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Smith, Patricia Mary

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An immunohistochemical technique was employed in the investigation of 138 cases of primary breast carcinoma, presented at Dryburn Hospital, Durham, and treated by mastectomy. The subsequent progress of these patients was then investigated, survival estimated by the life table method and comparisons made by the log rank test. No relationship was found between the presence of CEA in the tumour and patient survival for up to 8 years post surgery. Similar investigations detected no relationship between the presence of CEA and the age, weight and menopausal status of the patient or the presence of synchronous lymph node metastases, or the histological grade or size of the tumour.

It therefore appears that detection of CEA in breast carcinomas is of no prognostic significance.

CARCINOEMBRYONIC ANTIGEN IN BREAST CANCER:

A RETROSPECTIVE STUDY.

A thesis submitted to the University of Durham (Faculty of Science) in fulfilment of the regulations for the degree of Master of Science.

Patricia Mary Smith B.Sc. (Dunelm)

Department of Histopathology,
Dryburn Hospital,
Durham.

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Chapter 1: INTRODUCTION

Oncofoetal antigens

Hirzfeld, Halber and Rosenblat (1932) were the first to demonstrate that tumour tissues contained antigenic substances previously thought to be confined to embryonic tissues. Interest in such oncofoetal antigens was revived by Abelev and his co-workers (Abelev, Perova, Khramkova, Postnikova and Irlin, 1963) who discovered alphafetoprotein in chemically induced murine hepatomas. Subsequently several oncofoetal antigens have been described, and interest in this group of proteins has persisted due to their potential application in the detection, diagnosis, prophylaxis and therapy of cancer (see Alexander, 1972; Laurence and Neville, 1972; Costanza and Nathanson, 1974).

Carcino embryonic antigen (CEA) is an oncofoetal antigen which was first demonstrated in human colonic tumours after a directed search (Gold and Freedman, 1965a). These workers prepared extracts of human colonic adenocarcinoma, raised antisera to these extracts in rabbits and used an immunoprecipitation technique to compare these with extracts of normal colonic tissues taken from the same individuals. In all cases CEA was confined to the tumour tissues. Subsequently smaller amounts of CEA were found in primary carcinomas from other sites along the gastrointestinal tract (oesophagus, stomach and pancreas) as well as in the liver, but not in normal adult tissues or in benign tumours of the digestive tract. CEA was not detected in rectal and colonic tissues which were affected by non neoplastic diseases such as ulcerative colitis and

diverticulitis (Gold and Freedman, 1965b). In the same series of experiments CEA was detected in metastatic tumours originating from a primary tumour in the gastrointestinal tract, even though the secondary tumours were at sites remote from the gut (lung and liver). Conversely, CEA was not detected in metastatic tumours of the gut which were derived from primary tumours of the prostate and ovary. It was therefore concluded that the presence of this antigen reflected the site of the primary tumour rather than the site of secondary growth.

In experiments with foetal tissues Gold and Freedman (1965b) demonstrated that CEA was present in foetal gut, liver and pancreas between 2 and 6 months gestation. They concluded that CEA was a normal cellular component of certain foetal tissues whose production was repressed during the differentiation of the gastrointestinal tract. They proposed that the antigen reappeared during oncogenesis by a process of derepressive dedifferentiation (see Jacob and Monod, 1961). More recent studies of cell surface alterations in virally induced tumours have suggested that an equally appropriate mechanism may be the uncovering of surface antigens at "cryptic" sites which are not normally exposed in adult tissues (Hayry and Defendi, 1970; Burger, 1971). CEA was therefore regarded as having potential value in the diagnosis of certain tumours of the gastrointestinal tract.

Subsequently, CEA has been isolated and shown to be a water-soluble glycoprotein with a molecular weight of 200,000. The chemical properties of CEA varied little between different tumours (Krupey, Gold and Freedman, 1967,

1968). The cellular localisation of CEA was investigated using immunofluorescence, (Gold, Gold and Freedman, 1968; von Kleist and Burtin, 1969) and electron microscope immunohistochemistry (Gold, Krupey and Anson, 1970). It was found to be localised to the cell membranes and the superficial glycocalyx of reacting tumours.

Gold (1967) found that antibodies to CEA could be found in the sera of 70% of all patients presenting with primary tumours of the digestive tract, whilst it was invariably absent from patients in whom dissemination (dispersion of the disease) was known to have occurred. The author proposed that the anti-CEA activity was removed from the patient's serum either by being bound to the tumour cells, or by combining with CEA released into the circulation by the tumour. However, Collatz, von Kleist and Burtin (1971) found no evidence that there were any specific anti-CEA antibodies in patients' sera and suggested that this protein was not autoantigenic, and that the autoantibodies demonstrated by Gold (1967) were directed against normal tissue proteins present in perchloric acid extracts of colonic tumours.

The next development was the direct assay of CEA in the serum. Radioimmunoassay techniques were modified to provide a sensitive assay capable of detecting 1-2 ng of CEA per ml of serum (Thompson, Krupey, Freedman and Gold, 1969). Using this technique CEA was detected in the serum of almost all patients with adenocarcinoma of the large intestine, but was not detected in sera from patients with malignancies arising outside the digestive system, from patients with non-malignant diseases or from healthy

individuals. These findings supported the view that CEA was a system-specific oncofoetal antigen (Gold and Freedman, 1965a, 1965b).

Martin and Martin, (1970), using a modification of the extraction technique described by Gold and Freedman, (1965a, 1965b), were able to demonstrate that low concentrations of CEA were present in normal adult colon as well as non malignantly diseased colon. These findings suggested that the difference in CEA levels between cancerous and non-cancerous tissues may be quantatative rather than qualitative. Similarly, Moore, Kupchik, Marcon and Zamchek (1971), using the same technique as Thompson et al. (1969), detected raised CEA titres in patients with tumours arising outside the gastrointestinal tract. CEA levels were also elevated in patients with chronic renal failure and chronic liver disease. A new radioimmunoassay technique was used by Lo Gerfo, Krupey and Hanson (1971) who detected raised levels of CEA in most patients with adenocarcinoma of the large intestine. However, these workers also found that serum CEA levels were elevated in patients with primary tumours of other organs, including lung, prostate and breast. Lo Gerfo et al. (1971) proposed that their antiserum may react with a different antigenic site on the CEA molecule to that used by Thompson and his collegues (Thompson et al., 1969), and that this site is common to several different types of tumour cell. There was also evidence to suggest that the carcinoembryonic antigen system may be heterogenous (von Kleist and Burtin, 1969; Kleinman, Harwell and Turner, 1971).

The occurrence of CEA has since been extensively

investigated in numerous tumours, various diseased and normal tissues, as well as in the serum of such individuals. It has been studied in carcinomas of the lung (Sehested, Hirsch and Hou-Jensen, 1981; Sun, Edgington, Carpentier, McAffee, Terry and Bateman, 1983); ovary (Heald, Buckley and Fox, 1979; Takeda, Matsuyama, Kuzuyak, Chihara, Tsubouchi and Takeuchi, 1984); in hypoplasia and adenocarcinoma of the cervix (Speers, Picaso and Silverberg, 1983); in benign (Penneys, Nadji and Morales, 1982) and malignant tumours of sweat glands (Penneys, Nadji, Ziegels-Weissman, Ketabchi and Morales, 1982); in normal and hyperplastic thyroids, and medullary thyroid carcinoma (Lloyd, Sisson and Marangos, 1983; Calmettes, Caillou, Moukhtar, Milhaud and Gerard-Marchant, 1982), as well as in normal gall bladder epithelium and all histological types of carcinoma of this organ (Albores-Saavedra, Nadji, Morales and Henson, 1983). Exfoliated bladder tumour cells have also been found to have a wide range of CEA content (Wahren, Esposti and Zimmerman, 1977).

CEA has also been detected in body fluids, including the pancreatic juice from benign and malignantly diseased pancreata (Tatsuta, Yamamura, Yamamoto, Okano, Morii, Okuda and Tamura, 1983), amniotic fluid (Oehr, Bellman and Hamann, 1982), and the in fluid from benign breast cysts (Fleisher, Oettgen, Breed, Robbins, Pinsky and Schwartz, 1974).

CEA-related antigens, including one moiety distinctive for CEA, have been detected in normal adult feces (Kuroki, Koga and Matsuoka, 1981), and CEA has been detected in the

blood of asymptomatic adolescents (Tabor, Gerety, Needy, Elisberg, Colon and Jones, 1981). Elevated blood levels of an antigen similar to human CEA have even been recorded in gorillas and chimpanzees, (Haagensen, Metzgar, Swenson et al., 1982).

Breast cancer

Breast cancer is the commonest cancer in women, causing over 12,000 deaths a year in the U.K. at the present time. An individual woman has a 1:14 chance of contracting the disease during her lifetime and it is the commonest cause of death amongst women in the 35-55 age group (see Baum, 1981). In the USA 27% of all malignant neoplasms in women are breast cancers (see Ruddon, 1981). An untreated victim of this disease can expect only 17.2% of the normal duration of life (Greenwood, 1926). Among women in the United States there has been little change in the overall death rate from 1930 to 1967 (see Seidman, 1969), this being maintained by small increases in both incidence and survival. Breast cancer occurs about 100 times as frequently in women as in men (Seidman, 1969).

There is considerable variation in the progress of cases of breast cancer, even in patients of the same age, with the same duration of symptoms and with tumours of comparable clinical extent. Women presenting with the disease in an advanced state and a long history may survive for many years with only limited treatment, whilst patients who attend hospital early with what appears to be only a localized growth may die of metastases within months of radical surgery and a full course of post-operative

radiotherapy. Knowledge of the natural history of cancer affects therapeutic decisions, and this is especially true of breast cancer.

Traditionally it was assumed that a cure for breast cancer was possible provided that the disease was confined to the breast and axillary lymph nodes. Syme (1842) proposed a hypothesis to explain the biological behaviour of breast carcinoma. He wrote "...The result of operations for carcinoma of the breast when the glands are affected is almost always unsatisfactory, however perfectly they seem to have been taken away. The reason for this is that the glands do not participate in the disease unless the system is strongly disposed to it...".

Fifty years later Halstead (1907) took a contradictory stance, stating "...We believe that cancer of the breast, in spreading centrifugally preserves, in the main, continuity with the original growth, and before involving the viscera, may become widely diffused along surface planes...". This second hypothesis rapidly became the accepted medical dogma.

In the early part of this century radical mastectomy was the treatment of choice for operable primary carcinoma of the breast. The common failures of this approach were usually explained as being the result of new cancers rather than of a failure of surgical technique.

By the mid 1930s it was recognised that radical mastectomy cured only a minority of patients, although failures were ascribed either to the assumption that the patient did not present early enough, or to the operation not being sufficiently radical (see Baum, 1979). This

situation led to the adoption of screening programs to facilitate early diagnosis, to the use of super-radical surgical techniques, and to the use of radiotherapy in addition to surgical treatment. At the time there was no evidence to suggest that the introduction of radical mastectomy confered any advantage over the more conservative techniques employed before Halstead (see Baum, 1979).

McWhirter (1948) introduced a treatment program consisting of a simple mastectomy followed by radiotherapy of the axilla, the supraclavicular lymph node bearing areas and the chest wall. This author contended that any improved survival of patients treated by radical mastectomy was an artefact arising from the process of patient selection. He maintained that only 56% of patients were operable by radical surgery, and that adoption of his combination of radiotherapy with less radical surgery would result in local control of a larger proportion of patients with breast cancer. It has subsequently been shown that addition of radiotherapy to any surgical treatment of breast cancer results in a lower incidence of local or regional recurrence. This is most clearly seen in patients with involvement of the axillary lymph nodes (Weichselbaum, Marck and Halman, 1976; Brady, Fletcher and Levitt, 1977), although Brady et al. (1977) failed to demonstrate that radiotherapy significantly improved patient survival. It has yet to be demonstrated that local control cannot be equally well achieved if radiotherapy is given at the first sign of recurrence. (Easson, 1968; Chu, Lin, Kim, Hun and Garmatis, 1976; Madoc-Jones, Nelson and Montague, 1976).

Prospective randomized trials were set up to investigate the effect of radiotherapy on survival and the use of less extensive surgical procedures. These studies were of two types, either a comparison of a radical surgical procedure with a less radical surgical procedure plus radiotherapy (Brinkley and Haybittle, 1971; Hamilton, Langlands and Prescott, 1974; Burn, 1974;), or a comparison of two groups of patients in which the only difference in therapy consisted of the addition of radiation. (Fisher, Slack, Cavanaugh, Gardner and Ravdin, 1970; Easson, 1968). It was concluded that there was no statistical difference in survival between patients given one or another therapy. Numerous criticisms of these studies were made, (Fisher, 1973), the most important being the relatively small number of patients involved.

Devitt (1965) suggested that "...axillary lymph node metastases are an expression of bad prognosis rather than a determinant...". This implied that women with primary breast carcinoma without metastases in the axillary lymph nodes may not necessarily exhibit a chronologically early stage of the disease, but a form which is biologically favourable, the axillary nodes having successfully destroyed any cancer cells that arrived within their substance and perhaps playing a role in the maintenance of systemic immunity. Surgical removal or radiotherapy of the lymph nodes could therefore be detrimental to the patient (Stjersward, Jondal, Vanky, Wigzell and Sealy, 1972). It has been speculated that the lymph nodes may serve as a barrier to the spread of metastases (Baum and Coyle, 1977).

Therefore in recent years the popular hypothesis is a

restatement of that proposed by Syme, (1842), that in the majority of cases, carcinoma of the breast must be a systemic disease, however small the primary tumour may appear. The magnitude of the residual tumour burden following the mastectomy is indirectly reflected by the extent of involvement of the axillary nodes. The axillary node involvement therefore is not a determinant of prognosis but merely a symptom of the most aggressive forms of this heterogenous disease (Fisher, 1970). This theory better fits the available data concerning the behaviour of early breast carcinoma, and systemic options of therapy are now available for testing this hypothesis.

Since 1970 three investigations have been initiated on a large scale to determine if simple removal of the breast will result in a survival equal to that obtained with more extensive surgical and radiation therapy. (Cancer Research Campaign Working Party, 1976, 1980; Fisher, Montague, Redmond et al., 1977; Lythgoe, Leck, Swindell, 1978). Their findings are almost identical. There are no statistically significant differences in the survival or in the incidence of distant metastases among patients receiving the various treatments, although the patients in the Cancer Research Campaign study (1976; 1980) and the Manchester study (Lythgoe et al., 1977) who did not receive radiotherapy have a much higher incidence of local recurrence. There is no evidence from these trials that regional radiotherapy produces a systemic immunosuppression and prejudices survival, or that patients with intact lymph nodes have a better survival.

At present it is difficult to assess scientifically

the contribution of local therapy to the cure of patients with breast cancer. No single type of local therapy appears superior to all others, and it is not clear that more intensive surgical procedures or the addition of radiation to adequate surgical treatment improves the welfare of most patients. In the majority of women with cancers associated with extensively involved axillary nodes the disease will relapse however radical the primary treatment (Fisher and Slack, 1970; Fisher, Slack, Katrych and Wolmark, 1975).

It is now appreciated that breast cancer is a disease which disseminates early but recurs late, and that local treatment can be expected to achieve cure only if it is applied before dissemination has taken place, which probably means before a breast lump is clinically evident. Brinkley and Haybittle, (1975) question the concept of cure in breast cancer, in that after twenty years, although the survival curve of breast cancer patients runs parallel to the expected survival curve of a similar normal population, the probability of dying from cancer of the breast in this group is still much greater than that in the normal population.

Realization of the nature of this disease has changed the objectives of local treatment. The aim now is to control the local disease, prevent local recurrence, and reduce tumour bulk, facilitating the effect of systemic therapy. Systemic therapy is achieved by hormone manipulation or cytotoxic chemotherapy and may be administered as an adjuvant, or at the time of recurrence.

The original concept that endocrine therapy may cause regression of advanced breast cancer was proposed and used

in treatment by Beatson (1896). It was based on the known method of maintaining lactation in cows by oophorectomy. It remains the primary treatment for advanced or metastatic disease in pre-menopausal women.

Schinzinger (1889) proposed oophorectomy for treatment of primary breast cancer based on the supposition that post-menopausal breast atrophy would contain the tumour. Pelvic irradiation was developed as a technique for ovarian ablation by Taylor (1934), who recommended it as an adjuvant systemic therapy.

Several trials involving adjuvant oophorectomy have been undertaken, (Treves, 1957; Nevinny, Nevinny, Rosoff, Hall and Muench, 1967; Ravdin, Lewison, Slack et al., 1970). Many trials involving ovarian irradiation have also been undertaken, (Nissen-Meyer, 1967; Cole, 1970). However, because of inadequate controls or too few people, it is not clear whether adjuvant oophorectomy or ovarian irradiation will delay relapse or improve survival for patients with primary breast cancer. Further properly designed trials are needed, particularly in conjunction with steroid receptor analysis. Among large groups of patients unselected except for menopausal status, there is probably no meaningful difference in response-rates to exogenous administration of oestrogen, oophorectomy, adrenalectomy, or hypophysectomy, (see Henderson and Canellos, 1980). Androgens, progesterones and corticosteroids are usually reserved for second-line therapy. The better response rates to oophorectomy occurs in premenopausal women and to oestrogens in patients who are more than five years beyond the menopause. Adrenalectomy and hypophysectomy are thought

to be effective because they remove persistent low levels of oestrogens, and so probably cause tumour regression.

Newer types of endocrine therapy include antioestrogens, (e.g. Tamoxifen, which is in general use with few side effects). Approximately one-third of patients treated thus have evidence of objective regression, (Willis, London, Ward, Butt, Lynch and Rudd, 1977).

Breast cancer is known to be responsive to the cytotoxic effect of many antineoplastic agents (see Henderson and Canellos, 1980), including doxorubicin, cyclophosphamide, methotrexate, thiotepia, 5-fluorouracil, phenylalanine mustard, chlorambucil, and vincristine. In early clinical trials of adjuvant chemotherapy, patients were given cytotoxic drugs at the time of mastectomy in order to kill any metastatic cells released into circulation. Cyclophosphamide for six days (5mg/kg daily) after mastectomy was found to prolong survival significantly (Nissen-Meyer, Kjellgren, Malmio et al., 1978).

The large number of endocrine treatments and variety of drug combinations make the selection of a treatment plan for an individual patient a difficult decision. There have been two reports of patients randomized to receive either chemotherapy or endocrine therapy, (Nemoto, Rosner, Diaz et al., 1978; Priestman, Baum, Jones and Forbes, 1978). In both the response rate to cytotoxics was higher, although this was significant only in the first study.

Chemotherapy is associated with toxicity, notably acute nausea, vomiting, myelosuppression, thrombocytopenia, alopecia and increased fatigability. The effects are all

reversible when the drug is discontinued. Early systemic therapy can theoretically treat metastases before they are detectable by methods known at present, but because of the toxicity, the use of adjuvant chemotherapy has been limited to patients with axillary node involvement. Methods are needed which select the groups of women who may benefit from the currently available systemic therapies so as to avoid the unnecessary toxicity for those who will not benefit. Prediction of early recurrence is of immense value in assessing the need, and the appropriate therapy, for selected patients at risk. Cutler, (1968), states that "..it is only through identification and evaluation of factors associated with variation in patient survival that we can hope to develop a more rational and more effective approach to treatment..".

Various factors are known to influence the prognosis of patients who are treated for carcinoma of the breast, (Forrest, 1971; Cutler, 1968). Of particular importance is the status of the axillary lymph nodes at the time of mastectomy. However, patients with medial tumours cannot be assessed adequately, as these tumours drain into the internal mammary nodes which cannot be sampled at mastectomy. Also, once removed, involved nodes no longer provide an index of therapeutic effectiveness, and indeed some patients with involved nodes may survive for long periods.

Bloom and Richardson, (1957), devised a system to grade the primary tumour histologically. This is a general indication of whether or not a tumour is mature or well differentiated. The tumour is assessed by three criteria:-

the degree of structural differentiation as shown by the presence of tubular arrangement of the cells; variation in size or shape or staining of the nuclei; frequency of hyperchromatic and mitotic figures. The potential malignancy of the tumour is determined from the composite histological picture. The tumour is placed in one of three grades of malignancy:- low (Grade I), intermediate, (Grade II), or high, (Grade III). The prognosis of the patient worsens as the tumour becomes less well differentiated.

The nature of malignant breast epithelium has been extensively investigated for a more sensitive marker of disease extent or for some indicator of tumour behaviour. One factor undergoing intensive current research is the presence of steroid receptors in breast tissue. It was established that an important difference between tissue which is dependant upon the synthesis and secretion of oestrogens by the ovary for growth and functional activity, and non-dependent tissues, was that such oestrogen target tissue (eg. uterus, vagina or mammary tissue) were able to concentrate oestradiol. This effect was found to be due to intracellular oestrogen-binding receptors. (Jenson, Suzuki, Kawashima, Stumph, Jungblut, and de Sombre, 1968; Jensen and de Sombre, 1973). The implications here are not only for anti-oestrogen therapy. The report of one study (Knight, Livingstone, Gregory and McGuire, 1977) indicated that patients with receptor-negative tumours recurred earlier. The reports of two recent studies (Maynard, Blamey, Elston, Haybittle and Griffiths, 1978; Cooke, George, Shields, Maynard and Griffiths, 1979) indicate potential value of the oestrogen receptor status of the

primary tumour as a prognostic indicator independent of other established factors such as lymph node status, tumour size, or histological grade.

Many other biochemical "tumour-index" and "tumour-marker" substances have been investigated, and suggested as possible aids in the management of patients with breast cancer.

Purpose of the present study

It has been reported that a relationship exists between the presence of CEA in histological sections of breast carcinoma tissue and the subsequent survival of the patient (Shousa, Lyssiotis, Godfrey and Scheuer, 1979). Although similar claims have been made for other carcinomas, (eg. for lung by Sehested, Hirsch and Hou-Jensen, 1981), the special problems associated with breast cancer make this suggestion very important. Shousa et al. (1979) found that the absence of CEA from a tumour was associated with significantly improved patient survival at 5 and 10 years. However, not all studies have given the same result. Walker (1980) was unable to demonstrate correlation between recurrence within two years and the presence of CEA in the tumour. Similarly, Rolf Smith, Howell, Minawa and Morrison (1982) detected no relationship between CEA occurrence in the primary tumour and patient survival.

The present study was therefore undertaken in an attempt to clarify the relationship between histological demonstration of tumour CEA and patient survival for up to eight years following surgery. The relationships between

tumour CEA and the presence of nodal metastases; histological grade of the tumour; size of the tumour, and other factors which are known to influence the prognosis for this disease, were also investigated.

Chapter 2: MATERIALS AND METHODS

i) MATERIALS

Tumour tissues

These were available from storage in the Department of Histopathology, Dryburn Hospital, Durham. They had been removed at surgery, formol saline fixed, routinely processed and wax embedded.

Clinical information

Case notes were available through the Medical Records Department, Dryburn Hospital. Additional follow-up information was obtained through the Radiotherapy Department and Cancer Register, Newcastle General Hospital; the local Registrar of Births, Deaths and Marriages, and, in some cases the patients' general practitioner.

Animals

Four rabbits of different strains were obtained through, and housed at the Animal House, Department of Zoology, University of Durham. They all received a standard laboratory diet.

