1. INTRODUCTION

Breast cancer is a major health problem for women in many countries. In Western Europe and North America, one fifth of cancer deaths and one twenty fifth of all deaths are attributed to breast cancer (Logan, 1975). Breast cancer is common in India, being second to cancer of cervix uteri. It uniformly affects all social and religious groups with higher predilection in Parsis (see Vaidya, 1983). The incidence of breast cancer in India is midway between the high incidence in the USA and Western Europe and the low incidence in Japan (Shimkin, 1973). The risk factors for breast cancer may be classed generally as genetic, hormonal, nutritional and breast irradiation (Berg, 1984). Cancer of the breast is described as hormone dependent on the basis of two facts. It arises from a tissue that normally is responsive to endogenous hormones and its course often be influenced favourably or unfavourably, by administration of hormones or removal of hormones (see Zumoff, 1982).

Epidemiological studies have demonstrated that Caucasian women in Western world are at a much higher risk than Asian and African females, in terms of incidence and mortality (see Levin and Thomas, 1977). However, low risk immigrants to high risk countries display an increased incidence of breast cancer in succeeding generations (Buell, 1973). This suggests
that environmental factors play a more significant role than genetic factors in determining the risk in racial groups.

Breast cancer occurs more frequently in women of higher socio-economic status, as measured in the United States and in the United Kingdom, by education level and income (see Campbell, 1972). This relationship between breast cancer and education has been accounted for by the fact that education has an influence on marital status, parity and age at first pregnancy (Wynder et al., 1960). In Western India, the relatively more prosperous Parsis have more breast cancer than Hindus, Muslims, Christians and Jews. Compared to women of other Indian communities, the high risk Parsis tend to marry less frequently, have their first child at a later age and also have fewer children (Paymaster and Gangadharan, 1972).

As early as in 1713, it was recognized that nuns have more risk of breast cancer than lay women (Ramazzini, 1713) and is confined to their postmenopausal years. This observation was well supported by later studies (Lancaster, 1971; Paymaster and Gangadharan, 1972).

A positive relationship was found between early menarche and increased breast cancer risk in majority of the matched case control studies (see Juret, 1982).
Straszewski (1971) estimated that women with menarche before 16 years have 1.8 times the risk of developing breast cancer compared to those with later onset of menarche. Shapiro et al. (1968) found a two fold increase in risk for women who had menarche before 12 years compared to those who had menarche at 15 years or later.

There is also a positive relationship between the late menopause and the risk of breast cancer. Trichopoulos et al. (1971) estimated that the risk for women with menopause at 55 years or late is twice of those who had menopause before that age. Stavraky and Emmons (1974) reported a 2.5 times greater risk for women with menopause after 55 years than those who had earlier than 40 years. Case control studies showed that breast cancer patients tend to have a longer menstrual life. (Shapiro et al., 1968).

The case control study conducted by Mac Mohan et al. (1970) revealed that the protection against breast cancer afforded by pregnancy lies with the age at first child birth, rather than with the number of pregnancies. According to their findings, mammary cancer risk is reduced to about one third for a woman who had her first child at 18 years compared to the woman who had the first child at 35 years. With only minimal differences, these findings were confirmed by others (Hunt et al., 1980). Lactation has a protective
effect against breast cancer because breast feeding arrests the menstrual cycle and has an unfavourable effect on cancer risk (see Juret, 1982).

HORMONES

The relationship of breast cancer risk with age at menarche, age at menopause, oophorectomy and the time of first child birth, as well as to parity suggests endogenous hormones as an important factor in mammary carcinogenesis. The hormones most likely involved in such pathogenic cellular changes include estrogen, progesterone, prolactin and androgen (Thomas, 1984). These hormones are not mutagenic but exert their carcinogenic effect by altering the rate of proliferation, atrophy or differentiation of stem or intermediate cells (Thomas, 1984).

Among all the hormones associated with breast cancer, estrogens have been most extensively studied. Haddow et al. (1944) and Taylor et al. (1948) were the first to report the role of estrogen in the development of breast cancer in young women. When the ratio of estriol to other estrogen fractions is low, women have a greater risk for the development of breast cancer (Lemon, 1969).

