4. DISCUSSION

HORMONES:

The mammary gland is an integral part of the female reproductive system and is subjected to regulation by hormones from the pituitary, gonads, adrenals and thyroid during various phases of development. Under normal circumstances, the mammary gland is influenced predominantly by the interactions among estrogen, progesterone and prolactin (see Korenman 1981). Estrogens stimulate ductal proliferation, progesterone stimulates lobuloalveolar proliferation and prolactin stimulates lactation and mammary cell division. Apart from their involvement in the normal developmental, structural and functional physiology of the mammary gland, these hormones are also involved in the mammary tumor development.

In normal premenopausal women, estrogen secretion is mainly of ovarian follicular and luteal origin (Nocke and Leyendecker 1970). Analysis of ovarian vein plasma in fertile women showed that 90 to 95% or more of the estradiol entering the systemic circulation originates in the ovaries (Baird and Fraser 1974). Estrogen synthesis occurs in ovarian granulosa and thecal cells but the relative contribution of the various cell types to the total amount of estradiol secreted is not known (Hammond and Soules 1978).
In the present study, in 23% of the premenopausal women with carcinoma of breast, serum estradiol was found to be increased in follicular and luteal phases. Probably, the ovarian granulosa and theca cells might have been triggered or amplified in these women resulting in increased synthesis of estrogens. It may also be suggested that the sensitivity of ovarian cells to the available gonadotropins might have been enhanced, leading to increased production of estradiol.

Similar to premenopausal women, 34% of postmenopausal women with breast cancer also showed elevated level of serum estradiol. Since the postmenopausal ovary is not capable of synthesizing estrogens, peripheral conversion of androgens may be the major source of estrogens in these subjects (Grodin et al 1973). There is evidence that postmenopausal ovaries continue to secrete significant amounts of androgenic hormones (Judd et al 1974; Vermeulan 1976; Schenker et al 1979). Androgens are known to be synthesized in the interstitial tissue of the ovary (Rice and Savard 1966). High incidence of ovarian interstitial cell hyperplasia has also been observed in breast cancer patients (Sommers 1955). Apart from the ovarian interstitial tissue, these postmenopausal subjects may also derive androgens from adrenal (Vermeulan 1976). These adrenal androgens also could contribute to elevated serum estradiol through their extraglandular aromatization (Siiteri and Mac Donald 1973).
The peripheral conversion of circulating androgenic precursors to estrogens, by the aromatase enzyme accounts for nearly all of the estrone and estradiol produced by postmenopausal women (Grodin et al 1973; Poortman et al 1973). Although much of its conversion occurs in fat and muscle cells (Longcope 1982), certain human breast cancer have the capacity to convert androgens to estrogens locally (Li et al 1976; Abul-Hajj et al 1979). Millar and Forrest (1976) have found that breast carcinoma tissue is capable of converting testosterone into estradiol, but they were unable to demonstrate this conversion in normal breast tissue. Recently, Bezwoda et al (1987) demonstrated the aromatization of androstenedione by human breast cancer tissue and that the aromatase enzyme activity was higher in the breast tissues of postmenopausal women than premenopausal women.

England et al (1974) measured daily plasma estradiol level throughout the menstrual cycle in normal and breast cancer females and reported that the mean serum estradiol levels were significantly higher in breast cancer subjects during follicular and luteal phases. However, some investigators observed similar estradiol levels in normal and carcinoma breast cases (Jones et al 1977; Bird et al 1981). Drafta et al (1980) reported elevated serum estradiol in postmenopausal subjects with breast cancer.
Overall 31% of subjects including pre and postmenopausal age groups showed elevated level of estradiol in the present study. The remaining subjects exhibited almost normal values. The present study may suggest that at least in these 31% of subjects, estradiol may be implicated in tumor induction.

Since the classic experiments of Lactasse and which first demonstrated the ability of estrogen to induce mammary cancer in mice, it has become recognized that the estrogen possess a special ability to promote tumor formation in a variety of mammalian tissues (Gardner 1953; Muhlbock 1956).

Exposure of the breast to unopposed estrogen during premenarchial or perimenarchial years appears to increase the risk of breast cancer (see Korenman 1981). Whether estrogen itself functions as a carcinogen, or whether its effects predisposes the tissue to the carcinogenic action of other factors, is not clear. There is little evidence to suggest that endogenous estrogens are carcinogens and it is generally assumed that they are promotors (see Siiteri et al. 1981). Estrogens induce primary proliferation of the duct epithelium, the cell most susceptible to neoplastic transformation (see Korenman 1981). Thomas (1984) suggested that estrogens cause proliferation of the human breast tissues and would therefore be expected to increase the risk of breast cancer by stimulating the growth of stem and
intermediate cells. It has been suggested that the predominant carcinogenic effect of estrogens appear to promote cellular susceptibility to neoplastic transformation and it is possible to hypothesize that unopposed estrogenic stimulation is most likely to produce susceptibility to a transforming event (see Korenman 1981).

In normal human breast tissues, progesterone facilitates cell growth and alveolar proliferation (Kirschner 1977). It appears that estrogen priming is essential for progesterone to be fully effective; indeed progesterone receptor sites in animal breast tumor models are stimulated by prior estrogen priming (Kirschner 1977). The significance of progesterone as an important factor in the development of human breast cancer has been suggested by Sherman and Korenman (1974).

The observed low titres of progesterone in the luteal phases of premenopausal women may suggest inadequate corpus luteal function in these subjects. This might have created a favourable situations for the unopposed action of estradiol on the breast of these subjects. Sherman and Korenman (1974) proposed that estrogenic stimulation of breast cell growth in the absence of sufficient cyclic progesterone secretion may provide a setting favourable for the development of breast cancer. The relation between declining progesterone levels and increasing risk of breast cancer was reported by Bulbrook et al (1978). Normal level of progesterone was observed in the serum of women with breast cancer by Fishman

The results of this study show that serum gonadotropin levels are identical in normal women and in patients with breast cancer. Previous studies have shown that serum gonadotropin levels are unaltered in women with breast cancer (Malarkey et al 1977; Bird et al 1981; Secreto et al 1983). Subnormal level of luteinizing hormone was reported in mammary carcinoma (see Zumoff 1982). