucut' biopsy is taken at the first clinical presentation so that histological confirmation is available pre-operatively. When the 'Trucut' is negative, then excision biopsy with frozen section histology is performed.

The standard operation in this centre is a simple mastectomy with triple node biopsy viz. single node sampling of the low axillary, apical axillary and internal mammary nodes. A total of 620 women have received this therapy while the remainder have been treated by an even more conservative approach. One hundred women have been treated by subcutaneous mastectomy with delayed breast reconstruction while 80 have received tumour lumpectomy followed by radical radiotherapy (5000 rads in 15-20 fractions over five weeks). These more conservative procedures are performed for purely cosmetic reasons and best results are obtained in younger women. A choice of either a simple mastectomy or a lesser procedure is offered to all women below the age of 50 years. No other criteria of selection are employed. Only a double node biopsy (of low axillary and internal mammary nodes) is possible with either lesser procedure.

#### Measurement of Physical Tumour Bulk

##### a) Assessment of lymph node involvement

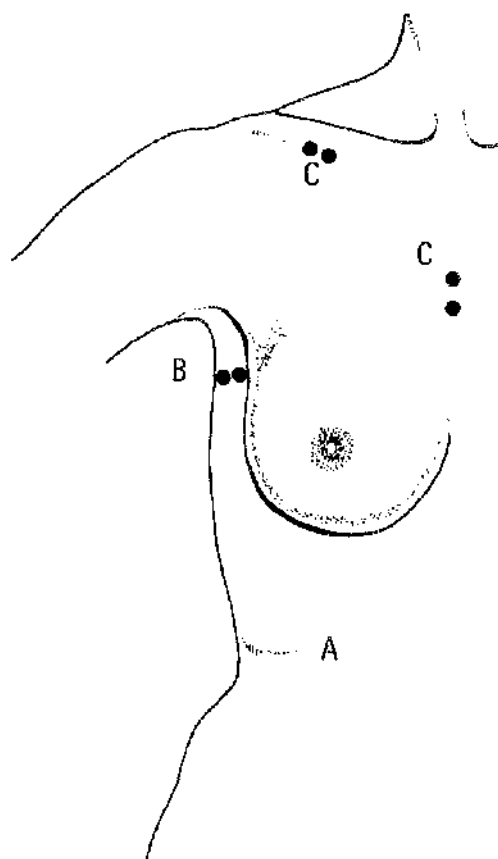
All lymph nodes excised at triple node biopsy are submitted for histological examination. Patients are 'staged' entirely on the basis of lymph node involvement (Fig. 3 : 1).

Stage A : Tumour apparently confined to the breast and all

nodes are histologically free of metastases.

Stage B : Metastatic involvement of only a low axillary node.

Stage C : Involvement of either apical axillary or internal  
mammary nodes.



TUMOURS 'STAGED' ACCORDING TO NODAL INVOLVEMENT :

- Stage A = All nodes histologically tumour free
- Stage B = Low axillary involvement only
- Stage C = Apical axillary or internal mammary involvement

Fig. 3 : 1      Nottingham triple node biopsy.

## b) Measurement of tumour size

At the completion of the operation, the primary tumour is excised from the mastectomy specimen and measured by calipers in three dimensions. The largest single diameter is taken as the tumour size.

## Measurement of intrinsic parameters in primary breast cancer

Adjacent specimens of the primary tumour are taken for evaluation of histological grade, steroid receptor and prostaglandin assays. The exception to this general rule occurs with the patient who requires frozen section histology, when the whole lump is submitted for examination and the only 'intrinsic factor' which may be measured, is that of histological grade.

### 1. Histological grading

Tumour samples are transported in formalin/saline and processed, sectioned and stained as for routine histology. Tumours are then graded histologically, by the criteria of Elston et al (1980) into three categories of differentiation. Fuller details are provided in the Appendix.

### 2. Steroid analyses

Tumour samples are 'snap frozen' and stored in liquid nitrogen at a temperature of  $-196^{\circ}\text{C}$  before being transported on dry ice, to the Tenovus Institute, Cardiff, where assays of oestrogen receptors and more recently progesterone receptors have been performed by the Dextran Coated charcoal method. Details are provided in the Appendix. Oestrogen receptor data is available on a total of 637 women in the series and progesterone receptor in 167 of the last 250 women in the series.

### 3. Prostaglandin E<sub>2</sub> radioimmunoassay

I have personally been responsible for this assay which is carried out in the Department of Surgery, University of Nottingham. Tumours are 'snap frozen' and stored at -196°C and the assay performed in batches. Full details are provided in the Appendix.

### Baseline clinical investigations

All patients have the following investigations at the time of their primary treatment.

a) Blood analyses: Full blood count, liver function tests, assays of plasma oestradiol and progesterone, and follicle stimulating hormone (FSH). Menopausal status is assessed on the basis of history and FSH values: a woman is considered to be postmenopausal if she is amenorrhoeic and has an FSH value in excess of 50 iu/l on two occasions.

b) Radiology Chest x-rays and bone scans are performed on admission routinely, in all patients.

### Clinical follow up

In Nottingham, a course of adjuvant chemotherapy has been given to a small number of patients. Thirty patients have received this treatment and have been excluded from any further analysis in this thesis. All other patients remained untreated until the development of recurrence. Patients were seen at a post mastectomy clinic for assessment at one month following surgery, then every three months until 18 months; from then every six months until five years and at annual intervals thereafter. Routine biochemical and haematological tests include a full blood count and ESR, liver function tests and serum and urinary calcium measurements which are performed at six

month intervals. Chest x-rays are performed annually. Annual bone scans were carried out in the first 500 patients but the practice has since been abandoned because of a poor diagnostic yield. Further investigations are performed as clinically indicated.

All follow up data in recurrence free patients is carefully documented and recorded in our 'Master Index' which is a file of all relevant information concerning every patient in the study. Categories of recurrence are defined thus:

'Spot' recurrence: - A small discrete skin metastasis which is confirmed histologically, and which does not recur after excision.

Local recurrence: - Multiple symptomatic or progressive metastases in mastectomy flaps which are confirmed histologically.

Regional recurrence: - Symptomatic metastases in ipsilateral axillary or supraclavicular nodes which are confirmed histologically.

Distant recurrence: - Any distant metastasis confirmed by clinical examination, abnormal liver function tests, appropriate x-rays, liver or brain scans or biopsy.

Palpable axillary nodes are not regarded as recurrences unless histological proof is available.

The likelihood of local recurrence is within limits related to the extent of initial local therapy for breast cancer (Paterson and Russell, 1959; Lacour, 1976). 'Spot' recurrences are completely eradicated by local excision and thus they may represent a complication of a conservative surgical approach rather than be a true manifestation of innate tumour biology. Consequently data relating to 'spot' recurrence (which has occurred in 50 patients)



has not been utilised in calculation of disease free interval which therefore refers only to the interval to a major recurrence. This category (of 'spot' recurrence) has been excluded from analysis for all groups and therefore ought to have no influence upon conclusions. All patients with "spot" recurrences continue to be followed up at the post mastectomy clinic following treatment by local excision or cryotherapy. All other patients who develop secondary disease are referred to the Advanced Breast Cancer Clinic for assessment and treatment.

#### Assessment of patients with advanced breast cancer

All patients with a major recurrence are evaluated at a clinic specifically held for that purpose (the Advanced Breast Cancer Clinic). Baseline data including the date and type of primary therapy and stage of disease at mastectomy is recorded for every patient.

#### History

A full history is taken with special attention to the following details:

- i Patient's age
- ii Date of last menstrual period
- iii Presence of concurrent disease and treatment
- iv General well being is estimated by the Karnofsky system in 5 grades of performance, viz.

#### Grade

- 0 - Fully active, able to carry on with all usual activities without restriction and without aid of analgesia.

- 1 - Restricted in strenuous activity but ambulatory and able to carry out light work or fully active with the use of analgesics.
- 2 - Ambulatory and capable of all self-care but unable to work. Up and about for more than 50% of working hours.
- 3 - Capable of only limited self care. Confined to bed or chair for more than 50% of working hours.
- 4 - Completely disabled, unable to carry out any self care, confined totally to bed or chair.

(Karnofsky and Burchenal, 1948)

#### Physical examination

A full physical examination, with measurements of height and weight, is carried out in all patients. All lesions are measured with calipers against a centimetre scale. Particular attention is paid to recurrent disease in the following areas.

1) Mastectomy site, regional lymph nodes and opposite breast:- All recurrences are confirmed by biopsy. Accurate measurements are taken of all lesions in two dimensions and a representative drawing denoting all recurrences is provided. Any new primary cancer in the contralateral breast is confirmed histologically, following a 'Trucut' biopsy and accurate measurements taken.

Up to eight discrete lesions are measured and colour photographs taken of more numerous deposits. Diffuse intracutaneous infiltration, which we have termed "Field change", is measured in two dimensions to cover its total surface area, and colour

photographs taken. Lymph node metastases are categorised as being ipsilateral or contralateral axillary, supraclavicular, deep cervical or other. Accurate measurements are taken in two dimensions. In the presence of lymphoedema, arm circumferences are measured at a point 15.0 centimetres above the olecranon.

2) Chest: Respiratory capacity is assessed by clinical examination and tests of respiratory function including Forced Vital Capacity (FVC) and Forced Expiratory Volume in one second ( $FEV_1$ ). Clinical abnormalities are confirmed by chest x-rays.

Pleural Effusions: Symptomatic pleural effusions are drained, the volume recorded and the fluid submitted for cytological examination. A pleural biopsy is taken for histology. A careful record is taken of any local agent which is instilled into the pleural cavity.

Nodular lung metastases Up to eight nodular lung secondaries are measured in two dimensions on chest radiographs. A representative number of six to eight are measured when the total number is greater.

Diffuse lymphangitis If localised, this abnormality is measured on chest x-rays in two dimensions. When generalised, no measurements are taken. When the shadow of lymphangitis encroaches upon the mediastinal shadow, then measurement in one dimension only is possible.

Mediastinal lymphadenopathy Abnormal opacities due to mediastinal lymphadenopathy usually encroach upon the normal mediastinal shadow. For this reason, measurement in one dimension only is possible, and is taken from the mid point of the sternum.

### 3) Abdomen

Hepatomegally is measured, using calipers against a centrimetre scale. The hepatic edge is measured, as a distance from the costal margin, in the mid clavicular line, in quiet respiration. Biopsies are taken when possible. Isotope or ultrasound liver scans are taken to support clinical impressions of metastases.

Other abdominal masses: These are measured in two dimensions and further evaluated by ultrasound abdominal scans, or appropriate contrast studies.

Ascites The abdominal girth is measured in centrimetres at the umbilicus and patient's weight carefully noted. Paracenteses are performed when ascites is symptomatic, the fluid volume recorded and specimens submitted for cytology. A careful record is kept of any installed cytotoxic or other agents.

4) Skeleton: Bone secondaries are confirmed and assessed primarily by skeletal survey radiographs. Radiological abnormalities are categorised as:

- i) Suspicious lesions with an explicable abnormality
- ii) Suspicious lesions with an inexplicable abnormality
- iii) Definite metastases.

Only patients with definite secondaries, receive systemic therapy, but those in the other 2 categories are closely scrutinized until suspicions can be confirmed or refuted. Bone secondaries are categorised as lytic, blastic or mixed, and the bony sites of involvement are carefully recorded. Measurements of up to eight lesions are taken in two dimensions. All x-rays are assessed by the

author. When there is difficulty of interpretation assessment is carried out by both the author and a consultant radiologist (Dr. A.H. Morris) and a consensus decision taken.

#### Central nervous system

Neurological deficits are evaluated by clinical examination and a record kept of the distribution and type of neuronal or central impediment. The duration of the impediment is also noted. Specific lesions are quantified by isotope or CAT (computerised axial tomography) scanning.

Skin: All skin lesions, other than in the mastectomy flap are measured in two dimensions and biopsied.

#### Non measurable lesions

The following lesions are regarded as being evaluable but non measurable, in accordance with the recommendations of the UICC (Hayward et al, 1977).

1. Diffuse pulmonary infiltration
2. Central nervous system abnormalities not detectable by scanning methods
3. Osseous metastases, in which marrow involvement is detected by bone biopsy only, or by biopsy and bone scan without x-ray changes.

#### Laboratory investigations

A full blood count, liver function tests, alkaline phosphatase, calcium balance measurements, (which involve serum and urinary calcium assays) CEA and urinary hydroxyproline estimations are

carried out routinely in patients referred to the advanced breast cancer clinic. Biopsies and colour photographs are taken as indicated. Abnormal blood or urine results are regarded as being suggestive, but not confirmative of metastases unless other objective evidence is available. All results are carefully recorded. An FSH assay is taken in order to define menopausal status.

#### Systemic therapy for advanced breast cancer

Following assessment, all patients with advanced breast cancer receive primary endocrine therapy. Generally postmenopausal women receive Tamoxifen (Nolvadex, ICI) 20 mg b.d. and premenopausal women have a bilateral oophorectomy. Within the past year, however, we have randomised 28 premenopausal women to receive either Tamoxifen or an oophorectomy for their stage IV breast cancer. A careful record is kept of any additional treatment which may be required for symptomatic reasons.

#### Advanced breast cancer assessment proforma

All relevant data, as outlined above, are carefully entered upon a standard proforma (Fig. 3 : 2) following the initial visit at the advanced breast cancer clinic. A summary of the date of onset, total distribution, sites of involvement by metastases and date of start and type of treatment is given for each patient.

#### Advanced breast cancer - Clinical follow up

Follow up intervals: All patients are seen at monthly or two monthly intervals depending upon their state of well being.

Follow up assessment: At every clinic visit, a full history is taken and a physical examination carried out. All lesions are

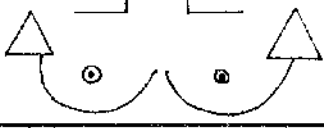
ABC PROFORMA - POST PBC GROUP			
FULL ASSESSMENT			
Name . . . . .		Age . . . . .	
Address . . . . .		Date . . . . .	
. . . . .		ABC No. . . . .	
. . . . .		PBC No. . . . .	
Stage of disease at presentation A <input type="checkbox"/> B <input type="checkbox"/> C <input type="checkbox"/> Histological grade at presentation I <input type="checkbox"/> II <input type="checkbox"/> III <input type="checkbox"/> ER Status Positive <input type="checkbox"/> Negative <input type="checkbox"/> Other <input type="checkbox"/>		Breast/Local Recurrence 	
Date of mastectomy Type of mastectomy <input type="checkbox"/> Simple mastectomy + TMB <input type="checkbox"/> Subcutaneous mastectomy + TMB <input type="checkbox"/> Lumpectomy + TMB + DXT Other <input type="checkbox"/> Right <input type="checkbox"/> Left <input type="checkbox"/> Tumor size Other treatment		Ipsilateral <input type="checkbox"/> Contralateral <input type="checkbox"/> Nodular <input type="checkbox"/> Diffuse infiltration <input type="checkbox"/> Intracutaneous <input type="checkbox"/> Subcutaneous <input type="checkbox"/> Multiple lesions <input type="checkbox"/> Number <input type="text"/> Dimensions <input type="text"/> Biopsy taken <input type="checkbox"/> Photograph taken <input type="checkbox"/> Lymph Nodes <input type="checkbox"/> Axillary <input type="checkbox"/> Number <input type="text"/> Biopsy taken <input type="checkbox"/> Dimensions <input type="text"/> Supraclavicular <input type="checkbox"/> Number <input type="text"/> Biopsy taken <input type="checkbox"/> Dimensions <input type="text"/> Mediastinal <input type="checkbox"/> Visible on CXR <input type="checkbox"/> Specific <input type="checkbox"/> Intra abdominal <input type="checkbox"/> Lymphangiothorax <input type="checkbox"/> Symptomatic <input type="checkbox"/> Other <input type="checkbox"/> Lymphoedema <input type="checkbox"/> Site(s) <input type="text"/> Are circumference <input type="text"/> Distant skin metastases <input type="checkbox"/> Site(s) <input type="text"/> Dimensions <input type="text"/> Photo taken <input type="checkbox"/> Biopsy taken <input type="checkbox"/> Chest <input type="checkbox"/> Pleural effusion <input type="checkbox"/> Tap <input type="checkbox"/> Malignant cells <input type="checkbox"/> Volume <input type="text"/>	
Menopausal Status Premenopausal <input type="checkbox"/> Postmenopausal <input type="checkbox"/> FSH <input type="text"/> LH <input type="text"/> LMP <input type="text"/> Current endocrine therapy <input type="checkbox"/> Concurrent disease <input type="checkbox"/> Concurrent treatment <input type="checkbox"/> Performance Karnofsky <input type="text"/> 0 <input type="checkbox"/> <input type="text"/> 1 <input type="checkbox"/> <input type="text"/> 2 <input type="checkbox"/> <input type="text"/> 3 <input type="checkbox"/> <input type="text"/> 4 <input type="checkbox"/> Self Assessment Score St. <input type="text"/> Weight <input type="text"/> Date first recurrence <input type="text"/> Site(s) <input type="text"/> Disease free interval <input type="text"/> Other <input type="text"/>		Modular metastases Dimensions <input type="text"/> Diffuse lymphangitis <input type="checkbox"/> Other <input type="checkbox"/> Respiratory function tests FEV <sub>1</sub> <input type="text"/> FVC <input type="text"/> Other <input type="checkbox"/> Abdomen Hepatomegaly <input type="checkbox"/> Dimensions (Distance below O/N) <input type="text"/> Quiet respiration <input type="checkbox"/> Liver scan <input type="checkbox"/> Biopsy <input type="checkbox"/> Asulites <input type="checkbox"/> ASD & Irt <input type="checkbox"/> Paracentesis <input type="checkbox"/> Malignant cells <input type="checkbox"/> Volume <input type="text"/> Abdominal mass <input type="checkbox"/> Dimensions <input type="text"/> U/S scan <input type="checkbox"/> Bone <input type="checkbox"/> Skeletal survey <input type="checkbox"/> Definite mets <input type="checkbox"/> Suspicious <input type="checkbox"/> Unexplained abnormality <input type="checkbox"/> Explained abnormality <input type="checkbox"/> Site(s) <input type="text"/> Dimensions <input type="text"/> Lytic <input type="checkbox"/> Blastic <input type="checkbox"/> Mixed <input type="checkbox"/> Other <input type="checkbox"/> Bone scan <input type="checkbox"/> Site(s) <input type="text"/> Definite mets <input type="checkbox"/> Suspicious <input type="checkbox"/> Other <input type="checkbox"/> CNS <input type="checkbox"/> Non Measurable Lesions Pulmonary infiltration <input type="checkbox"/> Osseous <input type="checkbox"/> Pleural effusions <input type="checkbox"/> Skin infiltration <input type="checkbox"/> CNS mets <input type="checkbox"/> Other <input type="checkbox"/> Photograph taken <input type="checkbox"/> Biopsy/Tap taken <input type="checkbox"/> Total No. of sites involved <input type="text"/> Reasons admitted ABC <input type="text"/>	

Fig. 3 : 2 Advanced Breast Cancer Assessment Proforma

Front Face (Reduced Scale)

Investigations (Abnormal)		Treatment	
Abnormal Hb.		Tamoxifen	
ESR		Doan	
WBC		Oophorectomy	
Alk. Phos.		Adrenalectomy	
Calcium		Other	
Phosphate		Chemotherapy	
SGOT		Regimen + Dosage	
GT			
Bilirubin			
U/E			
Urinary	Creatinine	DXT	
"	Hydroxyproline	Target area & dosage	
Duration of Abnormality			
Tumour Markers			
CtQ		Prednisolone	
CEA		Dosage	
α Lactalbumin			
p RCG			
Spermidine		Symptomatic	
		Specify	
Check List Current Investigations		Local surgery	
Phys. Exam.			
Dimensions of all lesions			
Photograph		Other	
Biopsy			
FBC		ESR	
LFT		Date commenced	
Alk. Phos.		Ca.	
Plasma proteins		Reason for choice	
Tenovus			
CtQ			
24 hr Hydroxyproline			
24 hr Urinary calcium			
RFT's			
Other			
Skeletal survey		Summary	
Bone scan			
CXR			
Other x-ray			
Brain Scan			
Other			

Fig. 3 : 2 Advanced Breast Cancer Assessment Proforma  
Reverse Face (Normal Scale)



measured in centimetres as outlined previously. A full blood count with ESR (erythrocyte sedimentation rate), liver function tests, respiratory function tests, calcium balance studies, CEA (carcinoembryonic antigen) studies are carried out at every clinic visit. Urinary hydroxyprolines are measured on every other visit. Radiographs of target lesions are taken at a minimum of two and then six months following the start of treatment. Other radiological or isotope investigations are carried out as clinically indicated. Chest x-rays and skeletal surveys are carried out routinely every six months. Any change of symptoms is noted and particular attention is paid to any change of the objective parameters. Full assessment, with a repetition of every investigation is carried out six monthly.

#### Evaluation of change

Regression or progression is assessed in measurable lesions by serial estimations of the lesions themselves, x-rays, etc. Clinical impressions of a reduction in size of hepatic or brain metastases are supported by serial scans. Changes in diffuse lymphangitis, or of asymptomatic pleural effusions which have not been drained are evaluated by comparison of serial chest films. Any sclerosis of lytic bone lesions, or regression of blastic secondaries are evaluated on serial films.

#### Advanced breast cancer - Follow up proforma (Fig. 3 : 3)

All of the details noted above are carefully entered into the follow up proforma at every clinic visit.

#### Definition of response to endocrine therapy

Response to therapy is defined by the criteria of the UICC (Hayward et al, 1977) with a minimum accepted duration of six months. Fuller details are given in chapter 8.

ABC PROFORMA		FOLLOW UP							
Name	Age	ABC No.							
Address	Hosp. No.	PAG No.							
Date			2/12	4/12	6/12	8/12	10/12	12/12	
			18/12	21/12	24/12	27/12	30/12	31/12	
			42/12	45/12	48/12	51/12	54/12	57/12	
Current therapy			Change from Previous						
Distant skin mets			Liver Scan						
Site									
Dimensions									
Other									
Symptomatic change			H/S Scan						
Abnormal investigations			Other						
RFT									
PEX									
Other			Measurable Lesions (New)						
Self assessment score									
Performance									
Karnofsky									
0									
1									
2									
3									
4									
Other			Photo Biopsy Trip						
Change(s) from Previous									
Rt.									
Lt.									
Breast/Local Recurrence			Duration of Change						
Tumour markers			Treatment Change						
CEA			Total Treatment						
CA15									
CA125									
CA199									
CA242									
CA2729									
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### Anti cancer therapy protocol

Primary endocrine therapy is continued indefinitely in patients showing an obvious response or until relapse occurs. Second line endocrine treatment in this centre is a surgical adrenalectomy which is performed in patients who have relapsed from first line endocrine control. Patients whose disease remains static despite a six month trial of endocrine therapy are referred for chemotherapy which is also given at any time to patients whose disease progresses, despite endocrine treatment. Very occasionally, patients who present with troublesome bone secondaries, are given radiotherapy in addition to endocrine treatment. If these metastases are the only deposits then assessment of response to systemic treatment becomes impossible. This thesis will be largely concerned with response to primary endocrine therapy.

The Nottingham treatment protocol is outlined in Fig. 3 : 4.

### Management of Cancer Related problems

Specific problems in patients with disseminated breast cancer are actively sought and if possible appropriate preventative steps are taken. These problems include:

#### 1. Complications of bone secondaries

a) Pain: Bone secondaries are the most common cause of the severe pain associated with advanced breast cancer. A pain specialist (Dr. Susan Mann, FFARCS) is in regular attendance at the clinic and bone pain is treated by oral analgesics, spinal and peripheral nerve blocks and by the use of nerve stimulators. Resistant cases receive radiotherapy.

## NOTTINGHAM ADVANCED BREAST CANCER TREATMENT PROTOCOL

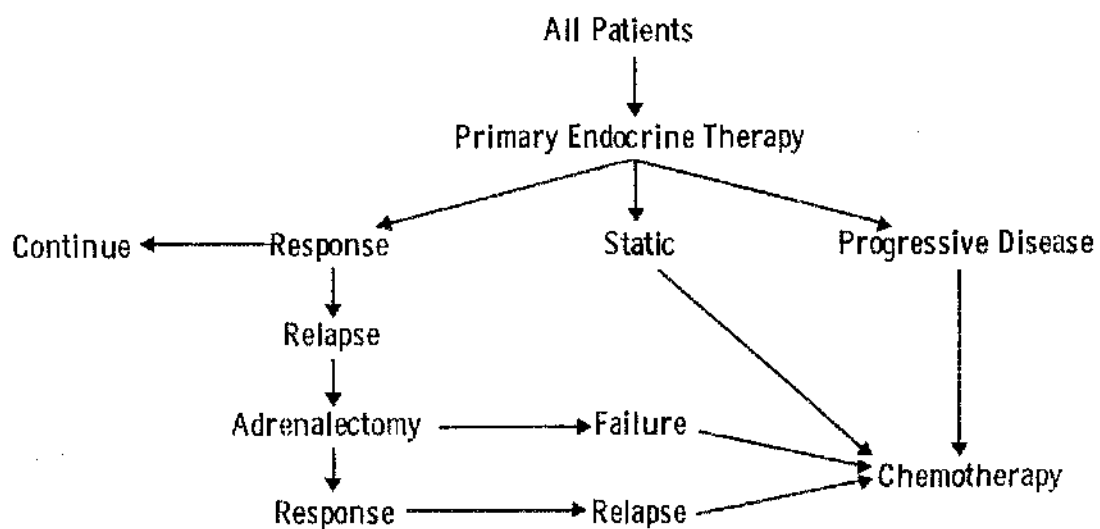


Fig. 3 : 4

b) Pathological fracture: This complication has its most serious consequences firstly in the vertebral column where cord compression and paraplegia may occur and secondly in the femora.

All patients with metastases in these areas have x-rays taken at every visit which are reviewed by both the author and a consultant orthopaedic surgeon (Mr. McKim-Thomas, FRCS). Prophylactic or therapeutic internal fixation is carried out as indicated.

c) Hypercalcaemia: Calcium balance is routinely monitored in all patients with advanced breast cancer. We have found the simple test of calcium excretion per nephron ( $\text{Ca}_E$ ) to be an extremely good indicator of progression of bone secondaries and a marker of patients at risk of hypercalcaemia. A sharp rise in  $\text{Ca}_E$  denotes a loss of calcium homeostasis and such patients are treated by rehydration.

Established hypercalcaemia is treated firstly by rehydration and secondly by an agent which suppresses bone resorption.

## 2. Complications of visceral metastases

These complications are impossible to prevent and difficult to treat. These include, nausea, vomiting, anorexia due to liver secondaries and dyspnoea due to lung parenchymal involvement.

## 3. Psychiatric disturbances:

Dr. Allan House (MRCPsych) lecturer in psychiatry provides much support. Antidepressants are prescribed as necessary from the advanced breast cancer clinic.

## 4. Terminal care

All patients who are insufficiently fit to carry out self care are admitted to hospital for terminal care. This is usually

provided by Dr. Anne Riley, director of Hayward House, the Nottingham terminal care institution.

#### The Advanced Breast Cancer File

All data as outlined above, concerning each patient was recorded in the assessment and follow up forms by the author. Together, these make up the advanced breast cancer file, in which sequential follow up data concerning more than 200 patients have been collected over the two year interval of my tenure. This file is available for inspection.

#### The Nottingham Primary Breast Cancer 'Master Index'

All events and details relevant to patients in the study are included in the master index, from the time of initial diagnosis through to the time of death. These details include patients name, hospital number, study number, date of birth, age, menopausal status, FSH value, date and type of operation, tumour size, extent of pathologically confirmed lymph node involvement, histological grade of the primary, oestrogen and progesterone receptor status and quantitative value, lymph node reaction, prostaglandin values in the primary tumour, date and description of first local and regional recurrence, date and site of first distant metastases, date of commencement and type of first systemic anticancer therapy, other therapies, response or failure, subsequent systemic therapies, response or failure, length of total follow up, date and cause of death, post mortem findings. I have tabulated the data concerning the first 750 patients in the study and it has recently been transferred to a computer, thus facilitating easy correlation of variables.

By these methods 'intrinsic' factors in the primary cancer, may be related to events which determine prognosis in patients with breast cancer, viz. disease free interval, site of metastases and response to systemic therapy.

#### Statistical Analysis

Time dependent events such as the onset of recurrences or deaths have been compared by life table analysis and evaluation carried out by the method of Mantel (1966).

#### Normally distributed data

Differences in quantitative data have been evaluated by Student's 't' test. Correlations have been tested by linear regressions. Differences in qualitative data were evaluated by chi-square or Fisher exact tests depending upon the numbers involved.

#### Data of a 'skewed' distribution

Non parametric statistics were employed. Specifically, differences in quantitative data were evaluated by Wilcoxon's Rank Sum and correlations have been tested by Kendall's Rank Test of Correlation.

By these methods, the influences of intrinsic biological factors in the primary tumour upon the natural progress of breast cancer have been studied in a scientific fashion.

## Chapter 4

### THE PROGNOSTIC SIGNIFICANCE OF TUMOUR BULK



### The Prognostic Significance of Tumour Bulk

Before we evaluate the influence of intrinsic tumour markers upon the clinical course of breast cancer, we must firstly consider the effect of tumour bulk and if necessary introduce a correction.

The prognostic significance of primary cancer size and the degree of lymph node involvement has long been recognised. Steinthal (1905) of Stuttgart first proposed a staging system based upon these parameters which categorised patients in three groups, viz:

- Group 1 Tumour not larger than a plum, confined to the breast, not involving skin or axillary nodes.
- Group 2 Obvious tumour adherence to the skin with clinically involved axillary nodes.
- Group 3 Most of the breast involved by tumour, extension to the skin and deep tissues, with clinically involved axillary and supraclavicular nodes.

At an undisclosed point in time during follow up, Steinthal reported a recurrence rate in 27% patients in Group 1 category, 76% Group 2 and 100% Group 3 category (Steinthal, 1905). Measurements of lymph node status and tumour size have formed the basis of every popular staging system every since (Cutler, 1967) and despite the advances in biochemical and scanning techniques, they still provide the best guide to prognosis (Forrest, 1976; Langlands, 1978). Each factor will be considered individually.

#### Primary Tumour Size

Eggers, De Cholnoky and Jessup conclusively demonstrated a relationship between the size of the primary breast cancer (which

had been measured after excision) and survival in a review of 278 patients. Tumours were rather large by today's standards (Table 4 : I) (Eggers, De Cholnoky and Jessup, 1941). Numerous others have reported similar findings (McWhirter, 1957; Berg and Robbins, 1966; Fisher et al, 1969) and Goldenberg et al (1961) described tumour size as the single most important prognostic factor in breast cancer.

Others have expressed a contrary view, however (Kunath, 1940; Hoopes and McGraw, 1942; Hamlin, 1968). Gorski was unable to demonstrate any significant difference in five or 10 year survival rates in women who had been designated TNM stages 2 ( $T_1 N_1$ ,  $T_2 N_1$ ) or 3 ( $T_3 N_0$ ,  $T_3 N_1$ ), a difference which was mainly related to tumour size (Gorski et al, 1968). Duncan and Kerr reported a and inverse relationship between tumour size and prognosis only for cancers measuring up to 3.0 cms diameter, but there was no progressive worsening of prognosis with further increases of size beyond 3.0 centimetres (Duncan and Kerr, 1976). As this thesis will demonstrate, factors other than tumour bulk influence survival in breast cancer. Bloom demonstrated the prognostic significance of three grades of histological differentiation in breast cancer and was unable to show any independent effect of tumour size within any given histological grade (Bloom, 1950). Thus it is possible that unrecognised variation of factors such as these, could mask the influence of tumour size upon prognosis.

An alternative explanation for the rather surprising negative findings of the above studies is that the methods of measurement could have been erroneous. All of the studies which failed to

Table 4 : ITumour size and prognosis

<u>Tumour size (cms)</u>	<u>Incidence %</u>	<u>5 year Survival (%)</u>
Less than 2	19	72
2 - 6	60	25
6 - 10	21	15

(Eggers, De Cholnoky and Jessup, 1941)

demonstrate a consistent relationship between tumour size and prognosis relied upon clinical measurements which are taken of the lesion, covered by its variable layers of fat, subcutaneous tissue and skin, in two dimensions only. Breast cancers, however, are three dimensional polymorphous lesions and clinical measurements provide only a crude estimation of their volume. Fisher et al (1975) have expressed the view that direct measurements of the pathological specimen after excision are more likely to be consistent, are more representative of tumour volume and ought to be used in any scientific study relating size to prognosis.

In the present study, tumour size measured in the excised cancer in three dimensions, will be related to recurrence free interval and survival of patients following mastectomy.

## The Present Study (Study 1)

### Patients and Methods

Tumour size has been measured immediately after mastectomy in 680 of a consecutive series of 750 women who have presented to Nottingham with operable breast cancer. The gross tumour was dissected free from the fresh mastectomy specimen and measured immediately in three dimensions. The single largest diameter was taken as the tumour size. No clinical measurements have been utilised. The first 550 women in the study have been followed for a minimum interval of two years and by far the majority of events eg. recurrences or deaths, have occurred in this group. Measurements have been taken of the primary tumour in the manner described in 504 of these patients in whom lymph node stage has also been evaluated. The patients who have presented more recently are relatively complication free at the present time and their data have not been included in the analyses pertaining to recurrence free interval and survival. For the sake of completeness the distribution of tumour sizes in the first group of 504 women are compared in 5 categories (less than 1 centimetre to greater than 5 centimetres), with that of the total study group of 680 women. However, tumour size has been categorised in only 3 groups ( $< 2$  cms, 2-4 cms,  $> 4$  cms) in the first 504 women to facilitate easy evaluation of the association of size with recurrence free interval and survival.

## Results

The distribution of different sizes of tumour in 504 of the first 550 patients is representative of the series overall (Table 4 : II). Further analyses will pertain only to the former group of patients.

### Tumour size and the interval to major recurrence

The effect of tumour size upon recurrence free interval up to seven years following mastectomy is shown in Fig. 4 : 1. Recurrence rates progressively increased with primary cancer size. A chi-square test for trend yielded 23.8 with one degree of freedom corresponding to  $p < 0.0005$ .

### Tumour size and survival

The prognostic effect is shown in Fig. 4 : 2. Survival progressively declined in cancers  $< 2.0$  cms, through 2 - 4 cms to those of more than 4.0 cms diameter. A chi-square test for trend yielded 32.9 with one degree of freedom which corresponded to  $p < 0.0005$ . Sixty percent of patients with small tumours remain alive at seven years compared to only 19% for those with large cancers.

Table 4 : II

<u>Size</u> <u>(centimetres)</u>	<u>Tumour size</u>			
	<u>All patients</u> <u>(n = 630)</u>		<u>Study Group</u> <u>(n = 504)</u>	
	n	%	n	%
< 1	52	7.6	31	6.2
1 - 2	263	38.7	183	36.3
2 - 3	222	32.6	163	32.3
3 - 4	76	11.2	62	12.3
4 - 5	40	5.9	38	7.5
> 5	27	4.0	27	5.4
	Median size 2.2 cms		Median size 2.3 cms	

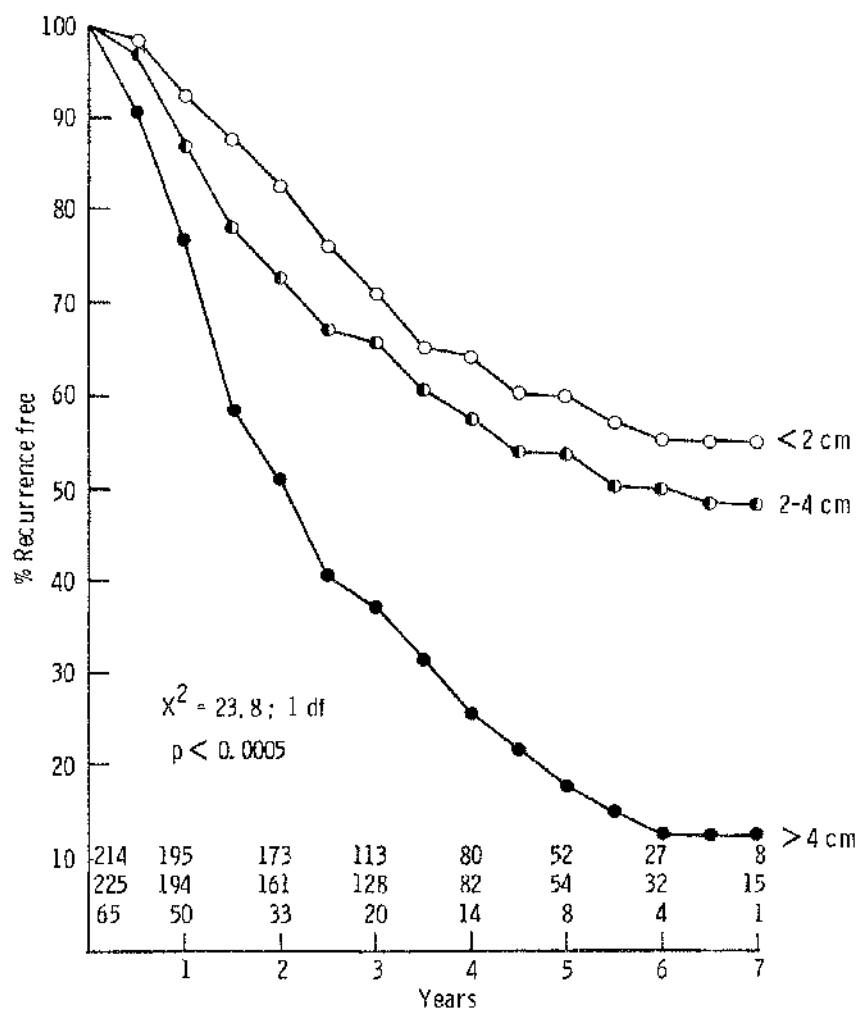
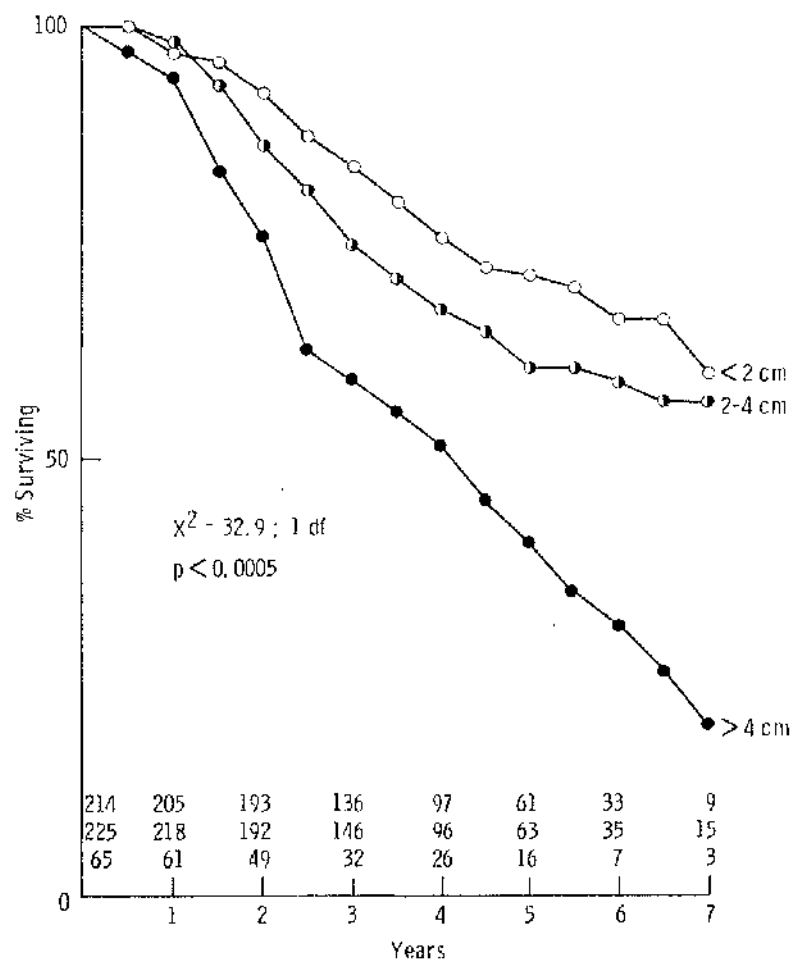


Fig. 4 : 1 Primary cancer size and major recurrence.





30.3.82

Fig. 4 : 2 Primary tumour size and survival.

## Discussion

This study conclusively demonstrates the prognostic importance of primary breast cancer size, when measured accurately following removal from the mastectomy specimen. The size of the primary tumour is clearly related to the time of onset of clinical metastases, which are the ultimate cause of demise.

Two possible explanations exist for these observations. Firstly, the dimensions of a breast cancer are likely to be related to the temporal stage of its development. Large cancers would be 'old' cancers and their disseminated malignant cells would have had ample time to become established and grow in the secondary sites where they have lodged. Alternatively, tumours which are large at presentation may simply represent aggressive, fast growing neoplasms which rapidly develop overt secondaries with a short subsequent interval until demise. In probability, both explanations are likely to be operative: the size of a tumour is likely to be related to both its chronological stage of development, from the instant of malignant transformation of the first cell and also its innate malignancy in variable degree.

If the variable malignancy of a cancer is important, is it possible that clinically detectable cancers may be truly confined to the breast without any dissemination? Clinical experience, ranging from cases with metastases from an occult primary (Ashikari, 1976) to patients with locally advanced cancers who have remained untreated for 30 years without developing metastases (Raum, 1980) dictates that such a possibility exists. Support for the concept that a proportion of detectable cancers may be confined to the

breast comes from the Health Insurance Plan (HIP) study of New York (Strax, 1976). Briefly, 62,000 women were randomised to receive screening (physical examination plus mammography on up to four consecutive occasions) or no screening (controls). Cancers detected in the 'screened' group were smaller than in the control population, were clinically impalpable in one third of cases and were also node negative in a higher proportion. The treatment strategy for cancers was identical in both groups. At five years of follow up, the mortality rate from breast cancer in the whole screened population of 31,000 women was one third less than that of controls. This difference achieved statistical significance (Strax, 1976). This important study provides evidence that early detection and treatment of breast cancer results in a reduced mortality from the disease possibly as a result of extirpation of the cancer in that proportion of cases where it is still confined to its site of origin. In this context the findings of the present study may also suggest that the smaller the size of a cancer, the lower its aggressiveness and the greater the likelihood of its complete extirpation by mastectomy.

It is of interest to compare the size distribution of cancers in this series with that of earlier studies. Only 19% of the patients in Eggers' study had a tumour diameter of less than 2.0 centimetres (Eggers, 1941), compared to 40% in the present study. Fisher reported a median tumour size of 3.0 cms in patients in the NSABP series (Fisher, 1975) but more recently Smart reported a median tumour size of 2.47 cms from the Surveillance, Epidemiology and End Results (SEER) programme (Smart, 1978) which compares with a median size of 2.2 centimetres in the Nottingham project. This data

suggests an encouraging improvement in the detection of smaller tumours.

In conclusion, the prognostic significance of tumour size is confirmed by this study. The 'intrinsic' parameters under study in this thesis will firstly have to be related to tumour size and if an association exists, then the prognostic influence of intrinsic factors will have to be investigated within a group of patients with a single tumour size.

#### Lymph Node Status

Nodal involvement, like tumour size has long been recognised as a prognostic discriminant in breast cancer. Sir James Syme wrote, "It appears that the results of our operations for carcinoma of the breast are almost always unsatisfactory when the glands are affected, however perfectly they may seem to have been taken away." (Syme, 1842). Today, nodal status is regarded by many as being the single most important prognostic factor in breast cancer (Forrest, 1979; Baum, 1980) and there is universal agreement that this guide ought to be obtained for every patient undergoing mastectomy. Despite the longstanding recognition of the significance of this prognostic factor certain practical questions remain unresolved viz.

1. What is the optimum method of determining nodal involvement; Clinical or Pathological?
2. What is the optimum surgical procedure of harvesting involved nodes for histological examination - Lymphatic Chain Dissection or Node Sampling?
3. What is the optimum method of grading the severity of nodal involvement?

There have been no randomised prospective studies to evaluate the points above, and we must therefore rely on retrospective comparative analyses.

#### 1. Evaluation of Nodal Status - Clinical versus Pathological

The advantages of clinical evaluation in determining nodal status include its simplicity and also that it provides prognostic information pre-operatively. Patients may be allocated to a high or low risk group and the total treatment strategy may be planned, and discussed with the patient in advance. Its main disadvantage is its inaccuracy. Cutler and Connelly found that 38% of clinically negative nodes were positive on histological examination and 37% of clinically positive nodes were pathologically negative (Cutler and Connelly, 1969). Fisher reported an overall error rate of 32% for clinical evaluation of the axilla, 24% false positive and 39% false negative (Fisher et al, 1975). Smart reported similar findings in an analyses of 6,628 cases from the Surveillance, Epidemiology and End Results (SEER) project (Smart, 1978) and Cutler, Zippen and Asire (1969) put the matter to the test in a comparative analysis of the prognostic significance of positive lymph nodes defined clinically or by histological examination. Pathological assessment allowed better separation of prognostic groups (Table 4 : III). Clearly this method is a superior index of prognosis and the inaccuracy of clinical evaluation rules it out from any scientific study concerning prognosis in breast cancer.

From the above data it is obvious that series which rely on clinical methods of evaluation are incomparable with those which

Table 4 : IIINode status and survival: Pathological versus clinical assessment

	<u>Pathological assessment</u>		<u>Clinical assessment</u>	
	<u>node (+)</u>	<u>node (-)</u>	<u>node (+)</u>	<u>node (-)</u>
5 year survival (%)	52	84	58	74
10 year survival (%)	33	70	38	58

(Modified from Cutler, Zippen and Asire, 1969)

utilise a pathological assessment of lymph nodes. It is noteworthy that the TNM system of staging which was originally an entirely clinical method (UICC, 1961) has recently been re-designed to facilitate a separate category for patients with histologically proven lymph node metastases.

## 2. Lymph Node Harvest - Axillary Clearance versus Node Sampling

A formal axillary clearance as is performed routinely in a radical mastectomy will determine with certainty the number of axillary nodes which are involved by breast cancer. This procedure increases postoperative morbidity and has a particular risk of lymphoedema, yet conveys no survival benefit (Fisher, Montague and Redmond, 1977) and an alternative method with fewer complications might be beneficial.

Forrest et al have demonstrated that pectoral node sampling combined with a thorough examination of the axillary tail of a simple mastectomy specimen is as effective in determining the presence or absence of positive nodes (nodal status) as a full axillary clearance of a radical mastectomy (Forrest, Roberts and Shivas, 1976). Fisher's findings that removal of a few axillary nodes ( $< 5$ ) was just as accurate in determining nodal status as dissection and removal of a larger number ( $> 26$ ) lend support (Fisher, 1981). This view has been challenged by Davies who reported false negatives in 46% (11/26) cases with axillary sampling alone and 14% (6/43) with examination of the axillary tail alone when compared to full axillary clearance (Davies, 1980). However, this study did not assess the accuracy of the combination of

axillary sampling and examination of the axillary tail as advocated by Forrest and in any case the numbers of patients involved in this series were small. While it is likely that sampling errors occur, there has been no attempt to evaluate their prognostic significance in any large study and the weight of evidence would suggest that sampling is a reliable means of determining nodal status in breast cancer.

### 3. Evaluation of the Extent of Nodal Involvement - The number of involved nodes versus the anatomical level of involvement

Nodal status of breast cancer, in terms of positivity or negativity is a useful oversimplification, which in many centres forms the basis of selection for adjuvant therapy (Fisher et al, 1975; Bonadonna et al, 1976). However, it is known that the number of nodes involved by breast cancer is related inversely to the recurrence free interval and survival (Fisher and Fisher, 1972; Nemoto et al, 1980). Fisher noted a five year survival of 62% in women with one to three nodes involved compared to only 35% when more than four nodes showed evidence of metastases (Fisher, Slack and Bross, 1969). Thus in view of the superior information obtainable from a quantitative assessment of all of the axillary nodes it might appear that node sampling is insufficient.

However, the anatomical level of nodal metastases is also of prognostic importance. Adair reported a five year survival of 65% for low axillary node metastases (lateral and inferior to the border of pectoralis minor - designated "level 1"), 45% for intermediate axillary node involvement (the group lying posterior to pectoralis minor - "level 2") and only 28% for high axillary nodal metastases



(superior and lateral to pectoralis minor - "level 3") (Adair, 1949). Handley reported a 67% five year survival in patients without nodal involvement, 42% in those with axillary involvement but only 6% for patients with involvement of both axillary and internal mammary node groups (Handley, 1969). Haagensen categorised patients with high axillary, or internal mammary node involvement as inoperable, on the grounds of poor prognosis (Haagensen, 1971). Leis has suggested that assessment of the anatomical level of nodal involvement provides equal prognostic information to an evaluation of the actual number of axillary node metastases (Leis, 1978).

#### The Nottingham Staging Method

The Nottingham staging procedure has been based on the principle that node biopsy from multiple 'levels' around a breast cancer, could provide optimum prognostic information with a minimum of morbidity. The methods will be described in fuller detail overleaf but briefly node biopsies are taken from the low axilla, the apex of axilla and from the internal mammary chain. Tumours are designated Stage A, if all biopsies are tumour free, Stage B if only a low axillary biopsy is positive and Stage C if apical axillary or internal mammary nodes are involved. A preliminary study from this centre has shown the value of this system when related to recurrence free interval: in the first 169 patients in this series, recurrence free interval was evaluated by life table analysis and the curves showed significant separation according to stage. The outlook for Stage A tumours was best and that for Stage C tumours worst ( $p < 0.001$ ) (Maynard et al, 1978a). However, lymph node stage has not

previously been related to survival. While the prospect of sampling errors have been raised, these have not previously been investigated in any large study and therefore the influence of any such errors upon the prognostic power of node biopsy remains unknown.

## The Present Study (Study 2)

### Patients

Five hundred and four of a total of 550 women have had lymph node involvement assessed at mastectomy and have been followed for a minimum interval of two years.

### Methods

Following the removal of the breast from the chest wall, single lymph nodes were sampled (1) from the low axilla, below the lateral border of pectoralis minor and (2) from the apex of the axilla which was approached anteriorly by dividing the pectoralis major in the line of its fibres between its clavicular and sternal heads. The clavipectoral fascia was incised, and the nodal group was located inferior to the axillary vein and superior to the upper border of pectoralis minor. Finally (3) a node biopsy was taken from the intercostal chain which was approached via the second intercostal space after dividing the pectoralis major and intercostal muscles. The biopsies were labelled in separate pots and fixed in 10% buffered formalin and stained with Ehrlich's haematoxylin and eosin. The biopsies were 'blocked' in paraffin wax at two levels, and histological sections were taken at three levels from each block. Lymph nodes were considered to be negative when examination of all sections revealed no evidence of tumour. Those biopsies which were found to contain no lymph node tissue for histological examination, were regarded as being tumour negative. Tumours were 'staged' A, B or C on the basis of histological examination of biopsy specimens:

Stage A - All biopsies tumour free

Stage B - Tumour involvement of the low axillary node only

Stage C - Tumour involvement of either the apical axillary or  
internal mammary nodes.

Fig. 3 : 1 Nottingham Staging Method

The procedure of triple node biopsy adds approximately 20 minutes to  
the operation time for a mastectomy.

## Results

### Distribution of involved nodes (n = 504)

The numbers of patients with tumours in different stages were as follows:

	<u>Stage</u>		
	<u>A</u>	<u>B</u>	<u>C</u>
<u>Patients</u>	274 (54%)	144 (28%)	96 (18%)

### Relationship of Lymph Node Stage and Tumour Size

The likelihood of cancers being node negative (Stage A) was inversely related to tumour size (Fig. 4 : 3). Conversely, the severity of nodal involvement was directly related to tumour size. Seventy-two percent of tumours measuring up to 2.0 cms diameter were node negative (Stage A), 20% Stage B and only 12% Stage C. In tumours measuring 5 centimetres or more the corresponding figures were 32% Stage A, 31% Stage B and 37% Stage C, and these differences were highly significant (Table 4 : IV).

### Lymph Node Stage and the Interval to Major Recurrence

The effect of stage upon recurrence free interval up to seven years following mastectomy is shown in Fig. 4 : 4. Recurrence free interval was progressively shorter from A through B to C and a chi-square test for trend yielded 111.2 with one degree of freedom which corresponds to  $p < 0.0005$ .

### Lymph Node Stage and Survival

The prognostic effect is shown in Fig. 4 : 5. Survival progressively deteriorated from A through B to C. A chi-square test for trend yielded 85.9 corresponding to  $p < 0.0005$ .

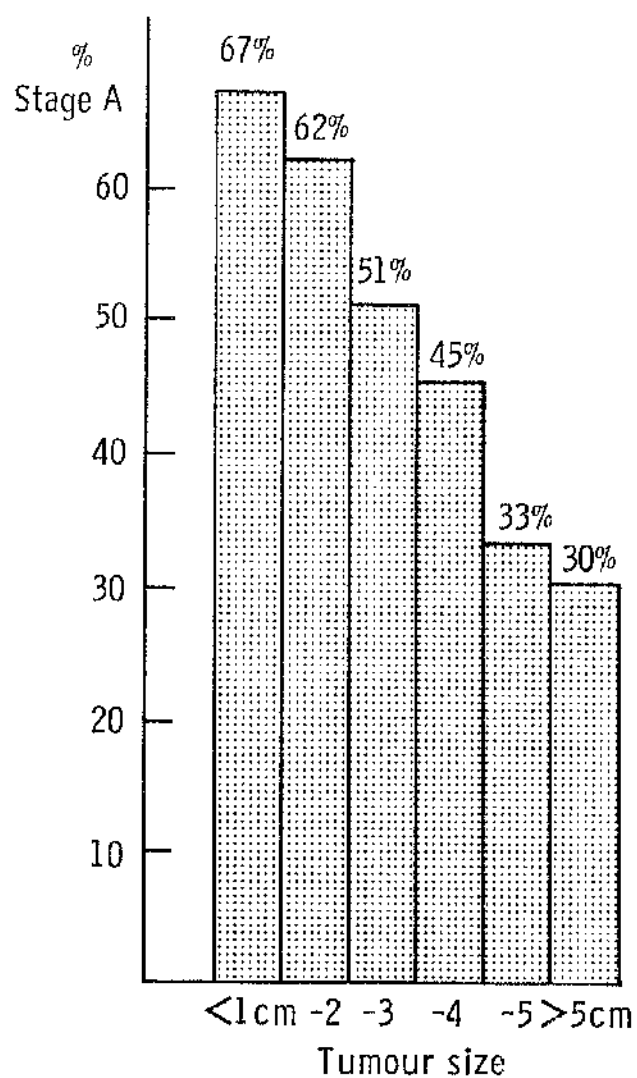


Fig. 4 : 3 Tumour size and node negative (Stage A) status:  
the likelihood of a cancer being node negative  
decreases as its size increases.

Table 4 : IVLymph node stage and tumour sizePrimary tumour size (centimetres)

<u>Stage</u>	<u>&lt; 1.0</u>	<u>1.1-2.0</u>	<u>2.1-3.0</u>	<u>3.1-4.0</u>	<u>4.1-5.0</u>	<u>&gt; 5 cms</u>
A	14	75	73	20	12	7
B	2	30	49	20	11	7
C	2	17	18	13	9	8
	—	—	—	—	—	—
	18	122	139	53	32	22

$$\chi^2 = 27.2$$

10 DF

$$p < 0.005$$

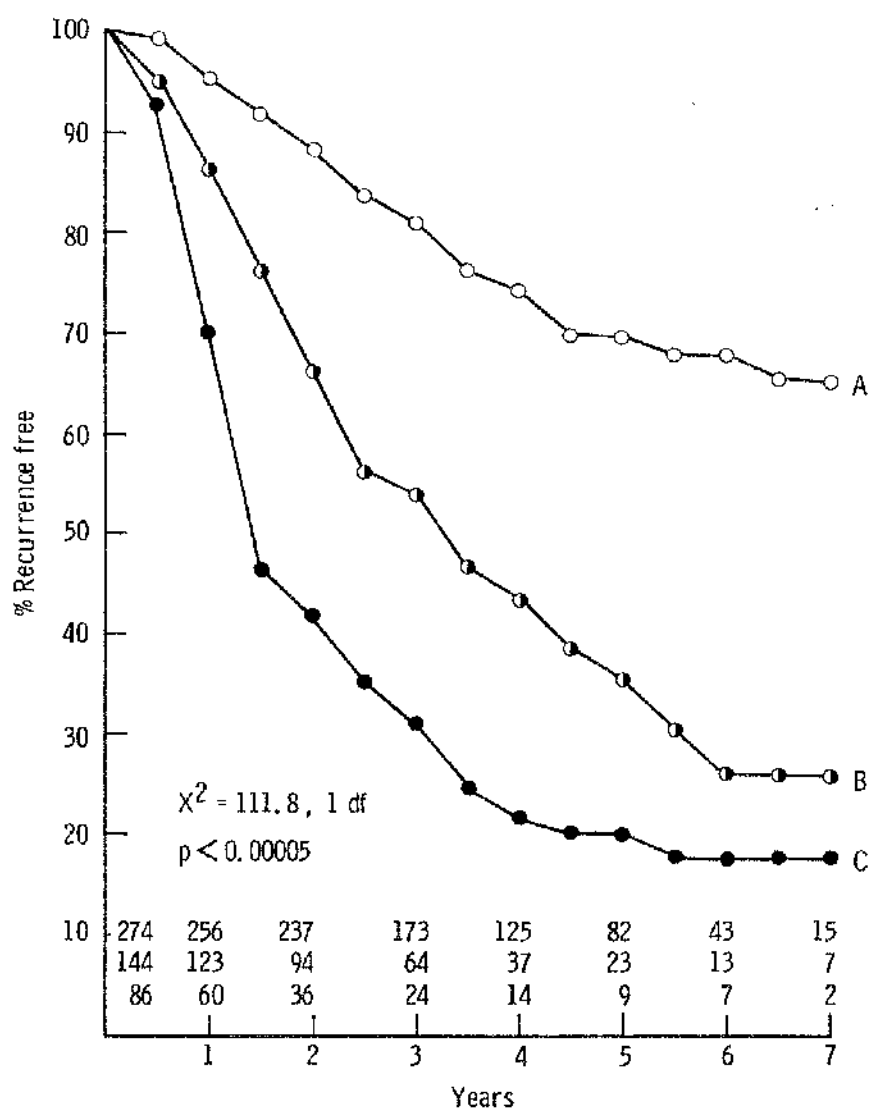


Fig. 4 : 4 Lymph node stage and major recurrence.



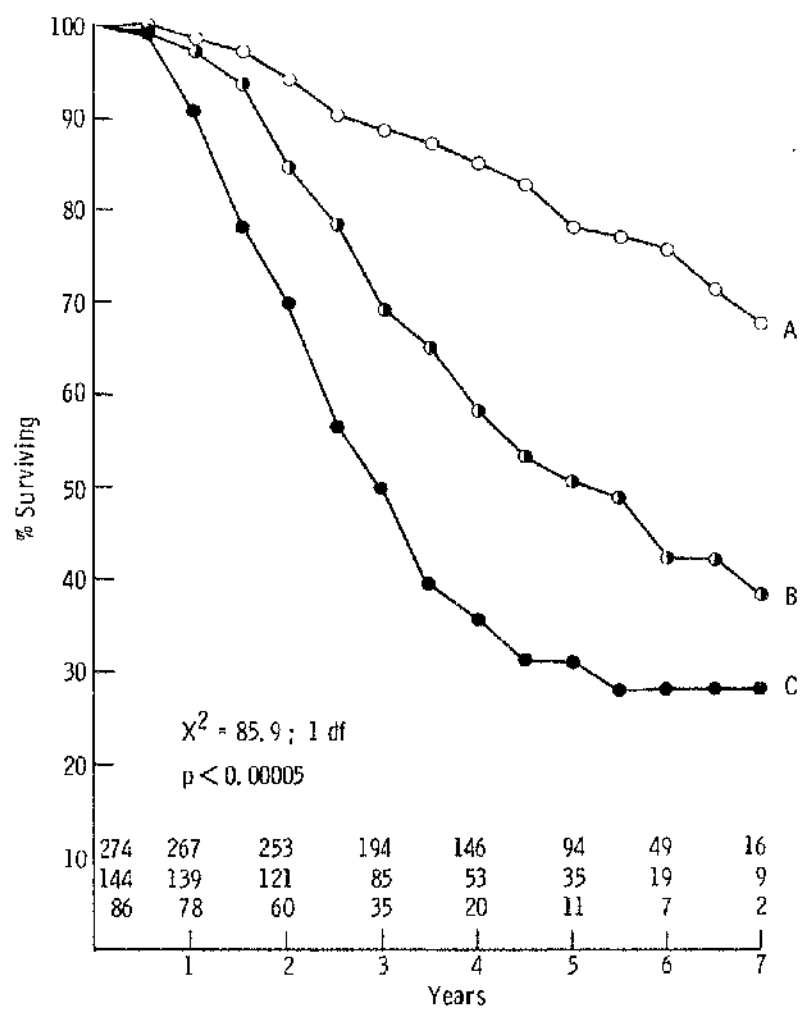


Fig. 4 : 5 Lymph node stage and survival.

### The Significance of 'missed' nodes in Lymph Node Staging

In this series, nodes were absent from one or more biopsies in approximately 30% cases. The effect of this factor upon the prognostic power of staging in terms of survival was evaluated in the first 389 patients in the study. Data has been compared in six groups (Table 4 : V).

The codes were arranged in a logical order of what seemed to be the likely prognosis, eg. Stage A with all 3 nodes identified and by definition histologically negative, ( $A_1$ ) might have a better outlook than A with one node missing ( $A_2$ ), or indeed A with 2 or 3 nodes missing ( $A_3$ ) since the 'missed' nodes could have been positive. Similarly, Stage B with all nodes identified ( $B_1$ ) might have a better prognosis than B with either of the high nodes missing ( $B_2$ ). A tumour which is designated Stage C is in this context, in the worst prognostic category and is unaffected by any of the other nodes which might have been missed.

The survival of each group has been compared by life table analysis. All the Stage A categories had a similar outlook irrespective of the number of nodes found at surgery and the prognosis of the two B categories was virtually identical although both were significantly better than C (Fig. 4 : 6).

Table 4 : VIncidence of 'missed' nodes

		numbers	Code
Stage A	All nodes present	123	A <sub>1</sub>
	1 node missing	47	A <sub>2</sub>
	2 or 3 nodes missing	31	A <sub>3</sub>
Stage B	All nodes present	76	B <sub>1</sub>
	1 or 2 nodes missing	43	B <sub>2</sub>
Stage C	Unaffected by 'missed' nodes	67	C

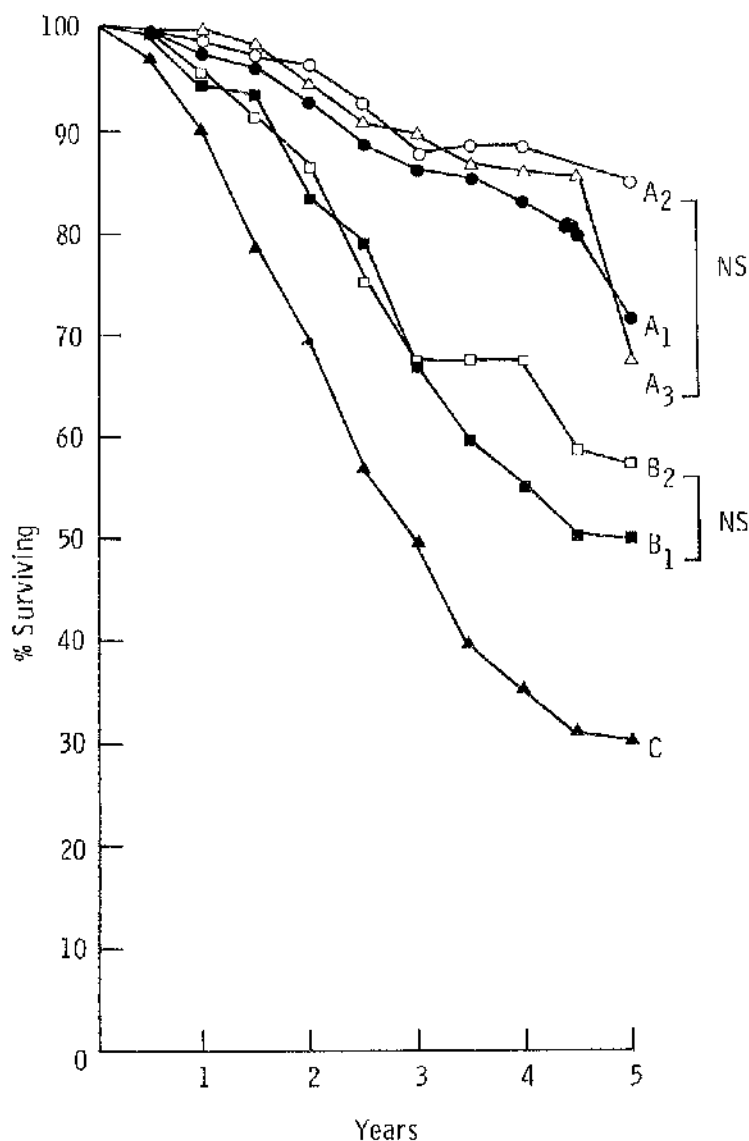


Fig. 4 : 6 Relationship of stage categories with or without 'missed' nodes to survival.

A<sub>1</sub> ~ All 3 nodes identified and tumour negative

A<sub>2</sub> ~ Two negative nodes identified, one 'missed' at surgery

A<sub>3</sub> ~ Either one negative node or no nodes found. 2 or 3 nodes 'missed'

B<sub>1</sub> ~ Axillary node positive, all nodes found

B<sub>2</sub> ~ Axillary node positive, one or two nodes 'missed'

C ~ Apical or internal mammary node positive

## Discussion

The present study has concentrated upon the determination of the status of multiple node levels in patients with breast cancer, by biopsy and histological examination. This method is not commonly employed in the United Kingdom, possibly as a result of reservations concerning its accuracy - that positive nodes might be missed at sampling with consequent false negatives. Fears such as these would only be allayed completely by a large randomised trial comparing the prognostic accuracy of axillary clearance with multiple node sampling but as stated in the introduction to this chapter, there has been no such study. The present study has shown that the prognostic power of staging as far as survival is concerned was unaffected by 'missed' nodes. Positive nodes are easier to find at surgery than uninvolved nodes and it seems unlikely on the basis of the evidence of this study, that these have been 'missed' in significant numbers of patients.

This study confirmed the relationship of stage to recurrence free interval as documented by a previous Nottingham treatise (Maynard et al, 1978), with greater patient numbers at a later point in the follow up interval. In addition, stage was significantly related to survival. Johnson used a similar method of staging in a study of 200 patients with breast cancer at the British Columbia Cancer Institute, with the exception that apical axillary and internal mammary node biopsies were performed prior to mastectomy and formed the basis for selection of therapy. If both nodes were negative, then patients were treated by radical mastectomy when axillary node status was also determined by axillary clearance, but

patients in whom either apical axillary or internal mammary nodes were involved, received radiotherapy alone. Thus Johnson defined three prognostic categories, which correlated with the three Nottingham Stages A, B and C. The incidence of these categories in the Vancouver study, were comparable to those of the Nottingham study, viz:

	Incidence	
	Vancouver	Nottingham
Lymph node negative (Nottingham		
Stage A)	- 44%	54%
Low axillary involvement (Nottingham		
Stage B)	- 29%	28%
Apical axillary or internal		
mammary involvement (Nottingham		
Stage C)	- 27%	18%

It is of interest to note the similarity of stage B cases in both series, determined by node sampling in the present study and by axillary clearance in the Canadian study. Five year survival of the various categories in this study showed a strong resemblance to the Nottingham data, despite the differences in treatment methods, viz:

	<u>Patients Correlating to Stages</u>		
	<u>A</u>	<u>B</u>	<u>C</u>
<u>% 5 year survival</u>			
Johnstone study	83%	59%	29% (Johnstone, 1972)
Nottingham study	78%	50%	31%

In the present study, nodal stage has proved a better prognostic factor than tumour size in that patients can be allocated

to specific prognostic groups with greater confidence (chi-square values for survival and disease free interval assessed by life table analysis were rather greater when groups were compared according to stage than by size). Thus the Nottingham staging system has the advantage of simplicity, with three clear categories, each with a predictable and well defined outlook.

While this method has a useful clinical application, there are prognostic limitations. For example, thirty percent of patients with Stage A tumours, whose disease was apparently "confined to the breast", had recurred within five years and twenty percent of patients with Stage C cancers were recurrence free at this time. Staging by this method appears more accurate than the TNM system which has shown prognostic overlap for different stages (Cutler, 1968; Gorski et al, 1968), but nonetheless the Nottingham stage alone incorrectly predicted prognosis in 25% patients. Thus some means which permits further refinement of our prognostic marker, without a reduction of the size of the patient groups to which the marker can be applied, is desirable.

### Conclusions

An assessment of the local extent of tumour at the time of mastectomy, by measurements of the primary cancer size and the degree of nodal involvement has a significant bearing upon prognosis in terms of both the interval to major recurrence and survival. It is likely that tumour size and lymph node involvement are related to both the chronological age of the cancer and its biology, in terms of its rate of growth and its degree of malignancy. A measure of the level of lymph node involvement provides a rather better prognostic discriminant than tumour size alone, but a strong relationship exists between the two. While the prospect of staging cancers by lymph node involvement is simple and practical it correctly predicts prognosis in only 75% patients with discrepancies in both lymph node positive and lymph node negative groups.

In any attempt to evaluate the prognostic significance of biological intrinsic factors, tumour bulk must first be evaluated, and if necessary a corrective factor applied.



## Chapter 5

### INTRINSIC BIOLOGICAL MARKERS ~ INCIDENCE AND INTER-RELATIONSHIPS

## Intrinsic Biological Markers - Incidence and Inter-relationships

### Introduction

As reported previously from this centre, a strong relationship exists between oestrogen receptor status and histological grade in primary breast cancer (Elston et al, 1980). Observations such as these imply that intrinsic parameters (ER, PgR status and histological grade) may simply represent different measures of the same aspect of tumour malignancy and it has been suggested that all these factors express themselves at the same prognostic level (Fisher et al, 1975). There is, however no evidence to support this contention. Later in this thesis, the predictive value of all of these parameters will be assessed in different situations but before carrying out such a comparison, we shall investigate the precise inter-relationships of the factors themselves.

### The Present Study (Study 3)

The first 550 patients in the Nottingham-Tenovus primary breast cancer series have been followed for a minimum interval of two years and the majority of analyses relate to these patients. Progesterone receptor assays are a more recent addition and have been performed in only 167 of the last 250 women in the survey.

### Histological grade

Primary cancers have been graded by a modification of the method of Bloom and Richardson (1957) (see Appendix) in 504 women of the first 550 who have also had tumour size and lymph node stage evaluated.

### Oestrogen receptor assay

Oestrogen receptor analyses have been performed on primary breast cancers by the Dextran Coated charcoal method (see Appendix) in 435 of the first 550 women who have also had tumour size, lymph node stage and histological grade assessed. Cancers were considered to be oestrogen receptor positive, when they contained more than 5 femtomoles specific oestradiol binding per milligram cytosol protein.

### Progesterone receptor assay

Progesterone receptor (PgR) analyses were performed on primary breast cancers by incubating with  $^3\text{H}$ -Progesterone with and without excess of free R5020 progesterone ligand (see Appendix). This procedure also utilised the Dextran Coated charcoal method. Progesterone receptor was assayed in 167 women who also had tumour size, lymph node stage, histological grade and oestrogen receptor concentration assessed. Tumours were regarded as being PgR positive

when they contained more than 5 fmoles specific progesterone binding per milligram cytosol protein.

#### Statistical analyses

Chi-square tests for goodness of fit were utilised for qualitative data. Evaluation of receptor values, which have a skewed distribution has necessitated the use of non-parametric statistics eg. Wilcoxon Rank Sum and the Kendall Rank test of correlation.

## Results Incidence and Inter-relationships of Intrinsic Factors

### a) Incidence

i) Histological grade Seventeen percent of tumours were grade I and 47% Grade III (Table 5 : I).

ii) Oestrogen receptor assays Fifty eight percent of tumours were ER positive and the measured receptor concentration ranged from 0 - 1500 with a median value of 58.5 fmoles/mg cytosol protein.

iii) Progesterone receptor assays Thirty one of 167 (18.5%) tumour were progesterone receptor positive. The receptor concentration ranged from 0 - 2740 with a median value of 251 fmoles/mg cytosol protein.

### b) Inter-relationships

#### Oestrogen receptors and histological grade

A highly significant relationship existed between oestrogen receptor status and histological grade. Receptor positive tumours tended to be well or moderately differentiated while receptor negative cancers tended to be poorly differentiated (Table 5 : II). Consideration of oestrogen receptor values and histological grade confirmed but did not strengthen the relationship (Table 5 : III). Indeed, within the receptor positive group, histological grade was unrelated to oestrogen receptor values (Table 5 : IV).

#### Oestrogen and progesterone receptors

No significant relationship existed between oestrogen and progesterone receptor status in primary breast cancer (Table 5 : V). However, when we considered quantitative values, a significant relationship emerged. Progesterone receptor positive tumours had significantly higher concentrations of oestrogen receptor than PgR

Table 5 : IHistological grades - Incidence

Grade	n	%
I	85	17
II	181	36
III	238	47
	—	—
Total	504	100

Table 5 : IIRelationship of ER status and histological grade

<u>Grade</u>	<u>ER status</u>	
	<u>Positive</u>	<u>Negative</u>
I	55	16
II	99	55
III	97	113

$$\chi^2 = 25.5 \quad 2 \text{ df} \quad p < 0.0005$$

Table 5 : IIIRelationship of ER values to histological grade

Grade	<u>All tumours</u>					<u>ER Negative</u>
	<u>ER Positive</u>					
	<u>ER value</u> <u>5-20</u>	<u>21-40</u>	<u>41-80</u>	<u>81-150</u>	<u>&gt; 150</u>	
I	8	13	12	9	12	16
II	15	22	19	16	20	55
III	21	21	11	17	21	113

$$\chi^2 = 31.0 \quad 10 \text{ df}; \quad p < 0.0005$$



Table 5 : IV

Relationship of quantitative oestrogen receptor concentration  
and histological grade

Oestrogen receptor positive tumours

<u>Grade</u>	<u>ER value</u> <u>5-20</u>	<u>21-40</u>	<u>41-80</u>	<u>81-150</u>	<u>&gt; 150</u>
I	8	13	12	9	12
II	15	22	19	16	20
III	21	21	11	17	21

$$\chi^2 = 4.5; \quad 8 \text{ df}; \quad 0.9 > p > 0.8$$

Table 5 : V

Relationship of oestrogen and progesterone receptor  
status of primary breast cancer

	<u>ER Positive</u>	<u>ER Negative</u>
<u>PR positive</u>	23	8
<u>PR negative</u>	82	54

$$\chi^2 = 2.01; \quad 1 \text{ df}; \quad 0.2 > p > 0.1$$

negative cancers (Fig. 5 : 1). The likelihood of a tumour possessing PgR was proportionately related to its ER concentration (Fig. 5 : 2).

#### Histological grade and progesterone receptors

Before considering the relationship of grade to progesterone receptors, we shall re-examine the relationship of ER and grade in the 167 patients, who have had PgR assays. As seen in Table 5 : VI, a highly significant relationship existed. Histological grade was unrelated to progesterone receptor status or to progesterone receptor values (Table 5 : VII). When considered in combination with oestrogen receptor status, progesterone receptor status did not strengthen the relationship with histological grade (Table 5 : VIII).

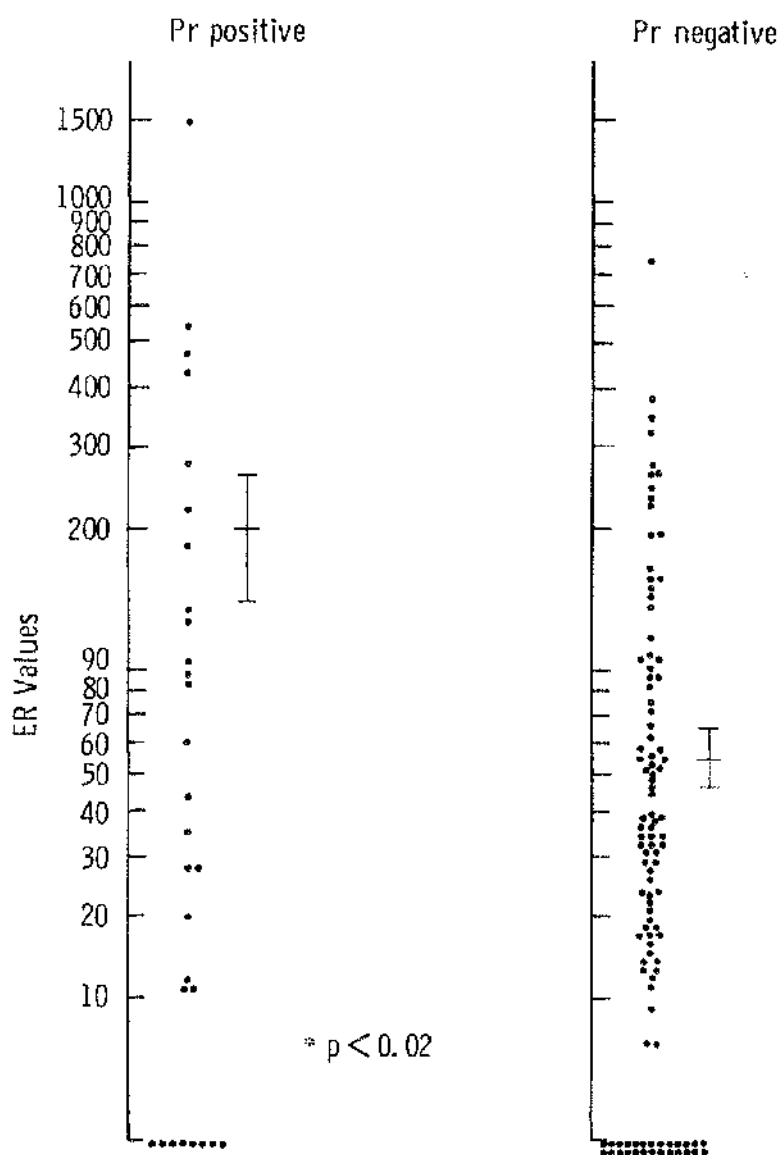


Fig. 5 : 1 Quantitative oestrogen receptor values in progesterone receptor positive and negative cancers ( $p < 0.02$  by Wilcoxon Rank Sum\*)

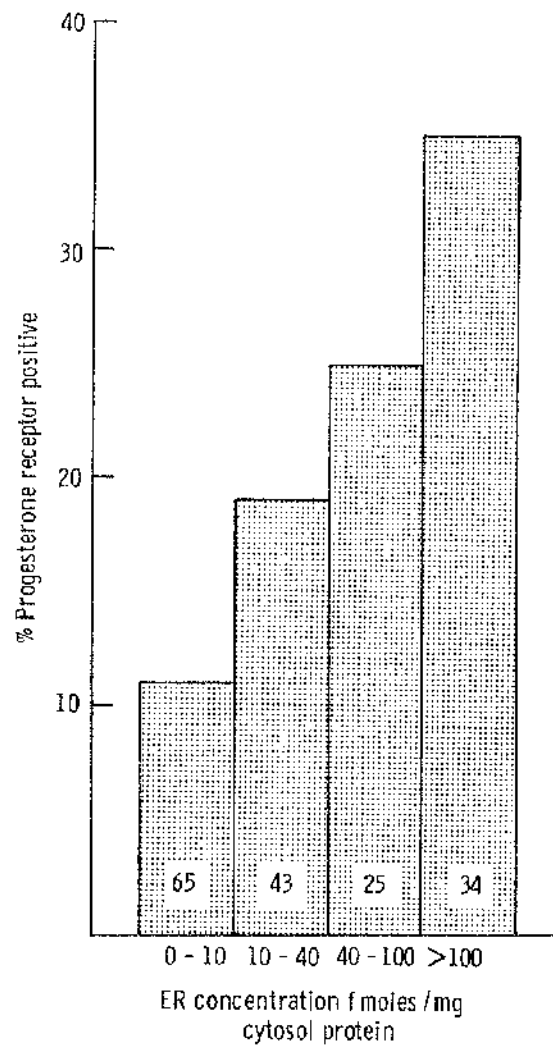


Fig. 5 : 2 Quantitative ER levels and progesterone receptor status of primary breast cancer.

Table 5 : VI

Relationship of oestrogen receptor status and  
histological grade in 167 women

<u>Histological</u> <u>Grade</u>	<u>ER Positive</u>	<u>ER Negative</u>
I	14	5
II	51	17
III	40	40

$$\chi^2 = 10.5; \quad 2 \text{ df}; \quad p < 0.01$$

Table 5 : VIIHistological grade and progesterone receptor status

<u>Histological Grade</u>	<u>PR Positive</u>	<u>PR Negative</u>
I	3	15
II	15	58
III	13	63

$$\chi^2 = 0.34; \quad 2 \text{ df}; \quad 0.9 > p > 0.8$$

Table 5 : VIIIHistological grade and steroid receptor status

<u>Histological</u> <u>Grade</u>	<u>Steroid receptor status</u>			
	<u>ER+</u> <u>PR+</u>	<u>ER+</u> <u>PR-</u>	<u>ER-</u> <u>PR+</u>	<u>ER-</u> <u>PR-</u>
I	3	11	1	4
II	12	39	2	15
III	18	32	5	35

$$\chi^2 = 11.3; \quad 6 \text{ df}; \quad p = 0.08$$



## Discussion

The incidence of various histological grades in this series differs from that of other studies, and in particular from the series reported by Bloom (1950) where 26% tumours were Grade I, 45% Grade II and 29% Grade III. Differences exist between Bloom's method and the Nottingham technique, particularly in the assessment of mitotic activity. Bloom regarded both mitotic figures and hyperchromatic nuclei as evidence of mitotic activity and evaluated the presence of these parameters in "slight, moderate or marked degree". In Nottingham, a more objective point count of mitotic figures was carried out at 100 x magnification. Hyperchromatic nuclei were disregarded. Methodological differences such as these may partly account for variation of results but it must be emphasised that grading is considered to be a subjective means of assessment of innate malignancy and has been criticised by some on the grounds of reproducibility (Cutler et al, 1966).

An oestrogen receptor assay is generally considered to be a more objective test of innate tumour biology, but scrutiny of the published data from different centres belies this assumption. A variety of techniques exist for receptor assay, and the 'cut off' point for positivity varies from 3 to 10 fmoles/mg cytosol protein. These factors partly account for the variation of results (King, 1980). However, even when definitive criteria for receptor positivity and methods of evaluation are similar, the incidence of oestrogen receptor positive tumours varies from 35 - 85% (Leclercq and Heuson, 1977). Thus it seems likely that the accuracy of oestrogen receptor determinations are limited by some factors which have not conclusively been identified.

The present study confirmed our previous reports of a highly significant relationship between oestrogen receptor status and histological grade (Maynard et al, 1978; Elston et al, 1980). It has been suggested that 'cut off points' for oestrogen receptor, for positive or negative status are artificial, and that hormone dependency is distributed along a continuous gradient assessed by the quantity of ER (Paridaens et al, 1980). Thus one might anticipate that quantitative ER values might bear a stronger relationship to histological grade than ER status alone. This study, however, has failed to demonstrate any stronger relationship of ER values to grade.

As with oestrogen receptors, there is a wide variation in reported results with progesterone receptors. The proportion of patients with PgR positive tumours varies from 17% to 41% (Hahnel et al, 1980; Brooks et al, 1980) and the incidence in the present study is at the lower end of the reported range. If the hypothesis that progesterone receptor is the end product of an oestrogen dependent pathway (Horwitz et al, 1975) is totally accurate then we would anticipate a strong relationship between progesterone and oestrogen receptor status and also we would expect progressive loss of progesterone receptor as tumours become less differentiated. However, contrary to the findings of Brooks et al, 1980 and Horwitz, 1980, we have failed to demonstrate a significant relationship between ER and PgR status, but in this, we are in agreement with Hahnel et al, 1980. Progesterone receptor assays in primary breast cancer remain at a relatively preliminary stage at the present time in all of the mentioned studies, and it is possible that with

greater numbers there will be closer agreement on this very elementary point. In the present study, we have certainly shown a significant relationship between quantitative ER values and progesterone receptor status: the higher the ER value, the greater the likelihood of a particular tumour being PgR positive. In this we are in agreement with Osborne et al, 1980. This study has shown no relationship between progesterone receptor and histological grade, irrespective of whether PgR values or PgR status were considered or whether ER and PgR status were considered in combination and thus we have been unable to validate the findings of King, 1980 or Pichon et al, 1980.

In conclusion, this study demonstrated a highly significant relationship between ER and histological grade. Despite the strength of this relationship we have begun to see some separation of endocrine markers (quantitative ER values; progesterone receptor status) from grades of differentiation in the primary cancer. Thus it is conceivable that endocrine markers may identify one specific aspect of tumour biology and histological differentiation another, with a degree of prognostic overlap.

### Clinical events and prognosis in breast cancer

In the preceding chapters, the prognostic significance of tumour bulk has been considered and the inter-relationships of intrinsic factors themselves have been investigated. The remainder of this thesis will be concerned with an investigation of the influence of intrinsic parameters upon the course of breast cancer. In this context a comparative evaluation of parameters will be carried out against three clinical events of prognostic importance, namely:

1. Disease free interval.
2. The anatomical site of secondary metastasis.
3. Response to endocrine therapy.

The significance of each of these three clinical events upon survival will be considered, and then 'intrinsic' variables will be evaluated against survival. A study of this nature is broad based and necessitates multifactorial analyses, which will be carried out in succeeding chapters.

## Chapter 6

PREDICTION OF RECURRENCE FREE INTERVAL IN BREAST CANCER:

TUMOUR GROWTH BEHAVIOUR

## Prediction of Recurrence Free Interval in Breast Cancer:

### Tumour Growth Behaviour

#### Introduction

The interval which elapses after mastectomy until the appearance of a major recurrence relates strongly to survival in patients with breast cancer (Cutler, Asire and Taylor, 1969). This interval provides a measure of growth behaviour (Devitt, 1976) : fast growing tumours will have a short disease free interval and vice versa. Since the rate of growth is difficult to measure in breast cancer, it has been impossible to predict the disease free interval which individual patients will enjoy following primary therapy, with accuracy. Measures of tumour bulk have certainly been used to identify risk groups, but as outlined previously these are not infallible. Intrinsic factors of grade and steroid receptors have been variously related to recurrence free interval in different studies (Maynard et al, 1978a; Cooke et al, 1979; Godolphin et al, 1981; Pichon et al, 1980) but there has been little comparative assessment of their relative value in the same group of patients.

Before considering the association of any intrinsic factor with recurrence free interval one must firstly take account of the influence of tumour bulk. In this study the relationship of histological grade, oestrogen and progesterone receptors in primary breast cancer will be related to tumour size and lymph node stage and thereafter to the intervals which elapse until the appearance of various types of recurrence: local, regional and distant and also to overall recurrence free interval.

## The Present Study (Study 4)

### Patients and Methods

Histological grade has been evaluated in 504 primary cancers, oestrogen receptors in 435 and progesterone receptors in 167, by the methods previously described (see Appendix). Oestrogen and progesterone receptor status have been considered both alone and in combination. Only a small number of tumours ( $n = 31$ ) are progesterone receptor (PgR) positive and therefore quantitative PgR values have not been considered. However, oestrogen receptor status and quantitative ER values have been related to recurrence free interval: tumours have been divided according to the quantity of ER into five approximately equal groups (Table 6 : I).

In this study, the term recurrence free interval refers to the period until the appearance of a major metastasis in any site as previously outlined. The categories of recurrence which have been considered in this study were defined thus:

Local recurrence: Multiple, symptomatic or progressive metastases in mastectomy flaps, which were confirmed histologically. 'Spot' recurrences in the flaps have been excluded.

Regional recurrence: Symptomatic metastases in axillary or supraclavicular nodes which were confirmed histologically.

Distant recurrence: Any distant metastases confirmed by clinical examination, abnormal liver function tests, appropriate x-rays, liver or brain scans or biopsy.

### Statistical Analysis

Intervals until the appearance of metastases were calculated by actuarial life table analysis and statistical evaluation performed by the method of Mantel (1966).

Table 6 : IQuantitative oestradiol receptor values

<u>ER concentration</u> (fmol/mg cytosol protein)	<u>n</u>
4 - 20	41
20 - 40	56
40 - 80	41
80 - 150	41
> 150	50



## Results

### I Intrinsic factors and tumour bulk

#### a) Histological grade

The histological grade of a primary breast cancer was significantly related to its size in centimetres: the greater the degree of anaplasia, as assessed by three grades, the larger the size of that tumour at presentation (Table 6 : II). Only a small percentage of Grade I tumours were large in size (Fig. 6 : 1). There was a trend for increasing severity of the stages of lymph node involvement with poorer histological grades, but differences were not significant (Table 6 : III).

#### b) Oestrogen and progesterone receptor status

Oestrogen and progesterone receptor status of primary breast cancer were unrelated to tumour size (Tables 6 : IV & V) or stage of lymph node involvement (Tables 6 : VI & VII).

### II Intrinsic factors and recurrence free interval

#### a) Histological grade

Histological grade of a primary cancer was significantly related to the recurrence free interval: the higher the grade, the shorter is the interval until a recurrence appears (Fig. 6 : 2). This relationship persisted for all types of recurrence: high grade tumours were significantly associated with a short interval until the appearance of local (Fig. 6 : 2b), regional (Fig. 6 : 2c) and distant (Fig. 6 : 2d) recurrences. However, poorly differentiated tumours tended to be large in size at presentation (Table 6 : II).

Table 6 : IIHistological grade and tumour size

Size (cms)	Grade		
	I	II	III
0 - 2	49	84	81
2 - 4	33	71	81
> 4	3	26	36

$$\chi^2 = 20.1; \quad 4 \text{ df}; \quad p < 0.0005$$

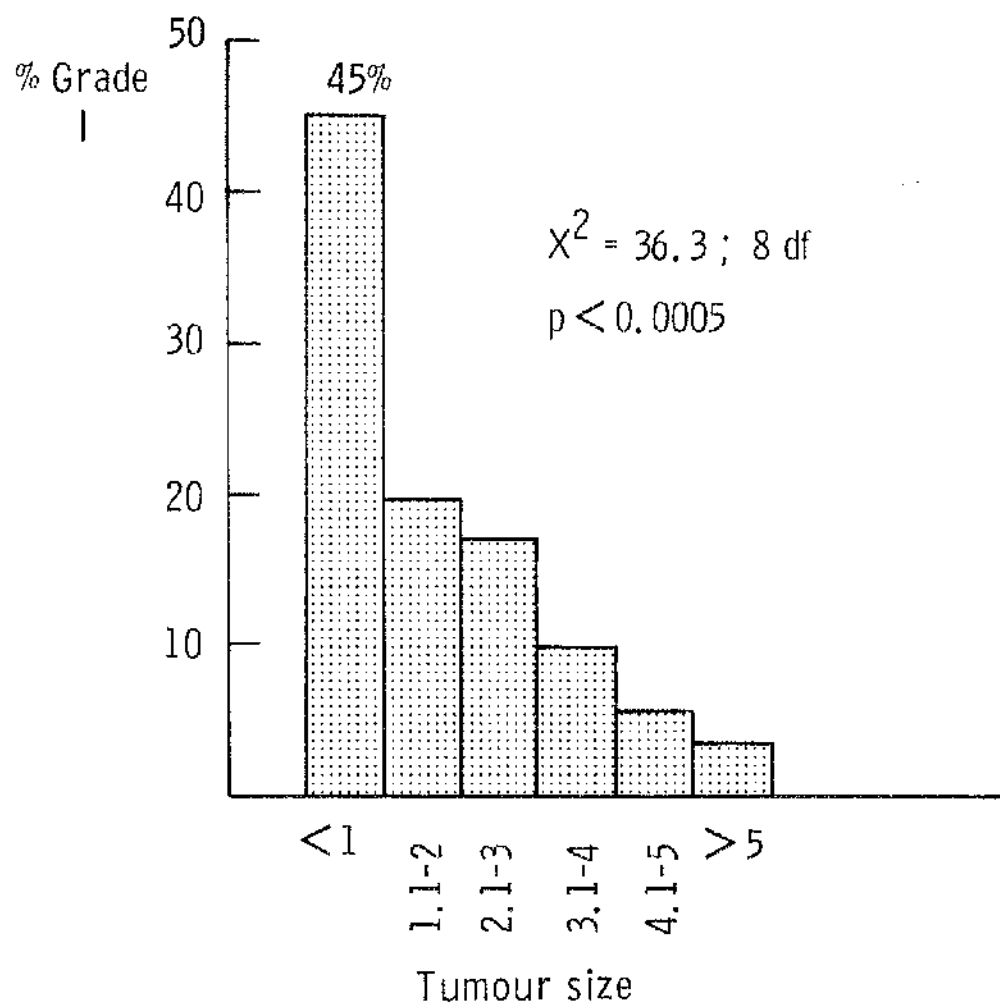


Fig. 6 : 1 Tumour size and grade I histological differentiation:  
Small tumours tend to be well differentiated (Grade I).

Table 6 : IIIHistological grade and lymph node stage

Stage	Grade		
	I	II	III
A	57 (67%)	93	124 (52%)
B	18	55	71
C	10 (12%)	33	43 (18%)

$$\chi^2 = 6.7; \quad 4 \text{ df}; \quad 0.2 > p > 0.1$$

Table 6 : IVOestrogen receptor status and tumour size

Size (cms)	ER Positive	ER Negative
0 - 2	106	72
2 - 4	120	84
> 4	25	28

$$\chi^2 = 2.8; \quad 2 \text{ df}; \quad 0.3 > p > 0.2$$

Table 6 : VProgesterone receptor status and tumour size

Size (cms)	PR Positive	PR Negative
0 - 2	16	69
2 - 4	15	62
> 4	0	5

$$\chi^2 = 1.2; \quad 2 \text{ df}; \quad 0.6 > p > 0.5$$

Table 6 : VIOestrogen receptor status and lymph node stage

Stage	ER Positive	ER Negative
A	124 (49%)	107 (58%)
B	83 (33%)	45 (24%)
C	44 (17%)	32 (17%)

$$\chi^2 = 4.2; \quad 2 \text{ df}; \quad 0.2 > p > 0.1$$

Table 6 : VIIProgesterone receptor status and lymph node stage

Stage	PR Positive	PR Negative
A	16	67
B	9	38
C	6	31

$$\chi^2 = 0.17; \quad 2 \text{ df}; \quad 0.95 > p > 0.9$$



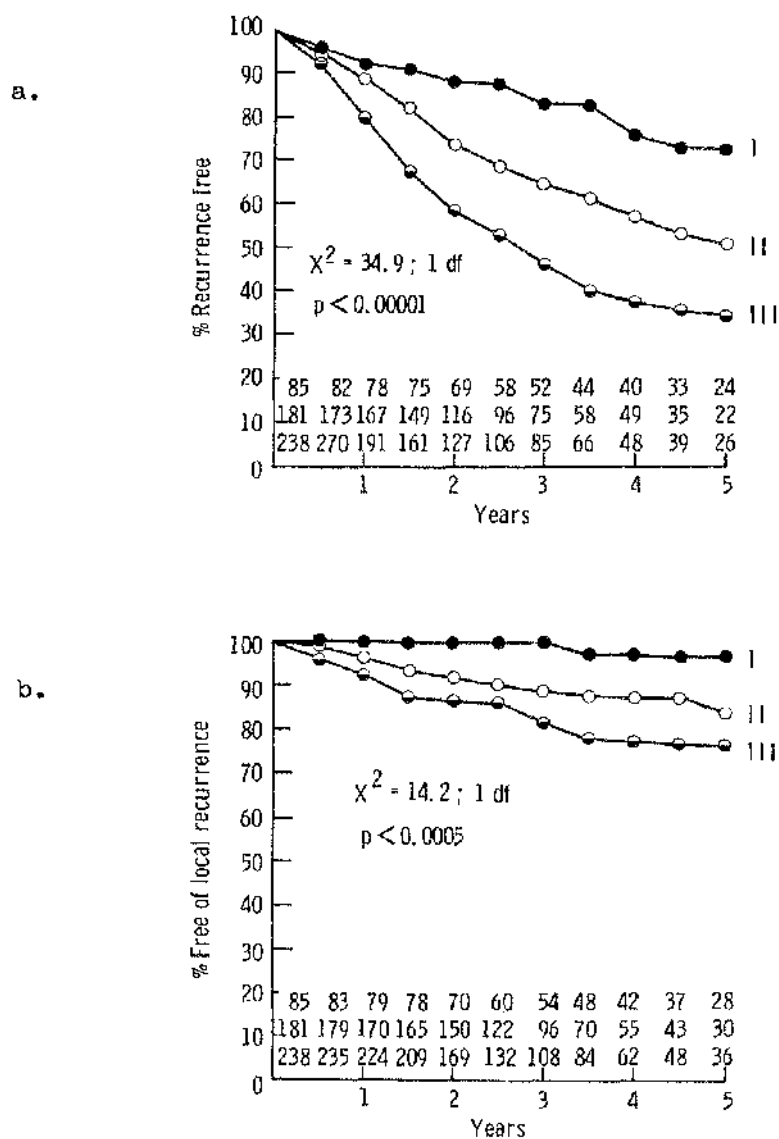


Fig. 6 : 2 Histological grade and recurrence free interval.

a. All recurrences

b. Local recurrence

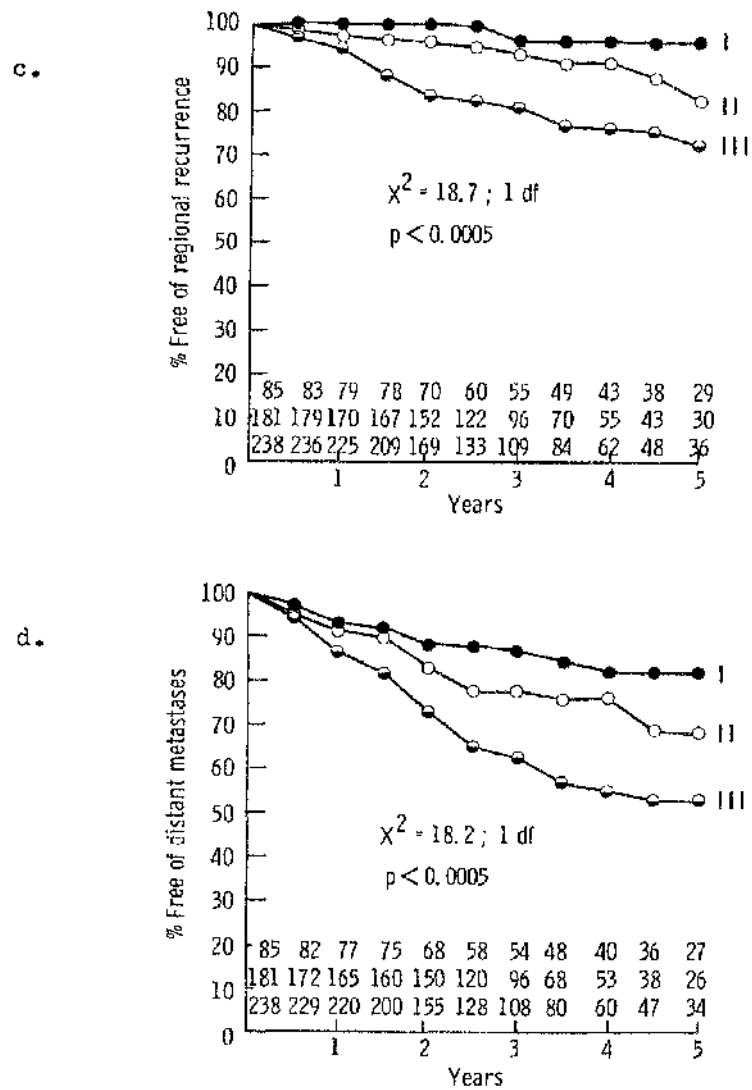


Fig. 6 : 2 Histological grade and recurrence free interval.

c. Regional recurrence

d. Distant metastases

To correct for this association, the independent prognostic influence of grade against disease free interval, has been assessed within a group of patients with a single tumour size (measuring < 2.0 centimetres). A highly significant relationship persisted (Fig. 6 : 3).

b) Steroid receptors and recurrence free interval

Oestrogen receptor status was related to the interval until regional recurrence (Fig. 6 : 4) but not to disease free interval overall (Fig. 6 : 5). Consideration of quantitative values of ER did not improve its predictive value (Fig. 6 : 6).

Progesterone receptor status was unrelated to disease free interval, irrespective of whether it was considered alone (Fig. 6 : 7) or in combination with oestrogen receptor status (Fig. 6 : 8).

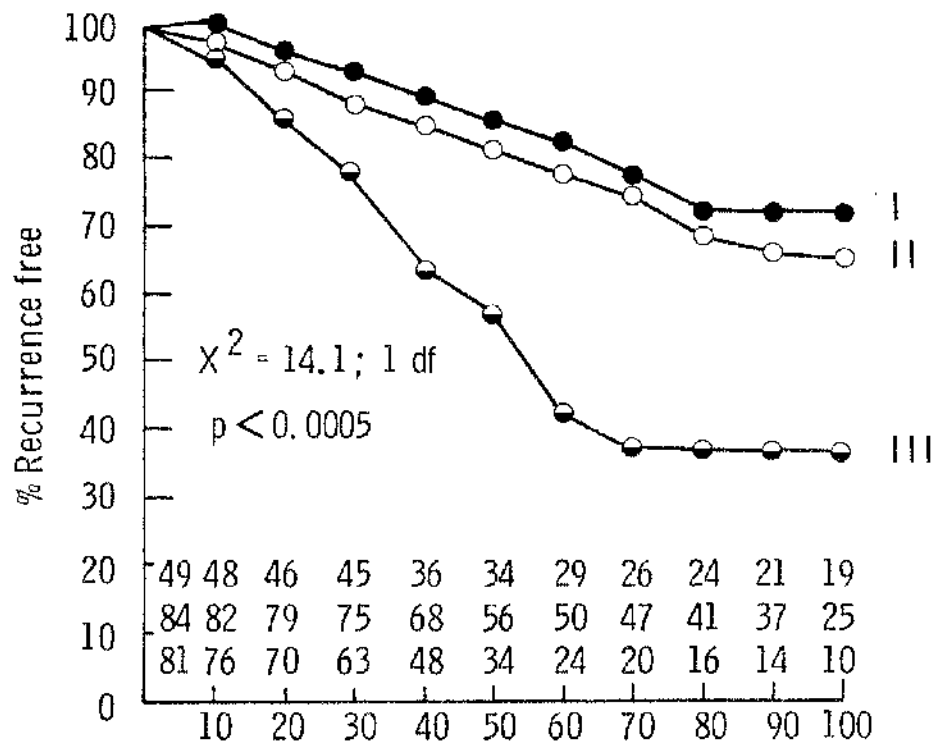


Fig. 6 : 3      Histological grade and recurrence free interval.  
 Small tumours (< 2.0 cms diameter) only.