Although estrogens have been linked to the etiology and development of breast cancer, no consistent difference has been observed in the plasma hormone levels between breast cancer patients and normal women (Ota et al., 1986). Premenopausal and
postmenopausal women from high and low risk groups had similar estradiol levels, but adolescents in the low risk group demonstrated significantly higher luteal phase estradiol and estrone (Bulbrook et al., 1976). However, few reports demonstrated that the mean estradiol levels were slightly but significantly higher in the breast cancer patients than normal subjects, during follicular and luteal phases as well (England et al., 1974; Cole et al., 1978).

Progesterone, which is largely produced during the luteal phase of the menstrual cycle causes alveolar cell growth and also differentiation, so it is unclear whether its net effect would be to increase or decrease the risk of breast cancer (Thomas, 1984). Because of its modifying effect on estrogen activity, it could be considered as a protective agent. Indeed, Sherman and Korenman (1974) speculated that breast cancer risk reflected inadequate corpus luteum formation and such unopposed estrogen stimulation may initiate breast cancer in some individuals. However, the fact that progestational agents promote mammary tumors in dogs (Nelson et al., 1972) and act as cocarcinogens with other hormones (Muhlbock and Boot, 1967) and chemicals (Poel, 1968) in rodents suggest that progesterone also constitutes a positive etiological factor.

Studies of plasma progesterone levels in human have failed to demonstrate any difference between high
and low risk populations (Bulbrook et al., 1978) or breast cancer patients and normal subjects (Malarkey et al., 1977). Daughters of breast cancer patients were found to have higher levels of plasma progesterone and urinary pregnanediol than controls (Pike et al., 1977).

Androgens may be protective against breast cancer as they depress mammary cell growth (Thomas, 1984). Bulbrook et al. (1971) found in a prospective study in the Island of Guernsey that women with breast cancer had a previous history of low urinary ketosteroids like etiocholanolone and androsterone. However, Hayward et al. (1978) reported low levels of plasma and urinary adrenal androgens in normal Japanese women when compared to normal British women. The contradictory results leave the role, if any, of adrenal androgens in the etiology of breast cancer, unclear.

The relationship between thyroid hormones and breast cancer if any, is not well understood. Repert (1952) suggested on epidemiological basis that breast cancer is associated with abnormalities of thyroid function. While the incidence of breast cancer is low in hyperthyroid patients (Humphrey and Swerdlow, 1964), it is high in women receiving thyroid therapy for hypothyroidism (Kapdi and Wolfe, 1976). Nevertheless, the incidence of breast cancer parallels that of endemic goitre due to iodine deficiency (Doll, 1969).
In recent years, there has been greater interest in the role of prolactin in breast carcinogenesis. Prolactin acts on the normal mammary gland to initiate and maintain lactation. Prolactin has been hypothesized to play a role in human breast cancer, largely because of its known physiological, mammotrophic effects and its influence on the induction of various types of mammary cancer in rodents (Chan et al., 1975). Multiple pituitary isografts, hypothalamic lesions and drugs which stimulate prolactin secretion, all increase the incidence of mammary tumors in rats and mice. On the other hand, drug induced suppression of prolactin release decrease the growth of established tumors (see Henderson et al., 1984).

Whereas the role of prolactin in experimental mammary tumorigenesis is fairly well established, its importance in human breast cancer is not well understood. Although prolactin has been connected with the development of breast cancer (Henderson et al., 1975; Kim and Furth, 1976; Cole et al., 1977), many experts deny such association (Boyns et al., 1973; Mittra et al., 1974).

There is no common agreement concerning plasma prolactin levels in breast cancer patients. While some reported high plasma prolactin levels in breast cancer patients (Rolandi et al., 1974; England and Sellwood, 1976) others could not confirm the same (Boyns et al.,
1973; Franks et al., 1974; Robyn, 1975; Mc Fadyen et al., 1976.

RECEPTORS

It has long been known that some human breast cancers are hormone dependent in that they undergo striking regression, when deprived of supporting hormone by removal of ovaries, adrenals or pituitary or on altering the hormonal 'milieu' by the administration of androgens or antiestrogens such as tamoxifen (see Seibert and Lippman, 1982).

Endocrine therapy affords the most effective treatment presently available for advanced breast cancer of the hormone dependent type (Jensen, 1981). However, only 25-30% of the patients have tumors of the hormone dependent type. Hence, there is a need for some means/marker to identify hormone dependent type, so that endocrine therapy can be restricted to persons to whom it can help and the majority of patients can be spared the trauma of useless surgery and therapy (Jensen, 1981).