Human CEA

This (1.3 mg) was the kind gift of Dr. G. Rogers, Department of Medical Oncology, Charing Cross Hospital Medical School, London. It had been extensively purified by gel filtration and was believed free from non-specific cross reacting antigen (NCA; Rogers, Searle and Bagshaw,

1976; Keep, Leake and Rogers, 1978).

Immunoglobulins

Rabbit anti-human CEA (commercial), soluble horseradish peroxidase-rabbit anti-peroxidase complexes (PAP) and porcine anti-rabbit immunoglobulins were obtained from 'Dako' Immunoglobulins, Copenhagen, Denmark. Normal porcine serum was obtained either from 'Dako' Immunoglobulins or from a local abattoir.

Stains and other reagents

Light green and basic fuchsin were obtained from Hopkins and Williams, Chadwell Heath, Essex; alcian blue 8GX from Raymond A. Lamb, Waxes and General Supplies, Alperton, Middlesex; eosin, yellow shade, water soluble and Mayer's heamatoxylin CI 75290 from British Drug House Ltd., Poole, Dorset; bactotrypsin and 'Polywax' from Difco Laboratories, Detroit, Michigan; diaminoethylaminoethane (DEAE) Sephadex A-50 and diaminobenzidine hydrochloride (DAB) from 'Sigma' Chemical Company, Poole Dorset, and Freud's complete adjuvent from Gibco Laboratories, New York, USA. All other reagents were of analytical grade and were obtained from British Drug House Ltd..

ii) PRODUCTION OF ANTI-CEA IMMUNOGLOBULIN

Immunization program

Rabbits were immunized with CEA following an adaption of the schedule of Burtin, von Kleist, Sabine and King (1973). Normal blood was taken prior to immunization and

innoculations carried out at two-weekly intervals as follows:

1) Intracutaneous injection of 0.1 mg of CEA in complete Freud's adjuvent.
2) Subcutaneous injection of 0.05 mg of CEA in 0.9% (w/v) saline.
3) Intravenous injection of 0.05 mg CEA in saline
4) Intravenous injection of 0.05 mg CEA in saline
5) Intravenous injection of 0.025 mg CEA in saline

The rabbits were bled via a lateral ear vein 10 days after the last injection.

Immunoglobulin purification

All operations were performed at 4 degrees C. The blood samples were allowed to clot and the serum separated by centrifugation. Solid ammonium sulphate was added slowly to the serum to give a final concentration of 25% (w/v) and the serum left stirring overnight. Precipitated proteins were then removed by centrifugation (Mistral HS18 centrifuge; 10,000 g for 15 minutes) and washed by repeated resuspension in 1.75 M (23%) ammonium sulphate. The washed precipitate was then dissolved in a small volume of distilled water and then dialysed against several changes of distilled water. Lipoproteins, precipitated by the low ionic strength, were removed by centrifugation (10,000 g for 15 minutes). The supernatant was then dialysed against 50 mM sodium acetate buffer (approx. 80 mM sodium acetate adjusted to pH 5 with glacial acetic acid and then diluted to 50 mM sodium acetate with distilled water), and then against distilled water again. The non-dialysable fraction

was then centrifuged and the supernatant again equillibrated with 50 mM sodium acetate buffer by dialysis. This was then passed through a 1.6 x 15 cm column of DEAE Sephadex A-50 which had been equillibrated with the same buffer. The eluate, which corresponds to the IgG fraction of the serum was dialysed against distilled water, lyophilised and stored desiccated at -18 degrees C. A full account of this method is given by Harbee and Ingild (1973).

Prior to use the antibody was absorbed overnight at 4 degrees C, and for 30 minutes at 37 degrees C, with human A and B erythrocytes as cross reaction of anti-CEA with red blood cell antigens has been observed (Holburn, Mach, Macdonald and Newlands, 1974; Isaacson and Judd, 1977).

iii) HISTOLOGICAL METHODS

Preparation of sections

All tissue blocks were re-embedded in wax and 5 micron sections cut as required.

General histology

Heamatoxylin and eosin and alcian blue / periodic acid Schiff's reagent (PAS) stained sections were prepared.

Tumour classification

The presence of ductal, lobular or medullary invasive carcinoma, and lobular in situ or intraductal carcinoma was recorded, as was the presence of Padget's disease.

Tumour differentiation

The tumours were graded according to the Bloom and Richardson (1957) system. From this 3 catagories were recognized: well (3 - 5 of the above system), moderately well (6 and 7) and poorly (8 and 9) differentiated.

Lymphocytic infiltration

This was recorded as none, moderate or extensive.

Macroscopic appearance of tumour

The size of the tumour and edge definition (poor or good) was obtained from the original description.

CEA localisation

This was performed immunohistochemically by the peroxidase anti-peroxidase (PAP) technique (Burns, 1975), a modification of the method of Sternberger, Hardy, Cuculis and Mayer (1969), as detailed below:

1) Dewaxed, hydrated sections were treated with 2% bactotrypsin in 0.2% $CaCl_2$ for 30 minutes at 20 degrees C to expose proteins (Curran and Gregory, 1977, 1978).
2) After washing with phosphate buffered saline (PBS), 0.5% of H_2O_2 in absolute methanol was applied for 30 minutes to destroy any endogenous peroxidase activity.
3) After washing with PBS the slides were flooded with a 1:10 dilution of normal swine serum (NSS) in PBS for 30 minutes at 20 degrees C to block any non-specific binding sites for IgG.
4) Either the partially purified rabbit anti-CEA (0.3

mg/ml), or control (non-immune) rabbit serum diluted 1:40 or the commercially available anti-CEA serum (1:40) was applied to the sections for 30 minutes at 20 degrees C. All dilutions were made in 5% NSS in PBS.

5) After washing with PBS, swine anti-rabbit immunoglobulin serum was applied to the sections at a dilution of 1:100 for 30 minutes at 20 degrees C.

6) PAP was applied at a dilution of 1:40 for 30 minutes at 20 degrees C.

7) The stain was then developed using DAB in 50 mM tris HCl buffer, pH 7.6 which also contained 1% H_2O_2. The peroxidase reacts with the DAB to give a brown stain.

8) Sections were counterstained with light green, dehydrated, cleared and mounted.

Initially the commercially available anti-CEA was used in this staining schedule. However, this antibody is known to be highly contaminated with antibody directed against NCA (Walker, 1980). NCA is known to occur in normal human tissue (von Kleist, Chavanel and Burtin, 1972) and has been found to have the same centroglandular localization as CEA in gastrointestinal tumours (Burtin et al., 1973). In preparation it shares the same extraction procedure as CEA, being finally separated by gel filtration (von Kleist et al., 1972). Sections of normal human spleen, a positive control for NCA (Burtin, Quan and Sabine, 1975) were therefore stained using either the antiserum obtained through 'Dako' immunoglobulins or the partially purified rabbit anti-CEA prepared by the author. The commercial

anti-CEA gave extensive reaction with these sections, whilst the antibody prepared in the laboratory gave no such staining (see plate 1). The commercially available antiserum to CEA was therefore considered unsuitable for the present study, and so the partially purified rabbit anti-CEA was used throughout.

Specificity of staining was checked by absorbing the anti-CEA with the CEA originally used as the antigen (0.3 mg CEA and 0.3 mg anti-CEA in 1 ml of phosphate buffered saline overnight at 4 degrees C). When sections known to be positive for CEA (human liver and breast tissue) were stained using this no staining was observed.

iv) SELECTION OF CASES FOR INVESTIGATION

All were women patients presented consecutively at Dryburn Hospital, between 1st January 1973 and 31st December 1975, with primary breast carcinoma. Those who had been treated previously for breast carcinoma and those who subsequently developed carcinoma in the other breast (10 cases) were withdrawn. This enabled any recurrence to be allocated to a particular primary tumour. Cases where there was no invasive component in the tumuor (3) were withdrawn as these have a different clinical course, and comparisons of survival could not be made fairly. Three cases where no clinical information could be found were also withdrawn. After withdrawals 138 cases were investigated.

v) ANALYSIS OF DATA

All information was collected and recorded onto prepared schedules for computer analysis. Throughout, any unavailable data was recorded as such. A copy of the schedule is enclosed in the appendix. The pathological data has been discussed. The clinical schedule was designed to establish the patient's condition at the time of mastectomy (1st page), variations in operation and treatment (2nd page) and the response or clinical course (3rd page).

The measurement of survival is of central importance in the study of diseases such as cancer, whose outcome is usually fatal. In common with most studies on survival rates for cancer the group of patients presented here require prolonged observation of each patient and exibit incomplete follow-up. That is, a large proportion of the patients are still alive at the time of analysis and so it would be inaproppriate to illustrate the survival rate by a simple graph showing "proportion alive" against "time since mastectomy". Instead survival rate is estimated by the life table or actuarial method. This is a graph showing an estimate of the proportion of a group of patients that will be alive at different times after mastectomy, calculated with due allowance for incomplete follow-up. A principal advantage of this method is that it makes the maximum use of all survival information accumulated up to the close of the study. The life table method was first described in a medical context by Greenwood (1926) and later by Berkson and Gage (1950), Merrell and Shulman (1955), Cutler and Ederer (1958) and Mould (1976). The method has recently

been well explained in a report to the Medical Research Council's Leukaemia Steering Commitee (Peto, Pike, Armitage, Breslow, Cox, Howard, Mantel, McPherson, Peto and Smith, 1977)

An "average" length of survival is expressed by the median survival time, that is, the length of time required for half the patients to have died, and can be derived from the life table by noting the time at which the survival curve crosses 50%. Although the median survival time is preferable to the mean survival time, which should almost never be cited (Berkson and Gage, 1950), median survival times can be unreliable unless the death rate around the time of median survival is high (Peto et al., 1977).

A comparison of the survival between any two subgroups of patients is achieved by employing the logrank test (Mantel, 1966). This test involves counting the number of deaths in each subgroup and comparing it with the extent of exposure to risk of death in that subgroup. The results of the numerical analysis is to arrive at a statistic, termed chi-squared, which may be compared with an appropriate chi-squared distribution in order to estimate a P-value. A full account of this technique is given by Peto et al., (1977). The life table method and logrank test have been employed to analyse survival in studies of the effectiveness of chemotherapy in advanced gastric (Rake, Mallinson, Cocking, Cwynarski, Fox, Wass, Diffey and Jackson, 1979) and pancreatic (Mallinson, Rake, Cocking, Fox, Cwynarski, Diffey, Jackson, Hanley and Wass, 1980) cancer.

A computer program embodying the above techniques and

implemented on an Apple II microcomputer was used to analyse the numerical data in the present study. The author wishes to thank Dr. B.L. Diffey, Department of Medical Physics, Dryburn Hospital, for his help with this part of the project.

Plate 1.

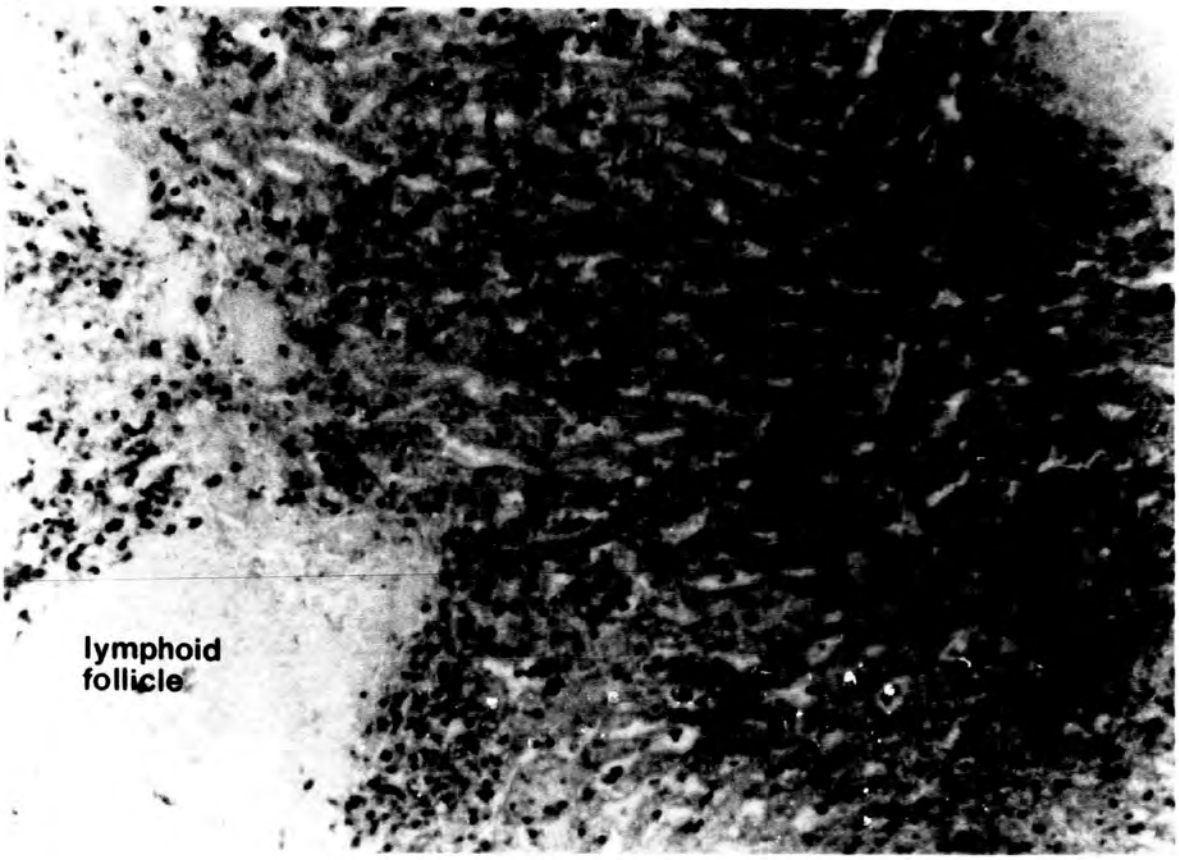
a) Section of spleen stained by the PAP technique using commercially available (Dakopatts) rabbit anti-CEA serum. There is extensive brown staining due to the reaction of this antiserum with NCA which is abundant in splenic tissue outside the lymphoid follicles.

b) Section of spleen stained by the PAP technique using the prepared IgG fraction of raised rabbit anti-CEA serum. There is no staining reaction using this antiserum.

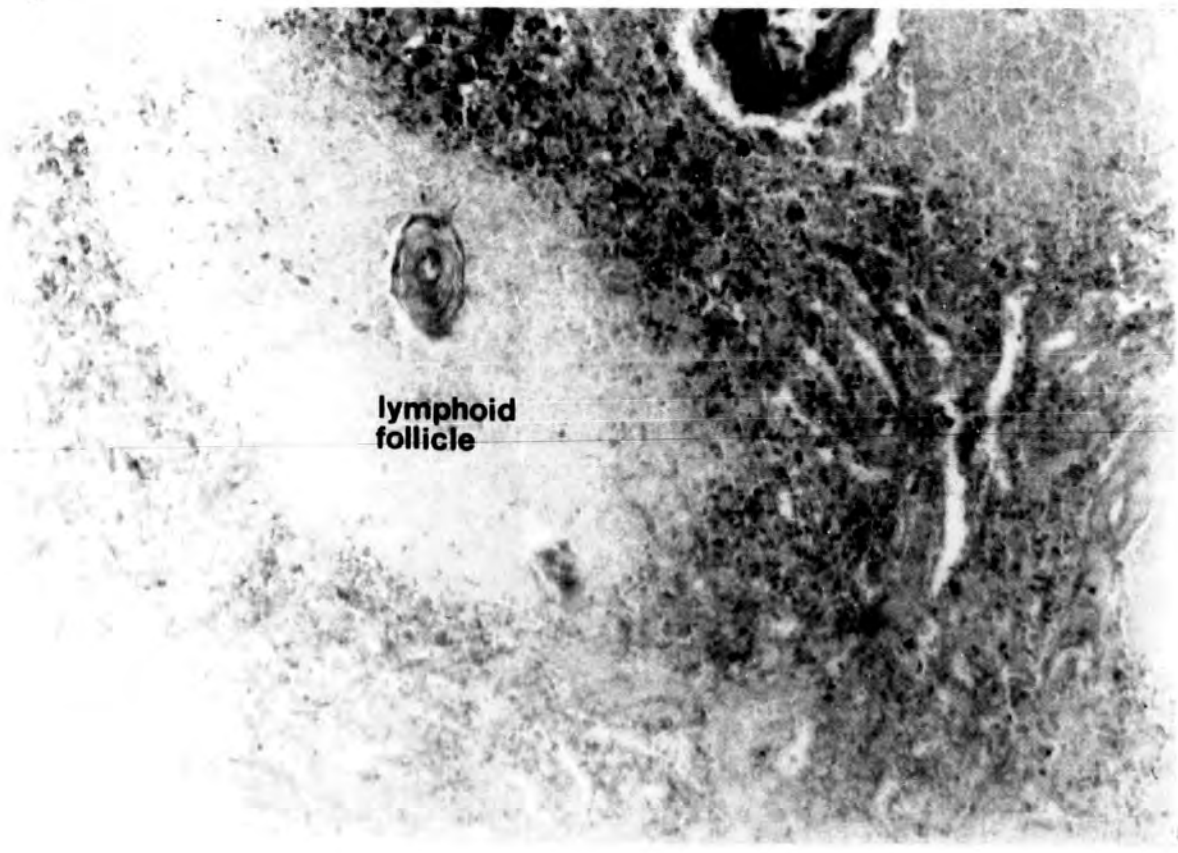
Light Green counterstain employed in both.

Plate 1.

a.



b.



50 μ

Plate 2.

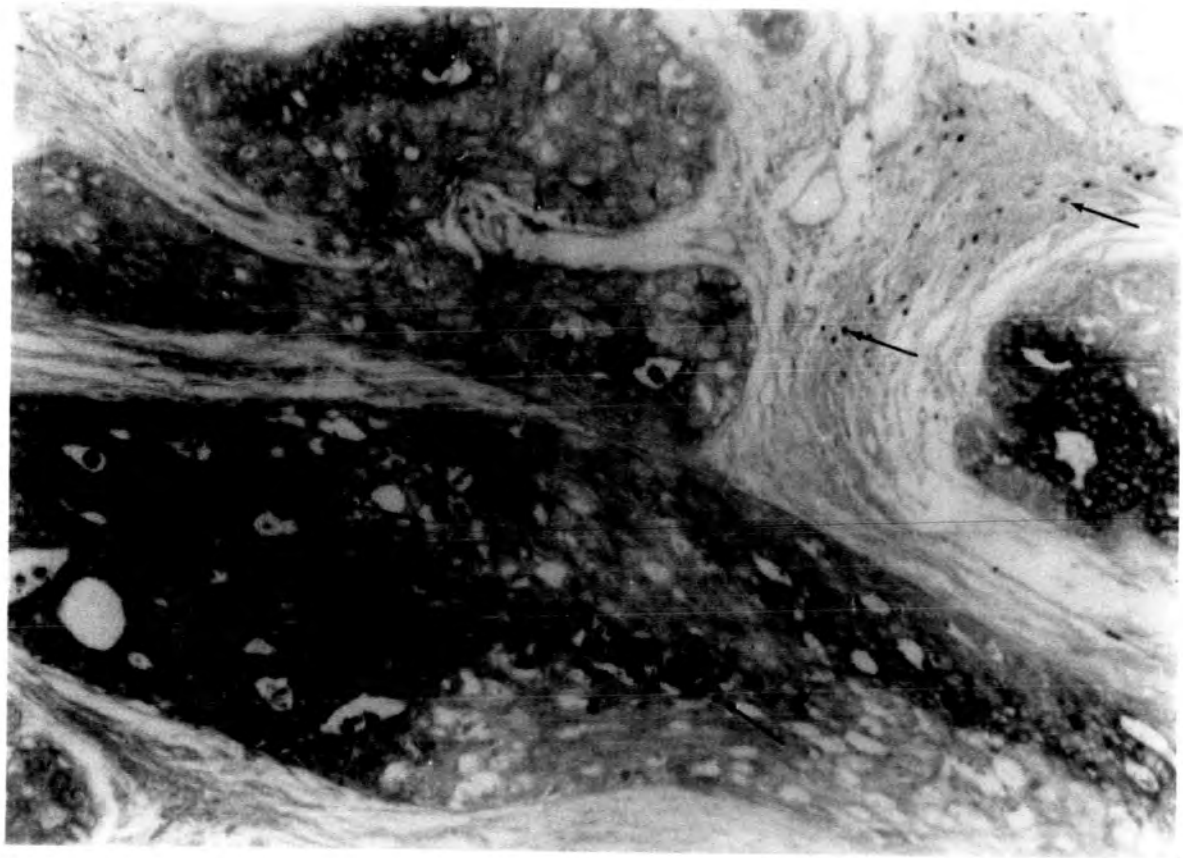
a) Breast tissue - positive control for CEA. Stained by the PAP technique using the commercially available anti-CEA serum.

b) The same breast tissue stained using the partially purified IgG fraction of the raised rabbit anti-CEA serum.

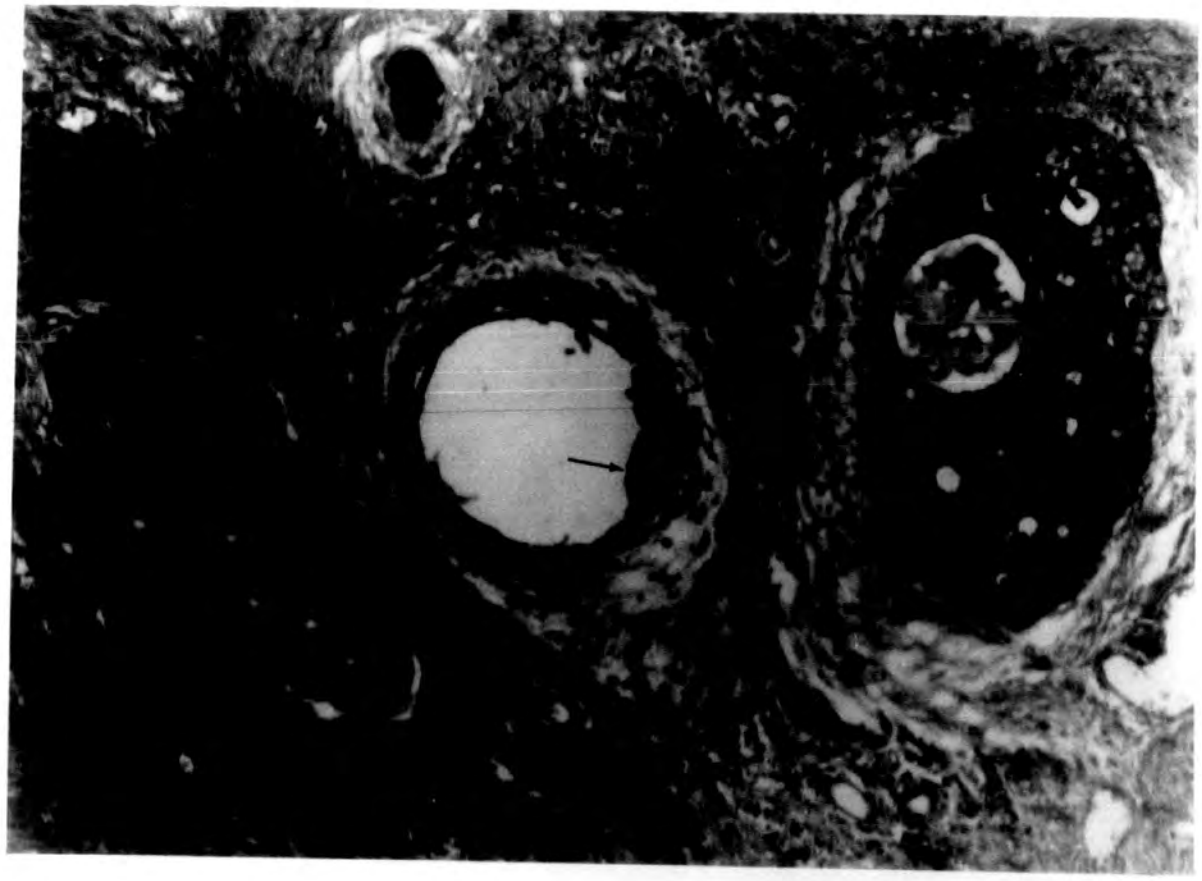
Light Green counterstain employed in both. Positive staining where dark deposition (arrows).

Plate 2.

a.)



b.)



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Chapter 3: RESULTS

GENERAL

The population of patients investigated in this study had, at the time of operation, an age range of 29 to 88 years, the mean age being 58.2 years (S.D. 12.43 years). They had a mean weight of 64.32 kg (S.D. 11.20 kg), the range being 43 to 105 kg, with six cases unknown.

Of these 138 cases of primary breast carcinoma 13 (9.4%) were of the invasive lobular variety, and one was a medullary carcinoma. Of the ductal invasive carcinomas 42 (30%) also had an intraductal component.

LOCATION OF CEA.

The PAP technique performed upon sections of primary breast carcinoma produced brown precipitation at the site of CEA occurrence.

In well differentiated carcinomas positive staining, when found, was located within the lumina of tubules and in the luminal border of the ductal epithelial cells. In less well differentiated carcinomas the staining was diffusely intracytoplasmic in areas where the cells formed solid groups.

Typical CEA localization in primary breast carcinomas is illustrated in Plates 3 a) and b), and 4 a) and b).

CEA was demonstrated in tissue sections from 60 of the 138 cases investigated, (43.5%).

Plate 3.

a) Case 95 - A ductal invasive carcinoma, histologically Grade 1. Nodal metastases were detected at mastectomy. Patient died 2yr 2mth after mastectomy. CEA staining grade 3+.

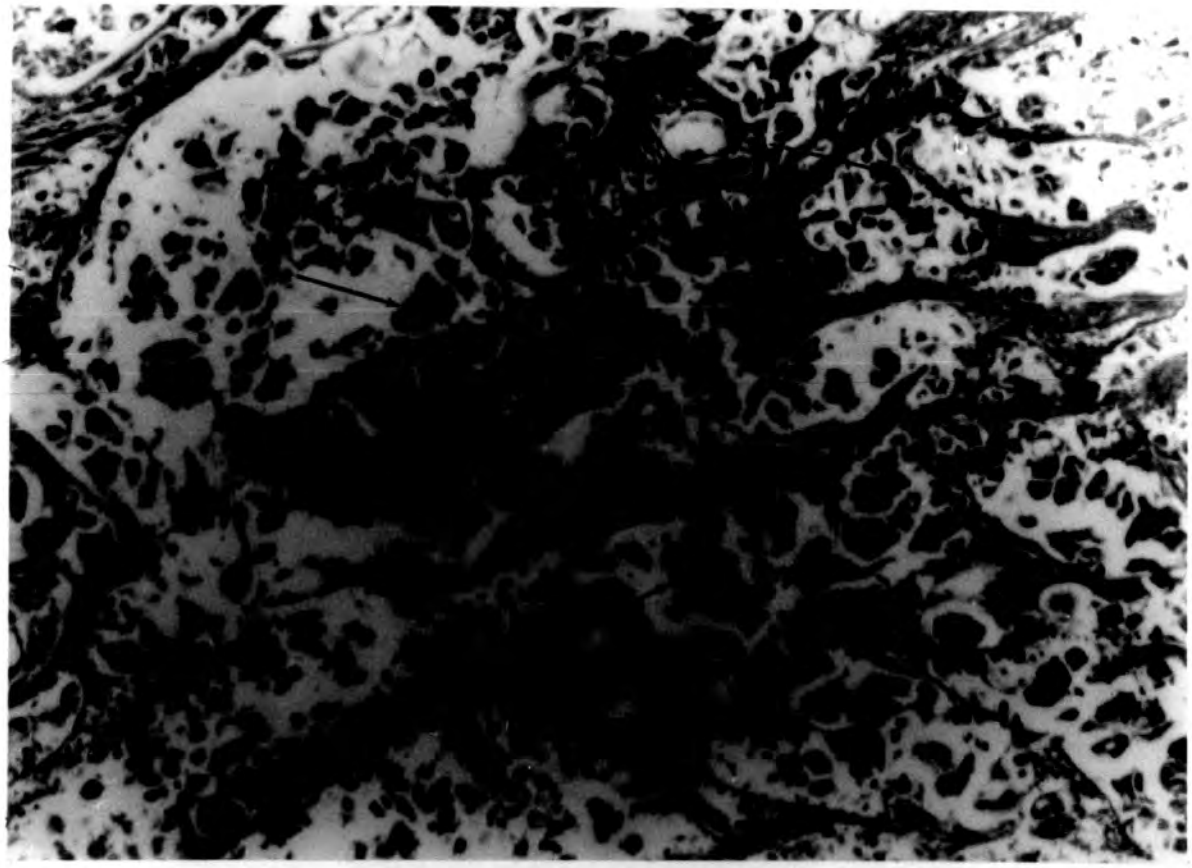
b) Case 141 - A ductal invasive carcinoma, histologically Grade 2. No lymph nodes were found at mastectomy for pathological investigation. Patient alive and well with no recurrence at 8yr post mastectomy. CEA staining grade 3+.

Light Green counterstain employed in both. Positive staining where dark deposition (arrows).

Plate 3.

a)

b)



50 μ

Plate 4.

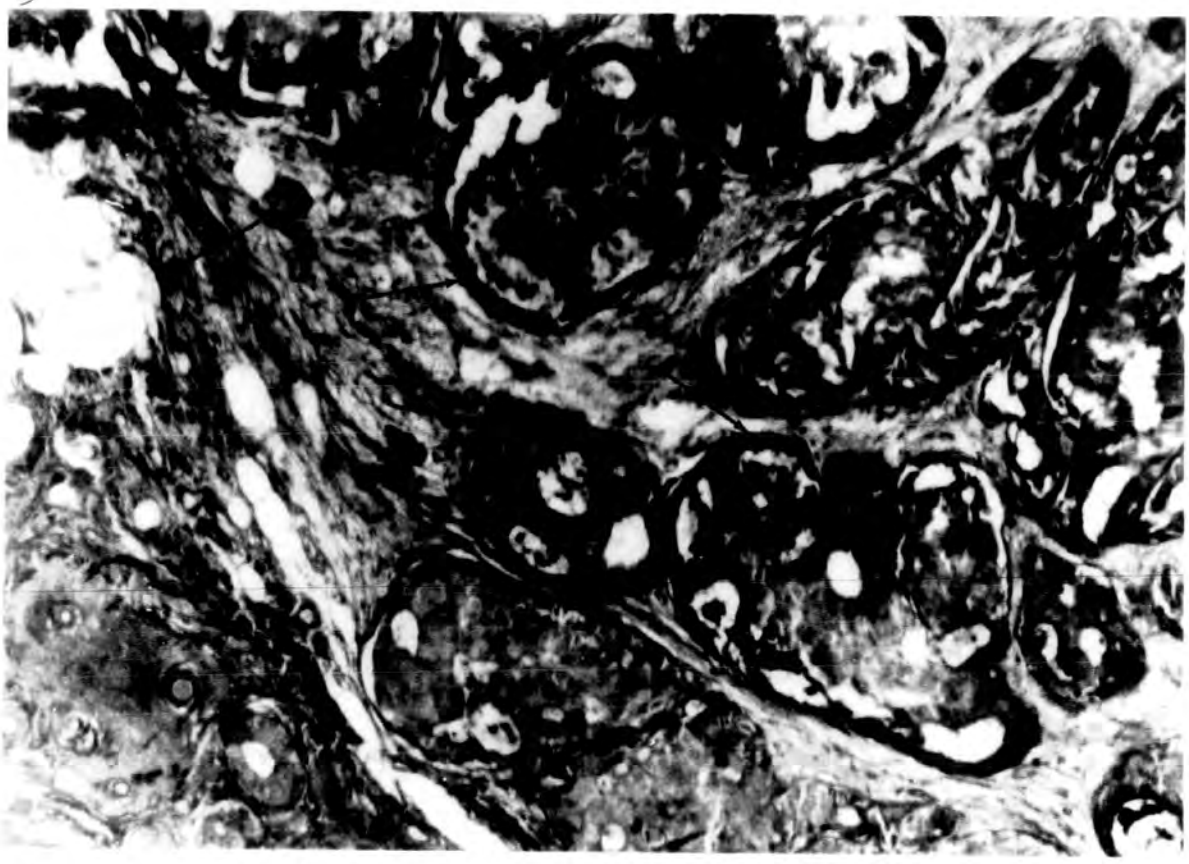
a) Case 99 - A ductal invasive carcinoma, histologically Grade 1. No pathological assessment of lymph node status was made at the time of mastectomy. Patient alive and well with no recurrence at 8yr post mastectomy. CEA staining grade 2+.

b) Case 92 - A ductal lobular invasive carcinoma, histologically Grade 1. Nodal metastases were found at the time of mastectomy. Patient alive and well with no recurrence at 8yr post mastectomy. CEA staining grade 3+.

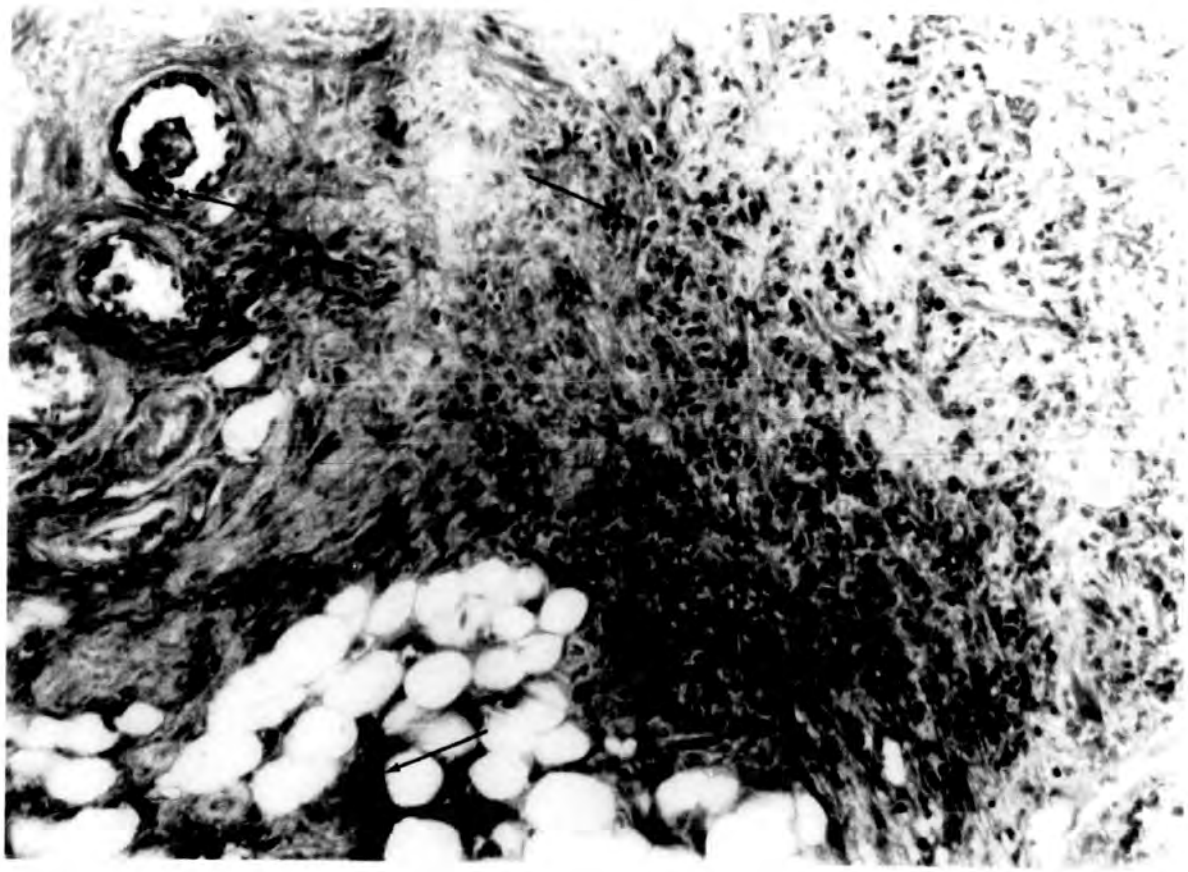
Light Green counterstain employed in both. Positive staining where dark deposition (arrows).

Plate 4.

a)



b)



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OCCURRENCE OF CEA.

Age and weight of patient.

Figure 1 shows the age distribution of the population studied, and also the age distribution in the CEA positive sub-population. The overall frequency of CEA occurrence (43.5%) was used to predict an expected number of CEA positive patients within each age group. These values did not differ significantly (chi squared test) from the observed values, indicating that patient age had no influence on CEA occurrence. The data for weight distribution (fig. 2) were treated in the same manner, and again the observed distribution of CEA positive tumours did not differ significantly from the expected assuming that the weight of the patient is incidental.

Menopausal status of the patient.

The relationship was investigated between the frequency of CEA detection in the primary tumour tissue and the menopausal status of the patient at the time of mastectomy. The results are given in table 1, and show that the presence of CEA is not related to the menopausal status of the patient.

Size of the tumour.

The frequency of CEA detection in the primary tumour tissue was investigated when the patients were grouped according to the size of that tumour. Table 2 shows that there is no reason to suppose that the presence of CEA is related to the size of the tumour. Fig. 3 shows the size

distribution of the tumours examined and of the CEA positive sub-population. No significant difference between these distributions was found.

Attachment of the tumour.

Table 3 shows the results of the comparison of the frequency of CEA occurrence when the patients are grouped according to any attachment of their tumour, either to the overlying skin or nipple or to the deep tissues. No relationship between CEA occurrence and attachment of the primary tumour was demonstrated.

Lymphocytic infiltration of the tumour.

The frequency of CEA detection was compared when the patients were grouped according to the extent of lymphocytic infiltration of their tumour, and the results are given in table 4. These results show that the presence of CEA is not related to lymphocytic infiltration of the tumour.

Intraductal component of the tumour.

The frequency of detection of CEA in the primary tumour was compared when the cases were grouped according to the presence or absence of intraductal carcinoma in the tumour tissue. The results given in table 5 show that the frequency of demonstration of CEA is significantly greater in invasive tumours which also have an intraductal component (chi-squared = 4.587, df=1, $0.05>P>0.02$).

Histological grade of the tumour.

The comparison was made between the frequency of CEA detection in the primary tumour when the cases were grouped according to the histological grade of differentiation of the primary tumour, (Bloom and Richardson, 1957). From the results shown in table 6 it is evident that the presence of CEA in the tumour is not related to the histological grade of the tumour. Fig. 4 shows the distribution of differentiation of tumours sub-grouped into components of the Bloom and Richardson grading system. It also shows the distribution of the CEA positive sub-population. These did not differ significantly.

Lymph node status of the patient.

The frequency of CEA detection in the primary tumour was compared when the patients were grouped according to their lymph node status at the time of mastectomy. The lymph node status was assessed clinically at patient presentation by palpation, and pathologically by microscopic examination of axillary lymph nodes sampled at the time of mastectomy. The latter assessment could not be made for every case, as although the present policy at Dryburn Hospital is to sample the axillary lymph nodes at every mastectomy, in this retrospective study only 66 of the 138 cases had undergone this investigation. The results do not indicate that the presence of CEA is related to the lymph node status of the patient, as assessed clinically (table 7), or pathologically for the presence of metastases (table 8), or the number of metastatic lymph nodes (table 9).

SURVIVAL AND THE PRESENCE OF CEA IN THE PRIMARY TUMOUR.

Patient survival and the presence of CEA in the tumour.

Survival following mastectomy was investigated when the patients were grouped according to the presence or absence of CEA in their excised tumour. The results are summarised in table 10. From these results it is evident that the presence of CEA in the tumour is of no overall prognostic guide to survival. This finding is shown graphically in fig.5, where it may be seen that the survival curves for patients with and without CEA in the tumour are virtually superimposed.

The patients were then grouped according to the extent of CEA occurrence in the tumour. Patients with CEA negative or weakly positive tumours (assessed as 0, +/-, +) were compared for survival with patients who had strongly CEA positive tumours (++, +++). These results are shown in fig. 6, and table 11, and indicate no significant difference between these groups.

Survival, CEA and menopausal status.

The relationship of patient survival according to the detection of CEA in the primary tumour was invesigated for patients who were pre-, peri-, and postmenopausal at the time of mastectomy independently. These comparisons are summarised in tables 12 for premenopausal, 13 for perimenopausal, and 14 for postmenopausal patients. The results offer no evidence that the presence of CEA in the tumour is of prognostic value within any of these subclasses. The survival curves shown in figs. 7 for pre-,

8 for peri- and 9 for postmenopausal patients illustrate these findings.

Survival, CEA and size of tumour.

The relationship of patient survival to the detection of CEA in the primary tumour was investigated for patients with tumours less than 5 cm. diameter, and for patients with tumours of 5 cm. or greater in diameter. The comparison of patient survival following mastectomy according to the presence or absence of CEA in the excised tumour is summarised in table 15 for the smaller tumours, and table 16 for the larger tumours. The survival curves are shown in figs. 10 and 11 respectively. The presence of CEA does not appear to be of prognostic value in groups of patients with large or smaller sized primary tumours.

Survival, CEA and histological grade of tumour.

The comparison of patient survival following mastectomy when the cases are grouped according to the presence or absence of CEA in excised, histologically grade 1 tumours is summarised in table 17. A similar comparison made for histologically grade 2 tumours is shown in table 18, and grade 3 tumours in table 19. The survival curves illustrating these relationships are given in figs. 12, 13, and 14 respectively. These results indicate that the presence of CEA cannot be used as a guide to survival even after patients have been grouped as having well, moderately well, or poorly differentiated primary tumours.

Survival, CEA and lymph node status of the patient.

The relationship of patient survival to the detection of CEA in the primary tumour was investigated for patients whose lymph node status was assessed pathologically at the time of mastectomy. The comparison of patient survival following mastectomy with the presence or absence of CEA in the excised primary tumour is summarised in table 20 where patients are known to have had metastatic lymph nodes at the time of mastectomy. Table 21 summarises the same comparison for patients who were found to have uninvolved lymph nodes at the time of mastectomy. These results indicate that the presence of CEA cannot be used as a guide to prognosis in either of these groups of patients. Fig. 15 shows the survival curves for CEA detection in patients with metastatic lymph nodes, and fig. 16 shows similar survival curves for patients with non-metastatic lymph nodes.

Survival, CEA and the presence of intraductal carcinoma.

A significantly greater rate of CEA detection was observed in invasive tumours which also possessed an intraductal component, (see table 5). The relationship of patient survival to the detection of CEA in the primary tumour was investigated for patients who were found to have an intraductal component to their tumour. The results in table 22, and illustrated in fig. 17 indicate that the presence of CEA in invasive tumours with an intraductal element does not influence patient survival within this group. Also, the results in table 23, shown graphically in fig. 18, imply that the presence of CEA in tumours without

an intraductal component cannot be considered to be a prognostic indicator.

OTHER FACTORS AND PATIENT SURVIVAL.

Patient survival and menopausal status.

The comparison of survival according to the menopausal status of the patient at the time of mastectomy is summarised in table 24, and illustrated graphically in fig. 19. These results provide no evidence to suppose that survival is influenced by the menopausal status of the patient at the time of mastectomy.

Size of tumour and patient survival.

Survival was compared when the patients were grouped according to the size of the primary tumour. The results, which are presented in fig. 20, and table 25, lead to the conclusion that patients having tumours of 5 cm. diameter or greater at the time of mastectomy have a significantly reduced median survival time compared with patients with tumours less than 5 cm. diameter. The chi-squared obtained for the trend was 5.29, giving a probability of 0.022.

Patients were grouped according to their menopausal status at the time of mastectomy, and the survival of those with large tumours was compared with that of those with smaller tumours for both pre- (table 26, fig. 21) and postmenopausal (table 27, fig. 22) groups. There is no significant difference in either group at the 5% level

(premenopausal, chi squared=3.03, P=0.082; postmenopausal, chi squared=0.01, P>0.5). However, taking into consideration the small numbers in the classes, these results could imply that the size of the primary tumour is of greater influence on the survival of premenopausal patients than on that of postmenopausal patients.

Attachment of tumour and patient survival.

The comparison of survival following mastectomy when patients were grouped according to any the attachment of their primary tumour, either to the overlying skin or to the deep tissues, was made and is shown in table 28. This relationship is shown graphically in fig. 23. The results imply that attachment of the tumour does not significantly reduce the median survival time.

Lymphocytic infiltration and patient survival.

Patient survival following mastectomy was compared when the cases were grouped according to the extent of the lymphocytic infiltration of the primary carcinoma. The results are summarised in table 29, and the survival curves are shown in fig. 24. No significant difference can be detected between the median survival times for patients whose tumours are infiltrated and those whose tumours show no lymphocytic infiltration. (However, the number of patients showing no infiltration of the primary tumour is small, with only 10 cases in this class.).

Intraductal component and patient survival.

Survival was compared when patients were grouped

according to the presence or absence of an intraductal element in their invasive carcinomas. Table 30 lists these results, and fig. 25 presents the survival curves for these two groups. It can be seen that there is no significant difference in survival.

Histological grade of the tumour and patient survival.

The comparison of survival when the patients were grouped according to the histological grade of their tumour is summarised in table 31, and illustrated graphically in fig. 26. These results support the hypothesis that the prognosis worsens as the tumour becomes less well-differentiated. There is a significant difference between the survival of patients whose tumours are grade 1 (median survival time >92 months) and those whose tumours are grade 3 (median survival time 47 months), with chi squared for the trend being 7.66, giving a probability of 0.005.

Lymph node status and patient survival.

Finally, survival has been compared for patients grouped according to their lymph node status at the time of mastectomy. The results, which are presented in table 32, and illustrated in fig. 27, lead to the conclusion that the presence of metastatic disease at the time of operation significantly reduces the median survival time. Patients with pathologically determined negative nodes had a median survival time of >90 months, whilst patients with pathologically determined metastatic nodes had a median survival time of 44.9 months. The chi squared for the trend

is 0.47, giving a probability of 0.029. No significant difference was demonstrated in comparison of the survival of patients with one or two metastatic lymph nodes, with the survival of patients with three or more metastatic lymph nodes. Table 32 gives these results, and the trend is illustrated in fig. 28.

Table 1-: Comparison of the frequency of CEA detection in the primary tumour of patients grouped according to their menopausal status.

Menopausal status	CEA positive	CEA negative
Pre-menopausal	16	21
Peri-menopausal	6	6
Post-menopausal	32	45

Chi squared = 0.30528
df = 2
0.9 > P > 0.8

Table 2-: Comparison of the frequency of CEA detection in the primary tumour of patients grouped according to tumour size.

Size of Tumour	CEA positive	CEA negative
< 5cm diam.	49	67
> 5cm diam.	11	11

Chi squared = 0.45298
df = 1
0.7 > P > 0.5

Table 3-: Comparison of frequency of CEA detection in the primary tumour of patients grouped according to any fixation of their tumour.

Fixation	CEA positive	CEA negative
Not attached	35	35
Attached	25	43

Chi squared = 2.45871
df = 1
0.9 > P > 0.8

Fixation	CEA positive	CEA negative
Attached to skin	18	35
Attached to deep tissue	7	11

Chi squared = 0.04751
df = 1
0.9 > P > 0.8

Table 4-: Comparison of the frequency of CEA detection in the primary tumour of patients grouped according to the extent of lymphocytic infiltration of the tumour.

Lymphocytic infilt.	CEA positive	CEA negative
0	5	5
+	39	52
++	16	21

Chi squared = 0.188194
df = 2
0.95 > P > 0.9

Table 5-: Comparison of the frequency of CEA detection in primary tumours of patients grouped according to the presence or absence of an intraductal element in the tumour.

Tumour type	CEA positive	CEA negative
Intraductal component	24	18
No intraductal component	36	60

Chi squared = 4.5874
df = 1
0.05 > P > 0.02

Table 6-: Comparison of the frequency of CEA detection in the primary tumours of patients grouped according to the histological grade (Bloom and Richardson, 1957) of their tumour.

Histological grade	CEA positive	CEA negative
1	12	12
2	29	50
3	19	16

Chi squared = 3.55241
df = 2
0.2 > P > 0.1

Table 7-: Comparison of the frequency of CEA detection in the primary tumour of patients grouped according to the presence or absence of clinically palpable lymph nodes at the time of mastectomy.

Palpable lymph nodes	CEA positive	CEA negative
Present	20	18
Absent	38	58

Chi squared = 1.88809
df = 1
0.2 > P > 0.1

Table 8-: Comparison of the frequency of CEA detection in the primary tumour where the patients have been grouped according to the presence or absence of metastatic axillary lymph nodes assessed pathologically at the time of mastectomy.

Lymph node metastases	CEA Positive	CEA Negative
Present	10	9
Absent	21	26

Chi squared = 0.34338
df = 1
0.7 > P > 0.5

Table 9-: Comparison of the frequency of CEA detection in the primary tumour of patients grouped according to number of metastatic lymph nodes detected pathologically at the time of mastectomy.

No. of metasteses	CEA positive	CEA negative
1 or 2	9	14
3 or more	12	12

Chi squared = 0.56140
df = 1
0.5 > P > 0.3

Table 10-: Comparison of survival when patients are grouped according to the presence of CEA in the primary tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	78	34	0.01	>0.5
Detected	60	27		

Median survival time in months (95% confidence interval)
CEA not detected 87.38 (62.3 to >92.25)
CEA detected >92.25 (46.73 to >92.25)

Table 11-: Comparison of survival when patients are grouped according to the extent of CEA detection in the primary tumour.

CEA staining	N	Obs. deaths	Chi squared	P
Negative or weak positive (0, +/-, +)	112	53	1.76	0.185
Strong positive (2+, 3+)	26	8		

Median survival time in months (95% confidence interval)
CEA (0, +/-, +) 86.88 (53.77 to > 92.25)
2+, 3+ > 92.25

Table 12-: Comparison of survival time for premenopausal patients when grouped according to the presence or absence of CEA in the primary tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	21	9	0.03	>0.5
Detected	16	7		

Median survival time in months (95% confidence interval)
Not detected >92.25 (48.48 to >92.25)
Detected >92.25 (39.94 to >92.25)

Table 13-: Comparison of survival for perimenopausal patients when graded acording to the presence or absence of CEA in the primary tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	6	4	0.01	>0.5
Detected	6	3		

Median survival time in months (95% confidence interval)
CEA not detected 62.61 (10.03 to >83.69)
CEA detected 53.17 (23.57 to >83.69)

Table 14-: Comparison of survival for postmenopausal patients when graded according to the presence or absence of CEA in the primary tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	45	19	0.07	>0.5
Detected	32	14		

Median survival time in months (95% confidence interval)
CEA not detected 87.45 (57.66 to >90.98)
CEA detected 68.03 (37.42 to >90.95)

Table 15-: Comparison of survival for patients with tumours <5 cm in diameter when grouped according to the presence or absence of CEA in the tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	67	27	0.03	>0.5
Detected	49	21		

Median survival time in months (95% confidence interval)
CEA not detected 87.46 (71.72 to >92.25)
CEA detected >92.25 (52.97 to >92.25)

Table 16-: Comparison of survival for patients >5 cm in diameter when grouped according to the presence or absence of CEA in the primary tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	11	7	0.47	>0.5
Detected	11	6		

Median survival time in months (95% confidence interval)
CEA not detected 19.69 (13.7 to >86.1)
CEA detected 40.59 (22.3 to >86.1)

Table 17-: Comparison of survival for patients with grade 1 histology when grouped according to the presence or absence of CEA in the primary tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	12	1	0.43	0.498
Detected	12	3		

Median survival time in months (95% confidence interval)
CEA not detected >88.93
CEA detected >88.93

Table 18-: Comparison of survival for patients with Grade 2 histology when grouped according to the presence or absence of CEA in the primary tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	50	22	0.47	0.482
Detected	29	15		

Median survival time in months (95% confidence interval)
CEA not detected 87.14 (61.18 to >89.38)
CEA detected 55.43 (36.33 to >89.38)

Table 19-: Comparison of survival for patients with grade 3 histology when grouped according to the presence or absence of CEA in the primary tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	16	11	1.5	0.223
Detected	16	9		

Median survival time in months (95% confidence interval)
CEA detected 19.58 (13.51 to 86.96)
CEA not detected 67.96 (37.47 to >91.05)

Table 20-: Comparison of survival for patients with lymph node metastases detected by histological examination at the time of mastectomy, when grouped according to the presence or absence of CEA in the primary tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	26	18	0.05	>0.5
Detected	21	15		

Median survival time in months (95% confidence interval)
CEA not detected 49.9 (18.25 to 86.98)
CEA detected 38.1 (22.46 to 55.62)

Table 21-: Comparison of survival for patients without lymph node metastases, by histological examination at the time of mastectomy, when grouped according to the presence or absence of CEA in the primary tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	9	4	0.14	>0.5
Detected	10	2		

Median survival time in months (95% confidence interval)
CEA not detected >87 (22.18 to >87)
CEA detected >87 (21.9 to >87)

Table 22-: Comparison of survival of patients whose primary tumours contained an intraductal carcinoma, when grouped according to the presence or absence of CEA in the tumour.

CEA	N	Obs. deaths	Chi squared	P
Not detected	18	12	0.61	0.431
Detected	24	12		

Median survival time in months (95% confidence interval)
CEA not detected 53.36 (20.47 to 71.8)
CEA detected 67.71 (35.78 to >92.09)

Table 23-: Comparison of survival of patients whose primary tumours did not contain an intraductal component when grouped according to the presence or absence of CEA in the tumour.

CEA	N	Obs. deaths	Chi squared	P
Detected	60	22	0.24	>0.5
Not detected	36	15		

Median survival time in months (95% confidence interval)
CEA not detected 88.03 (86.93 to >91.05)
CEA detected >91.05 (46.46 to >91.05)

Table 24:- Comparison of survival of patients grouped according to menopausal status at the time of mastectomy.

Menopausal status	N	Obs. deaths	Chi squared	P
Pre-	37	16	0.15	>0.5
Peri-	12	7		
Pre-	37	16	0.05	>0.5
Post-	77	33		
Peri-	12	7	0.14	>0.5
Post-	77	33		

Median survival time in months (95% confidence interval)

Premenopausal	>92.25 (50.62 to >92.25)
Perimenopausal	71.75 (23.1 to >92.5)
Postmenopausal	87.41 (55.45 to >92.25)

Table 25-: Comparison of survival of patients grouped according to the size of the tumour.

Size of tumour	N	Obs. deaths	Chi squared	P
>5 cm	22	13	5.29	0.022
<5 cm	116	48		

Median survival time in months (95% confidence interval)

>5 cm	27.93 (19.52 to >92.25)
<5 cm	89.05 (67.99 to >92.25)

Table 26-: Comparison of survival when patients, all premenopausal at the time of mastectomy, are grouped according to the size of the primary tumour.

Size of tumour	N	Obs. deaths	Chi squared	P
>5 cm	5	4	3.03	0.082
<5 cm	32	12		

Median survival time in months (95% confidence interval)

>5 cm	25.21 (14.83 to >92.25)
<5 cm	>92.25 (54.75 to >92.25)

Table 27-: Comparison of survival when patients, postmenopausal at the time of mastectomy, are grouped according to the size of the primary tumour.

Size of tumour	N	Obs. deaths	Chi squared	P
>5 cm	13	5	0.01	>0.5
<5 cm	64	28		

Median survival time in months (95% confidence interval)

>5 cm	>90.98 (19.84 to >90.98)
<5 cm	87.32 (54.51 to >90.98)

Table 28-: Comparison of survival of patients grouped according to any attachment of the primary tumour.

Attachment	N	Obs. deaths	Chi squared	P
None	70	28	1.1	0.297
To skin	50	24		
None	70	28	1.5	0.223
To deep tissues	18	9		
To skin	50	24	0.09	>0.5
To deep tissues	18	9		

Median survival time in months (95% confidence interval)

No attachment	89.19 (66.67 to >92.25)
To skin	72.18 (47.14 to >92.09)
To deep tissues	53.56 (16.52 to >92.52)

Table 29-: Comparison of survival of patients grouped according to degree of lymphocytic infiltration of the tumour.

Lymphocytic Infiltration	N	Obs. deaths	Chi squared	P
0	10	2	0.81	0.37
+	91	44		
0	10	2	0.32	>0.5
++	37	15		
+	91	44	0.06	>0.5
++	37	15		

Median survival time in months (95% confidence interval)

0	>89.38 (52.26 to >89.38)
+	87.09 (53.69 to >92.25)
++	>89.38 (46.08 to >92.25)

Table 30-: Comparison of survival of patients grouped according to the presence or absence of an intraductal carcinoma in the tumour.

Intraductal comp.	N	Obs. deaths	Chi squared	P
Absent	96	37	2.44	0.118
Present	42	24		

Median survival time in months (95% confidence interval)
Intraductal present 53.38 (37.76 to >92.25)
Intraductal absent 88.04 (86.9 to >92.25)

Table 31-: Comparison of survival of patients grouped according to the histological grade of the tumour (Bloom and Richardson, 1957).

Grade	N	Obs. deaths	Chi squared	P
1	24	4	5.14	0.235
2	79	37		
1	24	4	7.66	0.005
3	35	20		
2	79	37	1.21	0.237
3	35	20		

Median survival time in months (95% confidence interval)
Grade 1 >92.09
Grade 2 86.9 (54.04 to >92.09)
Grade 3 47.02 (19.44 to >92.09)

Table 32-: Comparison of survival of patients grouped according to their lymph node status at the time of mastectomy.

Node status	N	Obs. deaths	Chi squared	P
Negative	19	6	0.47	0.029
Positive	47	33		

Median survival time in months (95% confidence interval)
Negative lymph nodes >90.98 (43.84 to >90.98)
Positive lymph nodes 44.92 (22.17 to 55.36)

No. of involved nodes	N	Obs. deaths	Chi squared	P
1 or 2	23	14	1.38	0.242
> 2	24	19		

Median survival time in months (95% confidence interval)
1 or 2 involved nodes 55.38 (22.25 to 89.1)
> 2 involved nodes 27.83 (16.74 to 51.98)

Fig. 1.

Histogram showing age distribution of patients at time of mastectomy, and also showing sub-population with CEA positive tumours.

Fig. 2.

Histogram showing weight distribution of patients at time of mastectomy, and also showing sub-population with CEA positive tumours.

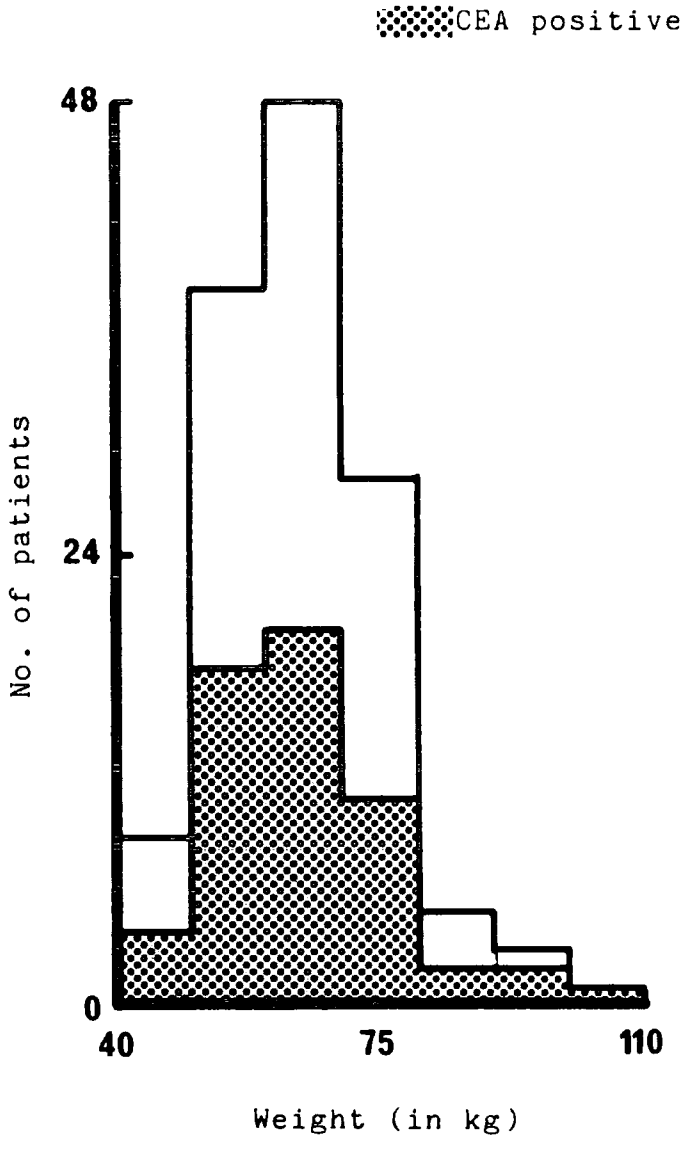


Fig. 3.

Histogram showing size distribution of tumours, and also showing sub-population with CEA positive tumours.

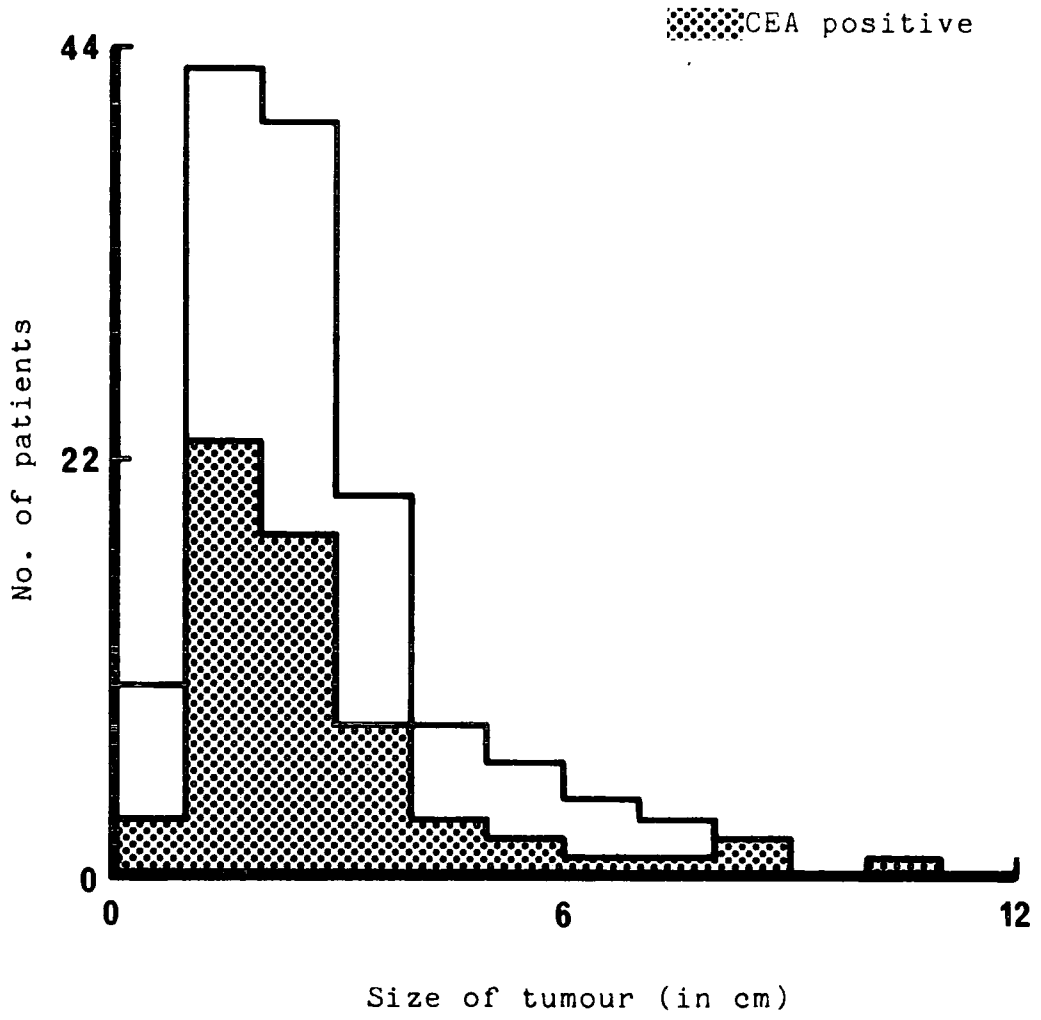


Fig. 4.

Histogram showing distribution according to histological sub-grade of tumour (Bloom and Richardson, 1957), and also showing sub-population which are CEA positive.

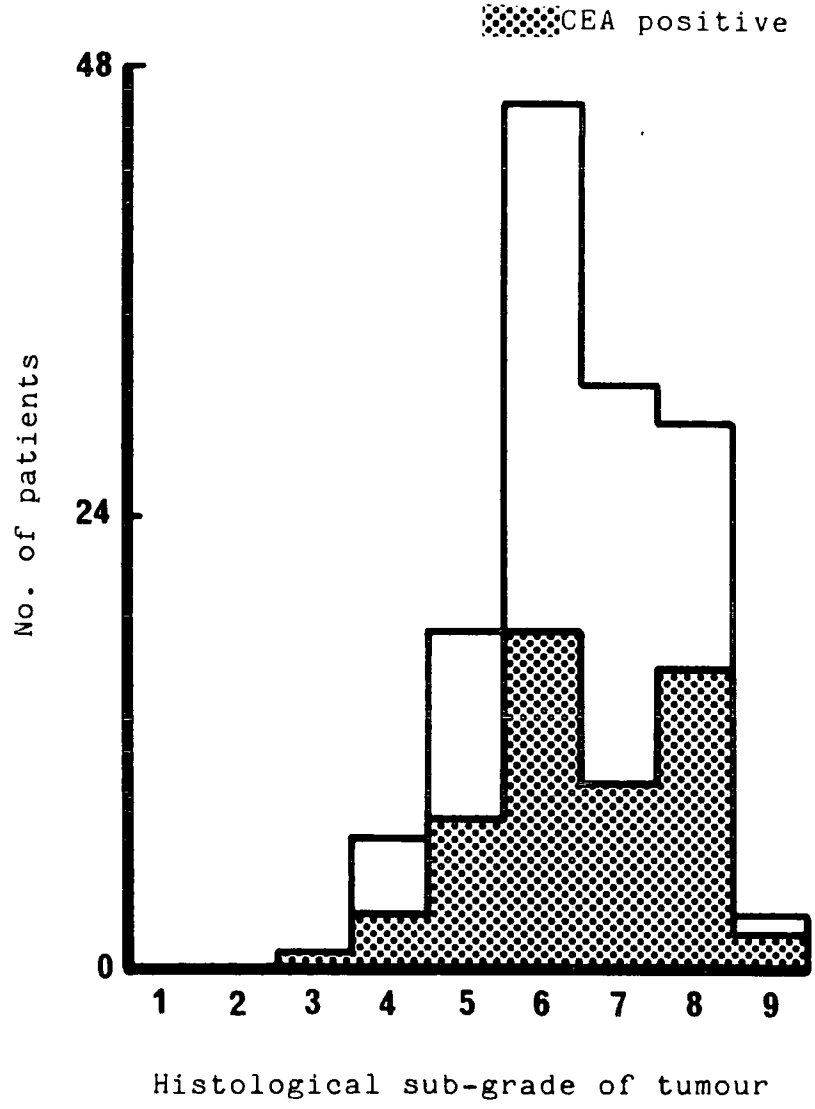


Fig. 5. Survival curves for patients grouped according to CEA occurrence in the primary tumour.

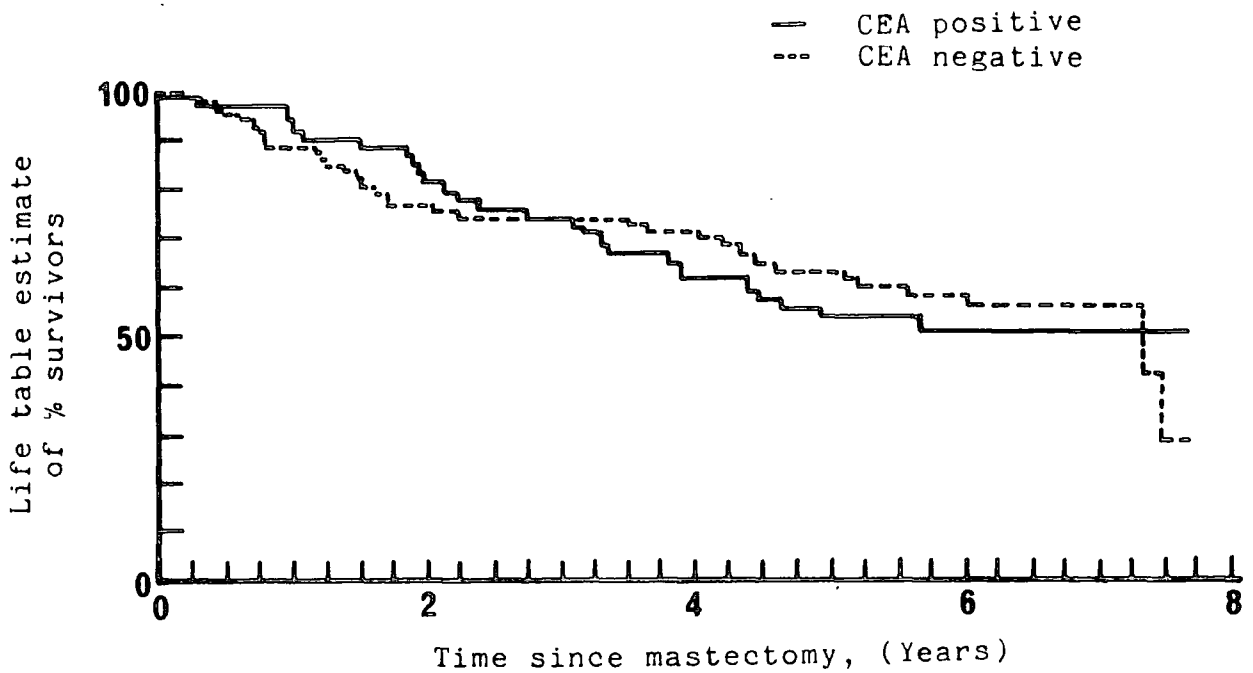


Fig. 6. Survival curves for patients grouped according to grading of CEA occurrence in the primary tumour.

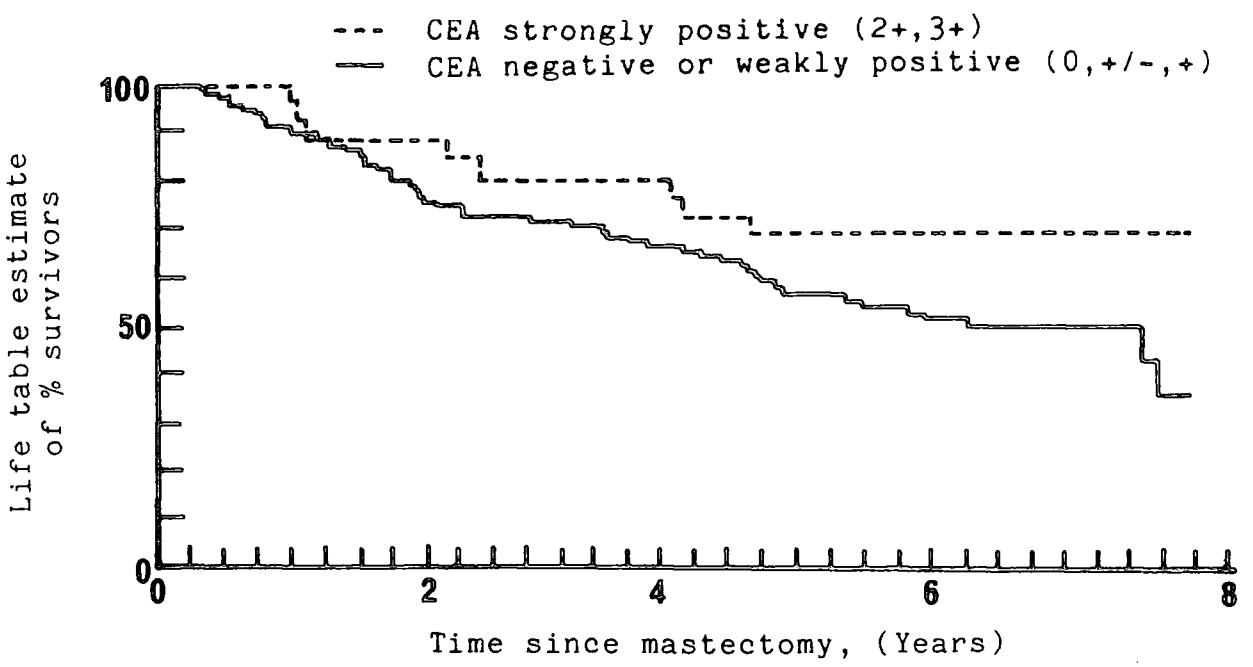


Fig. 7. Survival curves for pre-menopausal patients grouped according to CEA occurrence in the primary tumour.

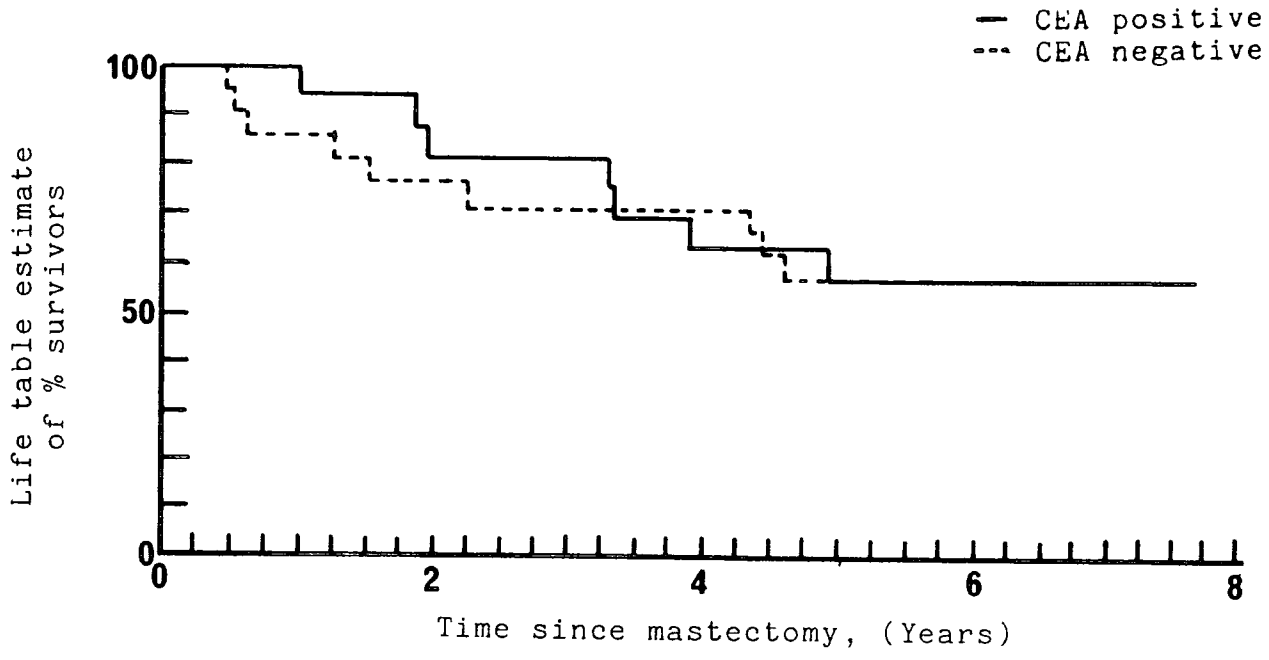


Fig. 8. Survival curves for peri-menopausal patients grouped according to CEA occurrence in the primary tumour.

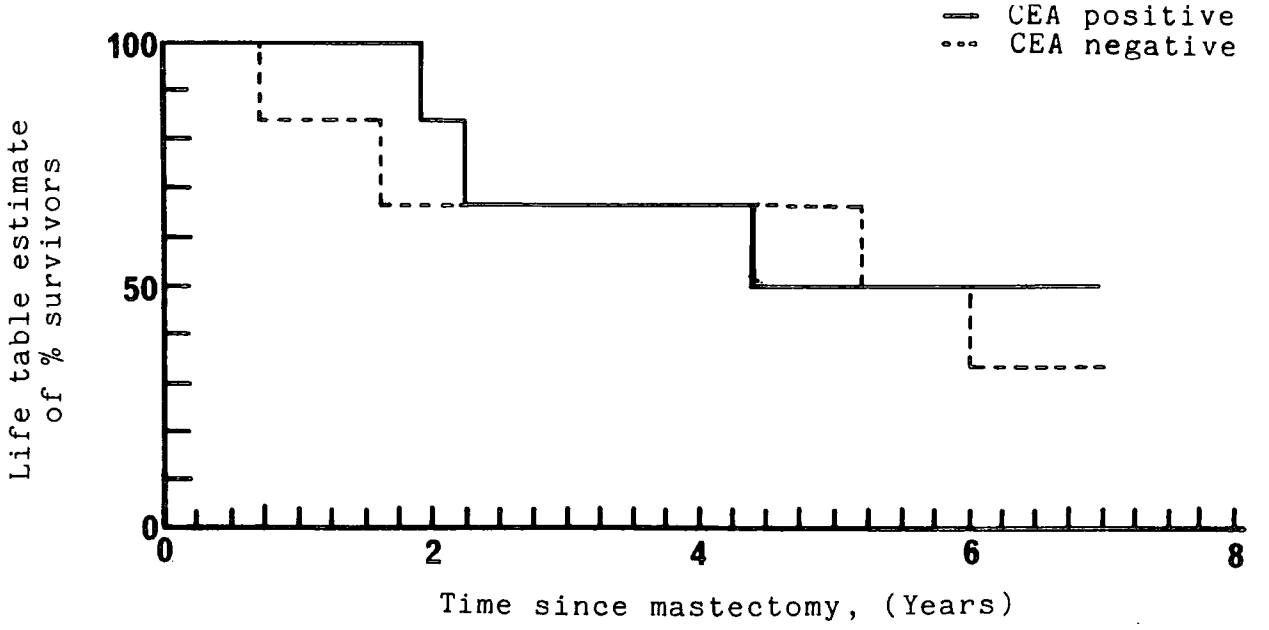


Fig. 9. Survival curves for post-menopausal patients grouped according to CEA occurrence in the primary tumour.

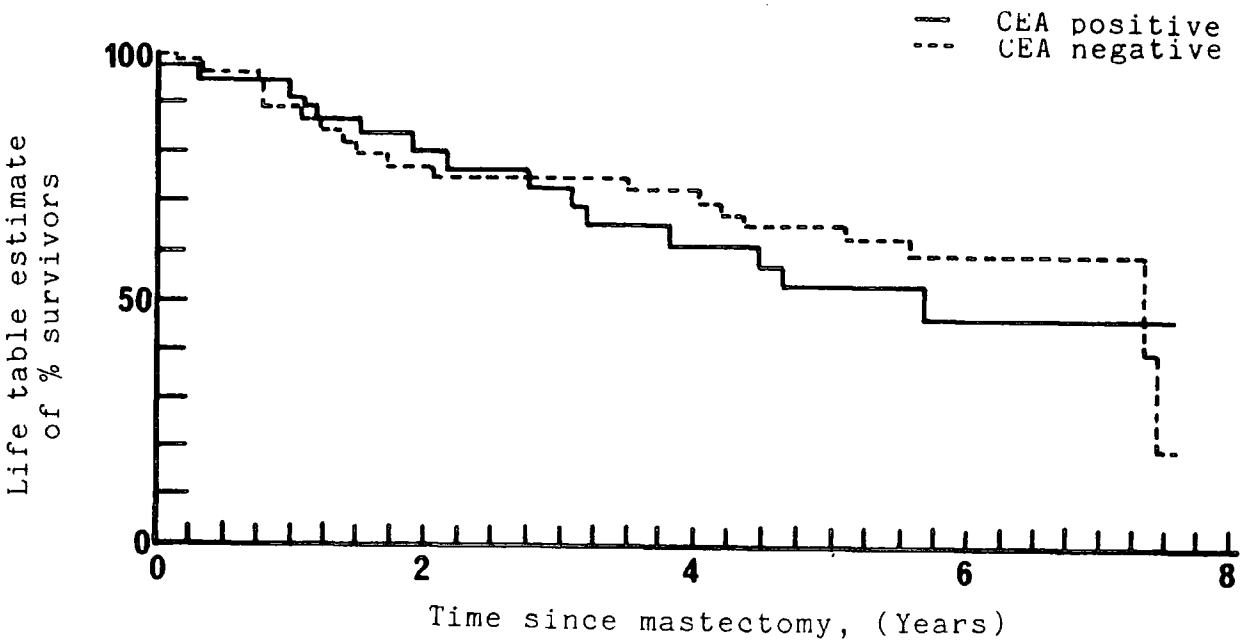


Fig. 10.

Survival curves for patients with tumours of <5cm. diameter, grouped according to occurrence of CEA in the primary tumour.

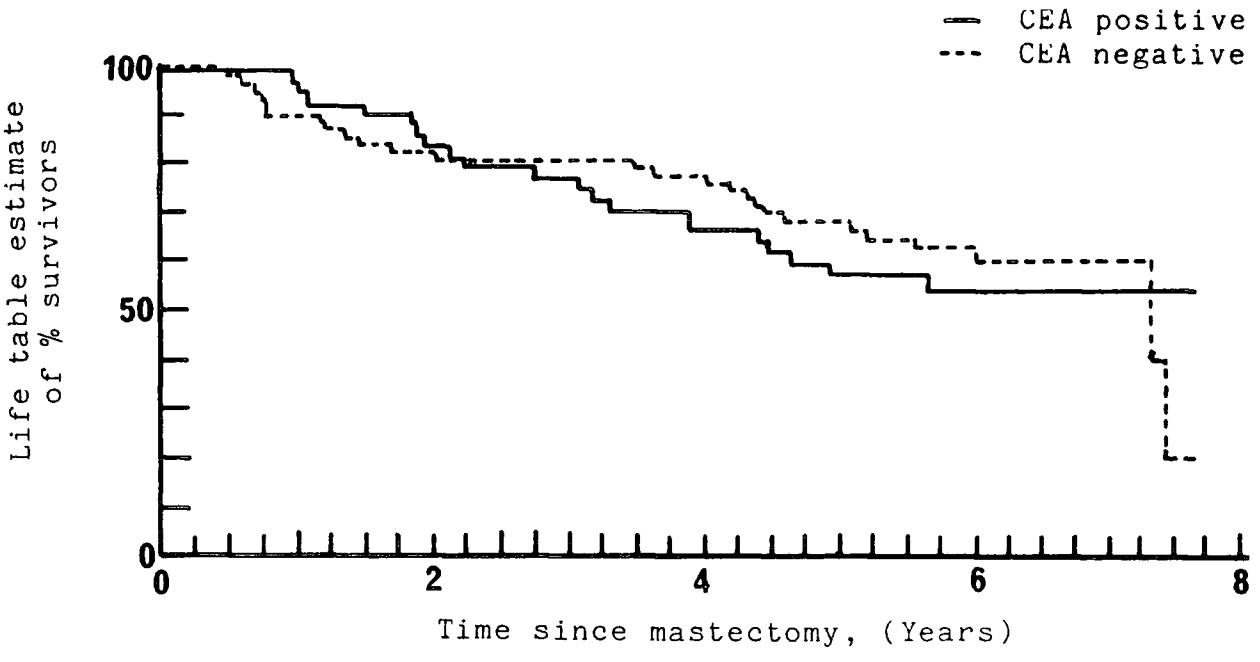


Fig. 11.

Survival curves for patients with tumours of >5cm. diameter, grouped according to occurrence of CEA in the primary tumour.

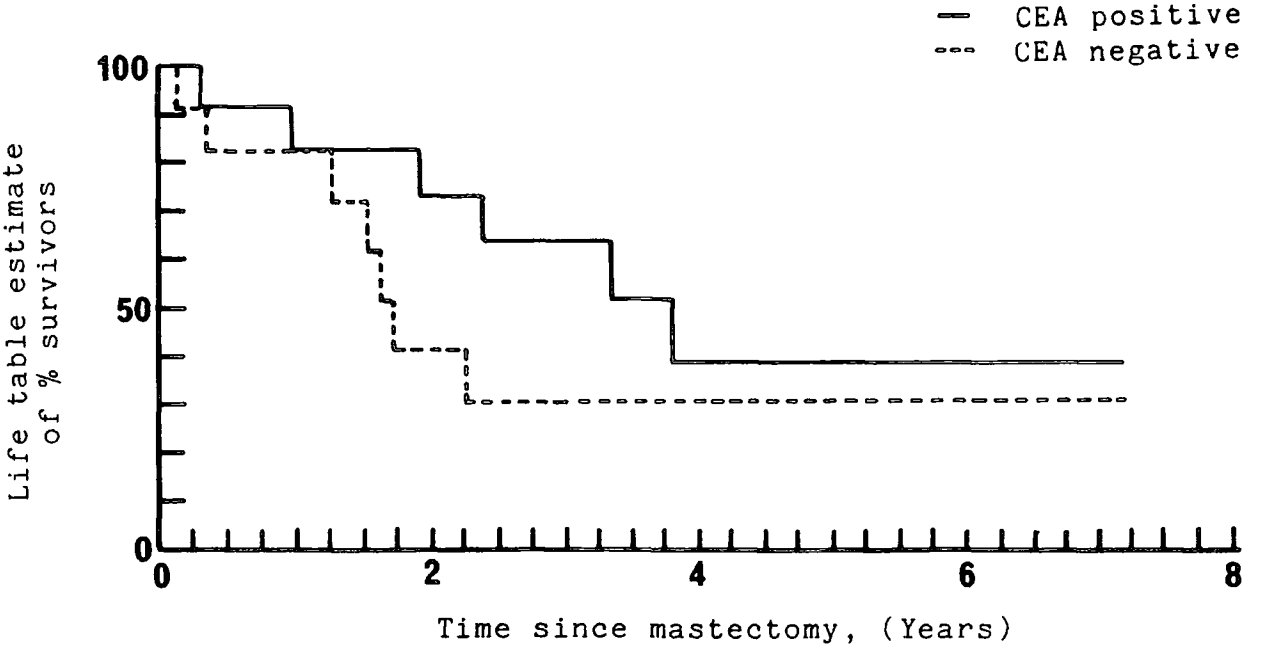


Fig. 12.
Survival curves for patients with tumours of Grade 1 histology, grouped according to occurrence of CEA in the primary tumour.

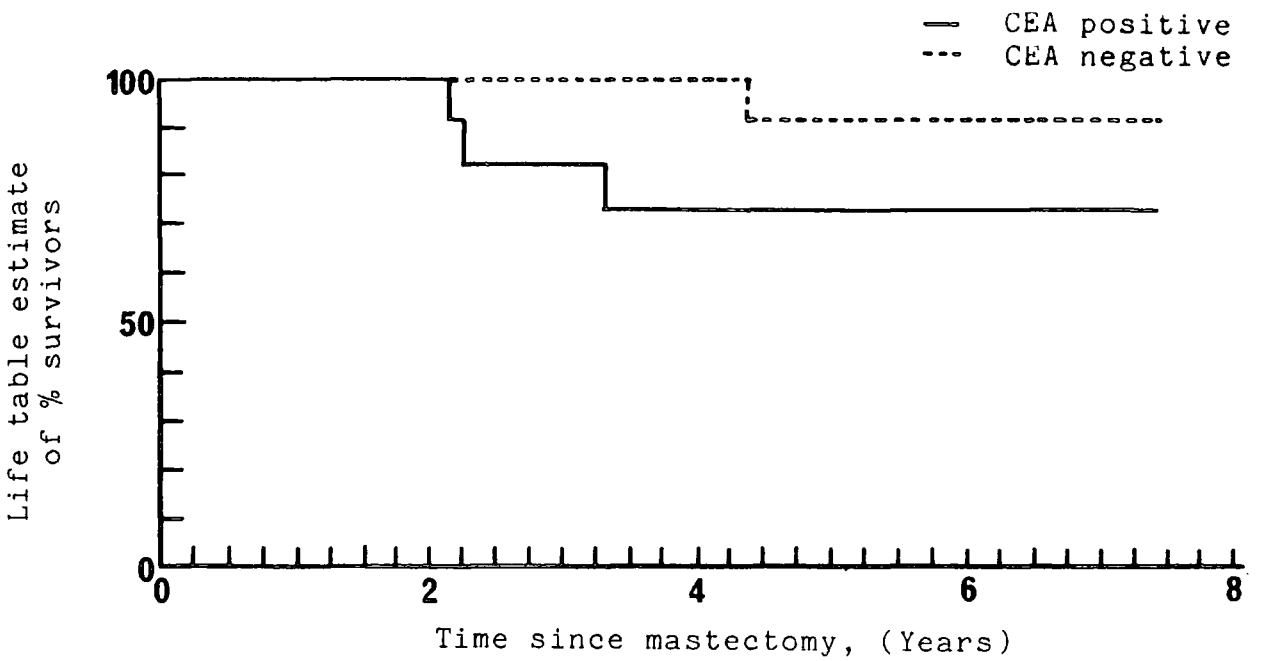


Fig. 13.
Survival curves for patients with tumours of Grade 2 histology, grouped according to occurrence of CEA in the primary tumour.

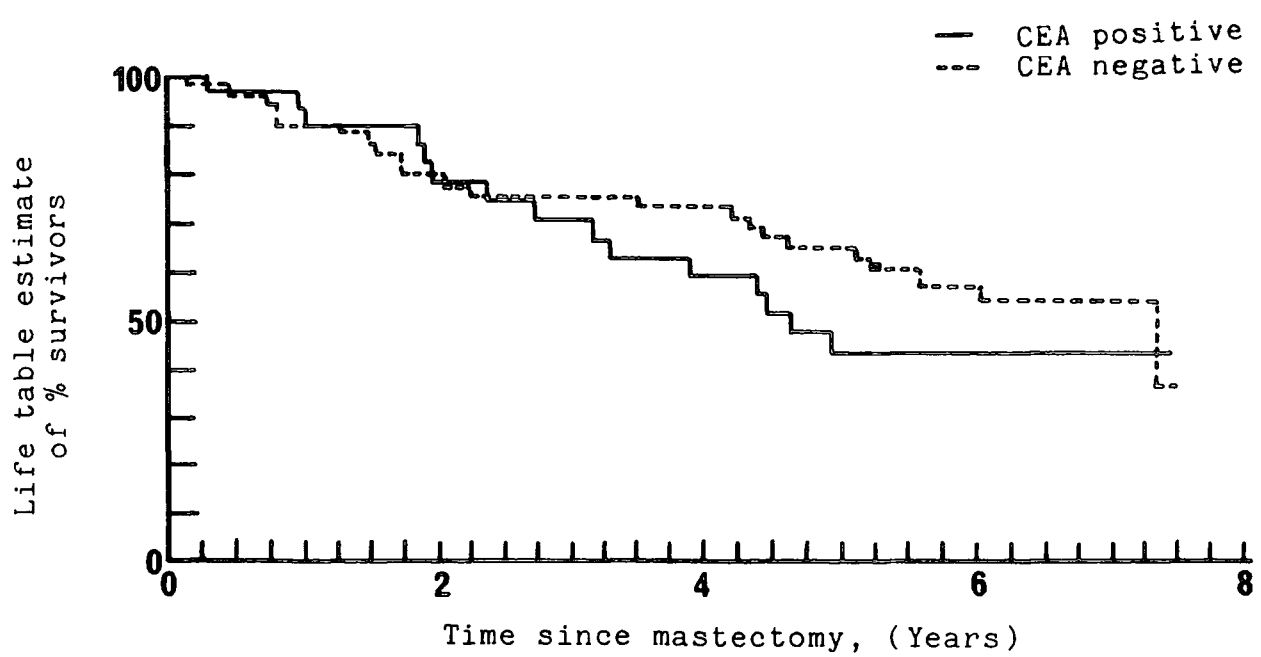


Fig. 14.
Survival curves for patients with tumours of Grade 3 histology, grouped according to occurrence of CEA in the primary tumour.

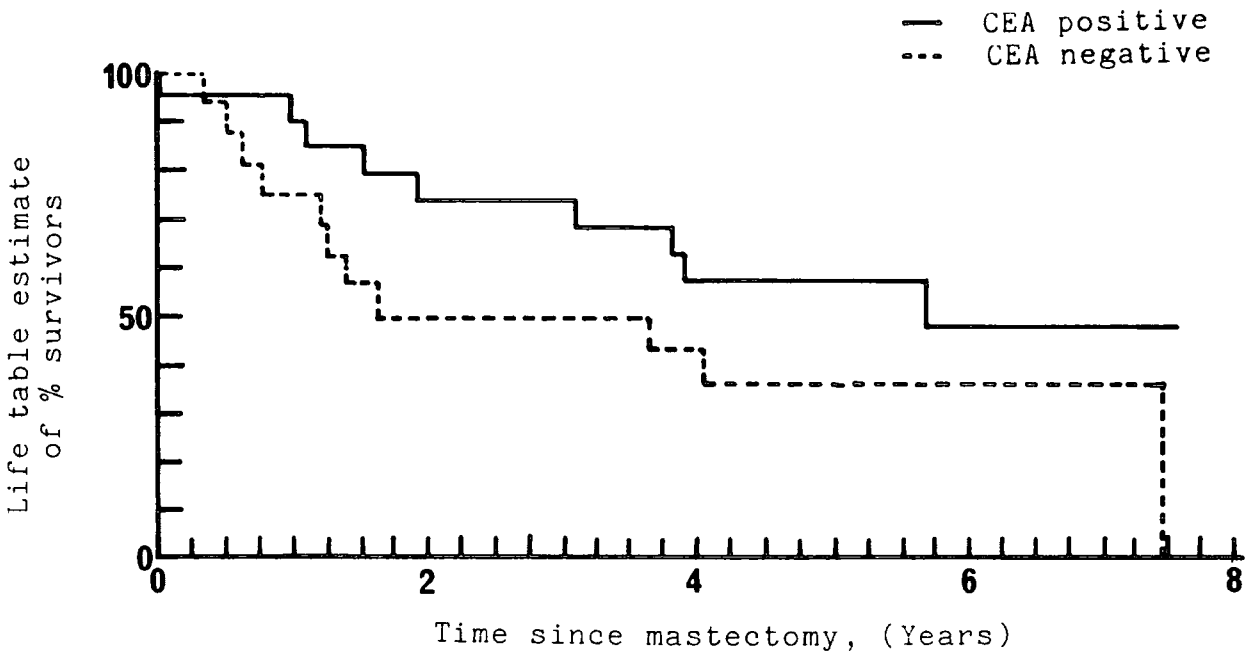


Fig. 15.

Survival curves for patients with lymph node metastases at time of mastectomy, grouped according to occurrence of CEA in the primary tumour.

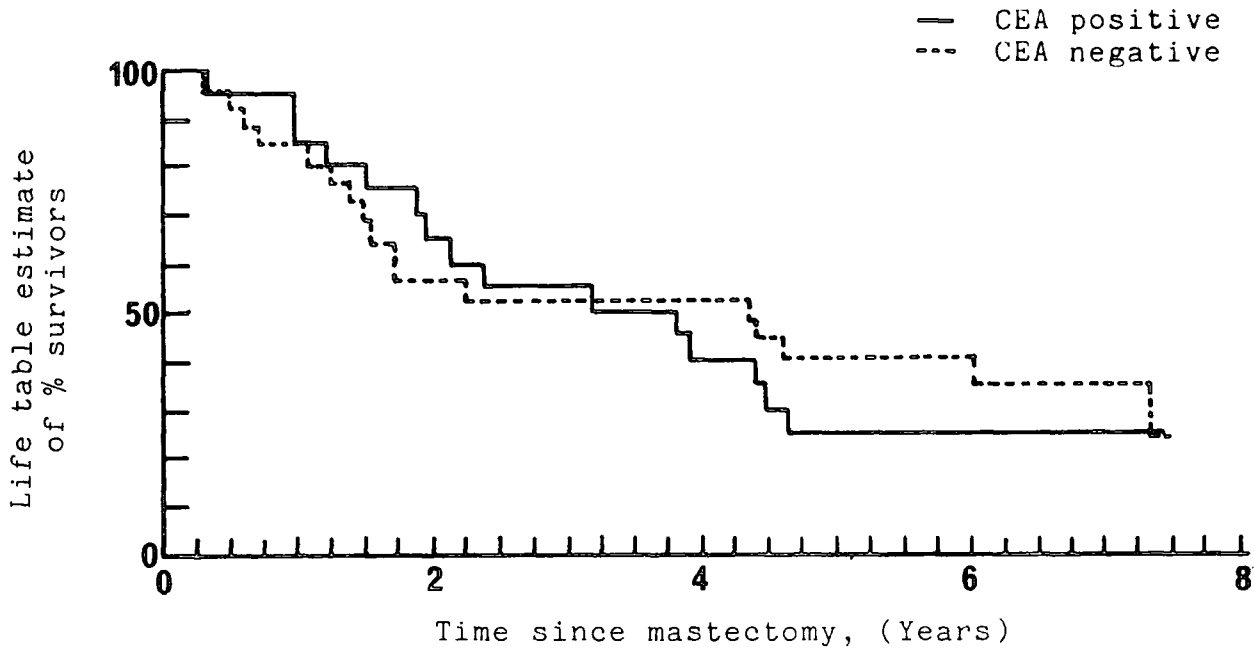


Fig. 16.

Survival curves for patients with uninvolved lymph nodes at time of mastectomy, grouped according to occurrence of CEA in the primary tumour.

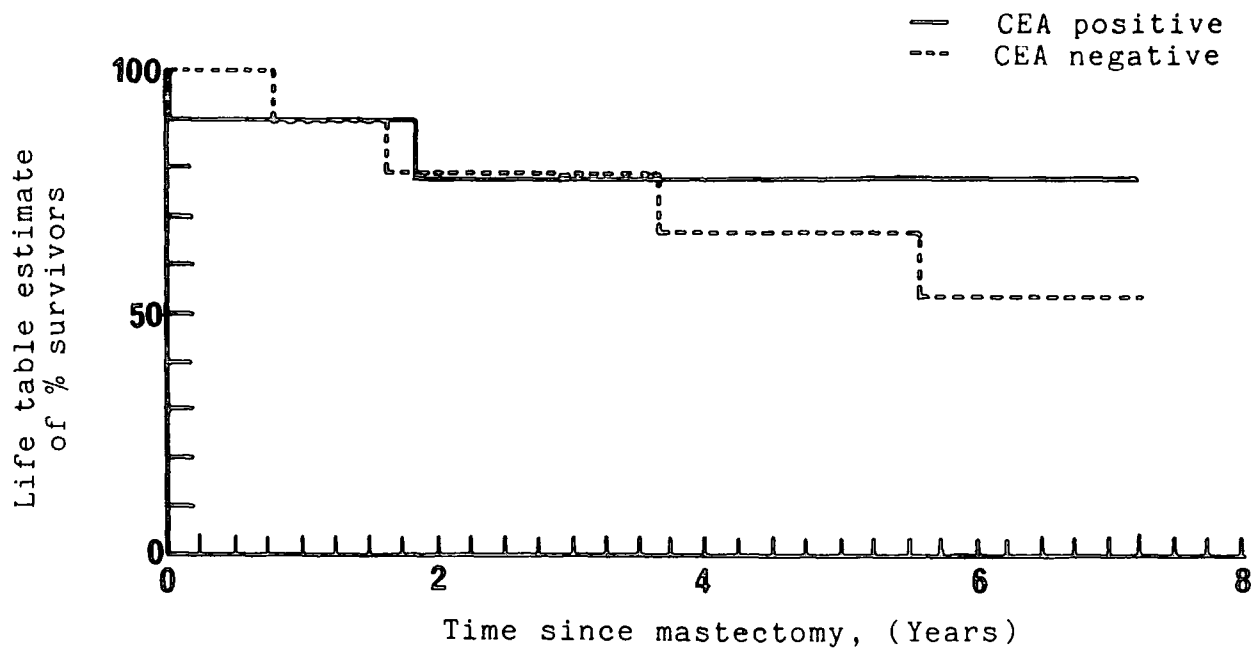


Fig. 17.

Survival curves for patients with an intraductal component to their primary tumour when grouped according to the presence or absence of CEA in the tumour.

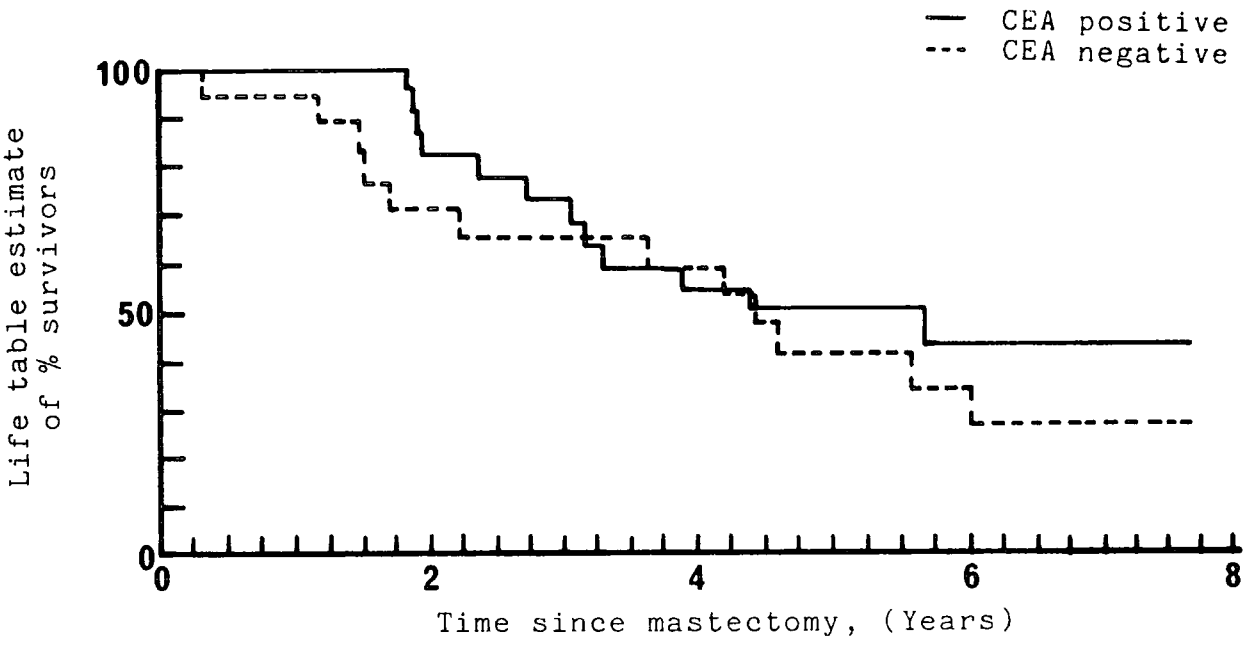


Fig. 18.

Survival curves for patients without an intraductal component to their primary tumour when grouped according to the presence or absence of CEA in the tumour.

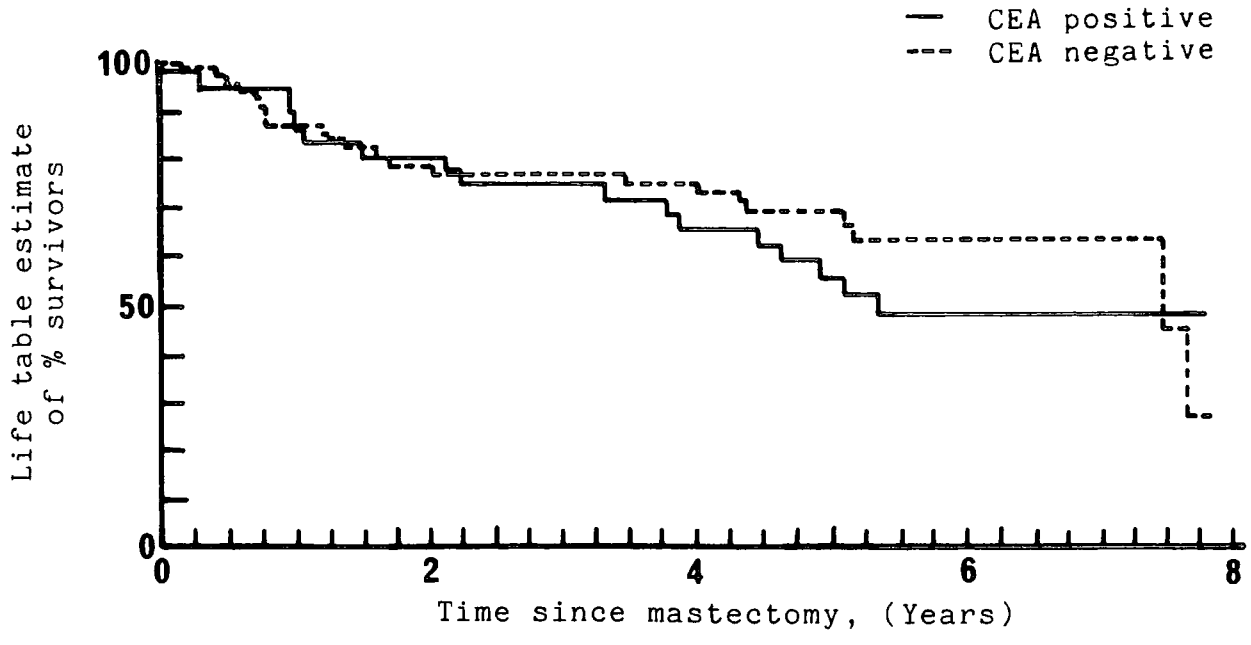


Fig. 19.

Survival curves for patients grouped according to their menopausal status at the time of mastectomy.

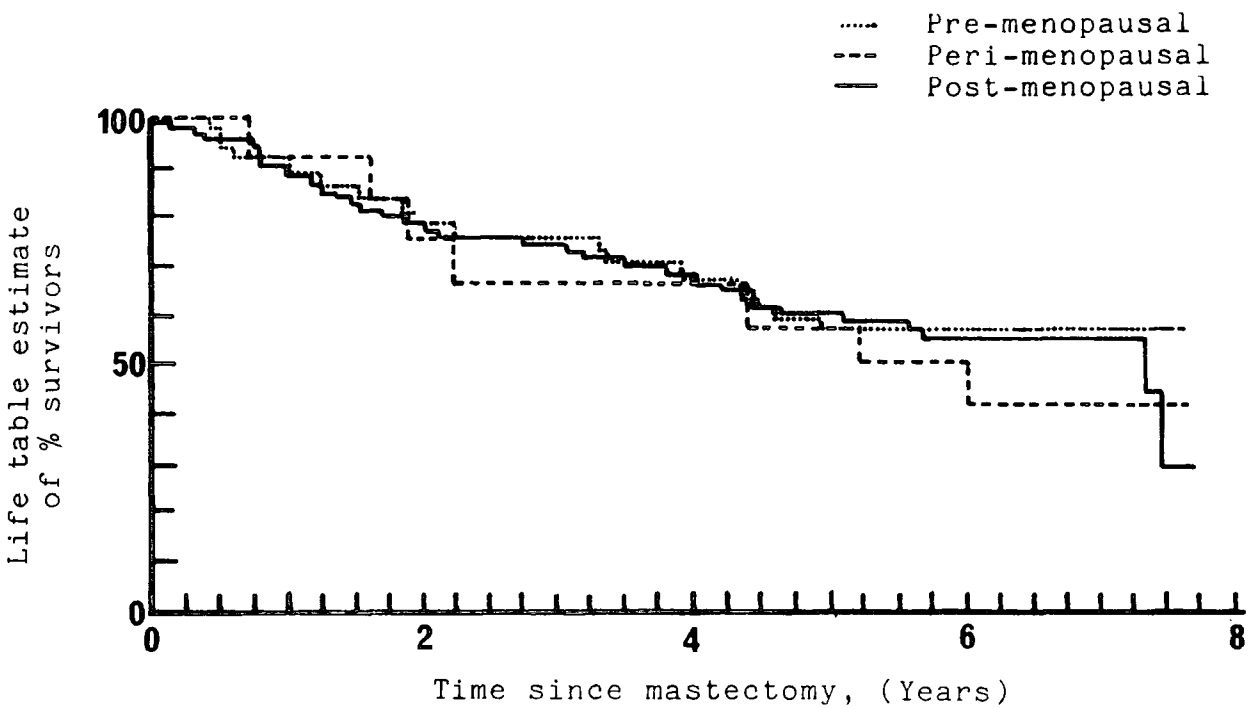


Fig. 20. Survival curves for patients grouped according to the size of the primary tumour.

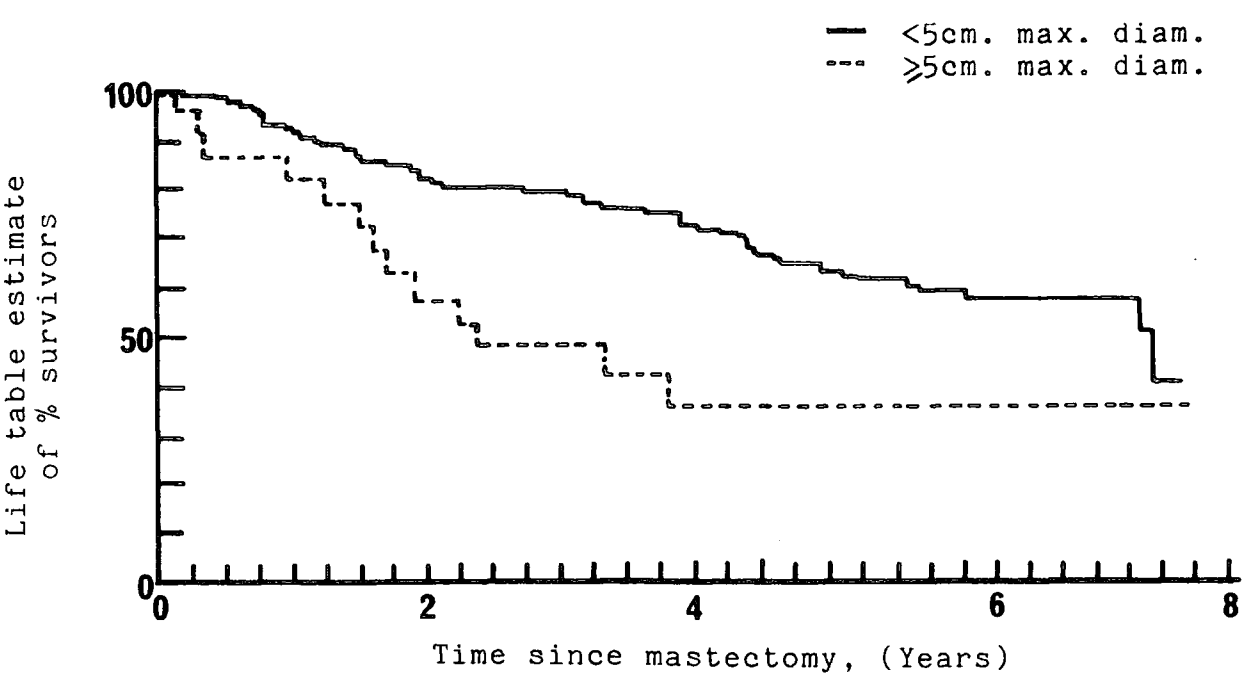


Fig. 21. Survival curves for pre-menopausal patients grouped according to tumour size.

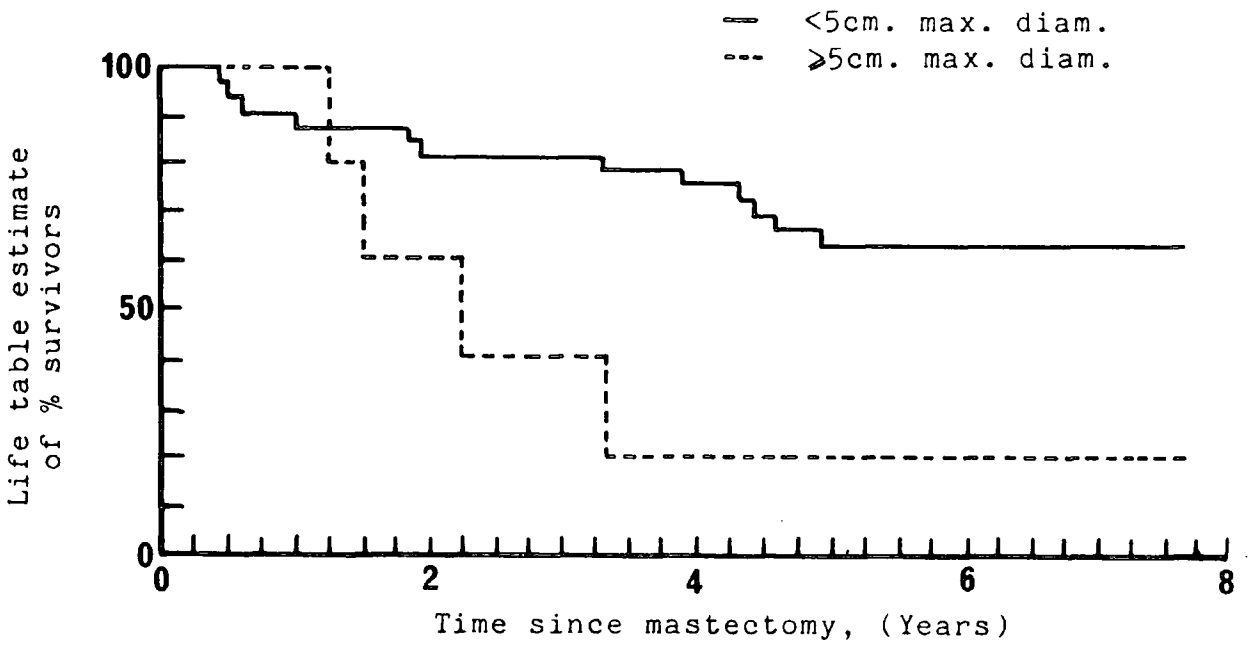


Fig. 22. Survival curves for post-menopausal patients grouped according to tumour size.

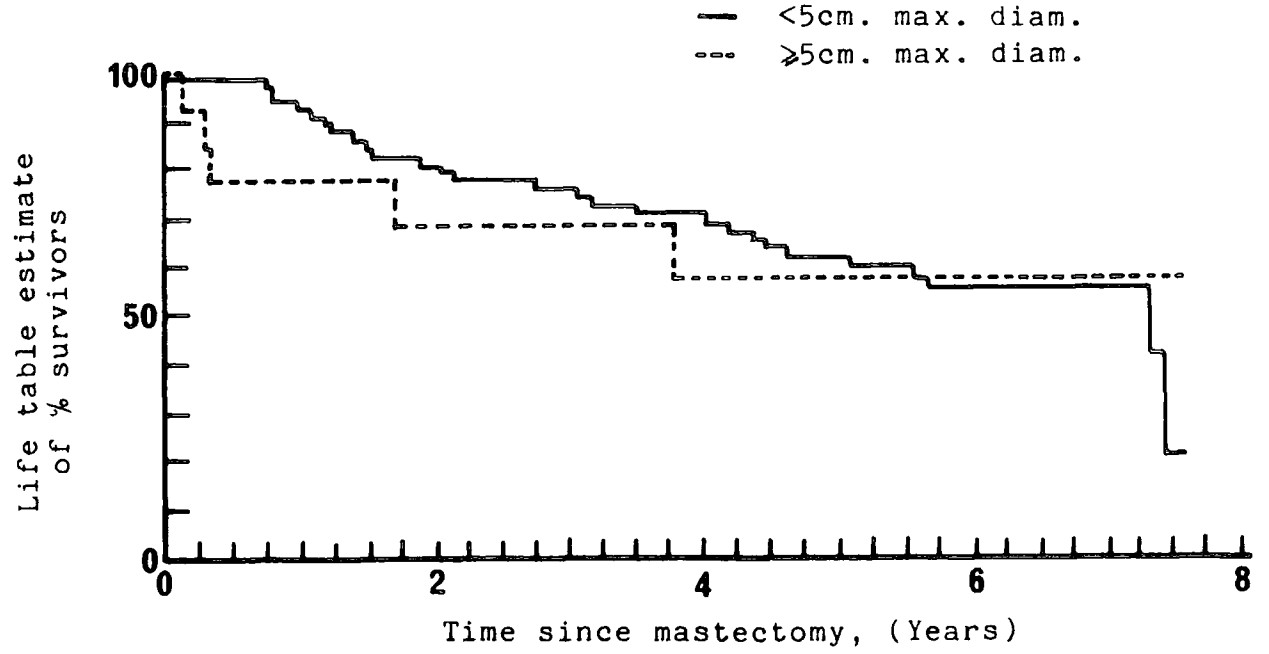


Fig. 23. Survival curves for patients grouped according to any attachment of the primary tumour.

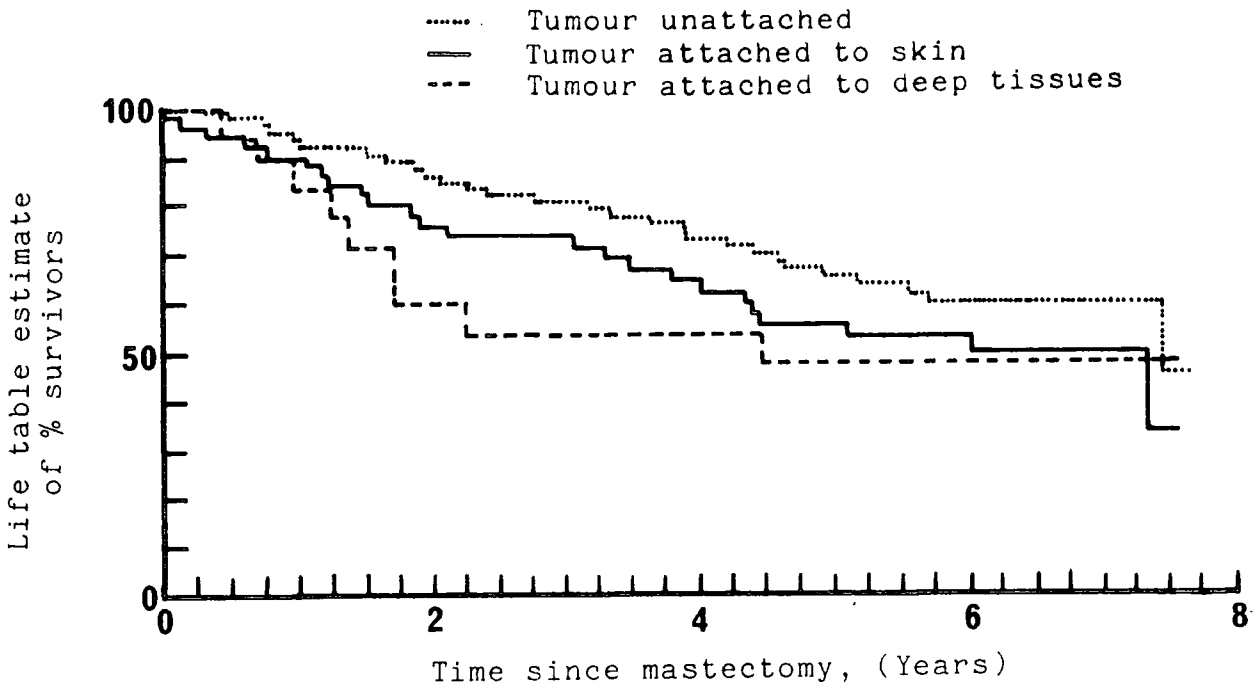


Fig. 24. Survival curves for patients grouped according to lymphocytic infiltration of the primary tumour.

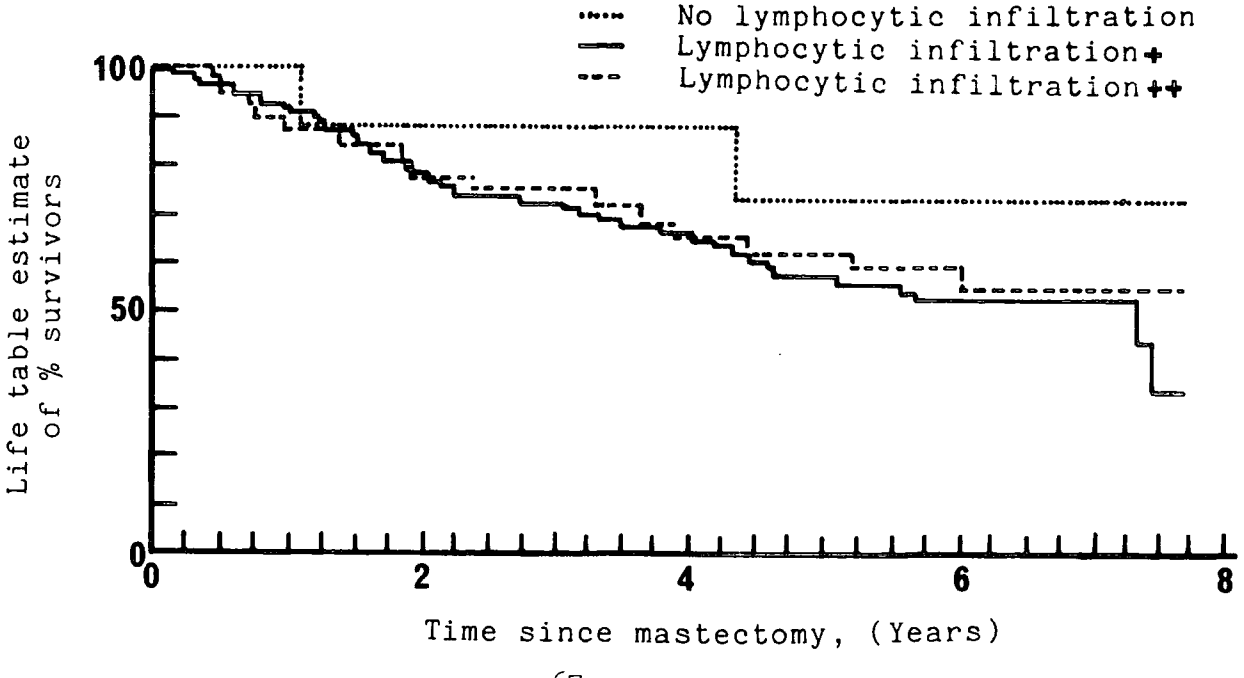


Fig. 25. Survival curves for patients grouped according to the presence or absence of an intraductal component in their invasive tumour.

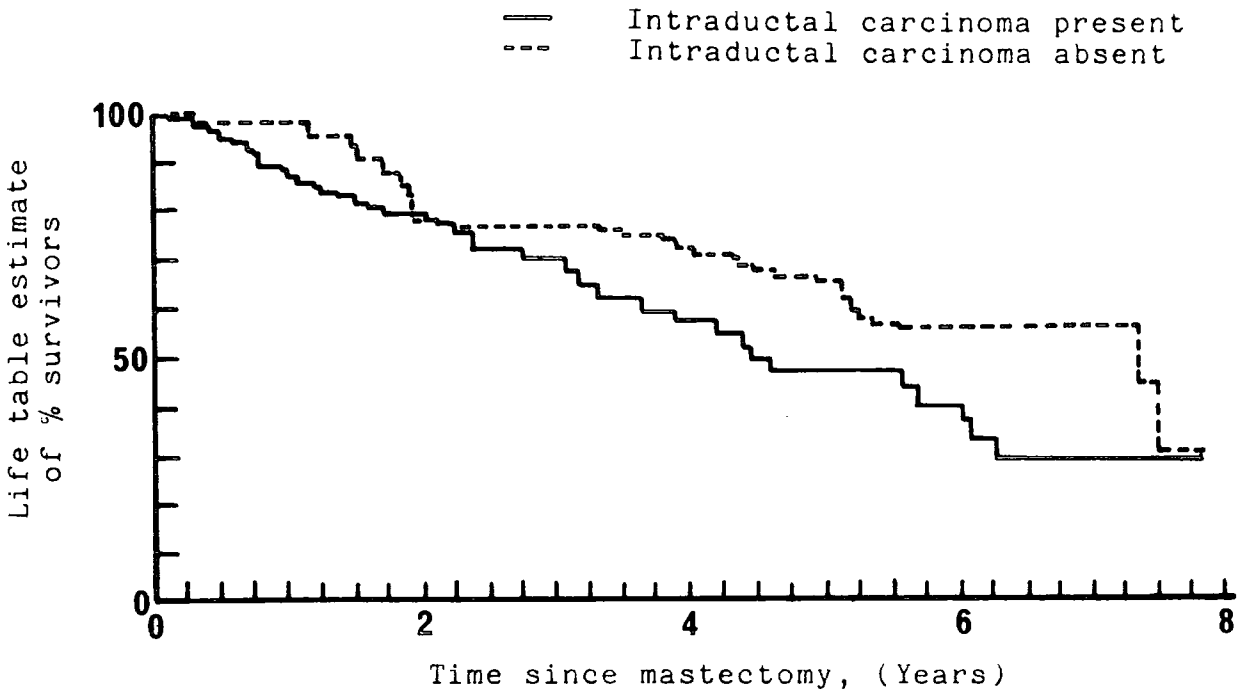


Fig. 26. Survival curves for patients grouped according to the histological grade of the primary tumour.

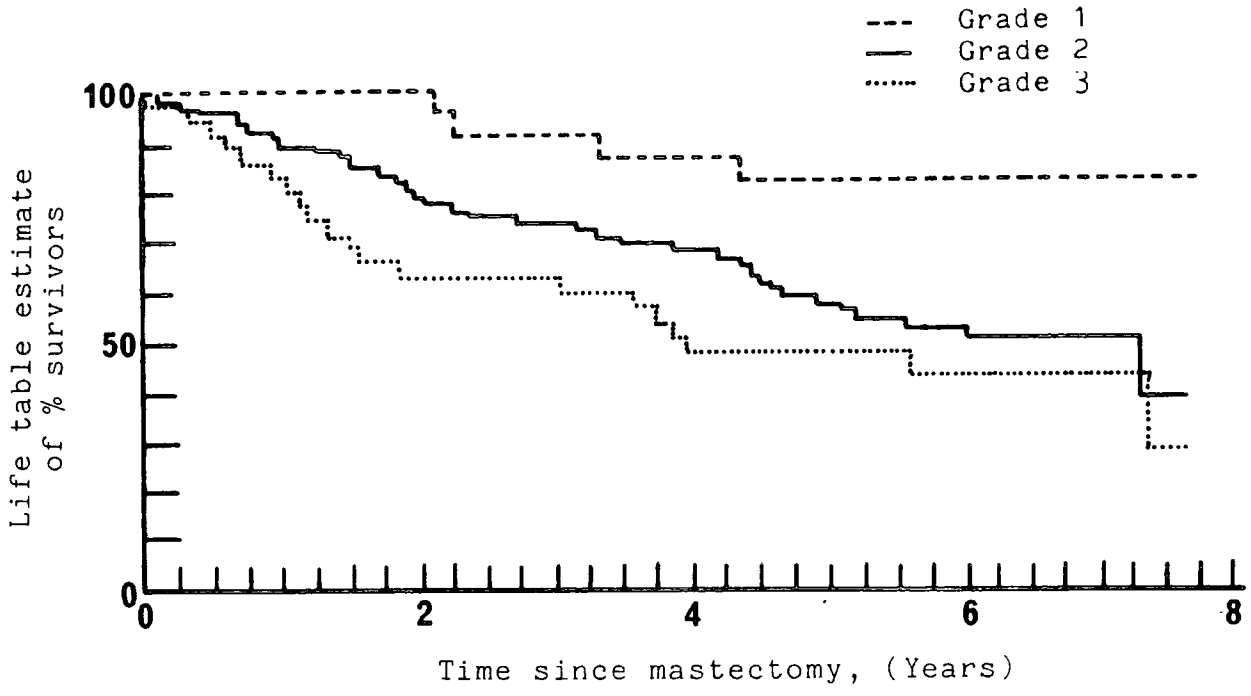


Fig. 27. Survival curves for patients grouped according to their lymph node status at the time of mastectomy.

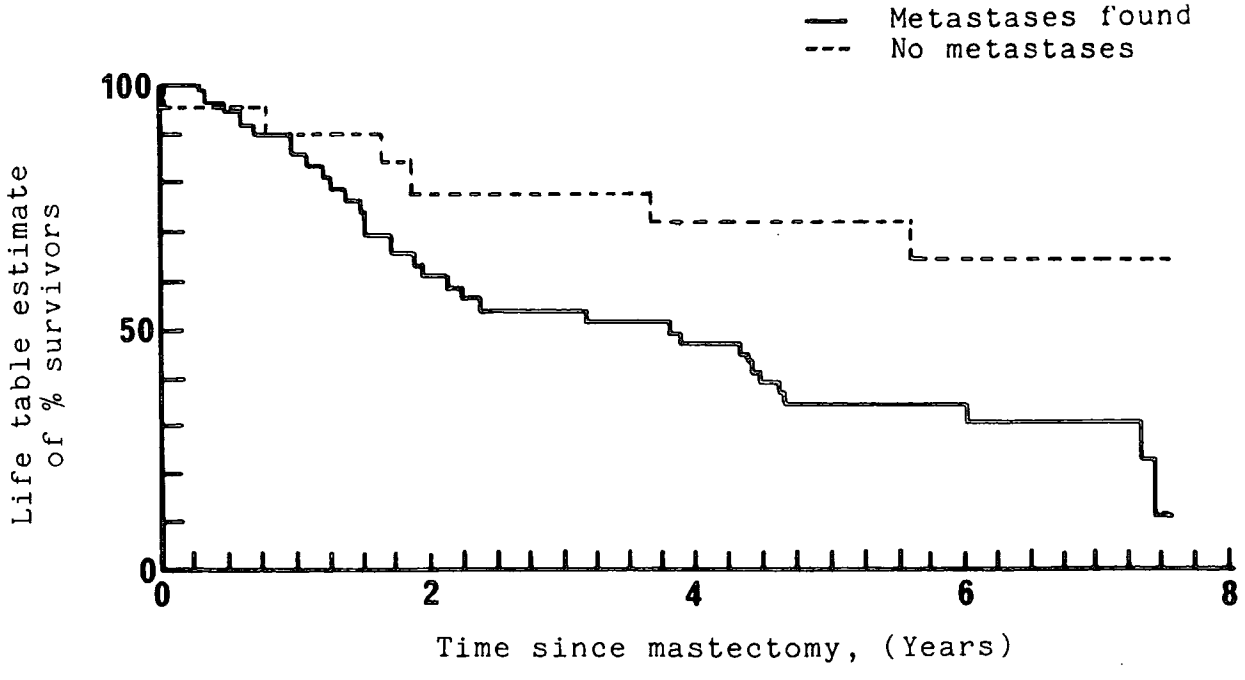
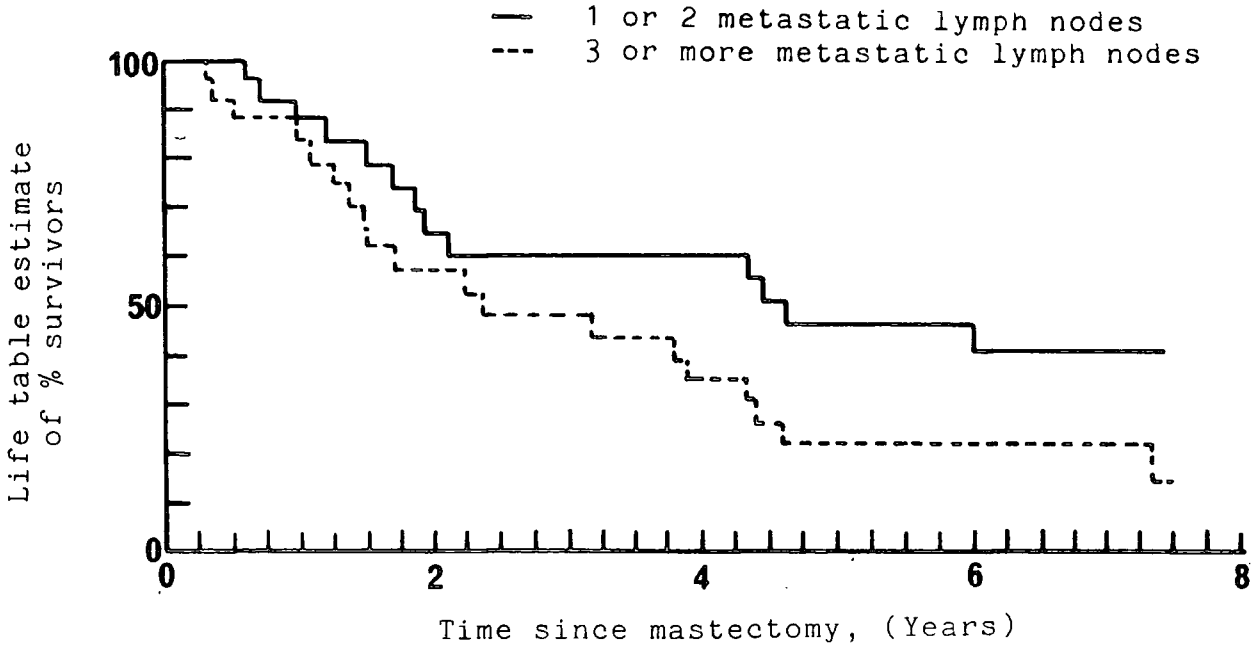


Fig. 28.

Survival curves for patients with lymph node metastases, grouped according to the number of positive lymph nodes found.



Chapter 4: DISCUSSION

The present study has demonstrated CEA by the PAP technique in 43.5% (60/138) of the cases investigated. No relationship could be detected between the occurrence of CEA in the primary tumour and the age, weight, menopausal status or the lymph node status of the patient at the time of mastectomy, or with the size, attachment, lymphocytic infiltration or histological grade of the tumour. A significantly greater number of CEA positive cases was detected for tumours which contained an intraductal element in addition to the invasive carcinoma than for those where intraductal carcinoma was not a component of the tumour. No relationship was found between the occurrence of CEA in the primary tumour and survival of the patient for up to eight years after mastectomy, and this remained the case when defined sub-populations of patients were investigated to exclude the possibility that other prognostic factors may mask or bias any relationship of CEA detection and survival.

It was shown in this study that survival was significantly influenced by the size and the histological grade of the tumour, and the presence of metastatic disease in the axillary lymph nodes of the patient at the time of mastectomy. The menopausal status of the patient at the time of mastectomy, the presence of intraductal carcinoma, attachment or lymphocytic infiltration of the tumour are factors which, in this series, cannot be shown to affect significantly the survival of patients.

The overall occurrence of CEA in primary carcinomas of the breast determined by other workers has varied from 0/12 and 1/62 by Goldenburg and his co-workers (Goldenburg, Sharkey and Primus, 1976; 1978) using PAP and peroxidase techniques respectively, to 10/12 by Heyderman and Munro Neville (1977), also using an immunoperoxidase technique. Shousha et al., (1979), by the same immunoperoxidase technique found 80% of 69 cases to be CEA positive. Kuhajda, Offutt and Mendelsohn (1983), again using peroxidase, demonstrated CEA in 68% of the 93 cases of breast carcinoma investigated, and Rolf Smith and co-workers (Rolf Smith, Howell, Minawa and Morrison, 1982) found 179/290 (62%) of the primary tumours they investigated by this technique to be CEA positive. Walker (1980) demonstrated CEA in 45/90 breast carcinomas also using the peroxidase method. Using an immunofluorescence technique on needle biopsies of primary breast tumours, 21/50 (42%) showed CEA containing cells in the tumour, and these results were confirmed using radioimmunoassay (RIA) of tumour extracts (Wahren, Lidbrink, Wallgren, Eneroth and Zajicek, 1978). Immunohistochemical staining (PAP) was performed upon cultured explants of histologically proven breast carcinomas by Miller and co-workers, (Miller, Sturgeon and Walker, 1983), in experiments on the detection and quantitative release of CEA. They obtained positive staining in 34/54 (63%) cancers, and found 41 (76%) to contain measurable amounts in replicate cultures by RIA. Work on cytosols from breast carcinomas (Duffy, O'Connell, O'Sullivan, McKenna, Allen and McDonnell, 1983) also by RIA found "CEA-like material" in 51/62 (82%) tumours.

These variations in incidence of CEA detection are attributable not only to the different techniques used, but also to the differences in the antisera employed. The character of the antiserum is critical in immunodetection studies. The related antigen NCA has already been mentioned (Ch.2) but the significance of this glycoprotein and the character of anti-CEA antisera should be discussed in more detail.

von Kleist and Burtin (1969) reported that perchloric acid extracts of tumour tissue (as used in the preparation of CEA for antibody production) contain several normal tissue proteins. Work by Kleinman, Harwell and Turner, (1971), who prepared antisera to extracts of colonic adenocarcinomata with perchloric acid, demonstrated two precipitation lines on immunodiffusion. It was suggested that two antigens were extracted, and, as these were not separated by gel filtration on Sephadex G200, that these antigens must be of similar size.

A glycoprotein was then characterized which was present in normal human tissue, and was neither organ or tumour specific, and cross-reacted with CEA (von Kleist et al., 1972). They termed this glycoprotein Nonspecific Crossreacting Antigen (NCA). The same workers found NCA to have the same centroglandular location as CEA in cancerous and non cancerous colonic and gastric mucosae (Burtin et al., 1973), and later produced a specific anti-NCA antiserum which identified many NCA positive cells outside the lymphoid follicles in sections of spleen. (Burtin, Quan and Sabine, 1975).

Other workers (Kuo, Rosai and Tillack, 1973) isolated

glycoproteins from membrane preparations of breast carcinomas. They found a CEA-like antigen sharing antigenic determinants with CEA, and termed it Breast Cancer Glyco Protein (BCGP).

Isaacson and Judd (1977) found that the cross-reactivity of anti-CEA with RBCs, vascular endothelium and Paneth cell granules could be eliminated with periodic acid, (a procedure incorporated into the present study), implying that the antigenic determinants shared with other glycoproteins belong to the carbohydrate portion. Holburn et al. (1974) using ^{125}I-labelled CEA and blood group antibodies concluded that the determinants of A, B and Lewis antigens and of CEA share the same glycoprotein carrier molecules. (Accordingly, the antiserum employed in the present study was absorbed with A and B erythrocytes before use.).

Ormerod (1978) employed RIA techniques with different antigen addition to produce inhibition curves from which he demonstrated the existence of at least five distinct antigenic determinants, establishing three on protein and two on the carbohydrate portion, one of which is shared with NCA (termed CEX by this author).

Kessler, Shively, Pritchard and Todd (1978) isolated and characterized a tumour-extracted, CEA-related antigen "TEX". Later claims were made that this antigen was superior to CEA as a tumour marker for breast and other cancers, (Shively, Spayth, Chang, Metter, Klein, Presant and Todd, 1982).

The evidence for "CEA" being a term for a heterogenous family of related glycoproteins with shared as well as

distinctive antigenic determinants is reviewed by Alpert (1978).

An immunological comparison of CEA extracted from tumours of various organs including colon and ovary, and from pleural and ascitic fluid of patients with cancer of the pancreas, lung and breast (Lamerz and Burtin, 1983), found no evidence for organ specificity of CEA. Other authors (Rogers, Rawlins, Keep, Cooper and Bagshawe, 1981) have suggested that different immunological forms of CEA may be expressed in the course of tumour progression. This work employed the double antibody RIA technique, using a mouse monoclonal anti-CEA which recognised CEA in patients with a variety of solid tumours, to measure CEA in human serum. However, no prognostic value was detected, suggesting that although expression may vary with tumour progression, it may not correspond to such progression.

The antisera employed in the previously mentioned studies concerning the frequency of CEA detection in breast tumours were of various sources. Duffy et al. (1983) used a commercial antiserum, but recognised the limitations of this in describing a "CEA-like material". Shousha et al. (1979), however, make no such distinction although the antiserum they employed (Dakopatts) is known to be contaminated with an antibody directed to NCA (Walker, 1980; present study, Ch. 2). Kuhajda et al. (1983) also employed the Dakopatts commercial antiserum and comment that "the results must be interpreted in the light of tumour marker heterogeneity and variability in both CEA and anti-CEA sera". In general these studies resulted in higher frequences of detection. In studies using this commercial

antiserum (Walker, 1980; Miller et al., 1983; Rolf Smith et al., 1982), attempts were made to abolish the anti-NCA activity by absorbing the antiserum with perchloric acid treated extracts of human spleen. Wahren et al. (1978) claimed no detectable reaction to normal cross-reactive antigen. The partially purified anti-CEA used in this study (as previously discussed) was believed to react only with CEA and reacted in comparable frequency to these studies.

Two reported findings are apparently out of consensus. Goldenburg et al. (1976; 1978) who also used absorption techniques to remove activity to what was termed CCA-III in two series failed to demonstrate staining in breast cancer in all but one instance, but succeeded in tumours of the GI tract, bronchus, ovary and cervix. Heyderman and Munro Neville (1977) employed an absorbed goat anti-CEA serum which detected a high proportion of CEA positive cases in breast cancer, however, only 12 cases were investigated.

No reference has been made in previous reports to investigations into the relationship between the frequency of CEA occurrence in the primary tumour and the weight of the patient with breast disease at the time of mastectomy, or the attachment of the tumour. The present study detected no relationship.

Kuhajda et al. (1983) found no correlation between CEA occurrence and the age of the patient or the size of the tumour. Rolf Smith et al. (1982) found no relationship between CEA occurrence and the size of the tumour either. The present study found no relationship between CEA occurrence and the age of the patient, or size of the tumour.

The frequency of CEA occurrence, either in the primary tumour or in lymph node metastases, was compared according to the menopausal status of the patient by Rolf Smith and co-workers (1982), and no correlation was found. The present study supports this finding for primary tumours.

Walker (1980) demonstrated no relationship between the presence of CEA and the extent of lymphocytic infiltration of the tumour, and no relationship could be detected in this present investigation. However, Duffy et al. (1983) found an inverse correlation of lymphocytic infiltration and levels of "CEA-like material" in cytosols from primary breast tumours.

No significant correlation between CEA and the histological type of the tumour was detected by Walker (1980), finding no difference between the proportion positive in infiltrating duct and infiltrating lobular carcinomas. Similar conclusions were drawn by Shousha and Lyssoitis (1978), and Duffy et al. (1983). Wahren (1978) found no correlation with the cytological type. Kuhajda and co-workers (1983) found intraductal and infiltrating duct carcinomas were more often positive for CEA than were in situ lobular and infiltrating lobular, but no significant difference between in situ lobular and infiltrating lobular, or between intraductal and infiltrating duct carcinoma. However, they observed that the strongest staining was often seen in the intraductal component (or at the advancing edge of the tumour). From a "dilutional immunoperoxidase study" of CEA employing the commercial antiserum, Wells and Hasleton (1981) suggested that as an intraduct lesion becomes invasive it loses its

ability to synthesize or store CEA. The antiserum employed in this work lacks specificity, and there is no mention of numbers of cases investigated. The present study found a significantly greater proportion of cases positive for CEA in invasive tumours which also contained an intraductal component than in invasive tumours without intraductal carcinoma. No investigation was made into pure intraductal carcinomas due to these producing a very different clinical course in a study concerned primarily with survival.

The present study detected no relationship between the occurrence of CEA in the primary tumour and the histological grade. This is in agreement with the findings of Wahren et al. (1978), Rolf Smith et al. (1982), and the findings of Duffy et al. (1983) for CEA-like material in cytosols from breast carcinomas. This latter investigation, however, found that when only considering tumours without, or with very little lymphocytic infiltration, concentrations of the CEA-like material correlated with grade. Only 10 tumours were assessed as having "0" lymphocytic infiltration in this current study, not allowing a similar comparison. In the study by Duffy et al. (1983), differentiation was assessed according to the frequency of mitotic figures, a modification of the Bloom and Richardson (1957) system of assessment. Walker (1980), using this same modification of assessment, detected an apparent relationship between histological differentiation and the presence of CEA, but although the chi-squared test gives a signif icant P value from the numbers given in the appropriate table, the totals quoted in the table for CEA positive and negative do not quite correspond to the

numbers given previously for CEA positive and negative cases.

There are conflicting results from previous studies concerning the significance of CEA occurrence in the primary tumour and synchronous lymph node metastases in the patient. Shousha and Lyssiotis (1978) reported a distinct correlation between the histological demonstration of CEA and the presence of metastases. Walker (1980) found no such relationship. A further study by Kuhajda et al. (1983) also failed to identify a correlation. These workers then investigated smaller tumours (3 cm or less) and reported a trend in this group for strong staining for CEA in the primary tumour to be associated with synchronous lymph node metastases, and suggested the influence of size on metastatic potential could explain this discrepancy between the two studies mentioned above. The tumours investigated by Shousha and Lyssiotis (1978) had a mean size of 2.8 cm. Walker (1980) did not report the mean size of the population of tumours which they investigated, and Kuhajda et al. suggest that this investigation may have included larger tumours. However, the trend they report is not significant ($P=0.09$). The present study found no relationship between CEA demonstration in the primary tumour and the presence of nodal metastases at the time of mastectomy. In this work, an investigation of tumours of 3cm diam. which stained strongly for CEA and had pathologically established patient lymph node status would have been meaningless due to small numbers, (the lymph node status was known for only 4 of the strongly staining tumours, although all 4 tumours were 3 cm diam. or less).

There have been three previous immunohistochemical investigations into the relationship between CEA occurrence in primary breast carcinomas and patient survival. The first study was conducted by Shousha et al. (1979) and involved 69 patients with breast cancer. These workers reported significantly lower 5 and 10 year survival rates for patients who possessed demonstrable CEA in their tumours, and that the difference was not related to the stage of the disease, postoperative treatment, or histological type of tumour, and suggested that this CEA assessment may provide more precise prognostic information. The antiserum employed in this investigation has been discussed previously. Further criticism of this work can be made regarding the method of statistical analysis employed. As discussed in the section on the statistical methods employed in the present study (Ch. 2), the use of chi-squared tests upon the proportion of patients alive at a certain time interval (as performed by Shousha et al., 1979) is inappropriate. Patients who are lost to follow-up, or are known to have died from causes not related to the tumour, are necessarily excluded from investigation with the loss of information and a certain amount of selection bias.

The second study was by Walker (1980) who employed an antiserum free from anti-NCA activity to investigate 90 cases. They found no correlation between the presence of CEA in the primary tumour and recurrence within two years of sugery, but do not mention how this was established. From the table presented it would appear that chi-squared tests were employed as in the previous study.

The third study is that of Rolf Smith et al. (1982). Here the CEA status of both primary and metastatic breast cancer was correlated with the clinical course of patients entered into a controlled trial of adjuvant chemotherapy for the treatment of operable breast cancer, in order to assess the value of CEA expression as a prognostic indicator and as a means of assessing which patients might benefit from adjuvant chemotherapy. This study is concerned exclusively with axillary node positive patients, and involved 290 primary carcinomas and 217 axillary metastases. The longest follow-up was 4 yrs 7 mths, the median duration being 2 yrs 7 mths. The anti-CEA serum employed was considered to be free from anti-NCA activity. These workers found no significant correlation between CEA status of the primary tumours and recurrence-free survival for patients in the untreated control group of patients, or for patients who had received adjuvant chemotherapy. No comparisons were made for actual survival and CEA status in the primary tumour. (All comparisons made were weakly or negatively CEA-staining against positively staining tumours). The present study compared survival in patients with positive lymph nodes when grouped according to the presence or absence of CEA in the tumour. The comparison of survival for patients with negative or weakly staining tumours against that for patients with more strongly staining tumours was not made for lymph node positive patients alone, but for the population as a whole.

When axillary node metastases were investigated by Rolf Smith et al. (1982), however, recurrence-free survival was significantly better in the untreated group

for those patients with strongly positive staining tumours than for those with weakly staining or CEA negative tumours. When actual survival was investigated the difference was not significant. No significant difference was detected for recurrence-free survival when the chemotherapy group was similarly investigated. These workers also found that patients in the treatment group with negative or weakly CEA positive nodes fared significantly better than similar patients in the untreated group. However, when actual survival was considered, the difference was not significant until only the weakly CEA positive nodes were considered alone. No difference in recurrence-free survival was found when the treated and control subgroups of patients with strongly CEA positive nodes were considered.

The authors of the above work state that these correlations were performed using life table analysis, and that the differences were demonstrated using the logrank method of Peto et al. (1977), as used and discussed in the present study. However, no median survival times or confidence intervals are quoted, and of the significant P values quoted three give these P value by direct application of the chi squared test, and the remainder, comparing recurrence-free survival for untreated and treated -/+ CEA subgroup, (giving a P value > 0.05 by the chi squared test) is exactly the same as the prece ding P value in the publication although the numbers concerned are very different and the graphs presented respectively are completely different also.

Various factors are known to influence the prognosis

of patients who are treated for carcinoma of the breast, (see Forrest, 1971; Cutler 1968; Bloom and Richardson, 1957).

The present study is in aggreement with these well accepted conclusions that the size and histological grade of differentiation of the tumour, and the presence of pathologically assessed lymph node metastases at the time of mastectomy are factors which significantly affect survival rates. It confirms that patients who have large or poorly differentiated carcinomas, or lymph node metastases at the time of operation have a significantly reduced rate of survival for up to eight years post surgery.

This investigation found no significant difference in survival up to eight years post surgery when attachment of the tumour was considered. A trend could be seen up to 4 years for patients with tumours attached to the underlying deep tissues having the poorest survival curve, then patients with tumours attached to the skin or nipple, and patients having unattached tumours having the best. However this trend diminished after 4 years. Cut ler (1968) found, in general, a significantly worse prognosis (10 years post surgery) for attached tumours.

No significant differences could be established in the present study for survival in patients grouped according to menopausal status, in patients grouped according to the extent of lymphocytic infiltration, or in patients grouped according to the presence or absence of an intraductal component in their invasive carcinoma.

In order to exclude the possibility of these known prognostic indicators from masking or biasing the

relationship of CEA presence in the tumour and patient survival in this study, survival was compared between CEA positive and negative cases within defined sub-populations. No reports of similar comparisons have been made in previous publications. The study of Rolf Smith et al. (1982) excluded the possibility of bias due to unequal distribution of known prognostic factors when significant differences in survival were found by assessing the distribution of these factors between the groups under comparison using the chi squared test. The present study detected no significant differences, implying that immunohistochemical demonstration of CEA in the primary tumour is of no value in prognosis for any selected group of patients investigated.

It would be inappropriate to discuss the significance of CEA in breast cancer without reference to the many clinical investigations which have been performed using measurements of CEA in the serum of patients with this disease. This approach has advantages and disadvantages. It is non-invasive to the patient, and therefore serial measurements can be made facilitating the monitoring of patients receiving treatment or during "follow-up".

However, it has been established that detectable CEA production is not a definitive feature of breast carcinomas, and, in the instances where a carcinoma does produce CEA, serum measurements may be influenced by many variables. Much work has been carried out in establishing these variables to include the rate of production by tumour cells, factors influencing release of CEA into the circulation, and excretion by the liver.

A study employing cultured human carcinoma cells by Drewinko and Yang (1976) indicated that CEA production is limited to the G1 phase of the mitotic cycle. Studies on cultured human colonic carcinoma cells (Rosenthal, Tompkins and Rawls, 1980) showed a stability of CEA expression which suggested that the level of CEA expressed by a cell is genetically controlled. Quayle (1982a) proposed that tumour lysis may be an important factor affecting blood levels of CEA, from work on a model tumour system employing immune-deprived mice bearing tumour xenografts (including the breast line S32). In later experiments using a similar system, Quayle (1982b) established correlation of tumour size with CEA blood levels in half the tumour lines investigated, and suggested the possibility of central necrosis of tumours to account for the variability and the contradictory findings (no correlation with tumour size) reported by Lewis and Keep (1981) in a comparable study. The latter authors discuss an array of factors which can potentially influence circulating CEA levels, including the kinetics of cell cycling within individual tumours, the degree of vascularization and lymphatic invasion of tumours, and host metabolism and degradation of circulating CEA. Zamcheck and Martin (1981) summarized factors influencing plasma CEA levels in patients with pancreatic cancer.

Initial clinical studies concerning serum CEA were structured to investigate this substance as a marker for cancer. Laurence, Stevens, Bettelheim et al. (1972) found raised plasma CEA levels in patients with many malignant tumours, including breast cancer. These workers also

detected raised levels in association with inflamation or regeneration, and concluded that this test could not serve as a routine screening test for cancer, but suggested a role in monitoring patients during the post therapeutic follow-up period.

Other workers (Steward, Nixon, Zamcheck and Aisenberg, 1974) proposed that the proportion of raised CEA levels increased with increased tumour spread, and that a good clinical patient response to therapy was mirrored by a fall in CEA level. An investigation by Wang, Bulbrook, Hayward, Hendrick and Franchimont (1975) found that the proportion of healthy women with measurable CEA was similar to that found in women with early breast cancer, and a similar proportion had an increased level after mastectomy. These workers also reported that a higher proportion of women with advanced disease had positive CEA titres, and proposed that raised post mastectomy levels implied a faster recurrence rate. This poposal was made again by Myers, Sutherland, Meakin, Kellen, Malkin and Malkin (1978). The study of Haagensen, Kister, Vandevoorde et al. (1978) reported elevated CEA levels in a post mastectomy population to be associated with development of metastases in 1/3 subjects. Tormey and Waalkes (1978) reported that the percentage of patients with elevated CEA levels increased with each clinical stage of breast cancer. Rimsten, Adami, Wahren and Nordin (1978), however, reported a similar frequency of raised CEA in patient with primary breast cancer and in healthy age-matched controls.

Subsequently, the implications for CEA in the serum of patients with breast cancer evolved from that of a marker

for the disease, to that of a monitor. McCarty, Cox, Silva et al. (1980) investigated CEA levels in the clinical response to hormone therapy, and found that the maximum functional decrease of elevated levels correlated linearly with the duration of response in patients who received hormone therapy. Other investigations (De Jong-Bakker, Hart, Persijn and Cleton, 1981) indicated that a pre-treatment CEA level was found to have no relation to prognosis, but a rise during follow-up was usually associated with progressive disease. Chatal, Chupin, Ricolleau et al. (1981) suggested the use of serial assays to detect relapses in "high risk" patients. Similarly, Doyle, Nicholson, Groome and Blamey (1981) concluded that CEA is of no prognostic value in primary stage breast cancer, but could be of value in monitoring treatment in patients with advanced disease, saving many patients from suffering side effects of several unnecessary cycles of chemotherapy. This argument was also put foreward by other workers (Falkson, Folkson, Portugal, van der Watt and Schoeman, 1982). Others (Staab, Ahlemann, Anderer, Hiesche and Rodatz, 1981) found that before treatment there was a higher incidence of raised serum CEA with distant metastases than in patients with local disease. Bezwoda, Derman, Bothwell, MacPhil, Levin and De Moor (1981) also found levels of serum CEA to be higher in patients with metastases. Lee (1983) observed a pronounced elevation of CEA most frequently in patients with hepatic and osseous metastases, supporting the similar previous findings of Tormey and Waalkes (1978). Other suggestions include a role for the serial measurement of cerebro-spinal fluid CEA in

the clinical management of meningeal carcinomatosis in patients with breast cancer.

Serum CEA has been extensively investigated in a similar manner in patients with a variety of malignant diseases, including those of the lung (Waalkes, Abeloff, Woo, Ettinger, Ruddon and Aldenderfer, 1980; Goslin, Skarin and Zamcheck, 1981; Woo, Waalkes, Abeloff, Ettinger, McNitt and Gehrke, 1981; Lokich, 1982), bronchial carcinomas (Paone, Kardana, Rogers, Dhasmanda and Jeyasingham, 1980), carcinoma of the cervix (Donaldson, van Nagell, Pursell, Gay, Meeker, Kashmiri and van de Voorde, 1980; Kjorstad and Orjasaester, 1982; Te Velde, Persijn, Ballieux and Faber, 1982), carcinoma of the pancreas (Zamcheck and Martin, 1981), hepatomas (Melia, Johnson, Carter, Munroe Neville and Williams, 1981), tumours of the thyroid (Rougier, Calmettes, Laplanche, Travagli, Lefevre, Parmentier, Milhaud and Tubiana, 1983), and especially colorectal carcinomas (Goslin, Steele, Macintyre, Mayer, Sugarbaker, Cleghorn, Wilson and Zamcheck, 1980; Rognum, Elgjo, Brandtzaeg, Orjasaeter and Bergan, 1982; Finlay and McArdle, 1983; Rognum and Brandtzaeg, 1983; Goldenberg, Kim, Bennett, Nelson and Deland, 1983).

A National Institutes of Health Consensus Development Conference was held in 1980 to address issues concerning the role of CEA in the management of cancer. The conclusions of this conference were that CEA should not be used in cancer screening, nor independently to establish a diagnosis of cancer, but that CEA may have value in assessing the extent of the disease in colorectal and bronchial carcinomas, and in monitoring the treatment of

colorectal cancer, whilst the arguments for assessing and monitoring CEA in other carcinomas were interpreted as much less convincing, (see summary NIH Consensus Statement, 1981). At present it would appear that clinical assessment by serum CEA lacks sensitivity and specificity.

The results of the present study suggest that assessment of histologically detectable CEA has similar limitations, and is of no value as an indicator of patient prognosis.

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SCHEDULE

IDENTIFICATION NO. 1 2 3

ITEM SHEET NO. 4 [1]

DATE OF BIRTH 5 6 7 8 9 10

AGE 11 12

DATE ADMITTED 13 14 15 16 17 18

MENOPAUSAL STATUS

Premenopausal	(last p.	6 months ago)	1
Perimenopausal	(last p.	6/12 to 5 yrs ago)	2
Postmenopausal	(last p.	5 yrs ago)	3
Uncertain	(inc.	60 + hysterectomy)	4
Not available			9

19

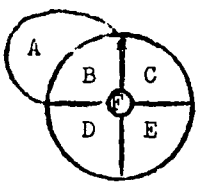
EXAMINATION

Weight in kgs. 20 21 22

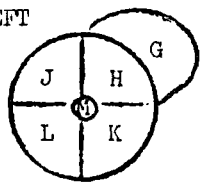
Weight in st. lbs. 23 24 25 26

SITE OF TUMOUR

RIGHT



LEFT



RIGHT

LEFT

YES = 1

A		27	J		35
B		28	K		36
C		29	L		37
D		30	M		38
E		31	N		39
F		32	P		40
G		33	R		41
H		34	S		42

SIZE OF TUMOUR

More than 5 cm. max. diam.	1	43
Less than 5 cm. max. diam.	2	

ATTACHMENT OF TUMOUR

Skin or nipple	1	44
Deep	2	

LYMPH NODES

Ipsilateral axillary nodes:-		
none palpable	1	45
palpable - clinically not involved	2	
clinically involved	3	
Supraclavicular nodes:-		
none palpable	1	46
palpable	2	

INVESTIGATIONS

leave blank if not done

Skeletal survey:-		
suspicious	1	47
not suspicious	2	
Bone scan:-		
suspicious	1	48
not suspicious	2	
Evidence of soft tissue metastases:-		
Yes	1	49
No	2	

IDENTIFICATION NO. 1 2 3

ITEM SHEET NO. 4 [2]

OPERATION DATE 5 6 7 8 9 10

METHOD BIOPSY:-

Excision biopsy	1	11
Incision biopsy	2	
Needle, drill, aspiration biopsy	3	
Other	4	
None	5	

FROZEN SECTION:-

Yess	1	12
No	2	

TYPE OF OPERATION:-

Local excision	1	13
Total simple mastectomy	2	
Simple mastectomy with total axillary clearance (inc. patey mastectomy)	3	
Classical radical	4	

NODE BIOPSY (only complete if total simple mastectomy performed):-

Nodes found for histology:-		14
Yes	1	
No	2	
Nodal metastases found:-		15
No	1	
One or two	2	
More than two	3	

RADIOTHERAPY:-

Given	1	16
Not given	2	

CYTOTOXICS:-

Given	1	17
Not given	2	

OTHER HORMONE THERAPY:-

Given	1	18
Not given	2	

IDENTIFICATION NO. 1 2 3

ITEM SHEET NO. 4 [3]

PATIENT ALIVE:

No recurrence detectable	1	5
Recurrent disease local	2	
Recurrent disease distant	3	

PATIENT DEAD:

Date of death		6 7 8 9 10 11
Autopsy carried out	1	12
Autopsy not carried out	2	

CAUSE OF DEATH:

Breast cancer	1	
Other causes - breast cancer present	2	13
Other causes - breast cancer not present	3	
Uncertain	4	
Not available	9	

IDENTIFICATION NO. [1][2][3]

ITEM SHEET NO. [4]

HISTOLOGY BIOPSY NO. [5][6][7][8][9][10]

MACROSCOPIC APPEARANCE OF BREAST TUMOUR:-

Maximum dimension in cms. [11][12]

Tumour edge: [13]
- well defined 1
- poorly defined 2
- not known 3

No. of axillary Lymph Nodes found: [14]
- none 1
- one or two 2
- more than two 3

HISTOLOGICAL APPEARANCE OF TUMOURS:-

Invasive carcinoma: [15]
- Ductal 1
- Lobular 2
- Medullary 3

Intraduct carcinoma Yes 1 [16]

Lobular in situ Yes 1 [17]

Paget's disease Yes 1 [18]

Grade of tumour: [19]
- well differentiated 1
- moderately differentiated 2
- poorly differentiated 3

Lymphocyte infiltration in and around tumour: [20]
- 0 1
- + 2
- ++ 3

IDENTIFICATION NO. 1 2 3

ITEM SHEET NO. 4 5

IMMUNOLOCALISATION - RETROSPECTIVE PARAFFIN

Carcinoembryonic antigen

Immunoperoxidase 0 1=Yes 7

± 0
1+ 1
2+ 2
3+ 3

8