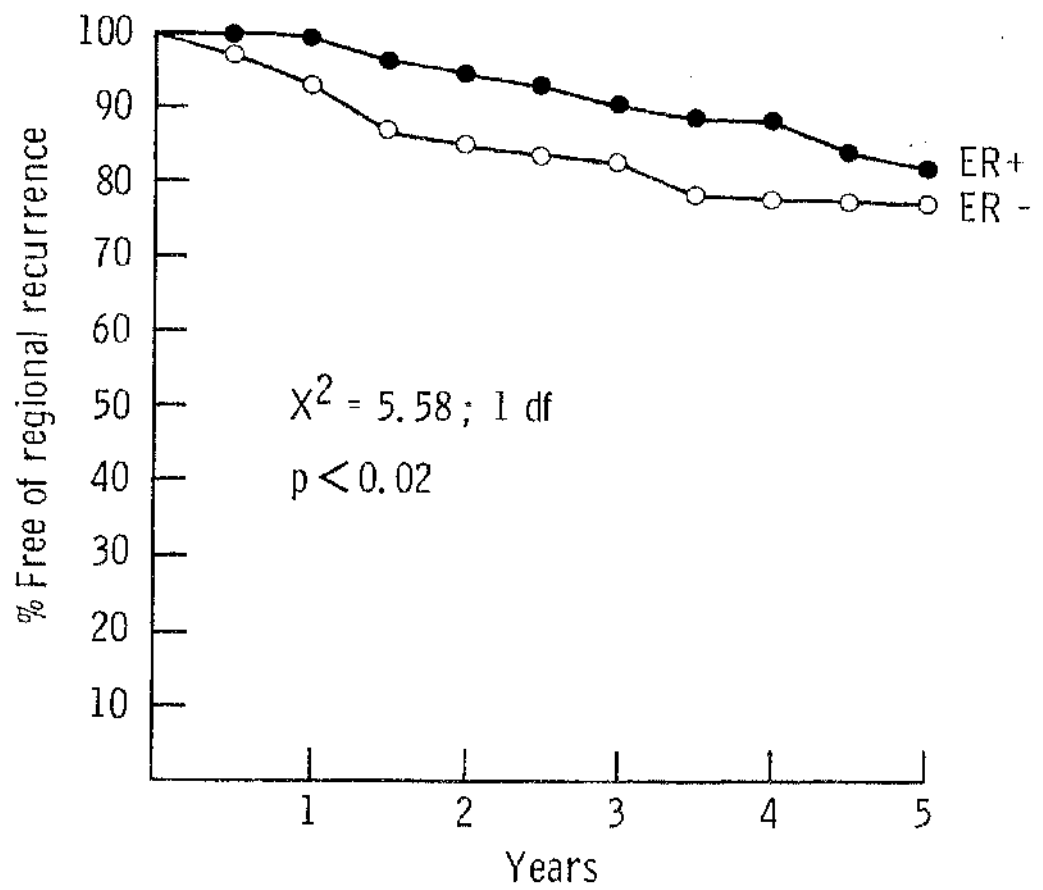


Fig. 6 : 4 Oestrogen receptor status and regional recurrence.

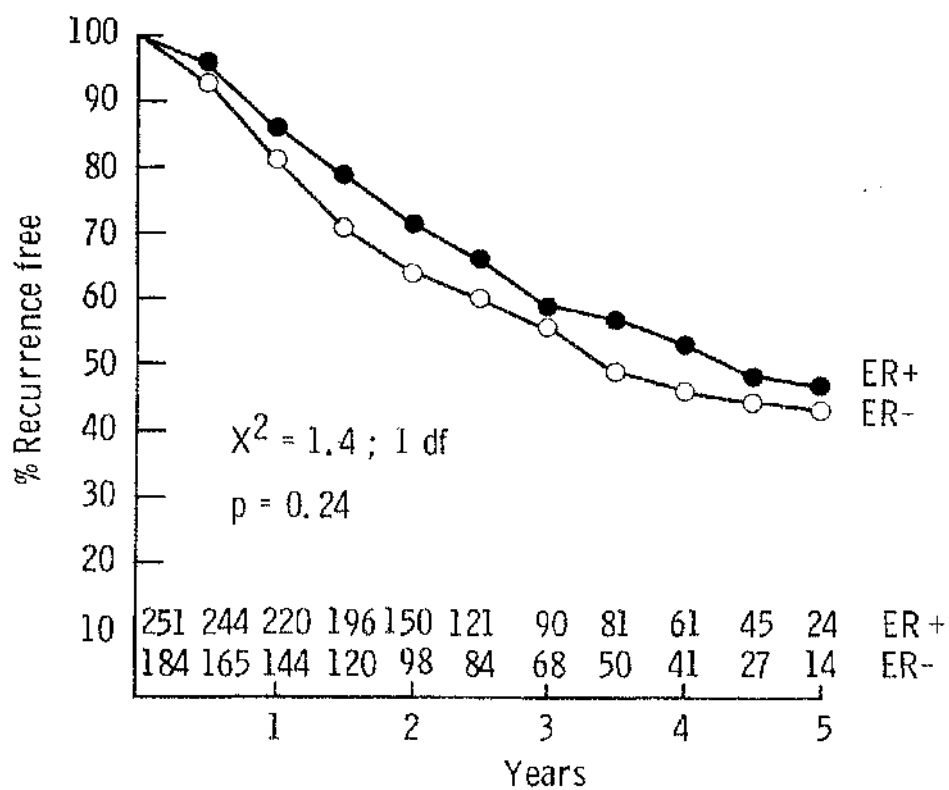


Fig. 6 : 5 Oestrogen receptor status and disease free interval: all recurrences.

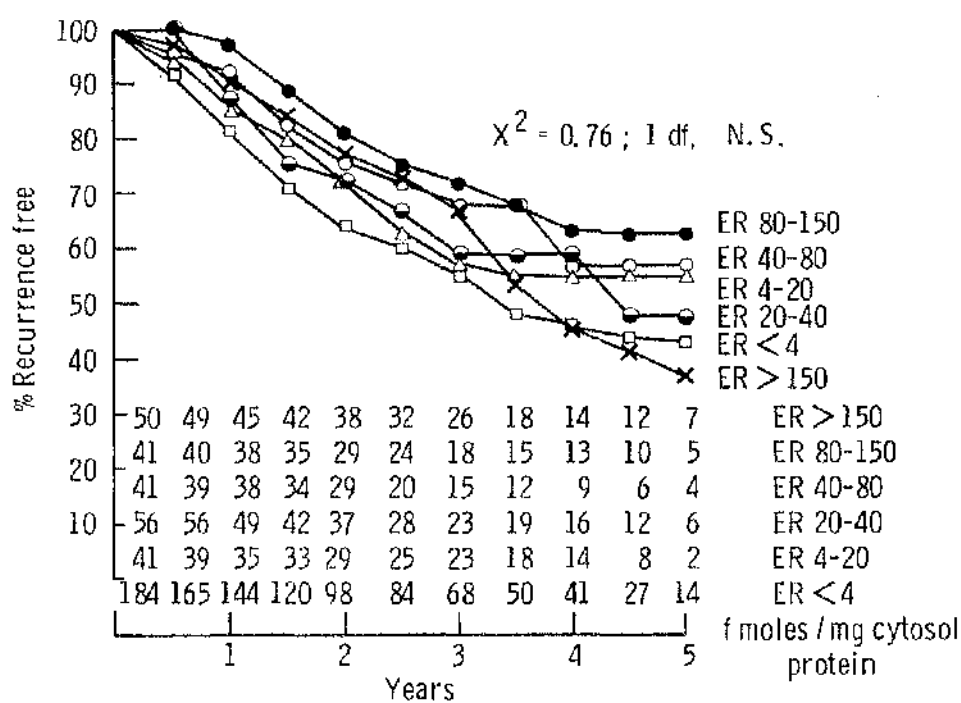


Fig. 6 : 6 Quantitative oestrogen receptor level and total recurrence free interval.

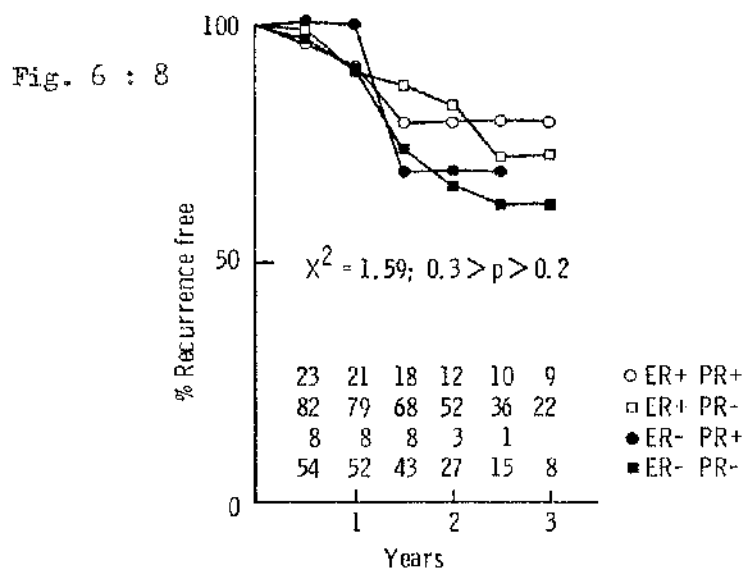
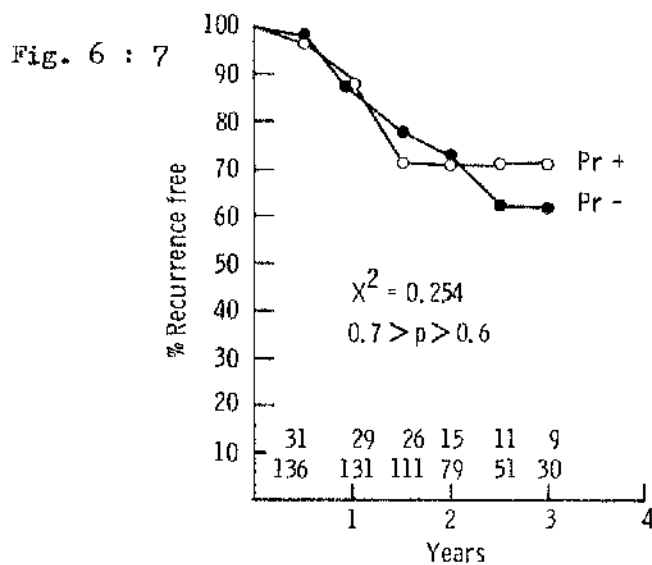


Fig. 6 : 7 Progesterone receptor status and recurrence free interval.

Fig. 6 : 8 Combined steroid receptor status and recurrence free interval.



## Discussion

In patients in this study, subclinical tumour growth following mastectomy was unimpeded by any form of treatment. Thus the interval from mastectomy until the clinical appearance of metastases (disease free interval) was likely to be determined by tumour growth rates. In this study, histological grading by the method described correlated well with disease free interval for all types of recurrence: local, regional and distant, and defined three clear risk categories. This parameter was a better marker of recurrence rates than either or both of the steroid receptors and was therefore the better marker of tumour growth rates. The relationship between grade and primary tumour size, that poorly differentiated tumours tended to be larger, lends support to this assertion, as one might have anticipated that fast growing tumours would reach a large size by the time of presentation.

Bloom (1950) noted a similar association between grade and clinical stage (which was largely determined by tumour size) but he also found that the prognostic yield of each of these measurements was synergistic. The findings of this study are in agreement: grade is related to tumour size but correlates well with recurrence rates even when 'corrected' for the prognostic influence of size, ie. within a group of patients with a single cancer size. Thus the prognostic yield of grade and measures of tumour bulk is likely to be additive.

Steroid receptors, only bore a tenuous relationship with disease free interval. Oestrogen receptor status was weakly related to recurrence rates in regional nodes, but not to recurrence free

interval concerning all types of metastases. It is noteworthy, that a relationship of ER status in the primary tumour and overall recurrence free interval, significant at the 95% level of confidence was previously reported from this centre (Maynard et al, 1978a). However, with greater patient numbers and longer follow up we have found that this is no longer the case and thus our data lends support to that of Hahnel et al, 1979 and Furmanski et al, 1980, who reported that the favourable influence of ER positive status upon recurrence rate was temporary and that no difference existed at five years of follow up.

We have not been able to validate the report of Godolphin et al, 1981 of an inverse relationship between ER concentration in the primary cancer and recurrence rates. It must be emphasised that although the Godolphin study used similar methodology for receptor assay to that of the Nottingham-Tenovus project, analyses pertaining to recurrence free interval were retrospective and were not carried out by any of the clinicians directly involved with patient care. Standards of record keeping were high (Personal communication, Elwood) but nonetheless the validity of that study is limited by errors of retrospective analyses.

Contrary to the findings of Pichon et al (1980) we have not found progesterone receptor status alone to be a useful marker of recurrences, but as outlined previously, the Pichon study was flawed by an uneven distribution of various tumour stages. Our findings are rather more in agreement with Skinner et al (1980) who demonstrated a non significant trend for ER+/PR+ tumours to have lower recurrence rates.

In conclusion, histological grade was the most useful marker of recurrence rates. Despite the highly significant association of grade and ER, the latter was only tenuously associated with tumour growth behaviour.

## Chapter 7

### PATTERNS OF METASTASES IN BREAST CANCER

## Patterns of Metastases in Breast Cancer

### Introduction

In the last chapter, we considered the recurrence free interval after mastectomy, which is an important clinical measure of growth behaviour of breast cancer and has prognostic significance (Devitt, 1976). Variable metastatic patterns are seen following mastectomy and these are of additional prognostic importance. Distant metastases are particularly important and the initial or predominant site of involvement is related to the outlook.

In a study of 1042 patients at the Radiumhemmet, Nohrman observed a 4% survival rate at five years following detection of metastases in 144 patients with predominantly bony secondaries, whereas none of 114 patients with visceral secondaries were alive at three years (Nohrman, 1949). In an elegant study of two series of patients who presented to the University of California Hospital, Shimkin et al (1954) demonstrated that patients with 'osseous' metastases (ie. whose initial site of involvement was in bone) enjoyed a longer disease free interval than those with 'generalised' secondaries (ie. initial secondaries in lung, liver, other intra-abdominal organs, distant lymph nodes) although differences were not significant (median = 27 months for osseous metastases versus median of 19 months for 'generalised' secondaries). In addition 'osseous' metastases were associated with a significantly better survival which in fact was as long as that associated with local recurrences. Papaioannou et al (1967) reported similar findings concerning both disease free interval and survival. A greater proportion of patients with bone secondaries had developed their first recurrence

more than five years following mastectomy than had patients with visceral metastases (35% for the former versus 23% for the latter). Average survival following detection of secondaries was 1.9 years for the bone secondaries compared to 1.0 years for the visceral metastases. Thus, the secondary site of involvement by metastatic breast cancer appears to bear a strong relationship to survival and a rather weaker relationship to recurrence free interval. Patients with skeletal secondaries fare better on both counts.

Factors which govern the distribution of distant metastases are unknown in human breast cancer but it seems unlikely that simple anatomical factors can be solely responsible for the observed patterns. Metastases from breast cancer are considered to be blood borne yet the observed distribution of clinical secondaries in breast cancer does not parallel the distribution of the cardiac output of blood. In one cardiac cycle, 100% of the cardiac output will traverse the lungs, 30% the liver, 20% the kidney, 15% muscle, 15% brain, 10% skin but only 10% will traverse the skeleton (Detweiler, 1973). As is well known, the skeleton is the most common secondary site of involvement by breast cancer (Cutler, Asire and Taylor, 1969).

In this study, intrinsic factors in the primary cancer have been related to the first distant site which has become involved by metastatic disease. In addition, the prognostic significance of metastases in different sites has been considered. Few of the 167 patients who have had progesterone receptor assayed have developed metastases and thus they have been excluded. Similarly, because the total number of patients with secondary metastases was rather small, oestrogen receptor status but not quantitative levels have been considered.

## The Present Study (Study 5)

### Patients

The first 550 women in the Nottingham-Tenovus series who have been followed for a minimum interval of two years, formed the basis of this study. In previous chapters, patient numbers have been confined to those with all parameters measured in their primary viz. stage, tumour size, histological grade and ER status, but in this study all patients with ER and all with grade have been included, irrespective of the other parameters. Thus, the numbers in each group were slightly different.

### Methods

Following mastectomy, all patients were followed up routinely and a clinical examination was performed every three months until 18 months and every six months thereafter until five years and at annual intervals from then. Routine blood tests including full blood counts, liver function tests, serum calcium and alkaline phosphatase were performed six monthly and chest x-rays at annual intervals. Bone scans were performed annually on the first two hundred patients for five years, on three hundred patients for four years, two hundred for three years and one hundred for two years. They have been abandoned as a routine follow up investigation because of a poor yield, but were still performed at the time of mastectomy and for the investigation of bone pain.

Additional investigations were performed for clinical indications:

Skeletal surveys were carried out for an elevation of alkaline phosphatase, or a fall of haemoglobin or for appropriate symptoms or signs. X-rays were taken to elucidate the nature of any 'hot spot' on bone scan.

Isotope or ultrasound liver scans were performed for symptomatic or clinical reasons or when gamma glutamyl transferase or alkaline phosphatase of liver derivation was elevated.

Isotope brain or cat scans were performed as clinically indicated.

No patient in this study received any adjuvant therapy prior to recurrence. When a major recurrence was detected at any site, then patients were referred to the advanced breast cancer clinic for assessment. All patients then received a full history, physical examination, full blood count, liver function tests, urinary calcium test, chest radiograph and skeletal survey, so that the exact distribution of metastases could be recorded. The site, number, time of onset of metastases were carefully recorded in both case notes and master index. Data concerning ER status and grade was documented only in the master index and not in case notes, so that this information was not available when the initial site of recurrence was recorded.

In this study, ER status and histological grade were related to the initial site of distant metastases which were considered in three categories:

- a) Bone - Initial distant recurrence in skeleton
- b) Viscera - Initial distant recurrence in lung, liver, brain or intra-abdominal organs
- c) Combined - When patients had initial distant recurrence at both (a) and (b) simultaneously or within a one month interval of first detection.

The prognostic significance of specific sites of metastases has been investigated. All patients received endocrine therapy on



detection of recurrence. In order to exclude the potential bias of this factor, survival rates according to sites of metastases have been compared (a) in all patients, (b) in treatment failures.

#### Statistical Analysis

Differences in the incidence of recurrence rates at different sites have been evaluated statistically by chi-square tests. Survival times were compared by life table analysis and statistical significance evaluated by the method of Mantel (1966).

## Results

### 1. Incidence of initial distant metastases at specific sites

#### Oestrogen receptor status

Oestrogen receptor status of a primary breast cancer was unrelated to the total incidence of distant metastases but showed a significant relationship to the initial anatomical site of involvement: oestrogen receptor positive tumours tended to metastasise to skeleton, while receptor negative tumours showed affinity for viscera (Table 7 : I).

#### Histological grade

The histological grade of a primary breast cancer showed a relationship to metastatic patterns: Grade III cancers had a higher total incidence of metastases and a disproportionate number recurred in viscera (Table 7 : II). The incidence of skeletal secondaries was unrelated to histological grade (Table 7 : II).

### 2. Prognostic significance of anatomical site of involvement

The anatomical site of the initial distant metastasis was related to survival, both from the time when metastases were discovered (Fig. 7 : 1) and from the time of mastectomy (Fig. 7 : 2). Median survival from the time of presentation with recurrence was 12 months for patients with bone secondaries and 4.5 months for patients with visceral metastases ( $p < 0.001$ ). This survival advantage for bone secondaries over visceral metastases persisted even when patients who have failed on endocrine therapy were considered (Fig. 7 : 3).

Table 7 : IOestrogen receptor status and initial distant metastasis

		ER Positive	ER Negative	p
Total distant metastases		69	55	$\chi^2 = 0.15; 1 \text{ df}$ NS
Site of metastasis	Bone	42	13	$\chi^2 = 21.8; 2 \text{ df}$
	Viscera	17	36	
	Combined	10	6	$p < 0.0005$

Table 7 : IIHistological grade and sites of distant metastases:Analyses at individual sites

<u>Site of initial distant metastases</u>	<u>I</u>	<u>Histological grade II</u>	<u>III</u>	<u><math>\chi^2</math></u>
Bone	7	21	26	0.26; p = 0.9 NS
Viscera	2	10	42	23.9; 2 df; p<0.00001
Combined	1	7	8	1.1; 2 df; p = 0.6 NS
Total recurrence	10	38	76	15.5; 2 df; p<0.0005
No recurrence				

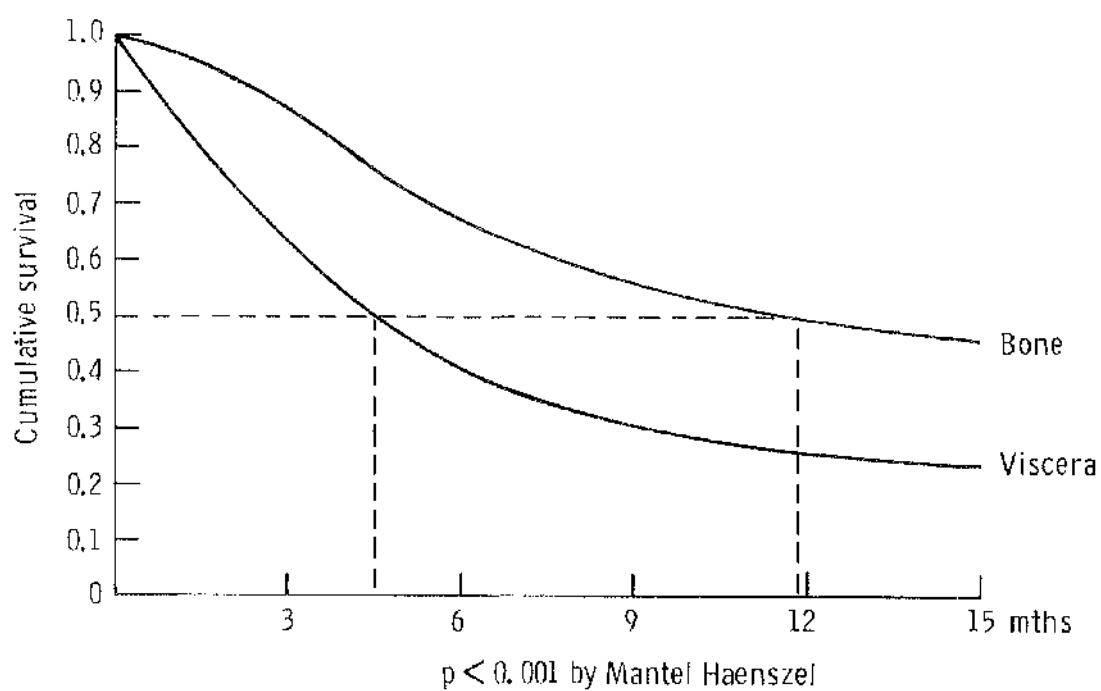


Fig. 7 : 1      Survival from onset of distant metastases  
Bone versus viscera  
Interrupted lines denote median survival interval  
for each group.

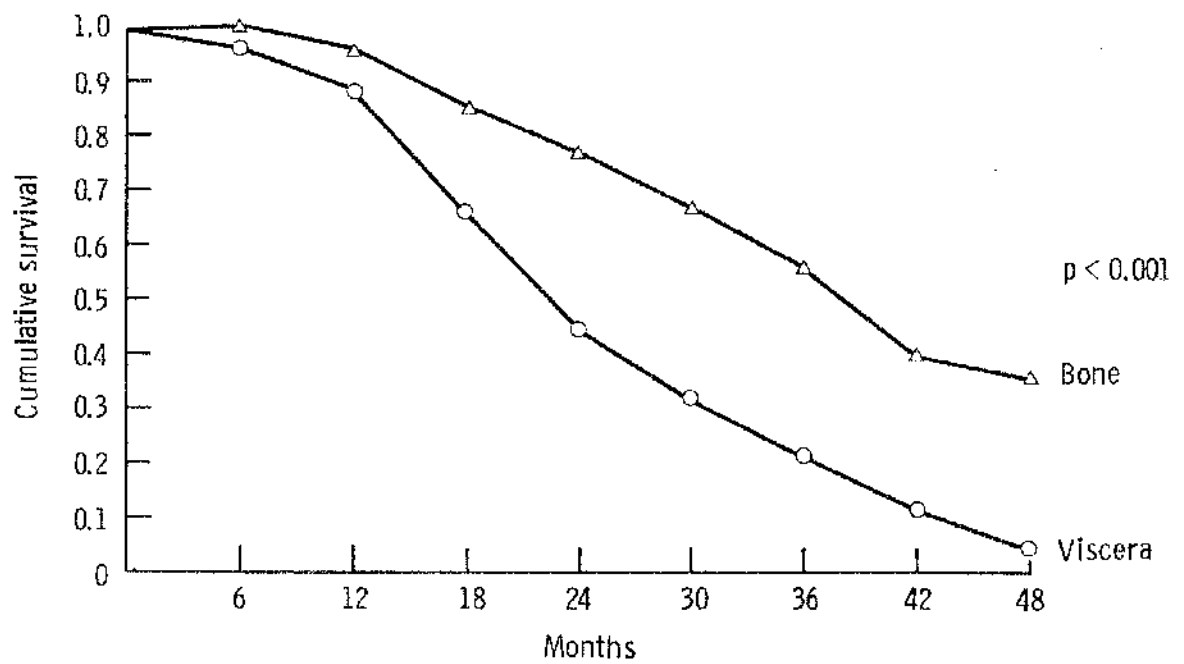


Fig. 7 : 2      Survival from mastectomy  
Bone versus visceral metastases  
p < 0.001 by method of Mantel.

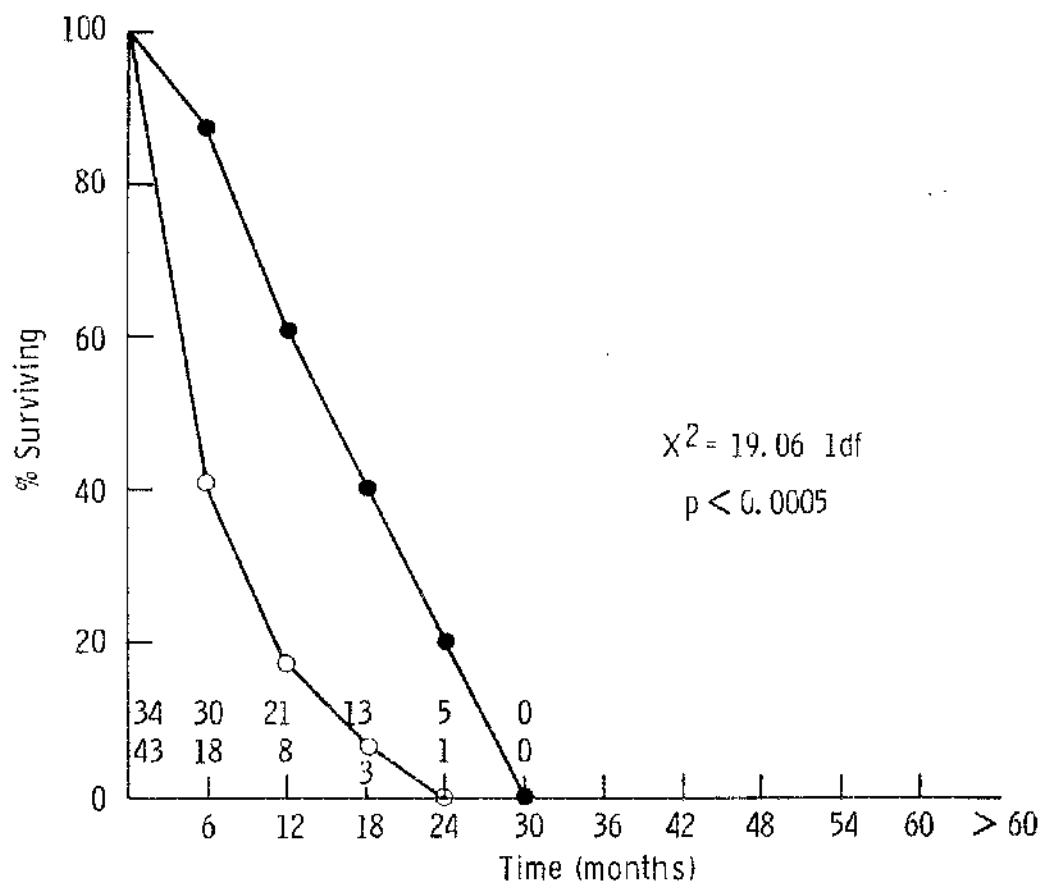


Fig. 7 : 3 Survival from onset of metastases in treatment failures.

Bone secondaries (● closed circles) versus visceral metastases (○ open circles).

## Discussion

The prognostic significance of specific anatomical sites of involvement is clearly shown by this study. Patients with initial bone secondaries may expect a longer survival than those with visceral recurrence. This survival difference exists not only from the time of clinical detection of secondary disease, but also when calculated from the time of mastectomy and it appears likely that it is the biologically more favourable cancer which develops bone secondaries. Support for this view comes from previous observations of a favourable relationship of skeletal metastases, to disease free interval and survival (Shimkin et al, 1954; Papaianou et al, 1967). In addition bone secondaries are known to be more susceptible to endocrine manipulations than visceral metastases. In a study of 381 premenopausal women with advanced breast cancer, Taylor (1962) reported an objective response in 38.7% of women with skeletal secondaries, but only 11% of those with visceral metastases, to oophorectomy.

Oestrogen receptor status of breast cancer has also been related to these same clinical variables viz. disease free interval (only in terms of regional recurrence in this study), response to endocrine therapy (Campbell et al, 1981) and to survival (Blamey et al, 1980). The findings of this study therefore, of a relationship between oestrogen receptor positive breast cancers and metastases in skeleton should cause no surprise. This observation is supported by that of Walt et al (1976) and Stewart et al (1981) who noted a high incidence of bone secondaries associated with ER positive metastatic tumour. It must be emphasised, however, that Hahnel et al (1979) found that sites of secondary metastases were unrelated to ER status.



Oestrogen receptor negative and poorly differentiated (Grade III) breast cancers show affinity for viscera, ie. every distant site other than the skeleton, which may become involved by metastatic breast cancer. As noted previously, a greater proportion of the cardiac output of the blood is distributed through viscera than through the skeleton and it is conceivable that the virulent cancer cells of these tumours, having been distributed through the bloodstream may grow well at whichever site that they happened to come to rest. The findings of this study emphasise that receptor positive cancer cells favour the bony skeleton. This preferential tendency may be mediated by steroid hormones acting via the receptors in the ER positive cancer cells. Conceivably, the hormone-cell interaction could, by some pathway, alter the environment in bone (metabolic or otherwise) to favour the growth of cancer cells. The exact mechanisms which are responsible have not been fully elucidated, but this question will be considered in more detail in the next chapter.

## Chapter 8

### BIOLOGICAL MECHANISMS OF METASTASIS - PROSTAGLANDIN E2 BIOSYNTHESIS

## Biological Mechanisms of Metastasis -

### Prostaglandin E<sub>2</sub> Biosynthesis

#### Introduction

The last chapter emphasised the significant tendency of oestrogen receptor positive primary breast cancers to metastasise to bone.

Prostaglandins of the 'E' series are potent agents which cause bone resorption in vitro (Tashjian et al, 1972) and they may be implicated in the development of bone secondaries from human breast cancer (Bennett et al, 1975, 1977 : Chapter 2). Prostaglandins are produced in greater quantities by breast cancers than by normal breast tissue (Bennett et al, 1975) but these agents can be synthesised by every mammalian cell and there is little direct evidence that it is the malignant cells of a neoplasm which comprise the main source of their production.

Prostaglandin production from primary breast cancer has been measured in Nottingham for two years, and to date we have complete data on 75 primary breast cancers. The present study will relate prostaglandin production firstly to the tumour cell fraction within the primary neoplasm and secondly to the oestrogen receptor status of these tumours cells.

## Materials and methods

In 75 primary breast cancers, adjacent tumour sections were taken immediately after surgery for oestrogen receptor analysis, prostaglandin  $E_2$  radioimmunoassay and for cellularity assessment.

### Oestrogen receptor analysis

Tumour samples were snap frozen and stored at  $-200^{\circ}\text{C}$  in liquid nitrogen before being transported on dry ice to the Tenovus Institute where the assay was performed, by the Dextran Coated Charcoal method (see Appendix). Tumours were considered to be ER positive when they contain more than 5 femtomoles specific oestradiol binding per milligram cytosol protein.

### Prostaglandin $E_2$ radioimmunoassay

Tumour samples taken at mastectomy were similarly frozen and stored at  $-200^{\circ}\text{C}$  in liquid nitrogen. Assays were performed in the Department of Surgery, Nottingham, by the author or under my supervision. The procedure is simple and is described in detail in the Appendix. Preparation of tissue (ie. cutting and dissecting specimens) liberates prostaglandins from damaged cells, and this is a recognised source of error. In order to minimise this error, three measured values of prostaglandin  $E_2$  were obtained for each tumour, viz.

1) Basal  $\text{PgE}_2$  This was obtained after incubation of tumour blocks with Ethanol, which totally inactivates prostaglandin production. 'Basal'  $\text{PgE}_2$  is a measure of extracellular prostaglandin as well as that liberated by tissue preparation, but does not represent  $\text{PgE}_2$  synthesis.

2) Total  $\text{PgE}_2$  This value was obtained after incubation of tumour blocks with Arachidonic acid (the prostaglandin E precursor) which promotes  $\text{PgE}_2$  synthesis. 'Total' values therefore consist of 'Basal'  $\text{PgE}_2$  plus any which is synthesised.

Both of the above measurements are subject to the errors of tissue preparation.

3) Synthesised  $\text{PgE}_2$  This value was calculated by deducting the 'Basal' from the 'Total' value for each tumour and the resulting  $\text{PgE}_2$  level was taken as the measure of tumour  $\text{PgE}_2$  synthesis under test conditions (Bennett et al, 1977). Furthermore when we deduct 'Basal' from 'Total'  $\text{PgE}_2$ , we subtract the inherent errors due to tissue preparation.

#### Cellularity assessment

Tumour epithelial cellularity was assessed by an objective histomorphic technique similar to that of Underwood (1972). Briefly, a proportional count of cancer cells was carried out using an eyepiece graticule, in all fields of 3-5 histological sections from each tumour and expressed as a percentage against non malignant material. The method is described in greater detail in the Appendix.

#### Statistical analyses

Distribution of all  $\text{PgE}_2$  values was extremely skewed. Thus, Wilcoxon Rank Sum tests were used to calculate the significance of frequency variation and Kendall's Rank test was used to calculate the significance of correlation.

## Results

Forty seven primary cancers were oestrogen receptor positive and the remainder ER negative. Tumour epithelial cellularity varied from 13 - 85% with a mean - se of 43 - 2.7% (Fig. 8 : 1). Cellularity was unrelated to ER status (Fig. 8 : 2).

No differences in 'Basal' or 'Total'  $\text{PgE}_2$  values were seen between ER positive and negative cancers. Synthesised  $\text{PgE}_2$  levels were higher in ER positive cancers, although differences were not significant (Fig. 8 : 3). A significant relationship between synthesised  $\text{PgE}_2$  values and tumour epithelial cellularity was seen (Fig. 8 : 4). This relationship was particularly strong in the receptor positive group of tumours (Fig. 8 : 5). The greater the cellularity, the greater the  $\text{PgE}_2$  production. Therefore, measured  $\text{PgE}_2$  values were related to the tumour cell fraction of every cancer by the formula:

$$\text{Tumour Cell } \text{PgE}_2 = \frac{\text{Measured } \text{PgE}_2 \times 100}{\text{Actual Percentage Tumour Cellularity}}$$

Thus an even 'correction' of  $\text{PgE}_2$  values for cellularity variation was applied across the whole range. When this was done, significantly higher synthesised  $\text{PgE}_2$  values were found in the tumour cell fraction of ER positive tumours (Fig. 8 : 6).

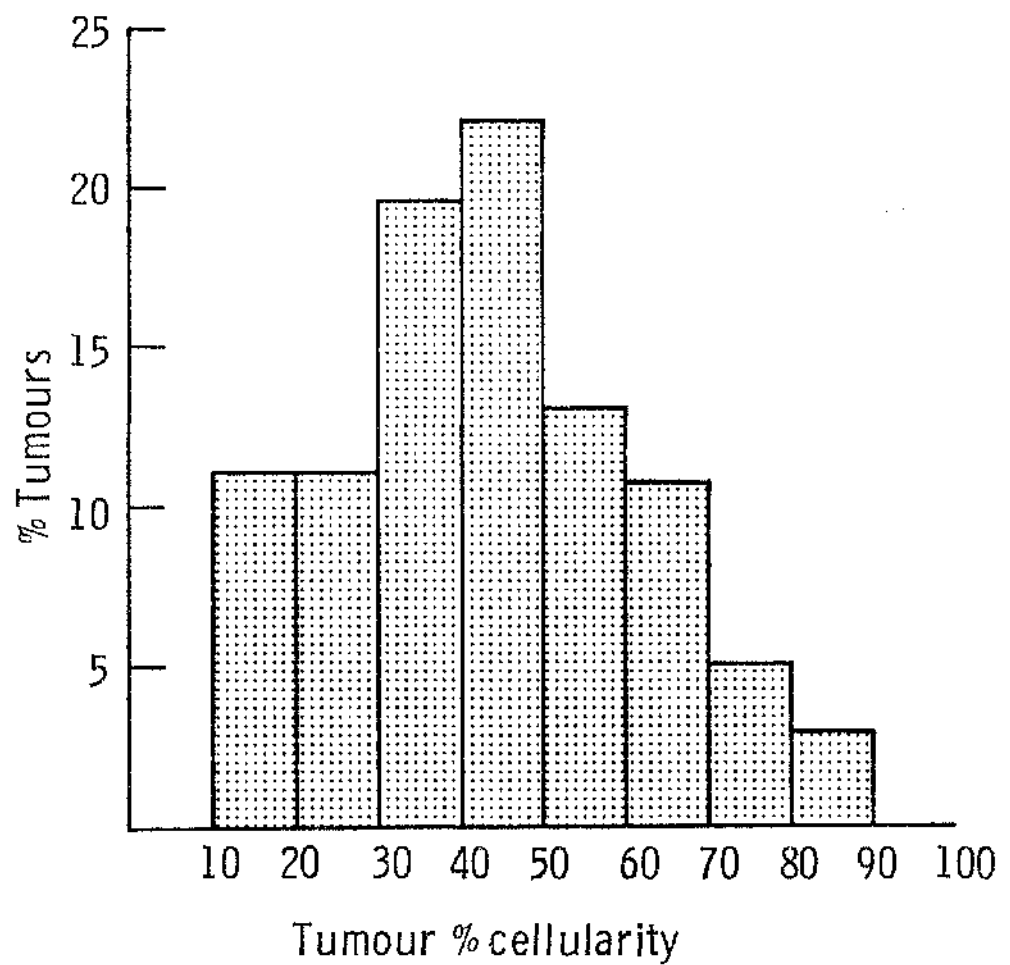


Fig. 8 : 1      Range of tumour epithelial cellularity:  
All tumours.

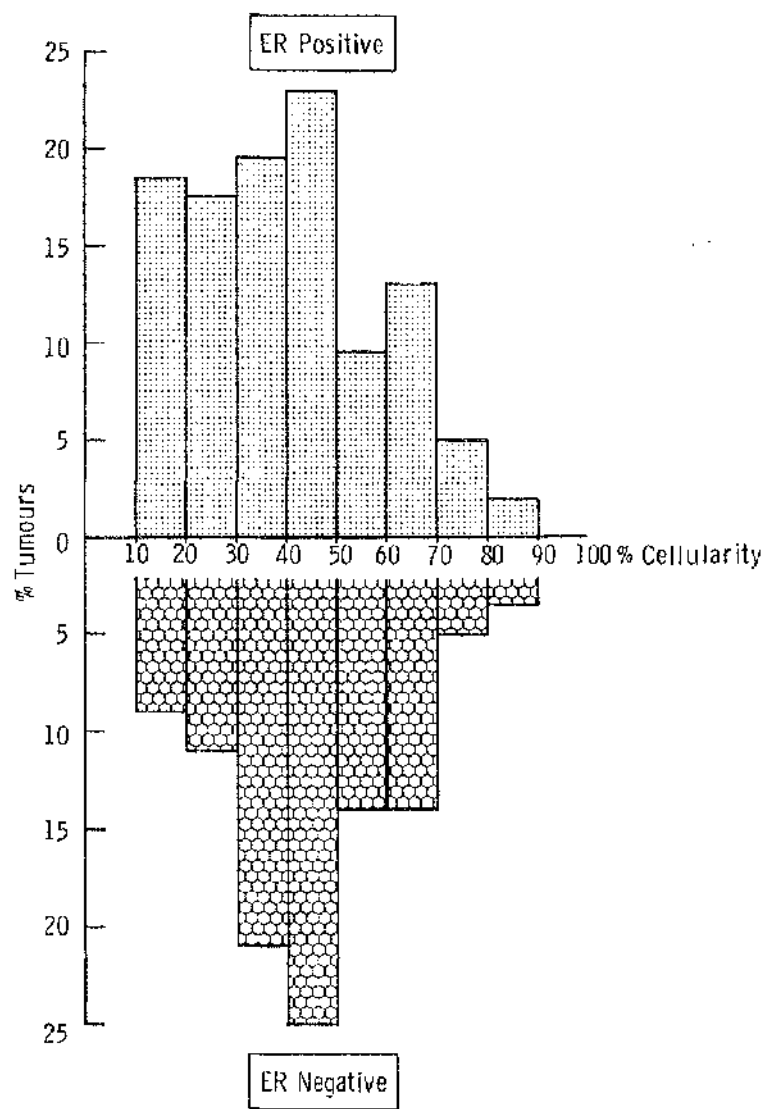


Fig. 8 : 2      Distribution of cellularity values in ER positive and negative cancers.



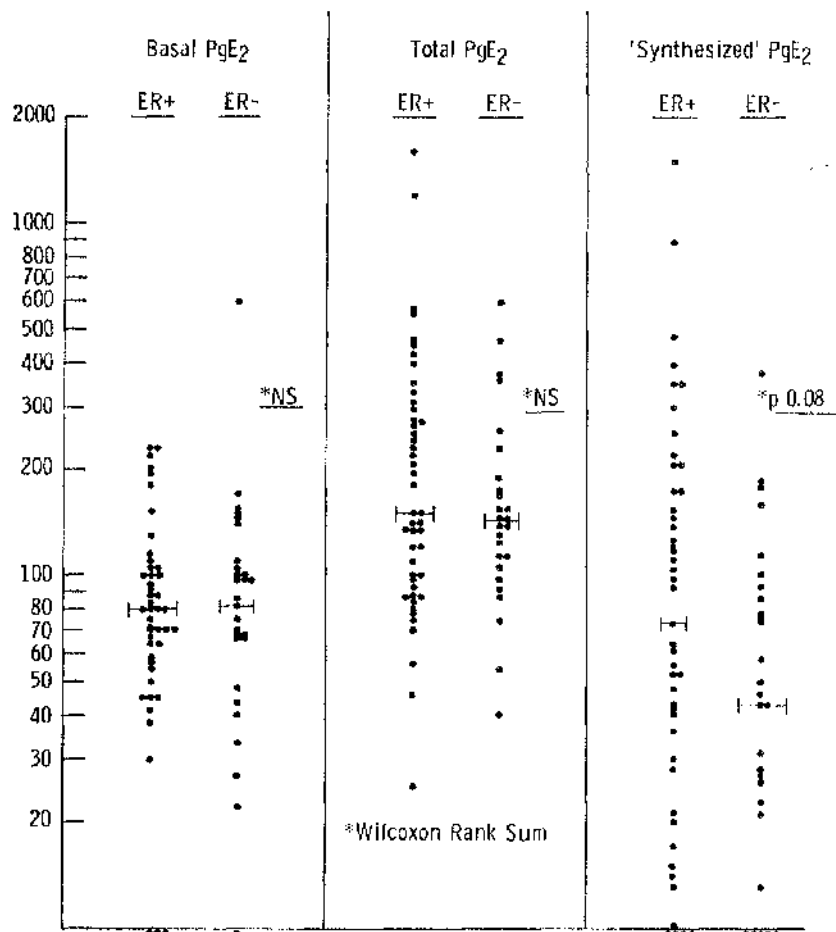


Fig. 8 : 3 Prostaglandin E<sub>2</sub> values (ng/mg wet tumour weight).  
ER positive versus ER negative cancers.  
Uncorrected for cellularity.

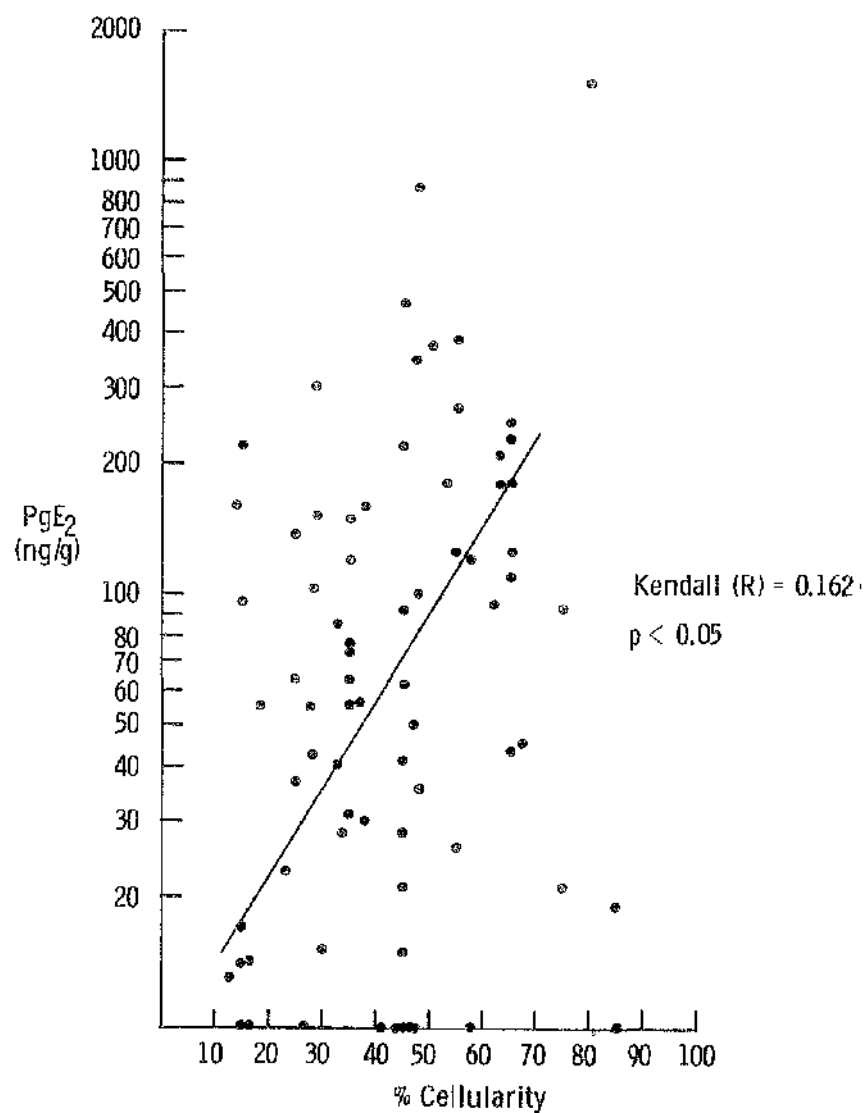


Fig. 8 : 4 Prostaglandin E<sub>2</sub> synthesis and tumour epithelial cellularity. All tumours (n = 75).

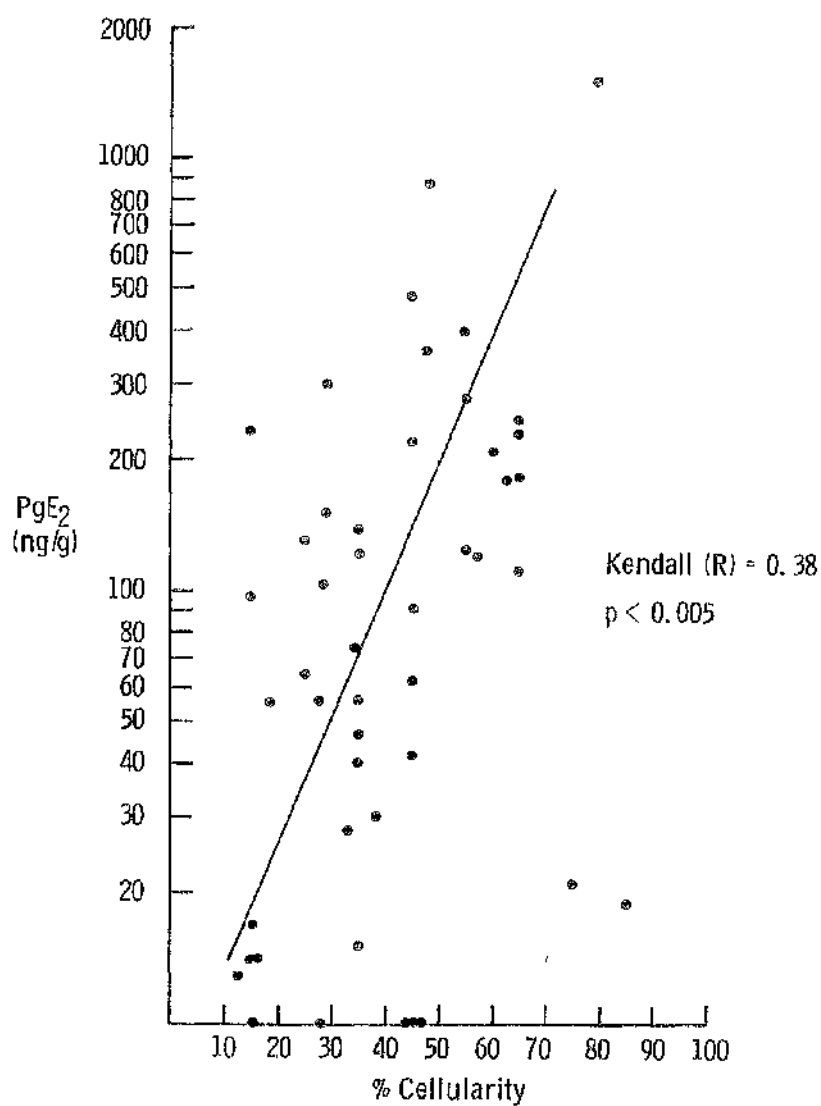


Fig. 8 : 5 Prostaglandin  $E_2$  synthesis and tumour epithelial cellularity. ER positive tumours (n = 47).

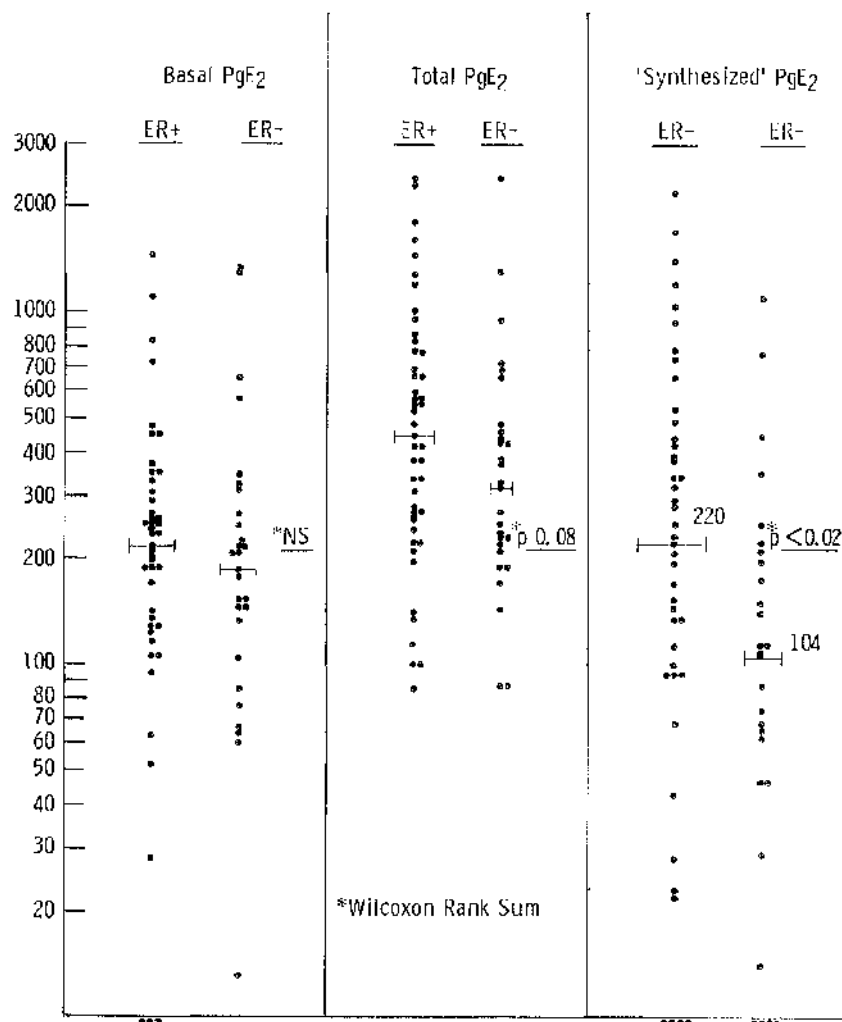


Fig. 8 : 6 Prostaglandin E<sub>2</sub> production by tumour cell fraction:  
ER positive versus negative cancers.  
Note 'Synthesized' values most representative of  
PgE<sub>2</sub> production.

## Discussion

This study had two main findings. Firstly, the magnitude of  $\text{PgE}_2$  synthesis was directly related to the proportion of tumour cells within a primary breast cancer. A weak relationship of  $\text{PgE}_2$  production to tumour cellularity was observed when all tumours were considered, but a much stronger association existed within the receptor positive group. These observations support the view that malignant cells and not stromal cells or other components comprise the main source of prostaglandin production within a neoplasm (Bennett et al, 1975, 1977). Thus it seems logical to relate measured  $\text{PgE}_2$  values to the tumour cell fraction of a primary breast cancer in other words, to express  $\text{PgE}_2$  values as units of production per tumour cell. When this was done, by the simple method of correction described, the second finding of this study emerged: that ER positive tumour cells synthesised higher  $\text{PgE}_2$  values than receptor negative cells.

The breast is an oestrogen target organ. It is well known that prostaglandin production by other oestrogen target tissues, is hormone dependent. Downie et al (1974) has shown that a two fold increase in prostaglandin secretion by human endometrium occurred with a four fold increase in oestrogen levels during the proliferative phase of the menstrual cycle. Demers et al (1974) demonstrated in experimental animals that oophorectomy caused a marked fall in prostaglandin concentration in uterine fluid. This hormone dependence of prostaglandin synthesis is retained even in malignant tumours of oestrogen target organs. Singh et al (1975) demonstrated a sharp rise in prostaglandin  $\text{E}_2$  production by human

endometrial adenocarcinomas which accompanied an elevation of circulating oestradiol and progesterone at different phases of the menstrual cycle. In breast cancer, Rolland et al (1980) failed to find any significant relationship of  $\text{PgE}_2$  synthesis and ER status but in this study prostaglandin values were expressed relative to units wet weight of tumour, and not corrected for cellularity. This same author, however, later demonstrated that natural oestrogenic hormones (particularly oestradiol) were the most potent stimulators of prostaglandin synthetase in tissue preparations of 261 human breast cancers and that Tamoxifen was a potent inhibitor (Rolland and Martin, 1981). Thus there is good evidence that prostaglandin production by oestrogen target tissues, and by tumours of these tissues, is hormone dependent. The findings of this study, therefore, of higher prostaglandin  $\text{E}_2$  synthesis by breast cancer cells which are likely to be hormone dependent (ER positive) accords with this experience.

As shown in the last chapter, ER positive primary breast cancers preferentially metastasise to bone and the present study demonstrates that ER positive cancer cells synthesise higher amounts of prostaglandin  $\text{E}_2$ . From the data in this study, it would be possible to postulate cause and effect between ER status, high prostaglandins and bone secondaries. Bone metastases are haematogeneous in origin and develop almost exclusively from within the sinusoids of red bone marrow. Once established, tumour cells spread into the marrow spaces and the canal system of compact bone, surrounding the trabeculae and subsequently invading the cortex (Willis, 1973). From the findings of the present study, I postulate

the hypothesis that oestrogen receptor positive cells, following haematogenous dissemination, find a hospitable environment in bone because of oestrogen mediated (via the receptor) prostaglandin production. The increased local concentrations of prostaglandin  $E_2$  would effect osteolysis and thus remove physical barriers to tumour cell replication and facilitate the appearance of clinical metastases.

It would of course be tempting to draw immediate therapeutic implications from the findings of this study, but such a course of action would be premature. This study has simply demonstrated an association between ER positive breast cancer and bone secondaries on one hand and ER positive breast cancer cells and high prostaglandin production on the other. This relationship may indeed be causal. However, it is worth remembering that ER positive breast cancers do have a better natural history than ER negative tumours. Before interfering therefore, it is necessary to ascertain whether high prostaglandin biosynthesis has any independent prognostic significance within the oestrogen receptor positive group.

## Chapter 9

### RESPONSE OF ADVANCED BREAST CANCER TO SYSTEMIC THERAPY



## Response of Advanced Breast Cancer to Systemic Therapy

### a) Assessment of Response

#### Introduction

Seventy five to eighty percent of patients with breast cancer eventually die of metastases, despite good local control (Brinkley and Haybittle, 1975; Mueller and Jeffries, 1975). Certain aspects of metastatic disease, such as its rapidity of onset following mastectomy (disease free interval) and the anatomical sites of involvement carry considerable prognostic significance and have been considered in previous chapters. A further important aspect is the susceptibility of metastases to systemic therapy, ie. a clinical response to treatment. This concept can be defined as some measurable reduction in the patient's tumour burden which results from treatment.

In some rare tumours, serum biochemical measurements give an indication of the total tumour bulk. For example, the magnitude of myeloma proteins and human chorionic gonadotrophin correlate well with the bulk of myeloma and choriocarcinoma respectively and low levels of these parameters after treatment can be taken to indicate a state of remission (Sullivan et al, 1972; Bagshawe and Searle, 1977). In metastatic breast cancer, no such reliable markers exists and we must rely upon direct measurement of visible or palpable deposits, radiographic shadows or occasionally of isotope scan abnormalities in order to quantify a response to therapy.

In recent decades, the practice of cancer medicine has reached progressively higher levels of scientific sophistication. Clinical investigation of cancer therapy has become more controlled and

objective, with meticulous treatment methodology and clear study objectives. The technology of experimental cancer therapy can isolate pharmacokinetic and physiological effects of a new treatment modality in considerable detail. However, the area of clinical assessment of response to anticancer therapy has been ignored until recent years. Until the middle of the nineteen seventies, there were no standard criteria by which a response to therapy could be assessed in patients with metastatic breast cancer. Well controlled clinical trials carried out previously are now valueless since the effects of their treatment methods were assessed by rule of thumb. The advantages of a uniform and stringent set of criteria for evaluation of response are obvious: results from different centres may be compared and the management of individual patients would be improved and set upon a more scientific basis. This is particularly true in patients treated by endocrine methods when the choice of an optimum second line therapy may rest with a prior response to the first.

A major advance in this area came with the formulation of a set of criteria, firstly by the British Breast Group (1974) and secondly by the UICC in 1976 (Hayward et al, 1977). In addition, the American Breast Cancer task force, formulated a rather similar set of criteria, but differences of definitions exist (Breast Cancer Task Force Committee, National Cancer Institute, 1977). The UICC method is most widely accepted and will be discussed briefly.

Definitions of response by UICC criteria (Hayward et al, 1977).

All lesions should be measured at each assessment, but it is recognised that this may not be possible when multiple lesions are

present. In such circumstances a representative number of 8 or more may be selected for measurement. Two categories of objective response are recognised.

1) Complete response: Disappearance of all known disease. Lytic bone metastases must have been shown radiologically to have calcified.

2) Partial response: A greater than or equal to 50% decrease in the sum of the products of the diameters of measurable bidimensional lesions or a similar percentage decrease in one diameter of unidimensional lesions such as liver involvement or mediastinal enlargement. Objective improvement must be shown to have occurred in evaluable but non measurable lesions (eg. osseous metastases, pulmonary infiltration, pleural effusion or skin infiltration), although no guidance is given in this context to the meaning of 'objective improvement'.

The report recommends that patients in whom one of the following is the sole manifestation of disease, should be excluded viz. lymphoedema, hilar enlargement, pleural effusion, ascites, metastases in the central nervous system, marrow suppression or osteoblastic skeletal lesions. It was recommended that a clinical impression of response should be confirmed by two observations, four weeks apart. The final recommendation in the report was that all patients under study should be assessed by extramural reviewers. These criteria represent a significant advance in treatment methodology. Nonetheless, many weaknesses exist and a brief review of potential error sources may be worthwhile.

### Errors in assessment by UICC criteria

Errors may arise in a variety of clinical situations, eg.

#### 1) Direct measurement of a palpable breast tumour

Serial measurement of a palpable tumour mass with calipers or a ruler is arguably the easiest assessment to make and ought to be foolproof.

There is little uncertainty about a complete response, but before contemplating the significance of a partial response we must firstly consider the composition of a breast cancer. The palpable mass is comprised of solid and fluid compartments. The former comprises both normal cells, eg. macrophages, endothelial cells, fibroblasts and inflammatory cells, which may be present in considerable numbers in a necrotic neoplasm, as well as malignant cells. The fluid component comprises intravascular and oedema fluid which may be considerable in an inflammatory neoplasm. Thus a reduction in tumour 'size' may be due to a decrease in any one or any combination of these constituents. Theoretically, one could argue that a short term reduction of tumour size by chemotherapeutic agents could be due to their cytotoxic action upon inflammatory cells and conversely that the actions of endocrine therapies upon a tumour cell fraction could be masked by their side effect of fluid retention.

Even with a manoeuvre as simple as that of measuring two diameters of a neoplasm, observer error is an important factor. Moertel and Handley (1976) designed an experiment to evaluate observer error in the serial measurement of superficial lumps. Twelve solid spheres measuring from 1.8 to 14.5 cms were placed upon

a soft mattress and covered with foam rubber. Sixteen experienced oncologists each measured the 12 simulated tumour masses by their usual clinical methods. Unknown to the oncologists two pairs of 'tumours' were identical in size. When a 50% reduction in the product of the perpendicular diameters was used as a criterion, the objective 'response' rate due to observer error alone was 7.8% by the same investigator and 6.8% by different investigators. Because of the ideal conditions the authors felt that the results could be viewed as a conservative estimate of measurement variations in real life conditions, where tumours are non spherical, of varying texture and where the bearer of the tumour is not often as immobile and compliant as a mattress.

## 2) Radiographic measurements

a) Lung metastases: It is recognised that evaluation of a 50% reduction of lymphangitis is difficult, and indeed this point is conceded in the UICC criteria, but no specific recommendations are made. Nodular lung metastases should be measured in two directions whenever possible. The physical size of an x-ray shadow is dependent on several factors, including the distance between the lesion, the photographic plate and the x-ray source, the degree of penetration and rotational differences. Patients with solitary circular lung metastases from breast cancer are rare and frequently we have to take several representative diameters to make an average. When magnification, orientation and penetration factors are taken into account, it is optimistic to hope that consistent measurements can be obtained even in the most favourable circumstances. It is noteworthy that each perpendicular diameter need decrease only by

30% to achieve a 50% reduction of their product (Moertel and Hanley, 1976). To take an example, a deposit measuring 5 mm x 5 mm need only decrease in size to 4 mm x 3 mm to be considered a response. In this area, observed error is also likely to be an important factor.

When a lesion encroaches upon the mediastinum, a reduction of 50% in one diameter is acceptable as indicative of a response by UICC criteria, but in these circumstances we have no means of ascertaining where the lesion begins and ends and indeed the visible abnormality may merely represent the tip of the iceberg. Similar difficulty arises when the shadow of the lesion being assessed encroaches upon an area of atelectasis or consolidation.

b) Skeletal secondaries: Assessment of response of skeletal secondaries from breast cancer is arguably the most difficult exercise of all. Osteoblastic metastases are extremely difficult to assess and indeed one part of the UICC report recommends that patients with this type of secondary as the sole manifestation of disease be excluded from assessment and another part includes "osseous metastases" as evaluable but not measurable disease (Hayward et al, 1977).

Lytic skeletal secondaries must be shown radiologically to have calcified. A complete response is easily assessed but unfortunately is relatively rare. A partial response would be assessed by the presence of some calcification of some lytic lesions with a reduction of their diameter. Breast cancer is characterised by three types of bony secondaries, osteoblastic, osteolytic and mixed. At a given point in time, lytic metastases with partial recalcification cannot be reliably distinguished from mixed skeletal secondaries in which the lytic component appeared first, to be

followed by the osteoblastic component. The radiological appearances of both categories are similar.

Assessment of lesions in vertebrae may be difficult. Gas shadows in bowel which appear superimposed upon lateral films of the vertebral column and may mimic lytic metastases. The significance of increasing density in a collapsed vertebra due to metastatic involvement is difficult if not impossible to evaluate.

### 3) Other

The UICC criteria permit the use of serial isotope scans in the assessment of a change of dimensions of a metastasis, both in skeleton and in liver. This is fraught with difficulty. In the former situation we cannot be certain, unless radiological evidence is also available that any 'hot spot' is a metastatic deposit. Furthermore, a metastasis which heals may either become 'cold' or remain 'hot'. In the liver, we cannot ascertain the margin by which the tumour deposit extends beyond the 'edge' of the image on our screen.

The UICC criteria were designed to facilitate objective evaluation of response to therapy, but in the common clinical situations listed above, they consist of no more than a series of subjective assessments.

Given the difficulties of assessment outlined above, the variations of reported response rates in different centres with similar treatment schedules can come as no surprise. The response rate to Tamoxifen has variously been reported between 22% and 49% in patients with advanced breast cancer (Henderson and Canellos, 1980) and similar difficulties have been encountered with chemotherapy.

In 1969, Cooper claimed an 88% complete response rate with CMF-VP (Cyclophosphamide, Methotrexate, 5-Fluoruracil, Vincristine and Prednisone) in an intensive weekly induction course followed by a less intensive maintenance schedule. Other authorities who have attempted to repeat the work have found a variable response rate between 20 to 70% with an average total response rate of 47% and a complete response rate of 20% (Carter, 1976).

One might anticipate that experienced clinical oncologists would take little notice of reported variations of response rates, but such is not the case. When the anti oestrogen drug Tamoxifen was first introduced, the dosage recommended was 10 mg b.d. However, following a publication by Ward (1973) of a higher incidence of "reversal or arrest of tumour growth" with 20 mg b.d., clinical practice has changed. It is interesting to reconsider Ward's data: 77% of 33 patients showed "reversal or arrest of tumour growth" with the higher dosage compared to 60% for the lower dosage. When a 50% reduction of tumour size was used as a criterion, the response rates were 40% for the higher and 36% for the lower dose regimes.

There are certain areas where the UICC criteria could be tightened. Of the many reports which claim to follow the UICC guidelines very few include the use of external assessors (eg. Roberts et al, 1978) and widespread acceptance of this recommendation could improve accuracy of assessment. A further dimension which could also improve the accuracy of response determination is that of time. The natural course for a breast cancer is to grow and when observations are carried out at a



specific interval after initiation of therapy, a 50% reduction in the product of the perpendicular diameters of the initial size actually represents a greater reduction of the size which the untreated tumour would have reached by the end of that specific interval. Thus a lengthy time interval of observation provides some natural assistance to the accuracy of response determination and serves as a counterbalance to the difficulties of measurement presented by the clinical circumstances previously outlined.

How long should the minimum duration of regression last before being considered a response? The UICC criteria recommend an interval of only four weeks. The median doubling times for breast cancers have been variously reported from 83 to 120 days (Spratt, 1977; Gershon-Cohen et al, 1963). Let us assume an average of 90 days (or three months) and for the sake of the hypothesis, ideal circumstances. If we had a tumour deposit measuring 10 mm x 10 mm, then its volume is  $523.5 \text{ mm}^3$ . At three months its volume would be  $1047 \text{ mm}^3$  and  $2094 \text{ mm}^3$  at six months. Thus at three months a 50% reduction of the product of the diameters to  $50 \text{ mm}^2$ , would represent a 68% reduction of the products of diameters of the size which the tumour would have reached by three months, and an 80% reduction of the size which the lesion would have reached by six months. The longer interval also enables the oncologist to distinguish clinical 'artefacts' from a true response. The original British Breast Group criteria (1974) include a recommendation that any tumour regression should last a minimum interval of six months before being considered a response, but few centres outside Nottingham have recognised this criterion.

In this study, the response rate to primary endocrine therapy in women with metastatic breast cancer who have been previously untreated by systemic means has been evaluated by UICC criteria with the additional proviso that all tumour regression must be maintained for a minimum interval of six months. External assessment has been carried out. Specific areas of difficulty have been investigated and the response rate and survival benefit of primary endocrine therapy has been evaluated.

## Patients and Methods

### Patients

Between 1973 and 1981, one hundred and seventy-seven of the first 620 women in the Nottingham-Tenovus series developed advanced breast cancer for which systemic therapy was considered necessary. These recurrences included histologically confirmed skin flap or lymph node metastases which were unresponsive to radiotherapy and distant secondaries confirmed by clinical examination, plain radiographs, liver function tests, brain or liver scans or biopsy. Chemotherapy was given as first line therapy to 20 patients and nineteen died without receiving systemic therapy. The remainder were treated by primary endocrine therapy. Premenopausal women were generally treated by surgical bilateral oophorectomy, whereas postmenopausal women were given the anti oestrogen drug Tamoxifen (Nolvadex, ICI) in a dose of 10 mg b.d. in the early years of the study and later increased to 20 mg b.d.

Seventeen patients, including five who had adjuvant chemotherapy, four who had co-existent primary tumours of another organ, two who had bilateral breast tumours of different ER status, two who were lost to follow up and four with non assessable disease, were excluded from the analysis. The four women in the last category included three who had had simultaneous Tamoxifen and radiotherapy to localised bony secondaries and one patient who had had a pulmonary lobectomy for a solitary lung secondary with subsequent Tamoxifen. Thus one hundred and twenty-one patients were assessable for response to primary endocrine therapy and these patients form the basis of the present study.

Patients with secondary breast cancer were assessed at monthly or two monthly intervals at the Advanced Breast Cancer clinic as outlined previously. Objective clinical measurements with relevant investigations and x-rays were performed at least of two and six months following commencement of endocrine therapy. All data concerning each patient was carefully documented in a standard form and entered into an advanced breast cancer file, before being transferred to the master index.

Response to therapy was assessed by UICC criteria (Hayward et al, 1977) but the British Breast Group recommendation (1974) of a mandatory regression interval of six months was observed.

All patients who failed to fulfill the strict criteria of response were considered to be treatment failures, irrespective of the length of time that they received endocrine therapy, eg. if a patient died only 24 hours after receiving this treatment she was considered to be a treatment failure. External review was carried out by Dr. A. Howell, Consultant Medical Oncologist, Christie Hospital, Manchester and Mr. J.M. Morrison, Consultant Surgeon, Selly Oak Hospital, Birmingham.

In this study, the predominant site of involvement by metastatic disease refers to that which was evident at the time of starting treatment. Survival rates have been calculated from the time of clinical appearance of secondary disease for all patients with distant metastases and from the time of commencing systemic therapy for patients with local or regional recurrence.

## Results

Twenty-two patients out of 121 responded to therapy by the criteria outlined, giving a total response rate of 18.2%. Responses were complete in 9 patients and partial in 13.

Of the 99 patients who failed to respond, disease was clearly static or progressive in 79. Twenty patients were initially considered to show tumour regression, but follow up to six months revealed 'clinical artefacts' which mimicked a response. In 10 patients, early assessment demonstrated significant resolution at one site with simultaneous but undetected progression at others (Table 9 : I). Condensation around lytic bone secondaries on x-ray, was initially misinterpreted as recalcification in six women but prolonged observation showed these to be progression of the osteoblastic component of mixed bone metastases (Fig. 9 : 1a,b,c). In four women, some regression of secondary disease occurred but did not meet the criteria viz. diminution was less than 50% in two, was not evaluable or measurable in one patient who had osteoblastic bone secondaries and regression of a pleural effusion, and in one patient marked tumour regression was not sustained for six months.

The predominant secondary sites of involvement in responders as opposed to treatment failures is shown in Table 9 : II.

Survival following the onset of metastatic disease was significantly better in responders than non responders (Fig. 9 : 2). Endocrine sensitive tumours recur in more favourable secondary sites and may be slower growing. Thus it may be misleading to attribute a survival advantage of an endocrine response to a therapeutic benefit. For this reason survival of responders against failures

Table 9 : I

Mixed progressive disease

	n
Regression of bone secondaries/progression visceral lesions	4
Regression some bone secondaries/progression others	3
Regression of lung secondaries/progression bone lesions	3



Fig. 9 : 1 False 'response' in bone metastasis.

- a) At presentation: large lytic defect involving whole of body of L5.

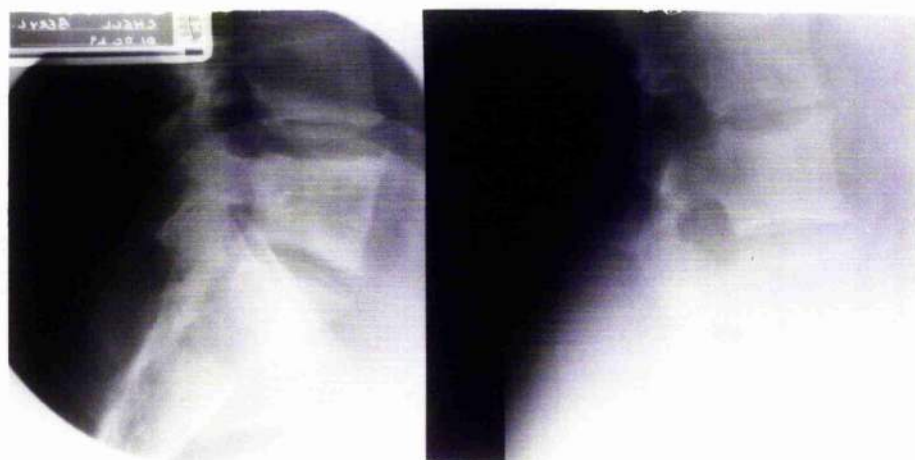


Fig. 9 : 1 False 'response' in bone metastasis.

- b) Two months after start of treatment. Increased density L5 with decreased size of lytic defect. An apparent response.



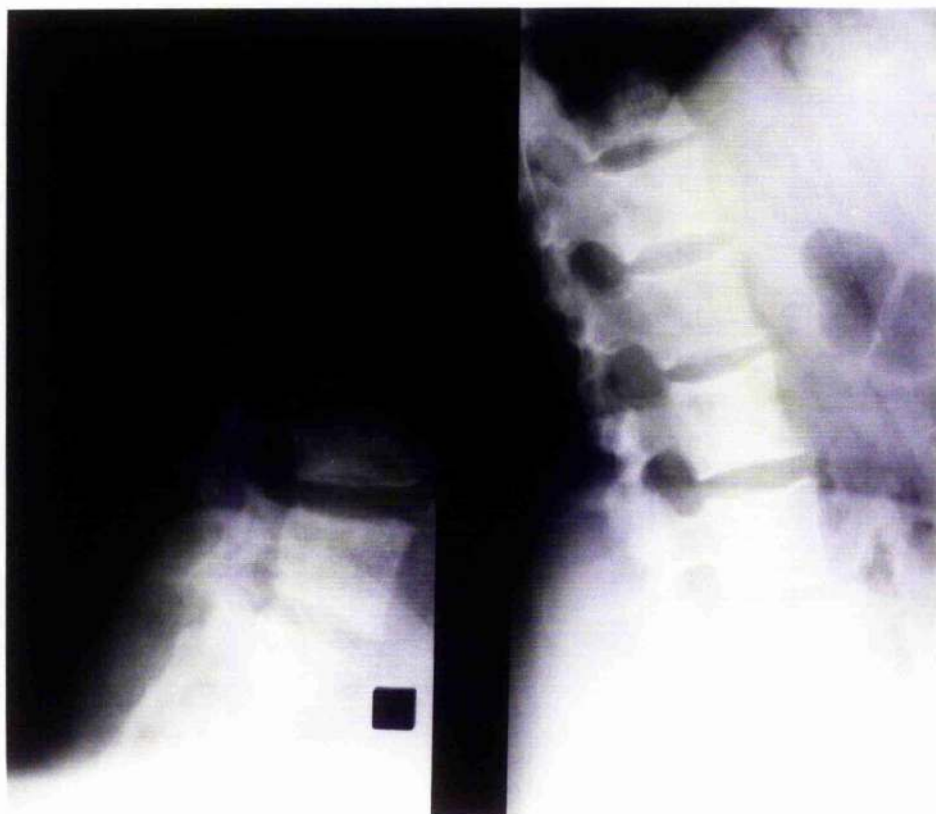


Fig. 9 : 1 False 'response' in bone metastases.

c) Six months after start of treatment. Increased density L5 persists but it is now clear that this has been due to progression of the osteoblastic component of mixed bone secondaries and not due to response to treatment. Note the appearance of new lytic and osteoblastic metastases in all of the lumbar vertebrae.

Table 9 : IIPredominant sites of involvement

## Endocrine responders versus failures

	Responders	Failures
Local/regional	6	12 (42)
Bone	11	34
Combined bone and other distant organs	3	11
Lung	2	24
Liver	0	12
Brain	0	4
Mixed viscera	0	3

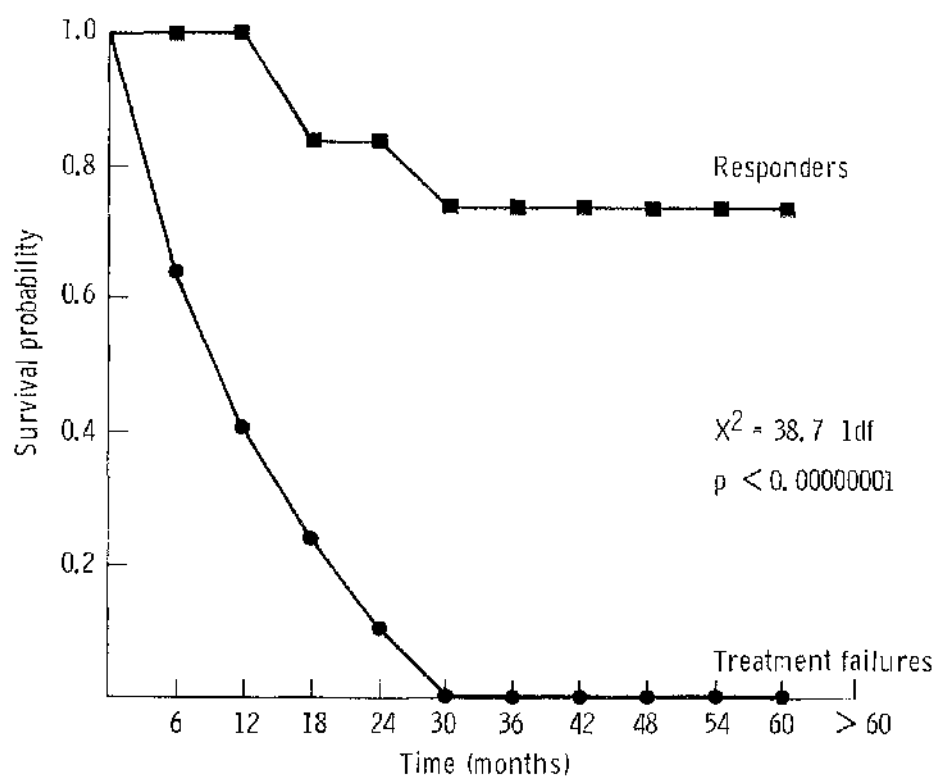


Fig. 9 : 2      Endocrine treatment responders versus treatment failures: all patients.

has been considered in a group of patients with a single secondary site of involvement (bone metastases). In this group the survival advantage of treatment responders over failures persists (Fig. 9 : 3). Within this group, no difference in the incidence of histological grades between responders and failures was seen (Table 9 : III).

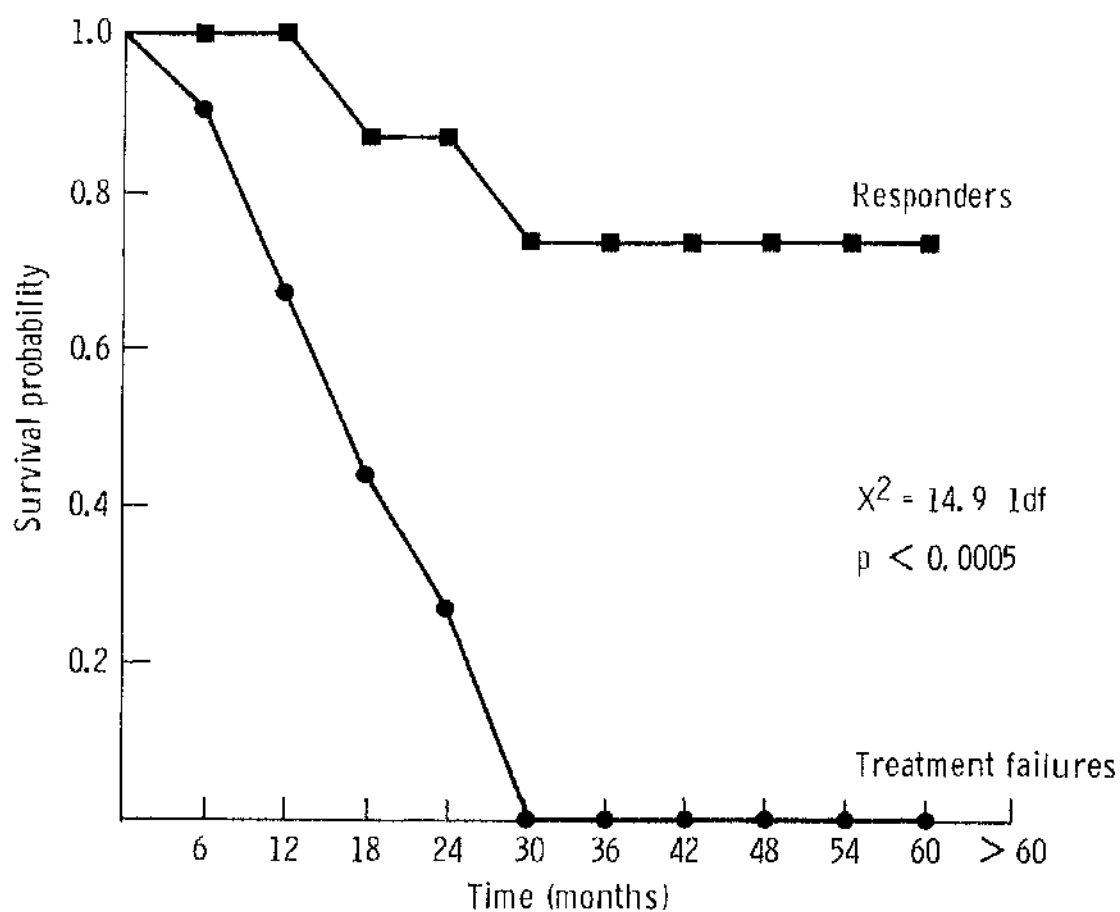


Fig. 9 : 3 Patients with bone metastases only.  
Endocrine responders versus failures.

Table 9 : III

Predominant bone secondaries: Incidence of  
histological grades

	I & II	III
Responders	9	4
Failures	18	16

Yates' correction  $\chi^2 = 0.2$ ;  $p = 0.7$

### Discussion

The response rate of 18.2% to hormonal therapy in this study is lower than that reported by others (Carter, 1976) but our criteria of assessment are unusual in that they include a mandatory regression interval of six months. Few centres recognise such terms. It might be argued that any tumour regression is beneficial irrespective of its duration and if recognition is limited to that which persists for a specific interval, then a proportion of patients with a short term response might be denied a second line endocrine therapy with a subsequent, albeit short lived remission. In this study, however, the main advantage of a six month period of observation has been our confident ability to exclude mixed progressive disease and other clinical 'artefacts' which mimic a response to therapy. In only one case was a true objective regression recorded which lasted less than six months. Thus, it seems likely that the higher response rates with endocrine therapy quoted elsewhere, may be erroneous!

It may be easily forgotten that response to therapy is not an end in itself. Few studies have analysed the relationship between response and survival and there is little hard data which shows that good quality life can be usefully extended by treatment schedules. Indeed, the Eastern Co-operative Oncology Group demonstrated that combination chemotherapy which induced a higher response rate conveyed no survival advantage over single agent chemotherapy in a randomised trial involving 687 patients with advanced breast cancer (Tormey et al, 1977). Thus a higher response rate had no apparent survival benefit.

The relationship between response to therapy and survival is complex but it does not totally defy interpretation. For example we have shown in the present study that responders to endocrine therapy enjoy a prolonged survival, in agreement with others (Taylor, 1962). However, this finding cannot of itself be considered adequate proof of the value of a regimen because tumours which are most apt to respond to endocrine measures are likely to be slow growing and as shown in previous chapters are more likely to recur in favourable secondary sites and thus have a better outlook irrespective of endocrine therapy. For that reason we have considered the impact of a remission with hormonal treatment only in patients with involvement of the most favourable secondary site ie. in the bony skeleton. As shown elsewhere in this thesis, metastases in bone are associated with a longer survival than metastases in viscera. As also shown in this thesis, the histological grade of a cancer is the best intrinsic marker of its rate of growth, and there is no difference in the incidence of histological grades between responders and treatment failures with bone secondaries, in this study. The only measurable differences in these two groups of patients were a response to endocrine therapy, on one hand and a significant survival benefit on the other. Thus, by the use of strict criteria for evaluation of response we have been able to identify a group of patients who have enjoyed a true benefit and a true survival advantage from endocrine therapy.

This study also shows that patients with skeletal secondaries have a relatively high response rate (28%) to endocrine therapy, in agreement with others (Taylor, 1962; Mouridsen et al, 1978). The



response rate of local and regional recurrences is high when these are the sole secondary sites of involvement (5/15), but when all patients with recurrences in these areas are considered, irrespective of metastases elsewhere, the response rate drops (4/41). The reason for this discrepancy is not clear.

In this study, we have grouped oophorectomy in premenopausal women and Tamoxifen in postmenopausal women together as primary endocrine therapy. The reported response rate with either modality is similar (Henderson and Cannellos, 1980) and this convenience appears justifiable. In the next chapter I shall relate a response to measurements of intrinsic parameters in the primary tumour.

## Chapter 10

### PREDICTION OF RESPONSE OF ADVANCED BREAST CANCER TO ENDOCRINE THERAPY

## Prediction of Response of Advanced Breast Cancer to Endocrine Therapy

### Endocrine therapy - Introduction

It is almost 90 years since the first effective oophorectomy was carried out in premenopausal women with advanced breast cancer. Beatson, working at Glasgow Cancer Hospital, reported successful results in three patients treated by oophorectomy and was moved to formulate his own theory of the pathogenesis of breast cancer and the mechanisms of hormonal therapy. He wrote,

"We must look in the female to the ovaries as the seat of the exciting cause of carcinoma of the mamma..... there seems evidence that the ovaries have control in the human body over local populations of epithelium and has an effect on carcinoma of the mamma..... removal of the tubes and ovaries helps carcinoma of the mamma in its natural tendency to fatty degeneration. This effect is best seen in cases of carcinoma in young people." (Beatson, 1896)

### Mechanisms of tumour regression with endocrine therapy

Our understanding of the mechanisms of hormonal action and in particular of the mechanisms of tumour regression following endocrine therapy have only progressed a little since Beatson's time and many aspects of the cellular events are poorly understood. Certain essential points are worthy of review.

In hormone dependent human breast cancers, the percentage of cancer cells which incorporate  $^3\text{H}$ -Thymidine into nuclear DNA, falls after initiation of endocrine therapy suggesting a decrease in the number of cycling cells. Endocrine therapy has no influence upon thymidine incorporation, however, in treatment failures

(Nordenskjoeld et al, 1976). Experimental DMBA (Di-Methyl-Benz-Anthracene) induced rat mammary tumours are hormone sensitive and recede following oophorectomy. When regressing tumour fragments are transplanted into non-ovariectomised rats, regrowth occurs which is indistinguishable from that of untreated tumours when transplanted into the same rats (Guillino et al, 1972). Oestrogen sensitive lines of human breast cancer cells eg. the MCF-7 cell line derived from malignant cells of a pleural effusion, grow very slowly in the absence of oestrogen but the addition of increasing molar concentrations stimulates cellular replication and nuclear trapping of  $^3\text{H}$ -Thymidine, until a plateau is reached. The addition of an anti oestrogen to the medium causes cell death (Lippmann et al, 1977). Interestingly, high levels of oestrogen also cause cell death, but this appears to be a non-specific steroidal effect, which also occurs even when inactivated oestrogens are used (Osborne et al, 1978). All of these observations suggest that the primary effect of hormone therapy is the removal of a stimulus for tumour growth. It is uncertain whether all hormone sensitive cells are killed by endocrine therapy, and there is certainly no evidence that all metastatic deposits have ever been eradicated in any given patient.

#### Prediction of response to hormonal therapy

Endocrine treatment measures, whether additive or ablative, have nonetheless been proven to be an effective palliation for advanced breast cancer (Taylor, 1962). Between 18 and 40% of patients will respond and enjoy a complication free remission lasting between 12 and 14 months (Henderson and Canellos, 1980).

Until recently, the choice of systemic therapy, whether endocrine therapy or chemotherapy, was based upon clinical criteria. Patients likely to respond to endocrine therapy would include those with a long disease free interval, with predominantly bony or soft tissue secondaries (Taylor, 1962) a prior hormonal response and a limited number of metastatic sites (Kennedy, 1974). Often a trial of treatment was the most feasible way of separating hormonally responsive from non responsive tumours.

It has become clear, however, that hormonal responsiveness is not a function of the patients internal hormonal milieu, as postulated by Beatson, but rather a function of the presence of hormone receptors in the tumour cell. It is over ten years since Jensen first reported the presence of oestrogen receptor in an experimental mammary tumour (Jensen et al, 1968) and he later demonstrated the value of this discovery in human breast cancers. In a group of 27 patients Jensen reported a response rate of 66% to endocrine therapy in tumours possessing the receptor compared to only 4% in tumours lacking the receptor (Jensen et al, 1973). This association between the presence of oestrogen receptor and response of a tumour to endocrine therapy, have been confirmed by other authors (McGuire, 1975; Roberts et al, 1978; Allegra et al, 1979). Oestrogen receptor status of the target tumour is a better marker of hormone responsiveness than the clinical criteria mentioned above (Allegra et al, 1980) and represents an important advance in therapeutics. Not only is the presence or absence of measurable receptor important in predicting a response, but the quantitative value of receptor is also significant. High levels of ER favour a response (Osborne et al, 1980; King, 1980).

Progesterone receptors represent the end product of the oestrogen dependent pathway in breast cancer cells (Horwitz et al, 1975) and the presence of both oestrogen and progesterone receptors may be indicative of a particularly high response rate (Osborne et al, 1980; Brooks et al, 1980) although others have failed to confirm this (Manni et al, 1980). Histological grade is related to receptor status (Elston et al, 1980) and this parameter has also been reported as a good indicator of tumour sensitivity to hormonal methods (Rubens et al, 1981).

All of the previous studies have evaluated steroid receptor concentrations or histological grade on biopsies of metastatic tumour taken immediately before the start of treatment. Thus the findings of these studies apply only to that selected group of patients with metastases which are accessible for biopsy, and the conclusions cannot be applied to the total breast cancer population. This point is also of practical importance. Patients in whom metastases are inaccessible have undergone major surgical exploration to obtain biopsies for receptor analysis (Leung et al, 1975) although the British Breast Group believes that such an approach is not justifiable (British Breast Group, 1980). Of obvious interest therefore is the relationship of receptor status or histological grade in the primary tumour to response of metastases to endocrine therapy. Data concerning this important point is lacking (De Sombre et al, 1980). This relationship is of particular interest where steroid receptors are concerned, as certain authorities have reported a change of receptor status usually from positive to negative, between the primary cancer and its recurrences

which raises the possibility that endocrine responsiveness may be lost with the passage of time (Leake et al, 1981). As pointed out in the last chapter the quantity as well as the quality of a response is important, yet few studies evaluate this.

In the present study, the relationship between intrinsic factors viz. steroid receptors (in the case of ER, receptor status and absolute levels have been considered) and histological grade, in the primary cancer and likelihood and duration of response of secondary metastases has been investigated. A comparative assessment of the predictive value of each parameter was carried out.

### Patients and methods

Patients, treatment protocols and methods of assessment were defined in the last chapter. Sixteen additional patients in whom ER or grade had not been evaluated were excluded, leaving 105 assessable patients.

### Duration of response

Duration of remission was defined as the interval between initial regression of metastases and subsequent relapse, either with regrowth of the same deposits or the appearance of new lesions.

### Intrinsic parameters

At the time of mastectomy, adjacent tumour samples were taken for evaluation of histological grade, oestrogen and progesterone receptors as outlined previously. To facilitate uniformity, analyses were confined to patients who had both ER and grade measured in the primary cancer viz. 105 patients. Tumours were considered to be receptor positive for both ER and PgR when they contained more than 5 femtomoles specific oestradiol or progesterone binding respectively, per milligram of cytosol protein.



## Results

### Response rate

Response rates to Tamoxifen (largely postmenopausal women) and to oophorectomy (largely premenopausal women) were similar (Table 10 : I). Significantly higher rates of response were seen in patients with ER positive primaries (Table 10 : II). Furthermore the likelihood of response increased proportionately with the measured ER value (Fig. 10 : 1). Response rates were higher in patients with well differentiated cancers (Grade I, II) than with poorly differentiated primaries, but differences did not reach statistical significance (Table 10 : III). Progesterone receptors had been measured in only 34 patients in this study. High response rates were seen in patients with ER+ PR+ primaries (Table 10 : IV).

### Duration of response

Only oestrogen receptors and grade were considered in these analyses. Preliminary data suggested that patients whose primary tumours contained high receptor values ( $> 60$  fmol/mg cytosol protein) or which were grades I or II enjoyed a longer duration of remission than those with low receptors ( $< 60$  fmol/mg cytosol protein) or grade III (Figs. 10 : 2 & 3). Differences however, did not achieve statistical significance.

### Change of receptor status between primary and metastases

This study referred to oestrogen receptor assays only. Biopsy specimens of metastatic tumour were taken from accessible sites in 24 patients (17 skin, 5 lymph node, 1 omentum, 1 liver). ER status was unchanged from that of the primary cancer in 18 patients; three, who had ER positive primaries developed receptor negative

Table 10 : 1Response to endocrine therapy

	No. treated	No. responding
Tamoxifen	76	14 (18.2%)
Oophorectomy	45	8 (17.7%)
	—	—
Total	121	22

Table 10 : IIER Primary versus response of metastases to endocrine therapy

	No. treated	No. responding
ER positive	57	16 (28%)
ER negative	48	2 (4%)
<hr/>		
No ER	16	4

$\chi^2 = 8.04$  (Yates' correction)  $p < 0.005$  for patients  
with tumour containing ER

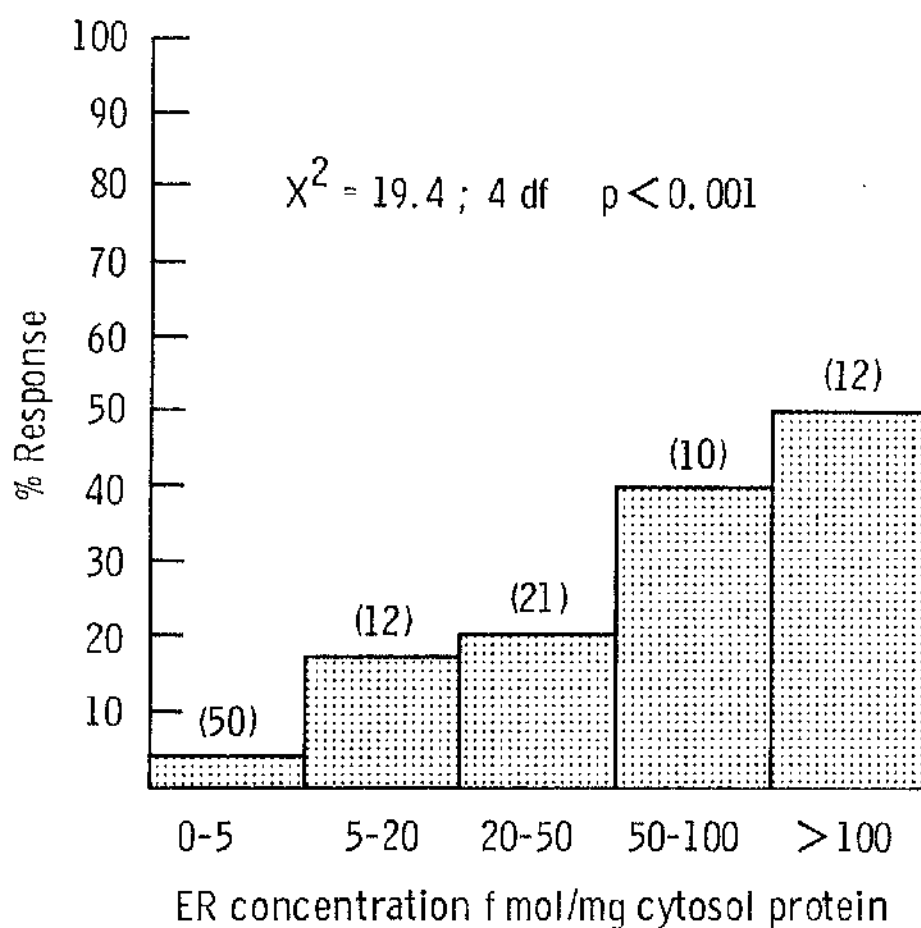


Fig. 10 : 1 Percentage of patients responding relative to ER concentration in primary cancer. Numbers in parentheses refer to the numbers of patients in each group.

Table 10 : IIIHistological grade of primary cancer: Response of metastases

	I	II	III
Response	3	8	7
No response	7	21	59
	—	—	—
	10	29	66

$$\chi^2 = 5.4; \quad 2 \text{ df}; \quad p = 0.07$$

Table 10 : IVProgesterone receptors of primary: Response of metastases

	No. treated	No. responding
ER+ PR+	5	3
ER+ PR-	12	1
ER- PR+	3	0
ER- PR-	14	1

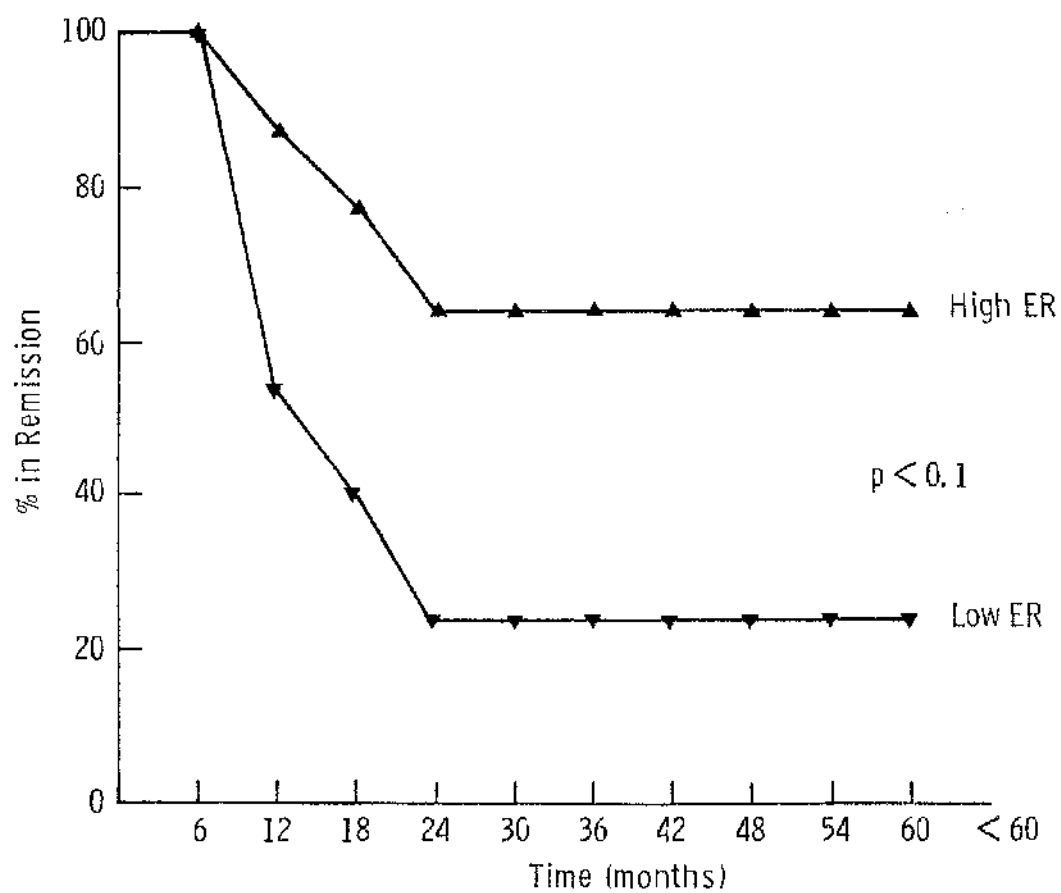


Fig. 10 : 2      Duration of response.  
High ER level (> 60 fmol/mg cytosol protein)  
versus low ER (< 60 fmol/mg cytosol protein).

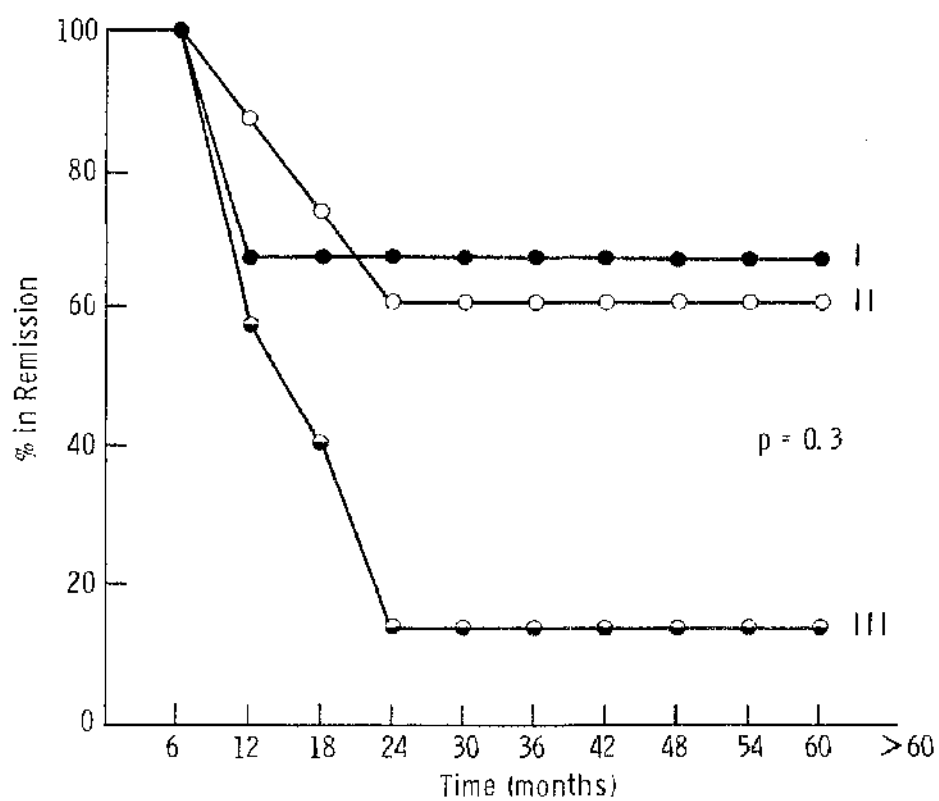


Fig. 10 : 3 Duration of response by histological grade.



secondaries and in a further three, a change in the reverse direction occurred. However, a change of ER status was associated, with one exception, with low values (Table 10 : V).

Table 10 : vChange of ER status

<u>Case No.</u>	<u>ER concentration (fmol/mg cytosol protein)</u>	
	<u>Primary</u>	<u>Secondary</u>
1	8	negative
2	11	negative
3	23	negative
4	negative	23
5	negative	33
6	negative	690

## Discussion

This study conclusively demonstrates that response of secondary metastases to endocrine therapy, can be predicted from measurable parameters in the primary cancer. Therefore this index is available to all patients undergoing mastectomy and need not be restricted only to those with secondaries which are accessible for biopsy. Clearly, reliance on clinical parameters such as disease free interval etc., or worse a trial of therapy which is certainly wasteful of the survival time of the non responder, will no longer be necessary. Patients with ER negative primary cancers are most unlikely to respond to hormonal measures and therefore an alternative therapy is preferable.

Of the parameters compared in this study, oestrogen receptor status and quantitative values appear to be the best indicator of hormone responsiveness. Both the likelihood and the duration of response of secondaries to endocrine measures may be reliably predicted in the individual patient on the basis of the ER concentration in the primary cancer. Absolute level of ER is important in this respect in addition to ER status. Preliminary data concerning progesterone receptors is disappointing: of eight patients with measurable progesterone receptor in the primary, only three responded. Certainly, the response rate is high when both receptors are present (ER+ PgR+) but this group tend to have a high concentration of ER in any case (Chapter 4, Fig. 4 : 4) and it seems unlikely that progesterone receptor assays have any advantage over quantitative ER. Others have failed to find any superiority of PgR over quantitative ER in metastatic tumour as a predictor of response

(Osborne et al, 1980). Histological grade is a crude indicator of endocrine responsiveness. Patients with poorly differentiated (grade III) cancers might not be expected to respond, yet 12% did so.

The effectiveness of measurable indices in the primary cancer as an indicator of responsiveness of secondaries suggests that these parameters remain stable with the passage of time, between the primary cancer and its daughter metastases. In this study, I have examined the stability of the best predictor (ER). In the small number of patients concerned, ER status remained unchanged in only 75% and a change occurred in the remainder. These figures are similar to those reported by others (Harland et al, 1982). However, in the majority of instances (5/6) a change was associated with low receptor values which could conceivably be attributable to laboratory error.

In conclusion, quantitative oestradiol receptor measurements in primary breast cancer provide the best index of hormone responsiveness of secondary metastases.

## Chapter 11

### INTRINSIC FACTORS AND PATIENT SURVIVAL.

## Intrinsic Factors and Patient Survival

### Introduction

The life survival curve of patients with operable breast cancer shows a steep early decline and then levels off progressively (Brinkley and Haybittle, 1959; Adair, 1974). Approximately 25% of deaths occur within the first two years after mastectomy and later mortality, although spread rather constantly over a prolonged interval, occurs at a lower rate than the early group (Fig. 11 : 1). Fox (1979) drew attention to this concept in an analysis of data concerning patients with operable breast cancer, treated between 1950 and 1973, compiled by the end results section of the National Cancer Institute of America. Initially, Fox noted that the mortality rate for 10 year survivors with breast cancer was only 2.5% per annum. He then went on to suggest that the survival curve of any given group of patients with breast cancer represented the behaviour of at least two populations, each with a characteristic mortality: Forty percent of the total population exhibit a relative mortality of 25% per annum with a median survival of 2.5 years while the remaining 60% have an annual mortality rate of 2.5% and an expected median survival of 30 years. A mixture of the survival data of these two populations would give rise to the observed curve for the group as a whole (Fig. 11 : 2). Fox estimated that 85% patients with stage I disease and 40% patients with stage II disease would fall into the good prognostic group, but beyond this, the two categories could not be reliably distinguished.

The limitations of prognostic stratification by tumour bulk alone, have also been shown by the Nottingham study when lymph node

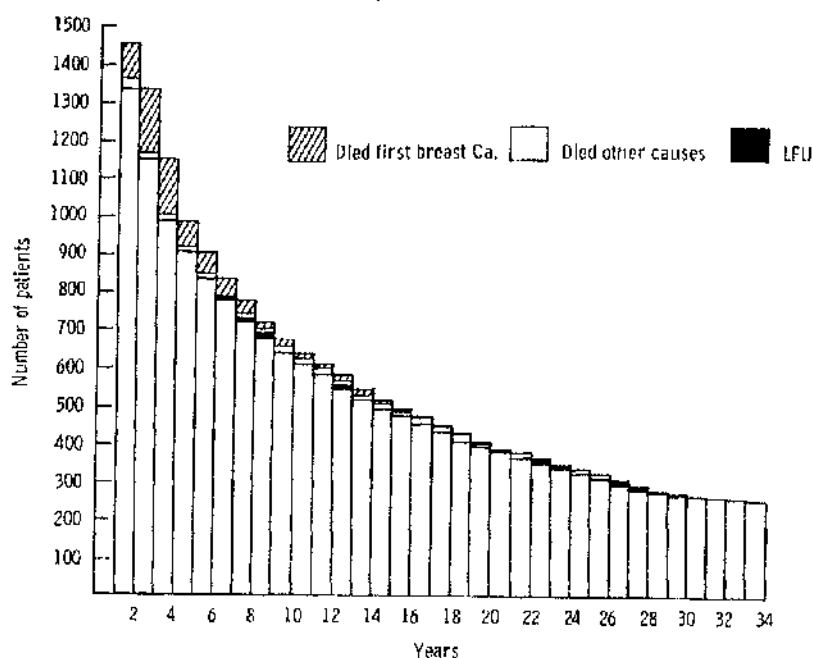


Fig 11 : 1 Survival curve of patients with breast cancer.

(Adair, 1974)

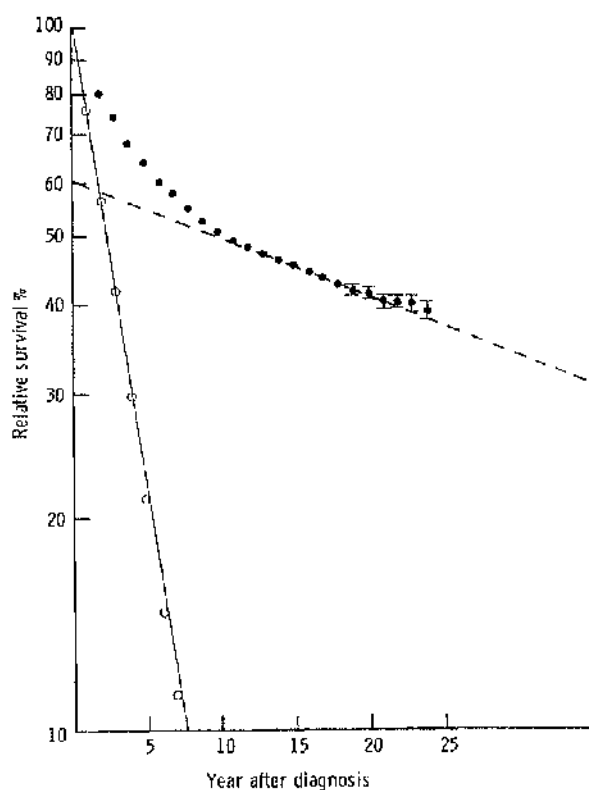


Fig 11 : 2 Survival curve of a population with breast cancer ●  
Composite graph of:

- a) Good prognosis group (Interrupted line)
- b) Poor prognosis group (Solid line/open circles)

Fox 1979

stage alone wrongly predicted the outlook in a sizeable minority of patients (Chapter 4). It is also noteworthy that 25% of the 21 year survivors in the Cambridge study, had involved axillary lymph nodes at mastectomy and would have been expected, on the basis of conventional staging, to have a poor outlook (Brinkley and Haybittle, 1975). Thirty years ago Bloom and Richardson (1957) noted that "The system of clinical staging provides a guide to the obvious extent of tumour bulk but fails to take account of the nature of the tumour itself and thus fails to indicate the likelihood of occult lymphatic and blood borne metastases nor the speed with which such metastases develop." Fox (1979) recognised a dilemma in our approach to cancer therapy; that a uniform approach cannot be appropriate for both (a) patients who are likely to have a rapidly fatal outcome, and for (b) those with an outcome only modestly different from the cancer free population, but in view of the limitations of prognostic stratification by tumour bulk alone he conceded that there was no alternative.

In previous chapters in this thesis, the prognostic influence of intrinsic tumour factors has been clearly demonstrated (when related to recurrence free interval, sites of metastases and response to endocrine therapy). The ultimate test of any prognostic parameters in breast cancer is, of course, against survival and in this chapter a comparative assessment of the predictive value of each parameter against survival has been carried out.



## Study 9

### Patients and methods

Studies relating to histological grade concerned 504 women and to oestrogen receptor status (ER) concerned 435 women of the first 550 in the Nottingham-Tenovus series. Quantitative ER values were available in only 237 of 253 patients with ER positive cancers and were considered in three groups viz. 0-4 fmoles/mg cytosol protein (ER negative,  $n = 180$ ), 5-79 fmoles/mg cytosol protein ( $n = 100$ ), more than 80 fmol/mg cytosol protein ( $n = 137$ ). Progesterone receptors had been evaluated in 167 patients and prognostic significance was considered singly and in combination with ER. Treatment of patients at the time of recurrence was as defined in chapter 9. Survival curves were derived from life table analyses and statistical evaluation carried out by the method of Mantel.

As shown in chapter 6, histological grade of breast cancer is significantly related to tumour size, and thus a 'correction' must be applied. The prognostic capacity of grade has been considered within a group of patients with tumours of a single diameter. No other parameters are related to tumour bulk and no other similar 'correction' was necessary.

## Results

### 1. Histological grade and survival

A highly significant relationship was demonstrated between histological grade of primary breast cancer and survival. Survival became progressively shorter from grade I, through II to III ( $X^2 = 38.6$ ; 1 df;  $p < 0.00001$ ) (Fig. 11 : 3). When patients with small tumours only were considered ( $< 2.0$  cms maximum diameter), the highly significant relationship persisted ( $X^2 = 21.1$ ;  $p < 0.0005$ ) (Fig. 11 : 4).

### 2. Oestrogen Receptors

Patients with oestrogen receptor positive breast cancers enjoyed a longer survival than those with ER negative cancers ( $X^2 = 4.3$ ; 1 df;  $p < 0.05$ ) (Fig. 11 : 5). When quantitative ER values were considered three patient groups were identified with progressively shorter survival from higher ER values through moderate to ER negative ( $X^2 = 8.08$ ; 1 df;  $p < 0.005$ ) (Fig. 11 : 6).

### 3. Progesterone Receptors

Progesterone receptor status of primary breast cancer is unrelated to survival irrespective of whether it was considered alone ( $X^2 = 0.07$ ; 1 df;  $p = 0.9$ ) (Fig. 11 : 7) or in combination with ER (Fig. 11 : 8).

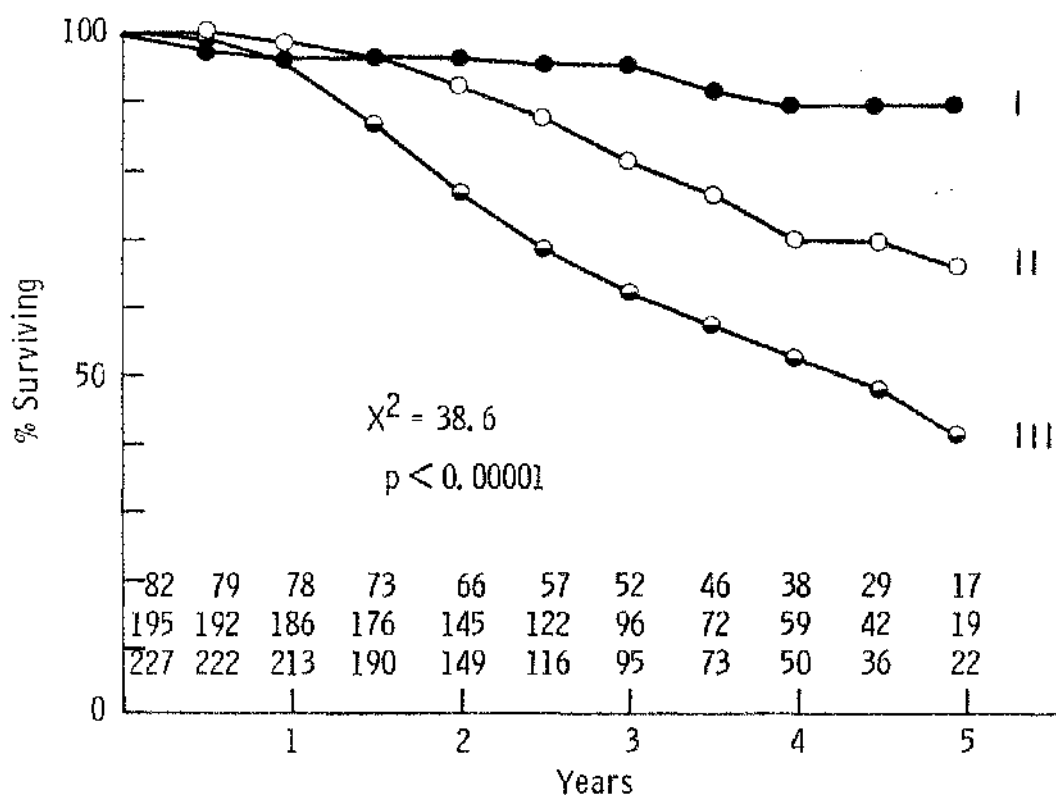


Fig. 11 : 3 Histological grade of primary breast cancer  
 and survival: All cases.

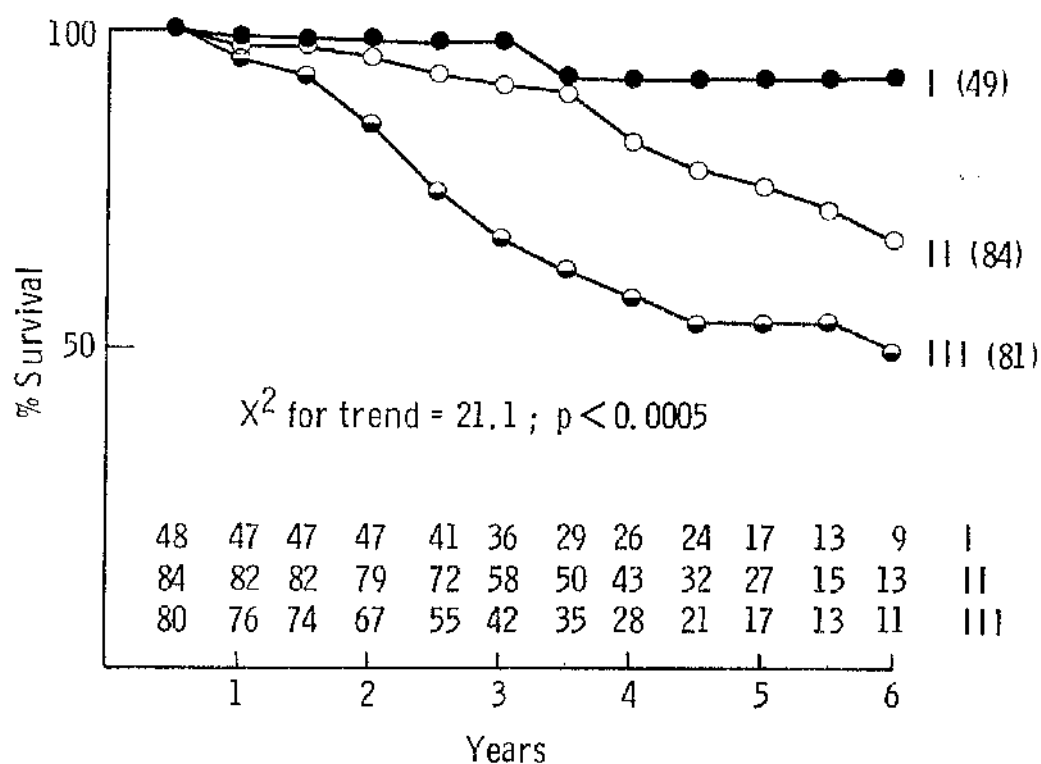


Fig. 11 : 4 Histological grade and survival: Small tumours (< 2.0 centimetres diameter).

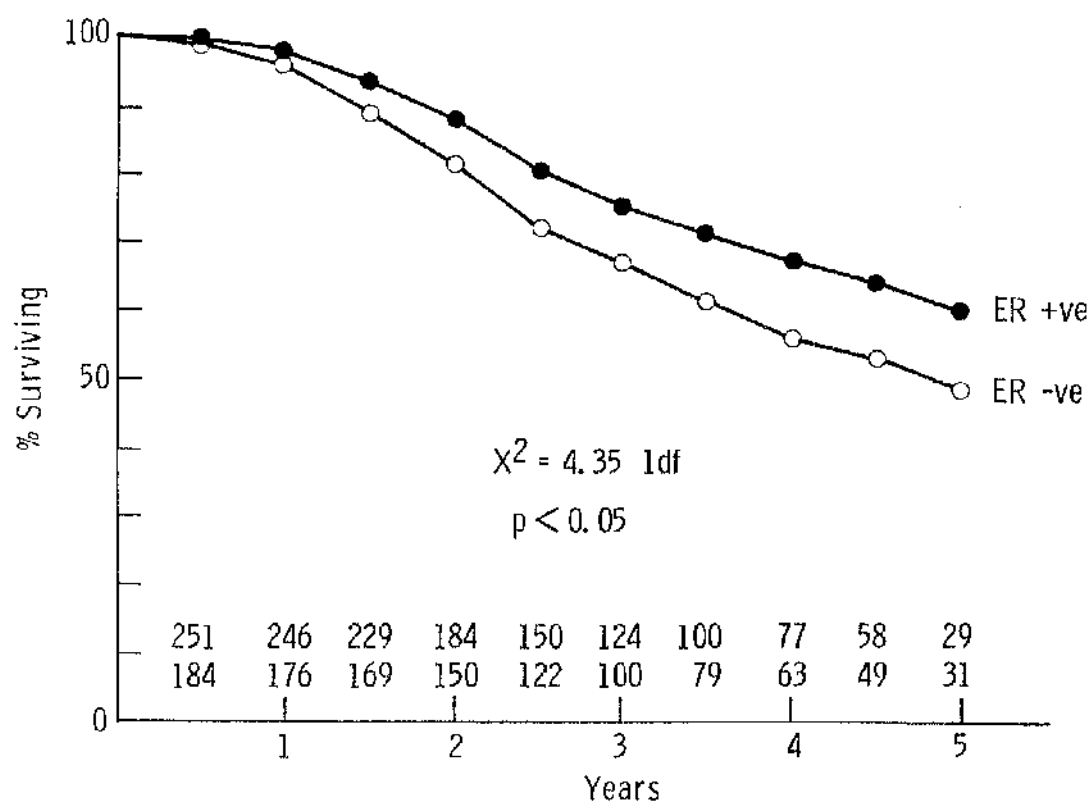


Fig. 11 : 5 Oestrogen receptor status of primary breast cancer and survival.

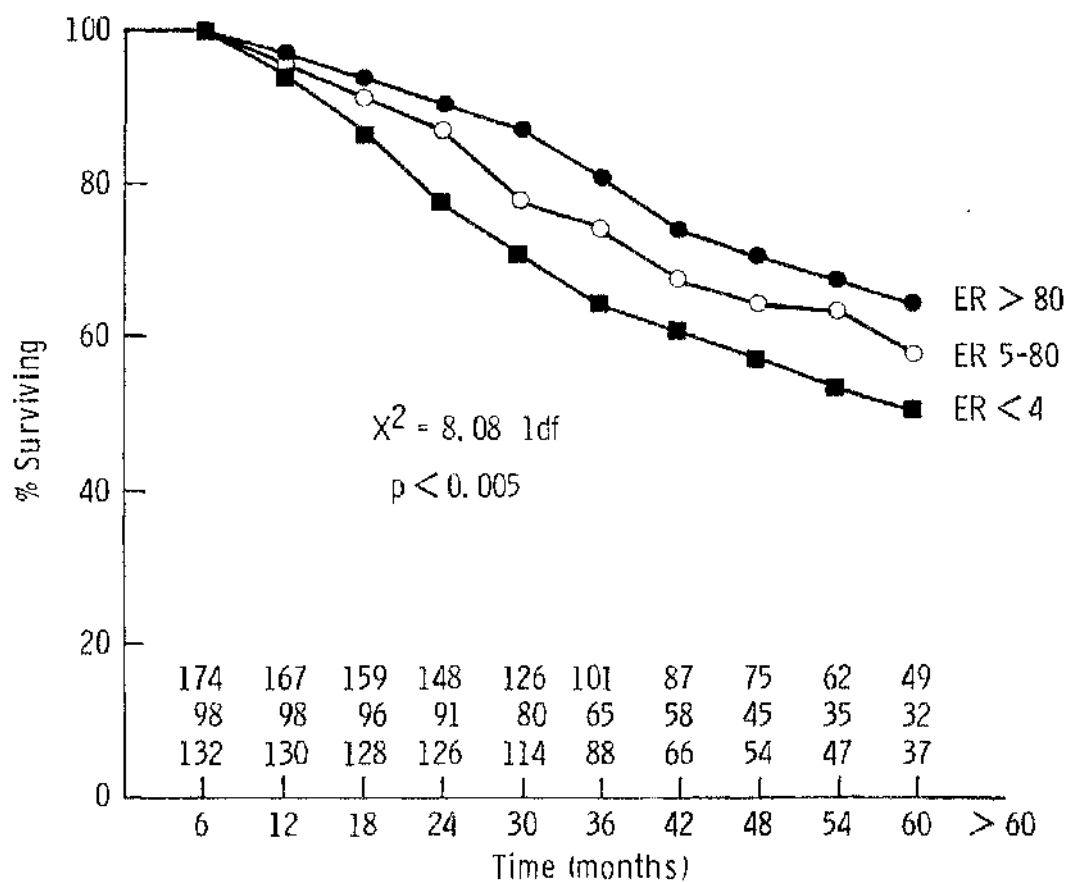


Fig. 11 : 6 Quantitative oestradiol receptor levels (fmol/mg cytosol protein) of primary breast cancer and survival.

Fig. 11 : 7

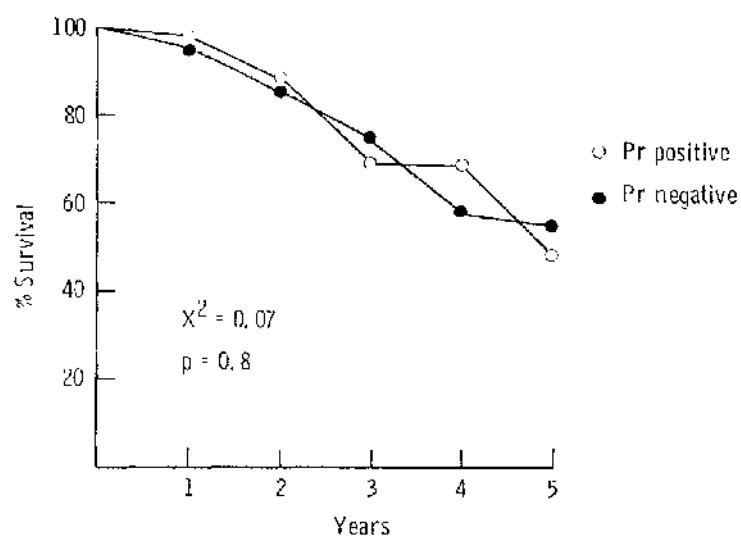


Fig. 11 : 8

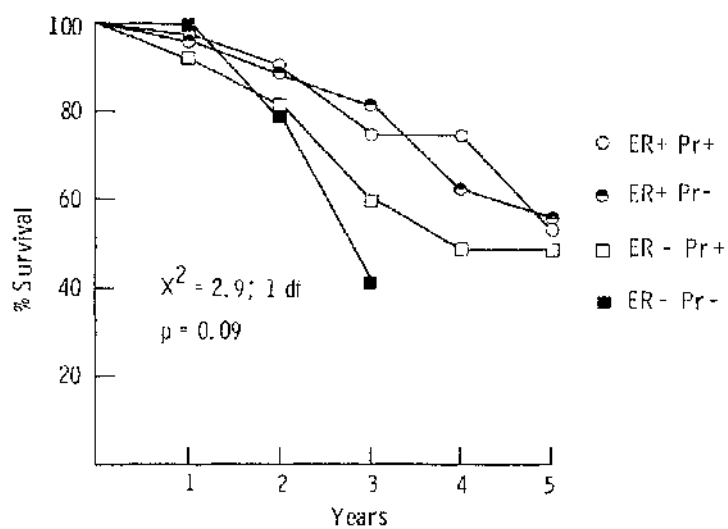


Fig. 11 : 7 Progesterone receptor (Pr) status of primary breast cancer and survival.

Fig. 11 : 8 Combined steroid receptor status of primary breast cancer and survival.

## Discussion

This study has clearly shown that of the parameters considered, histological grade was the best predictor of survival. As shown in chapter 5, grade was related to primary cancer size, but in this context its prognostic power was entirely independent of the influence of tumour size. Similar significant survival differences were seen between the three grades even when patients with a single tumour size ( $< 2.0$  centimetres) were considered. Thus the predictive power of grade and tumour bulk are complementary, and these factors may be taken together to provide a more accurate index of prognosis. Indeed, this point has been demonstrated in the Nottingham series (Haybittle et al, 1982).

Oestrogen receptor measurements relate to survival but are clearly weaker in this respect than grade. However, the association of oestrogen receptors with survival is stronger than that of ER with recurrence rates: patients with ER positive tumours survive significantly longer than those with ER negative primaries, when the whole population was considered. Similarly quantitative ER levels defined three prognostic groups with an inverse relationship between risk of early death and ER value. Clearly, this favourable effect of ER on survival must be related more to the favourable site of metastases and favourable response to treatment rather than to slow tumour growth rates. We have been unable, however, to confirm the findings of Godolphin et al (1981) of a strong relationship between quantitative ER and survival.

Progesterone receptors have been disappointing in the prediction of survival.



This study has clearly demonstrated that 'intrinsic markers' have prognostic significance in the ultimate test against survival, which is independent of the influence of tumour bulk. The implications of this finding will be considered in the next chapter.

## Chapter 12

### CONCLUSIONS

### Conclusions

This thesis commenced with a critical appraisal of the principles and results of present day treatment for early breast cancer. Local treatment methods which are based upon anatomical principles have progressed from one extreme to the other, from radical mastectomy to extended radical and 'super radical' mastectomy to lumpectomy with or without radiotherapy, without influencing mortality rates. There is clearly little hope of any survival improvements with new developments of local treatment methods. Systemic 'adjuvant' regimes are essentially based upon a physical principle and today appear disappointing despite the promise of their initial findings. One glimmer of optimism has emerged from cancer screening programmes with early detection and treatment. One such study has shown very promising findings and has managed to achieve a reduction in mortality rate within a defined population (Strax, 1976). However, it is unlikely that screening programmes will eradicate breast cancer and they provide no guidance for treatment of established disease.

Where, then does our hope for the future lie? Advances in medicine occasionally occur accidentally, by some chance finding but the main part of medical progress by far has resulted from the painstaking application of scientific method to a problem disease, starting with a true understanding of its innate nature and of the biological influences which govern its behaviour. While we do recognise great variation in the clinical behaviour of breast cancer, our understanding of its nature has been limited in the extreme.

A study concerning markers of the behavioural traits of breast cancer might allow appropriate therapy to be tailored to appropriate disease but more importantly might lead to an improvement in our comprehension of the innate biology of this disorder. Thus the objectives of this thesis have been two fold:

- a) The identification of good markers of specific traits of malignancy.
- b) An advance of our understanding of the biology of breast cancer.

These objectives will be considered separately.

#### Markers of Specific Traits of Malignancy

Rapid growing tumours will tend to be large in size at clinical presentation and be associated with a short disease free interval. This treatise has clearly shown that the histological grade of a primary breast cancer is significantly related to its size in centimetres: poorly differentiated tumours tend to be large in size and well differentiated cancers small, at the time of clinical presentation.

In the interval in which patients remain untreated following mastectomy, grade relates very well to recurrence rates. Poorly differentiated cancers have more rapid rates of local, regional and distant recurrence than those of moderate differentiation, which are next in order, through to well differentiated cancers, which have the slowest recurrence rates. This prognostic effect of grade upon recurrence free interval is entirely independent of the influence of tumour size, because exactly the same differences are seen when a group of patients with a single tumour size are considered. There

can be little doubt, then, that the histological grade of a primary breast cancer is an excellent marker of its rate of growth.

Histological grade is related to oestrogen receptor status of primary breast cancer, but not to quantitative ER levels nor progesterone receptor status. As we might expect oestrogen receptor status shows some association with recurrence rates: ER positive have a lower incidence of regional recurrences than ER negative cancers but the association is weak. Neither quantitative ER levels nor progesterone receptor status are related to recurrence free intervals. None of the steroid receptor parameters, neither ER, PGR status nor levels, is related to tumour bulk. In summary, steroid receptors show some relationship with growth rates, but are much less accurate in this context than histological grade.

However, the ER status of a cancer is strongly related to the anatomical site of its secondary metastases: ER positive cancers preferentially metastasise to bone. As previously shown, ER positive cancer cells synthesise significantly greater amounts of prostaglandin  $E_2$  and it is logical to suggest that these two properties are related. It is conceivable that hormonal stimulation of prostaglandin  $E_2$  synthesis by endocrine responsive malignant cells which lodge in bone following dissemination causes breakdown of bony physical barriers and thus facilitates the growth of these malignant cells and the appearance of metastases. Thus endocrine influences upon compliant breast cancer cells could facilitate the development of metastases in bone, which as shown, have a survival advantage over visceral secondaries.

Definition of response of advanced breast cancer to systemic therapy is not easy but it must be accurate before reliable

conclusions can be drawn concerning the relationship between measured variables in the primary tumour and response rate. In this thesis, specific areas of difficulty in assessment of response have been considered and errors or 'clinical artefacts' have been identified and excluded. The response rate to endocrinotherapy reported by this thesis (approximately 18%) was therefore low, but all patients categorised as responders had a minimum of six months of tumour regression, were externally assessed and confirmed and enjoyed a prolonged survival advantage over non responders. There can be little doubt that these patients enjoyed a true response from endocrine therapy. Oestrogen receptor status of the primary predicts the likelihood of response of metastases to endocrine therapy and, in addition, consideration of quantitative ER levels or progesterone receptor status improves the accuracy of prediction. However, progesterone receptor measurements offer no advantage over ER level in this context. Histological grade is a poor predictor of responsiveness.

In conclusion, a relationship exists between the morphological and endocrine 'markers' in primary breast cancer, but each relates very well to one specific 'trait of malignancy' with little overlap and clearly additional prognostic information will be available when both are measured. Thus, this thesis disproves Fisher's hypothesis that all 'intrinsic markers' represent different measurements of the same aspect of tumour malignancy (Fisher et al, 1980). This treatise has clearly shown that histological grade of primary breast cancer is an excellent marker of its growth rate. Oestrogen receptor status and quantitative levels provide good markers of endocrine mediated aspects of tumour behaviour, viz. anatomical sites of metastases and susceptibility to hormonal therapy.

The Biology of Breast Cancer - A Hypothetical Evaluation

What determines prognosis in breast cancer? If on one hand, we consider the traits of malignancy of this disease which our markers identify;

- a) Tumour growth rates
- b) Endocrine compliance

and then on the other hand consider the clinical variables which are seen during the natural course of breast cancer and which are most strongly related to prognosis;

- i) Tumour bulk
- ii) Disease free interval
- iii) Anatomical sites of metastases
- iv) Response to systemic hormonal therapy

then it seems likely that a strong relationship exists between them. As the findings of this thesis have suggested, tumour growth rates (a) will determine the primary cancer bulk (i) and disease free interval (ii) while 'endocrine compliance' (b) will determine (iii) sites of metastases and (iv) response to hormonal therapy. Thus the findings of this thesis suggest that the two traits of malignancy to which our markers relate (which I shall term 'essential characteristics' of breast cancer) will determine the size of the lesion at presentation and the entire clinical course of the disease thereafter, including the interval until a metastasis appears, the site at which it will appear and its susceptibility to hormonal treatment.