Studies during the past 15 years have established that estrogen receptor content of an excised tumor specimen can provide useful information in selecting the optimal endocrine therapy (Jensen, 1981). There are some cases with substantial level of estrogen receptor but still do not respond to hormonal therapy. The reason for this is not clearly known but probably due
to tumor heterogeneity in some cases and in others, receptor may be present but nonfunctional (see Jensen et al., 1975).

Subsequent experience has shown that breast cancers that contain substantial amounts of both estrogen and progesterone receptors show a significantly high response to endocrine therapy, suggesting that measurement of both receptors can increase the accuracy of the hormone dependency. However, some cancers with high levels of both receptors still do not benefit from hormonal therapy (see Mc Guire et al., 1978).

Recently prolactin receptors have also been identified in human breast cancer, suggesting a potential role of this hormone in mammary tumorigenesis. (Peyret et al 1984).

Inspite of the extensive investigations on hormone receptors the prognostic importance and the predictive value are low sometimes. The definition of other biochemical parameters which may be of prognostic value would appear to be desirable (Hilf et al., 1980, 1982). Of potential interest in this regard are the differences in the activity of a number of glycolytic enzymes between benign and malignant breast tissues (Hilf et al., 1973) and also between tissues responsive and nonresponsive to chemotherapy (Hilf et al., 1982). Premalignant changes could be detected biochemically before histologic changes were evident and the elevated
activities of glycolytic enzymes may be indicative of cancer (Balinsky et al., 1984). A logistic regression model has been developed in which raised activities of a number of these glycolytic enzymes were correlated with response to chemotherapy, while estrogen receptor assay determination did not have any such predictive value (Miller et al. 1982).

**GLYCOLYSIS**

Tumors display aberrant gene expression and this accounts for the appearance of new proteins and altered metabolic activity. The most remarkable and consistent metabolic abnormality of neoplastic tissue is the high glycolysis, as first described by Warburg (1931). Cancer develops as a result of an irreversible alteration in oxidative systems of the cell and mere anaerobiosis would be sufficient to induce the malignant transformation of normal cells (Warburg, 1931). It has been postulated that during the transformation of a normal cell into a malignant one, the cells are exposed to anaerobiosis, as a result of which permanent damage to the electron transport system occurs (Warburg, 1956). Carbohydrate metabolism in cancer cells is characterized by the predominance of glycolysis over gluconeogenesis, presumably to meet increased energy requirements and to facilitate the production of ribose 5' phosphate for increased DNA synthesis (Webber, 1977 a, b).
It has been shown that the destruction of the coenzyme NAD is faster in homogenates of cancer cells than in normal tissues (Wenner and Weinhouse, 1953). Probably, the respiration of cancer cells is essentially normal but the production or subcellular distribution of coenzymes and cofactors are altered/modified (Weinhouse, 1955). Branstor and Morton (1956) reported that the synthesis of NAD is less active in nuclei isolated from breast cancers than in nuclei of normal mammary glands. Such a result is in agreement with the view that in tumors, the biochemical lesions involves enzymic systems linked with NAD. This conclusion was also supported by the experimental work of Glock and McLean (1957) in animal tumor system.

The existing literature on the biochemistry of human breast cancer is limited. Goldman et al., (1964) reported a correlation between total lactic dehydrogenase (LDH) activity and the degree of histological malignancy. A significant elevation of the anaerobic isozymes of LDH (M type) was found in malignant than in benign breast cancer. The increase in glucose-6-phosphate dehydrogenase (G-6-PDH) activity is associated with intraductal proliferation, whether benign or malignant (Cohen, 1964).

One of the most significant metabolic differences between normal and malignant cells appears to be the disproportionately high LDH activity in the malignant cells resulting in the lowering of the G-6-PDH/LDH
ratio (Deshpande et al., 1977). A relationship between histological grading and increased phosphohexose isomerase (PHI) activity was reported by Muir and Fawcett (1965).

PLASMA MEMBRANE

Another aspect of tissue which may give a lead to predict the cancer is the membrane biochemistry. The cell surface plays a crucial role in the regulation of cell metabolism in general and in the regulation of cell proliferation in particular (see Moolenaar, 1981). The plasma membrane receives and transduces growth stimulating signals which trigger a cascade of physiological and biochemical events in the cell. Ultimately, these events lead to the initiation of DNA synthesis and cell division (see Moolenaar, 1981). Membrane modifications are consistent features of the transformed neoplastic cells. During last decades numerous changes have been described in isolated plasma membranes and in the intact surface of tumor cells as compared with homologous normal cells. These changes differ widely in character and concern various chemical classes of membrane components and also the functional activities related to enzymes, antigens, hormones, receptor proteins and transport processes (see Emmelot, et al., 1981).

Of the recorded membrane changes, none is explicitly known to mediate the tumorigenesis directly.
If some changes were to have such an effect, it would represent the direct expression at the cell surface of the primary events in tumorigenesis. Changes can also represent secondary expressions associated with or derived from primary events (see Emmelot et al., 1981).

A number of cellular functions, such as cellular adhesiveness, contact inhibition of growth and movement, and antigenicity are regulated by the cell plasma membrane (Chatterjee et al., 1981). Transformed or malignant cells, aberrant in these biologic characteristics differ from their normal counterparts with respect to structure and composition of their plasma membranes (see Wallach, 1975). Some attempts to elucidate the role of cell surface in transformation involved the determination of alkaline phosphatase activity as a plasma membrane marker enzyme. The differential behaviour of this and related membrane enzymes may provide a monitor for indicating the biological behaviour of tumor cell (Larner and Rutherford, 1977).

The ectopic production of alkaline phosphatase by human neoplasm is very well known. The isozymes of alkaline phosphatases are coded by three separate structural loci: one for placental alkaline phosphatase, one for liver/bone/kidney alkaline phosphatase and at least one which controls the
intestine (McKenna et al., 1979). Placental type of alkaline phosphatase has been reported in the tumors of the breast (Wada et al., 1979; Mc Dicken et al., 1983).

5' nucleotidase is an established plasma membrane marker in many mammalian cells where it exists as an ectoenzyme (Oseroff et al., 1973). In rat mammary adenocarcinoma, it was demonstrated that the activities of a number of plasma marker enzymes, including 5' nucleotidase were significantly reduced in the homogenates and purified plasma membrane preparations from the metastasizing tumor (Chatterjee et al., 1981). Kim et al., 1975). found that 75% of the 5' nucleotidase activity was membrane bound in nonmetastasizing rat mammary tumors as compared to 20% in the metastasizing tumors.

Gamma glutamyl transferase (GGTP) is another membrane bound enzyme which catalyzes the transfer of gamma glutamyl groups between peptides or amino acids (Tate and Rose, 1977). Numerous investigators have shown that increased activity of GGTP is associated with skin neoplasm, liver carcinoma and mammary carcinoma in animal tumor system (Cheng et al., 1978; Deyoung et al., 1978). Increased GGTP activity has been detected in neoplastic tissue compared to that in the corresponding normal tissue, in rat mammary gland (Jaken and Mason, 1978) and human breast cancer (Dawson et al., 1979).
LYSOSOMES

The importance of lysosomes in cellular digestion is very well known. Lysosomes degrade cell organelles (Segal et al., 1969). Lysosomal enzymes can digest and modify the ground substance of connective tissues, thereby favouring growth or migration of both normal and malignant cells (see Poole, 1973). The extracellular fluid of tumors has a high content of viable cells detached from both tumors and surrounding tissues, facilitated by lysosomal hydrolases. Exogenous administration of glycosidase inhibitors inhibited tumor spread under some conditions (see Poole, 1973).

Increased lysosomal enzyme activities have been found in processes characterized by tissue breakdown, as in hormonal, spontaneous, chemotherapeutic and irradiation induced tumor regression, in muscle breakdown and in postpartum mammary gland (Lanzerotti and Gullino, 1972). Likewise, chemotherapy, radiation or hormone induced tumor regression was accompanied by increased acid hydrolase activities in whole tumors or cells and tissues surrounding the tumors (Lanzerotti and Gullino, 1972).

Acid phosphatase, usually considered as the classic lysosomal enzyme, has long been used in the diagnosis and evaluation of prostatic carcinoma (Murphy et al., 1969). Elevation of this enzyme has also been observed in the course of breast cancer, and its
by cancer cells (Bates and Longo, 1955). A variety of substances, including enzymes, hormones, antigens and proteins may be called as tumor markers. The detection and quantitation of these tumor markers, by serologic examination has potential applications for diagnosis, assessment of tumor burden, evaluation of therapy and monitoring of disease progression (Staal et al., 1935). A variety of tumor markers have been identified in breast carcinomas.

Carcinoembryonic antigen (CEA) is an oncofetal antigen normally found in the embryonic and fetal gut and sometime produced by malignant cells. CEA was discovered in 1965 by Gold and Freedman in patients with adenocarcinoma of the colon. Since its initial description, this antigen has been detected in patients with gynaecologic neoplasm (Di Saia et al., 1975). In the carcinoma of the breast, as in colon cancer, the incidence and the levels of CEA elevation correlates with the stage of the disease. The highest levels are seen in patients with liver and bone involvement (Tormey et al., 1977). Serial assessments of CEA levels during therapy for metastatic breast cancer have shown a rise with disease progression and a stabilization or decline with response (Tormey et al., 1977; Haagensen et al., 1978). Post operative elevations of CEA correlate with disease recurrence (Haagensen et al., 1978).
Human chorionic gonadotropin (hCG) is a glycoprotein hormone secreted by the placenta and normally present only in pregnancy, and it has been found to be associated with a variety of trophoblastic and non-trophoblastic malignancies (Bates and Longo, 1985).

Plasma membrane constituents are shed into the surrounding media, in vitro and in vivo, as cell replicate (De Broe et al., 1977). It is conceivable that solid tumors growing in a host would release these constituents into the systemic circulation. With proliferation and metabolic rates of the tumor cells being higher than most normal cells, the rate of shedding of plasma membrane constituents into the circulation of a tumor-bearing host would also be expected to be higher (Chatterjee et al., 1981). Detection of released plasma membrane constituents in the circulation in amounts higher than normal might thus indicate the presence of tumor. Changes in blood parameters in malignancies may reflect the effects of proliferation of cells with growth potential and metabolic turnover dramatically different from those of normal cells (see Stefanini, 1985).

Serum 5' nucleotidase is a superb marker in differentiating the presence of liver metastasis in a patient with elevated alkaline phosphatase activity. If the nucleotidase is normal with elevated alkaline phosphatase, the patient may have bone metastasis. If
both enzymes are elevated, there is a very strong probability of liver metastasis. Gamma glutamyl transferrase has also been used to further confirm the liver metastasis (Schwartz, 1984).

Serum phosphohexose isomerase, lactic dehydrogenase and its isozymes also reflect the clinical status of the patient (Schwartz, 1984).

In the search for a battery of biomarkers useful for diagnostic screening of breast cancer, the serological estimation of these tumor markers could be of more importance to know the extent of the metastasis and to know the progression or regression of the disease.
SCOPE OF THE PRESENT INVESTIGATION

Taking into consideration of all available information on breast cancer, the present study has been planned to provide additional information which may be useful in the prognosis and therapy. Women with fibroadenoma and carcinoma of the breast were investigated in the present study.

Most of the available studies on hormonal profiles have been carried out in breast cancer subjects irrespective of the menstrual status and even the menopausal status. This provided inconsistent and controversial results. In the present study the breast cancer subjects were divided into premenopausal and postmenopausal age groups. The premenopausal subjects were further classified according to their menstrual status. Circulating hormonal profiles were studied in these subjects to know whether any consistent differences would be observed, if so whether they could be implicated as markers or etiological factors in the disease.

The studies of Morris and Novikoff hepatomas have shown that the growth rates of these animal tumors are related to the enzymes associated with carbohydrate metabolism. Attempts have been made to form a similar concept for human breast cancer on the basis of similarities in enzyme patterns between these neoplasms and animal tumors whose growth rates and hormonal
dependence are well established. It was therefore decided to test whether there is any correlation between enzymes of carbohydrate metabolism and the clinical course of the disease.

Knowledge of the functional status of the lysosomal hydrolytic enzymes is an important parameter for the understanding of their participation in tissue regression following injury. So far investigation on lysosomes have been carried out only on tumors of experimental animals. Very limited information is available on human subjects. To validate further the lysosomal theory in relation to human breast cancer, the pattern of distribution of various acid hydrolases were determined.

It has been well established that biochemical characteristics of the hormone dependent cancers are different from those of hormone independent cancers. Differences in sensitivity to hormones by various neoplastic tissues may be a reflection of their differences in metabolic characteristics. Hence, a detailed investigation on both the hormonal and metabolic changes in the same group of patients will throw more light in recognizing the nature of the unresponsive tumors. Hence, an attempt has been made to evaluate the circulating hormonal profiles and tissue biochemical parameters and their interrelationships in breast cancer patients. This may provide an insight
into the understanding of the prolife
proliferative changes of the disease or circu-
changes of the disease or circulating factors to influence the changes.

The present study will provide a better idea about the ocular changes associated with different stages of the disease. This may help to improve the current diagnosis, prognosis and management.