Only a minority of cancers within any given population is endocrine responsive. Within this minority, the two 'essential characteristics', ie. growth rates and endocrine compliance, may be related. Endocrine responsive tumours generally grow slowly and at the more benign extreme of the spectrum of malignancy where there is total hormone dependence, tumour growth rates may be restrained by endocrine influences. However, progressive loss of susceptibility to endocrine moderation may occur through to the other extreme of complete hormone independence when growth rates would be totally uncontrolled. Thus, within a random population of patients with breast cancer, hormonal effects could determine the outlook for the small group which is totally 'endocrine compliant' and govern the rate of growth, site of metastases and response to hormonal therapy while at the opposite end of the spectrum, rapid uncontrolled tumour growth rates would determine outlook for the larger group of hormone independent types, which would metastasise early, grow well at whichever secondary site that they happened to come to rest and be unaffected by hormonal therapy. Therefore, tumour growth rates would be the most important determinant of survival of a total population with breast cancer.

As with all things biological, there is likely to be a progression, with varying degrees of overlap between the two extremes described above. Thus the variable influence of the 'essential characteristics' of cancer cells could entirely account for the diversity of clinical behaviour of breast cancer, from slow growing hormone dependent tumours which only metastasise after a prolonged interval, through rapid growing hormone dependent types



which develop bone secondaries, to large rapid growing tumours which are hormone independent and quickly metastasise to viscera.

Thus, in summary, the findings of this treatise suggest that two elementary types of breast cancer exist within any random population with variable degrees of overlap.

a) Endocrine compliant, the smaller group, whose clinical course would be governed by hormonal influences.

b) Endocrine non responsive, the larger group, whose clinical course would be entirely governed by tumour growth rates.

These hypotheses concerning 'essential characteristics' of breast cancer have been based on the scientific observations of this thesis which have concerned large numbers of patients who have presented to a single surgeon (Professor Roger Blamey) and have been treated in a uniform manner and in particular, have received no additional therapy between mastectomy and recurrence which might have distorted the natural history of their disease. Clinical follow up and documentation has been meticulous.

All biological observations and hypotheses must be tested, however, and if those of this thesis are corroborated, then we shall have achieved a significant advance in our understanding of breast cancer.

How do our hypotheses bear up in the ultimate test against survival? As anticipated, the marker of growth rates, histological grade is the best predictor of survival. Survival time is longest with well differentiated cancers but progressively decreases through tumours of moderate differentiation to high grade cancers. Markers of endocrine compliance, as previously noted, apply to a minority of

cancers and as anticipated are less strongly related to survival of the total population.

Oestrogen receptor status and quantitative levels show stronger relationships to survival than they do to recurrence rates probably as a result of their favourable associations with secondary sites of metastases and response to endocrine therapy. Thus the markers of endocrine compliance are associated with favourable survival not only because of their association with slow tumour growth rates, but also because of their relationship with favourable secondary sites of metastases and favourable response to therapy. Progesterone receptor assays have been disappointing.

Chemotherapy is given to patients on failure of endocrine therapy in this centre. The response to chemotherapy is unrelated to any 'intrinsic factor' (Blake et al, 1982) and no attempt has been made to estimate its influence upon survival.

#### Breast Cancer - Two Diseases

As noted in the previous chapter, Fox (1979) reported that the survival curve of any random group of patients with breast cancer represented the behaviour of at least two distinct populations. One group had an annual mortality rate of 25% and a median survival of two and a half years while the other had a favourable outcome, only modestly different from that of a breast cancer free population. While recognising the existence of these subgroups, Fox was unable to identify them with accuracy and thus justified the continuation of a uniform 'blanket' approach to breast cancer therapy. Fox's model may represent an oversimplification and, as outlined, there may be considerable variation between the extremes which he described. However, as outlined in this thesis it has become

possible to identify two diseases which lie at either end of the spectrum of malignancy of breast cancer by the use of intrinsic markers as shown in Table 12 : I.

#### Therapeutic Implications of this Project

Three immediate therapeutic implications arise from this project:

i) Histological grade is the best predictor of recurrence rates and survival. This factor may be used, preferably in combination with measures of tumour bulk, to define high risk groups, for whom adjuvant chemotherapy may be appropriate. Prolongation of disease free interval by a toxic therapy is arguably more justifiable in a group of patients with an extremely poor prognosis.

ii) Hypercalcaemia is a relatively common but rapidly fatal complication of bone metastases from breast cancer and it may precede their clinical detection. This complication may be prevented by early treatment. In Nottingham, oestrogen receptor assays in the primary taken together with measures of tumour bulk and to a lesser extent, histological grade have been used to define a group of patients at high risk of bone secondaries. These patients are being screened for any imbalance of calcium homeostasis, so that early effective therapy may prevent hypercalcaemia (Campbell et al, 1982).

iii) Oestrogen receptor measurements in primary breast cancer may be used as a basis for selection of systemic therapy at the time of recurrence. Thus the empirical selection of therapy for the majority of women whose metastases are inaccessible for biopsy may be abandoned.

Table 12 : ITwo diseases in breast cancer

<u>Oestrogen receptor positive/ moderate or well differentiated cancers</u>	<u>Oestrogen receptor negative/ Poorly differentiated cancers</u>
Small primary cancers	Large cancers
Slow growing	Fast growing
Long disease free interval	Short disease free interval
High prostaglandin $E_2$ synthesis	Low prostaglandins
Bone secondaries	Generalised dissemination
Progesterone receptor positive	Progesterone receptor negative
Response to endocrine therapy	No response to endocrine therapy
Favourable survival	Poor survival

## Recent Developments

Progress has occurred in two areas, where this thesis has left off.

### a) Construction of a prognostic index

As noted, the prognostic yield from measures of tumour bulk and intrinsic factors are complementary. A recent study from this centre has devised an index for stratifying prognostic groups based upon the composite prognostic yield of nine measured factors: age, menopausal status, tumour size, lymph node stage, oestrogen receptor status, histological grade, cellular reaction, sinus histiocytosis and adjuvant chemotherapy. The optimum combination concerns three parameters:

Tumour size, lymph node stage and histological grade

Using this index, patients may be allocated to low, intermediate and high risk groups with considerable accuracy. The index holds an advantage over measures of tumour bulk alone, in that it allows greater proportions of the patient population to be allocated to high and low risk groups with accuracy.

### b) Oestrogen receptor values and tumour epithelial cellularity

Errors may occur with oestrogen receptor assays. As shown in this project, ER status changes between the primary tumour and its daughter metastases in up to 25% cases, but particularly where ER values are low. It has been suggested that oestrogen receptor values are related to the number of tumour cells in a cancer (Feherty et al, 1971), and that cellularity variations between primary lesion and daughter metastases could account for the ER variations between primary and secondary.

A further study from this centre has investigated the relationship of tumour epithelial cellularity and measured oestrogen receptor values in 100 primary breast cancers (Mumford et al, 1982). Cellularity was measured by an objective histomorphologic cell count (Underwood, 1972) and oestrogen receptor values were expressed in femtomoles per milligram of cytosol protein. A positive relationship was found in the tumours of postmenopausal women: approximately 20% of the variation of oestrogen receptor values were due to cellularity variations. No relationship was found in tumours of premenopausal women.

The method of expressing oestrogen receptor values in terms of femtomoles per milligram of cytosol protein provides a crude biochemical correction for cellularity variation (Blamey et al, 1980) but it is clearly inadequate. A better biochemical correction for cellularity might improve the predictive accuracy of oestrogen receptor measurements.

#### Future Prospects

Studies such as those of this thesis have begun to scratch the surface of breast cancer. We have been successful in identifying biological and morphological markers in breast cancer, which associate with its behavioural characteristics. In addition, we have identified some of the 'biochemical rules' which govern the natural course, of the receptor positive, well differentiated type of cancer. In due course we may be able to influence these processes.

Research along similar or related lines has proceeded in other parts of the world and there are new developments in two areas which are very exciting.

#### a) Oestrogen receptors

Jensen, in Chicago has purified the oestrogen receptor molecule, from human breast cancer. In addition, a monoclonal antibody has been raised against the molecule and immunoperoxidase stains have been developed which will shortly become available for use with both frozen and paraffin sections of breast cancers. Thus, oestrogen receptor determination and quantitation will become possible in a routine laboratory (Jensen, 1982).

It seems likely, that the next logical step will involve the development of a vaccine with either passive or active immunisation against the oestrogen receptor. This may offer some improvement over the systemic anti oestrogens or ablative procedures currently available.

#### b) Cellular differentiation and control

Studies in this area have concerned myeloid leukemia cells, or sarcoma cells, but certain parallels exist with breast cancer.

Like breast cancers, a range of malignancy exists in myeloid leukemias, with well differentiated, intermediate and poorly differentiated leukemia cells. Well differentiated leukemia cells, possess the same genes that regulate the control of growth and differentiation as normal cells. Non malignant myeloid precursors can be induced to grow and differentiate to mature macrophages and granulocytes by a low molecular weight protein (termed MGI). Well differentiated leukemia cells are responsive to this protein, whereas poorly differentiated cells are not. It is possible to reverse malignant change in leukemia cells by this protein, and convert the well differentiated leukemia cells into a normal

macrophage or granulocyte (Sachs, 1980). Similarly it has been possible to reverse the malignant phenotype of sarcoma cells to a non malignant one (Rabinowitz and Sachs, 1970). Other agents can induce differentiation of leukemia cells including steroid hormones (prednisolone, oestradiol and dexamethazone) and prostaglandins., Prostaglandin  $E_2$  can halt the growth of malignant cells completely (Sachs, 1982).

As shown in this thesis, oestrogen receptor positive breast cancer cells, which tend to be well differentiated also tend to be associated with high prostaglandin production. If similar control mechanisms exist for different types of tumours, it would seem possible that the high prostaglandin levels could have a role in holding the tumour in check and maintaining the relatively well differentiated nature of the cancer. Thus, in years to come, it may be possible to modulate tumour growth, induce differentiation or possibly reverse the malignant change by influencing prostaglandins or similar agents.

This author makes no apologies for the speculative nature of the last section of the present thesis. A greater understanding of the genetic and biochemical controls of breast cancer is the only way for the development of newer more selective therapeutic approaches.



## Summary

This thesis evaluates oestrogen and progesterone receptors and histological grade, measured in primary breast cancer as 'markers' of innate tumour malignancy. A comparative assessment of these 'intrinsic markers' has been carried out against three main characteristics of a cancer which determine prognosis viz. tumour growth behaviour, its predisposition for metastasis to specific anatomical sites and its susceptibility to endocrine therapy at recurrence.

Inter-relationships of 'intrinsic factors' have been investigated before a comparative assessment of their predictive value in any clinical situation was carried out.

## Inter-relationships of 'Intrinsic Factors'

Oestrogen receptor (ER) status is strongly related but quantitative ER levels are unrelated to histological grade. Quantitative ER levels are, however, proportionately related to progesterone receptor status: the higher the concentration of oestrogen receptor, the greater the likelihood of a tumour possessing progesterone receptor. Progesterone receptor status is unrelated to histological grade. Thus, despite the overlap between ER status and grade, a degree of separation is beginning to emerge between endocrine and morphological markers.

## Tumour Growth Behaviour

Histological grade is the best predictor of tumour growth rates, as assessed clinically by recurrence free interval. The higher the histological grade the shorter the interval from mastectomy to any type of recurrence, local, regional, distant or

combined. Rapid growing tumours may be expected to be large in size at the time of clinical presentation and the findings of this study are as anticipated: the poorer the differentiation the greater the size of the primary at presentation. Grade remains the best predictor of growth behaviour, even when tumours of a single size are considered. Thus the prognostic yield of measures of tumour bulk and histological grade are synergistic.

Oestrogen receptor status correlates weakly with the interval from mastectomy until regional recurrence, but is a poor marker of growth rates. Consideration of quantitative ER levels and progesterone receptors offers no advantage in prediction of recurrence free intervals. Neither endocrine marker is related to tumour bulk.

#### Selectivity of Metastasis to Specific Anatomical Sites

Bone secondaries are associated with a longer survival than visceral metastases. Oestrogen receptor positive cancers show a preferential tendency to metastasise to bone, whereas ER negative carcinomas have a predisposition to generalised dissemination. Histological grade is a crude marker of patterns of metastases.

Oestrogen receptor positive breast cancer cells synthesise greater amounts of prostaglandin  $E_2$ , a potent bone resorbing agent, than receptor negative cells, which could account for observed patterns of metastasis.

#### Susceptibility of Metastases to Endocrine Therapy

The quantitative oestrogen receptor level in primary breast cancer is proportionately related to the likelihood of response of metastases to endocrine therapy. In addition, duration of response

appears longer in cancers with high ER values. Progesterone receptors constitute a good predictor of endocrine sensitivity, but offer no advantage over quantitative ER level. Histological grade is a crude predictor of hormonal sensitivity of metastases.

### Survival

It is likely that the rate of tumour growth is the most important contributory factor to survival. Histological grade of the primary cancer is the best predictive factor of survival. Oestrogen receptor status and quantitative levels are significantly related to survival, but this effect is not only related to slow tumour growth rates but also to favourable secondary sites of metastasis and favourable response to endocrine therapy. Progesterone receptor status is unrelated to survival.

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From these studies it has become evident that histological grade and steroid receptor analyses provide markers of the 'essential characteristics' of breast cancer cells which determine the entire clinical course of the disease:

Histological grade offers a reliable measure of growth rates.

Oestrogen receptor measurements offer a good marker of their 'endocrine compliance' and predict the anatomical site of metastasis and susceptibility to hormonal therapy.

## APPENDIX

## Appendix I

### Oestrogen Receptor Assay

#### a) Preparation of Cytosol

The tumour specimen was cleared of fat before being placed in liquid nitrogen. The tumour tissue was then powdered in the frozen state in a Thermovac automatic frozen tissue pulveriser. The powdered tumour was added to 3 mls Tris Buffer (10 mmoles Tris [1.21 grams] 1 mmole EDTA [0.3725 grams] 10% Glycerol 100 mls, and 5 millimoles Dithiothreitol in 1 litre). pH was adjusted to 7.4 with Hydrochloric acid. The mixture was then homogenised using an all glass homogeniser and centrifuged at 105,000 g for 60 minutes, to obtain the cytosol fraction. All procedures were carried out at a temperature of 4° C.

#### b) Incubation with Tritiated Oestradiol

Aliquots of cytosol (200 ul each) were incubated with an equal volume of tris HCL buffer, as had been prepared above, containing increasing concentrations (0.2 - 5.0 nanomoles) of tritiated oestradiol and left to incubate for 16 hours.

#### c) Separation of Bound and Free Steroid and Scintillation Counting

A suspension of 400 ul of charcoal (0.5% w/v) in tris HCL buffer containing gelatin (0.1% w/v) and Dextran T70 (.005% w/v) was then added and the tubes agitated for 90 minutes. The charcoal was precipitated by centrifugation at 100 g for 10 minutes. The supernatant was removed and its radioactivity was determined in a Nuclear Isocap Scintillation Spectrometer.

d) Scatchard Plot

To obtain the value for receptor concentration a linear plot of the data as devised by Scatchard (1949), was carried out. In this method, the ratio of oestrogen bound to receptor to free (unbound)  $^3\text{H}$  oestrogen was plotted against the concentration of  $^3\text{H}$  oestrogen bound to receptor. The intercept on the abscissa on the line so obtained, gives the concentration of oestrogen receptor binding sites. Non specific binding was accounted for by inclusion of a saturating concentration of tritiated oestradiol in one tube which was used as a correction for the other point.

e) Protein Estimation

Protein content of the cytosol was determined by the method of Lowry et al (1951).

Standards were made of Bovine Serum Albumin in duplicate from 0 - 0.1 g/litre water. Each cytosol was diluted to various concentrations (10 - 100 times). Solutions in both standard and control tubes were heated to  $50^{\circ}\text{C}$  and cooled to room temperature. Folin's reagent was added to each tube and colour intensity of test solutions measured upon a Guildford Spectrophotometer. Protein content of cytosol was calibrated against BSA standards.

Receptor content of the tumour is expressed in femtomoles per milligram of cytosol.

## Appendix II

### Progesterone Receptor Assay

Preparation of cytosol and protein determination were performed as for oestrogen receptor assay.

#### Incubation

One hundred microlitres of cytosol were incubated with 100 ul tritiated progesterone in various concentrations (0.5 - 10 millimoles). Excess R 5020 progesterone ligand was added. Incubation was at 4° C for three hours.

#### Separation

A suspension (400 ul) of charcoal (0.25% w/v) in tris HCL buffer containing gelatin (0.5% w/v) and Dextran T70 (0.05% w/v) was agitated for 10 minutes at 4° C. Charcoal was precipitated by centrifugation at 100 g for 10 minutes. The supernatant was carefully removed. Its radioactivity was determined in a Nuclear Chicago Isocap Scintillation Counter.

#### Scatchard Plot

The ratio of bound  $\frac{{}^3\text{H Progesterone}}{\text{free } {}^3\text{H Progesterone}}$  bound  ${}^3\text{H Progesterone}$  was plotted as for oestrogen receptors.

The intercept of the line obtained gives the concentration of progesterone receptor binding sites.

### Appendix III

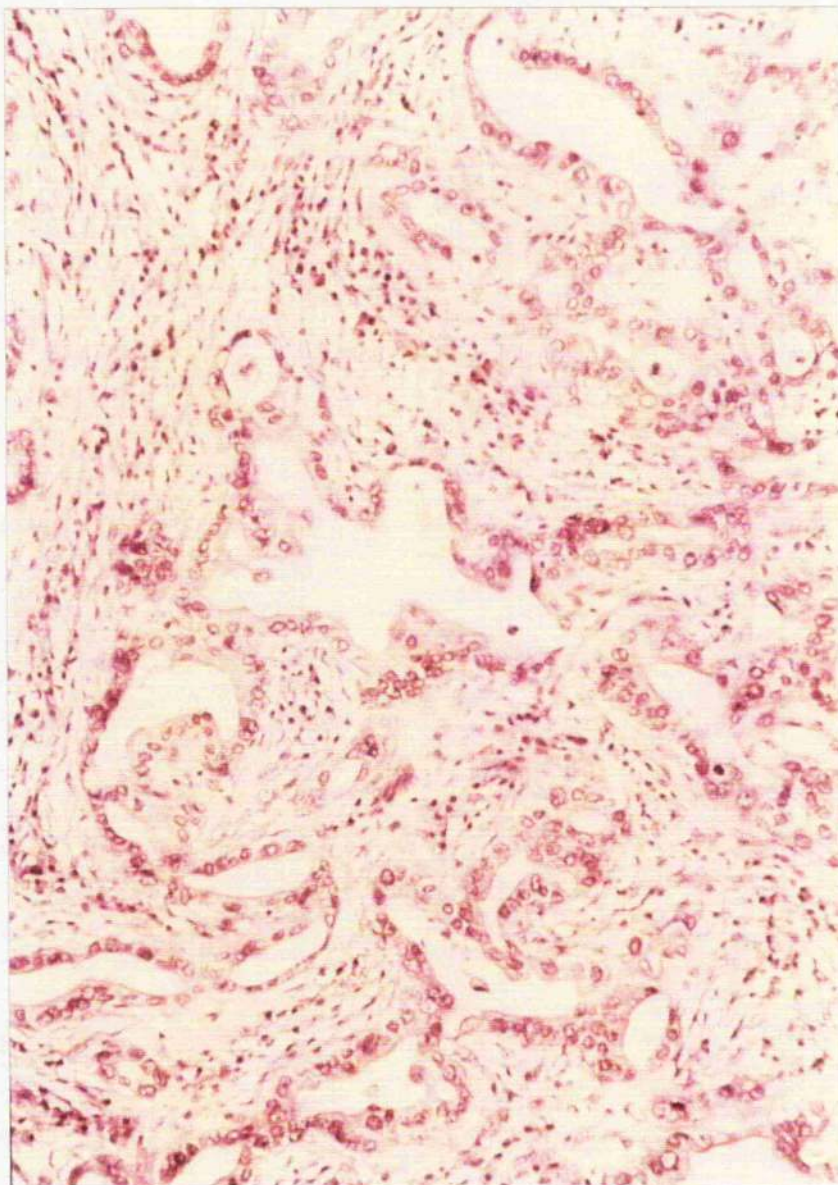
#### Histological Grading

All tumour specimens were fixed in 10% buffered Formalin. Between one and four blocks were cut from each tumour and paraffin sections of 4-6 um thickness were taken and stained with Ehrlich's haematoxylin and Eosin. Where necessary, multiple sections were examined. Histological differentiation was assessed independently by Dr. C.W. Elston and Dr. Jane Johnson in every tumour, by the method of Elston et al (1980). Histological grade was scored on the basis of three features; the degree of tubule formation, the degree of variation of size and shape of nuclei and the number of mitotic figures. Each feature was scored from 1 to 3 in ascending order of abnormality. Thus each tumour was given a composite score of 3 - 9, divided as follows:

Grade I	(well differentiated)	3, 4, 5	(Fig. 1)
Grade II	(moderately differentiated)	6, 7	(Fig. 2)
Grade III	(poorly differentiated)	8, 9	(Fig. 3)

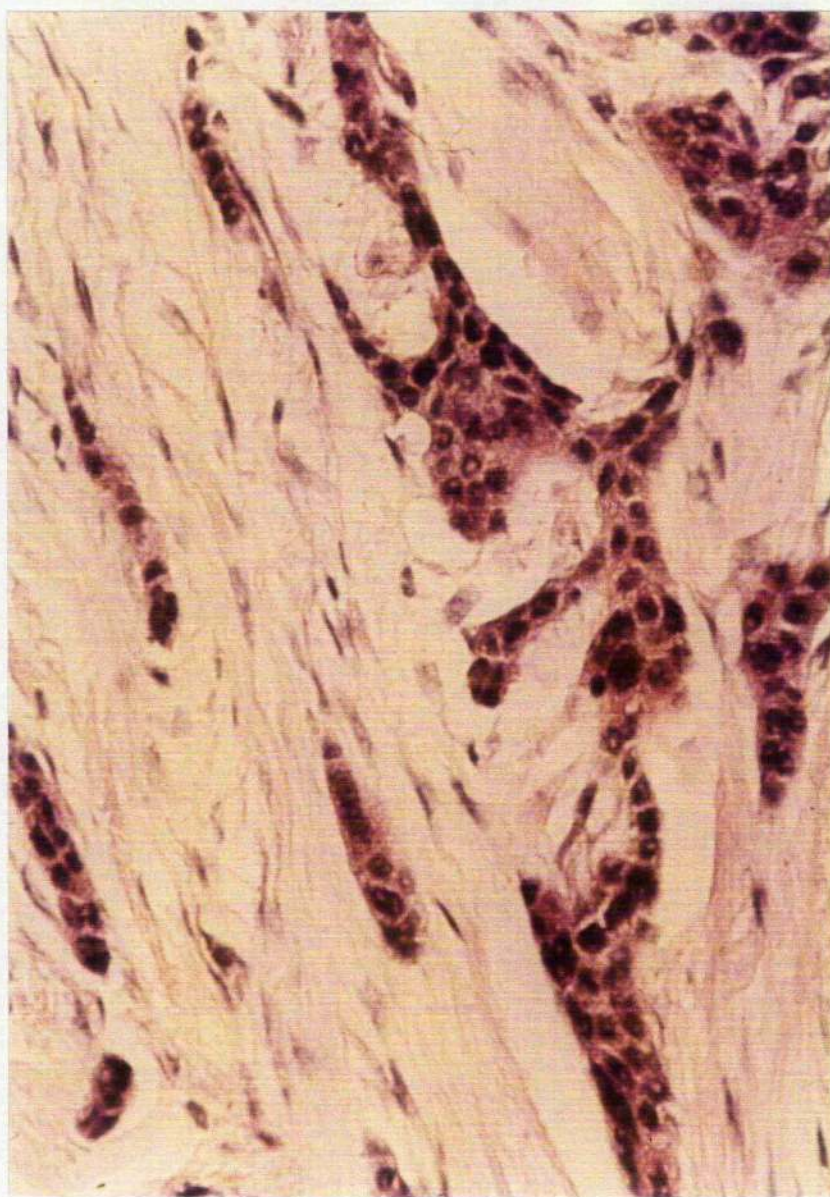
There was initial agreement between the two examiners in 90% cases and a consensus decision made upon the remainder.





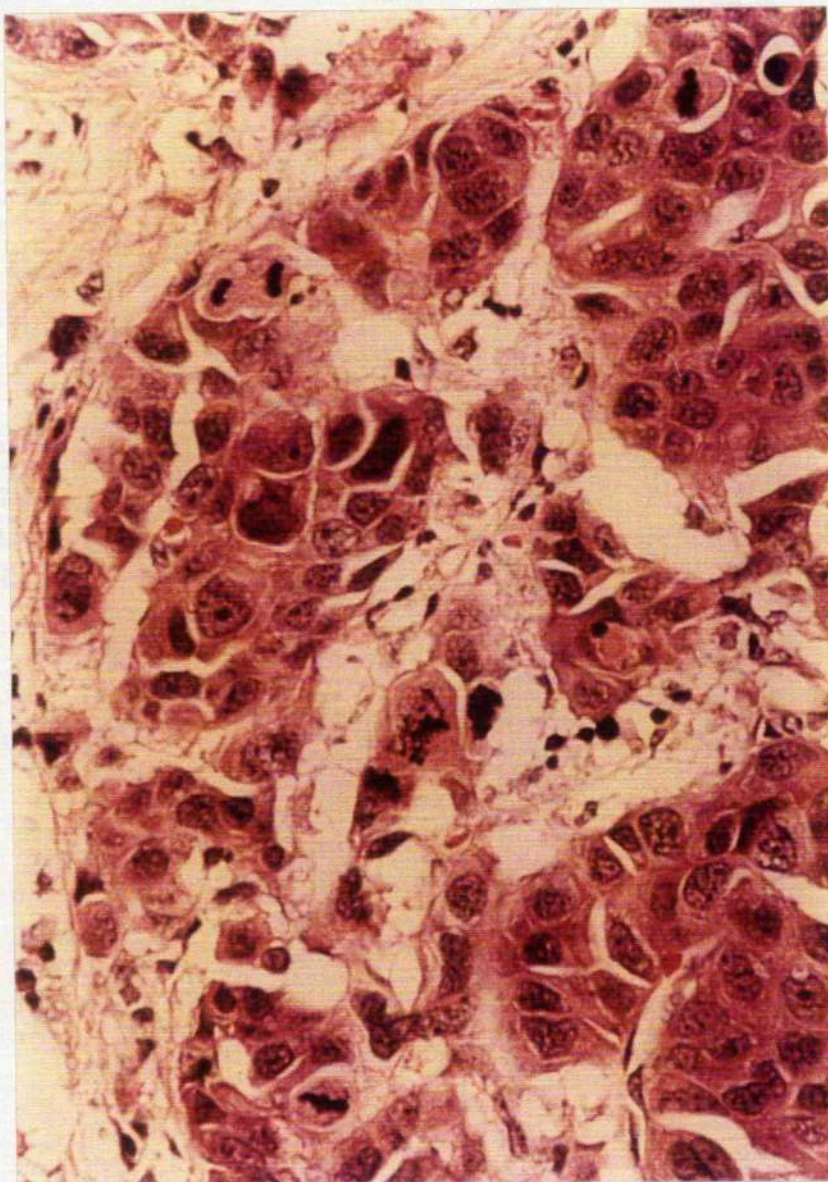
Appendix Fig. 1      Breast cancer: Histological Grade I  
Note regular nuclei, tubule formation,  
scarcity of mitoses.





Appendix Fig. 2      Breast cancer: Histological Grade II  
Note moderate nuclear pleomorphism and the  
rudimentary 'attempt' at tubule formation.





Appendix Fig. 3

Breast cancer: Histological Grade III

Note gross nuclear pleomorphism, widespread mitotic activity, total lack of tubules.

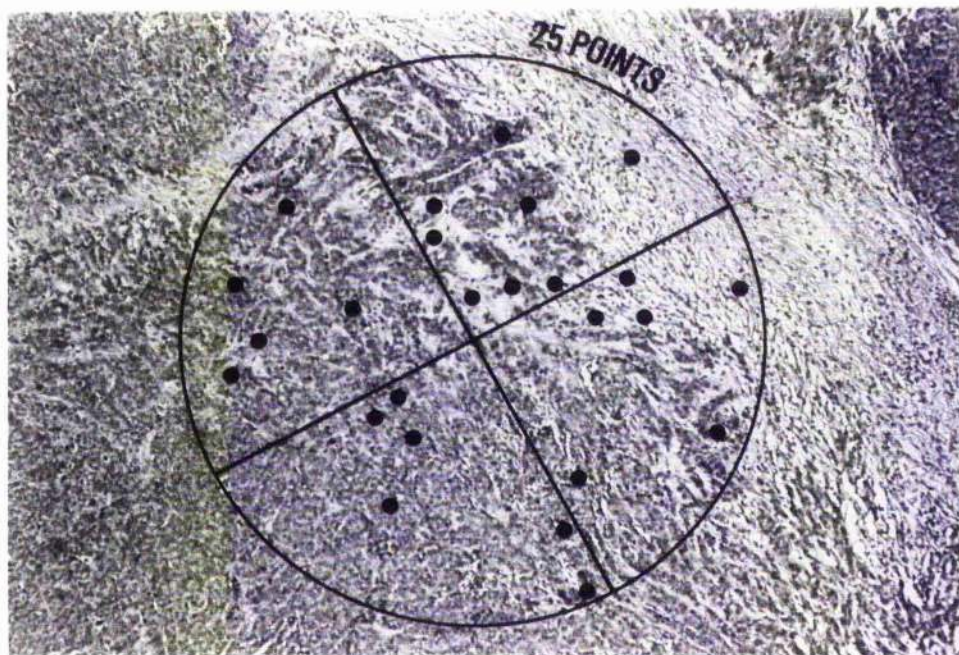
## Appendix IV

### Cellularity Assessment

The method used is essentially that of Underwood (1972). Tumour blocks were taken and fixed in 10% buffered Formalin. Paraffin sections of 4-6  $\mu$ m thickness were cut and stained with haematoxylin and Eosin. Tumour cellularity was evaluated by a proportional count of cancer cells against non malignant background material in all fields of 3-5 histological sections at 63 x magnification. An eyepiece graticule with an array of 25 randomly allocated points was incorporated into a microscope. The points of the graticule appeared superimposed on the field under examination (Fig. 4). In each field, if  $n$  points fall upon tumour cells and  $m$  fall upon non malignant cellular material, then the ratio  $\frac{n}{n + m}$  represents the proportion of tumour cells in that field. All fields covering the whole surface area of 3-5 histological sections were counted. A mean of 87 fields were counted, with a mean point count of 2,200 per tumour, which is representative of the proportion of malignant cells in the whole three dimensional tumour with a low standard error (Dunnill, 1968).

The total ratio  $\frac{n}{n + m}$  for all fields was summated in each tumour and expressed as a percentage. That value was designated the percentage tumour epithelial cellularity.





Appendix Fig. 4

Cellularity assessment.

Single field of highly cellular tumour with graticule superimposed. Note that 22 of the 25 points fall on tumour cells.

Haematoxylin and Eosin x 63.

## Appendix V

### Prostaglandin E<sub>2</sub> Radioimmunoassay

Tumour samples taken at mastectomy were frozen and stored at -200°C in liquid nitrogen and assays were performed in batches. There were three steps to the assay procedure.

#### 1. Incubation

Four 50 mg tumour blocks were weighed out accurately and placed in four labelled tubes.

- a) 900 ul of Acidified Krebs Ethanol solution which is a potent inhibitor of prostaglandin synthesis, was added to two of the tubes. Incubation with this solution provides a 'BASAL' prostaglandin value which is indicative of extracellular Pg and amounts liberated by tissue preparation and does not represent prostaglandin production.
- b) 900 ul 5% Arachidonic Acid (the precursor of the 'E' series prostaglandins) in Krebs were added to the remaining two tubes to promote Pg synthesis to give a 'TOTAL' level. This was the maximum value of prostaglandins produced by that tissue.

All tumours were homogenised and incubated at 37° C for 15 minutes and then, reactions were stopped by the addition of 100 ul at 3% Formic Acid.

#### 2. Extraction

Prostaglandins of all classes are extracted into Chloroform. Chloroform was mixed into each tube and centrifuged and the heavier Chloroform and prostaglandin layer was removed by pipette into a second tube. The Chloroform was removed under a stream of nitrogen and samples were diluted with radioimmunassay buffer to obtain prostaglandin concentrations within the calibration curve 0.03 - 5 ng/ml. (see below)

### 3. Radioimmunoassay

Each assay included standards of pure prostaglandin  $E_2$  for its own calibration curve ranging from 0 to 5.0 ng prostaglandin  $E_2$ /ml. Fifty microlitres of \*Miles Yeda anti prostaglandin E antisera which has 100% cross reactivity with  $PgE_2$ , 85% reactivity with  $PgE$  and only 2% reactivity with  $PgF$  was added to each unknown sample and incubated at  $4^\circ C$ , to equilibrium. Then 100  $\mu$ l of tritiated  $PgE_2$  (30,000 - 50,000 cpm/ml) was added to each tube and incubated for one hour to cause a shift of equilibrium and competition with sample  $PgE_2$  for binding sites on the antibody. Unbound tritiated  $PgE_2$  was then absorbed onto Dextran coated charcoal, the mixture centrifuged and supernatant removed, leaving the charcoal pellet with the unbound  $^3H - PgE_2$ , which was discarded. Liquid scintillant was added to supernatant and radioactivity counted over 5 minutes. Prostaglandin  $E_2$  values were obtained by plotting the radioactivity count against the standard calibration curve. Three measured  $PgE_2$  values were obtained for each tumour: Total, Basal and "Synthesised" (synthesised  $PgE_2$  = Total activity minus Basal values).

\* Miles Yeda Ltd., Kiryat Weizman, Rehovot, Israel.

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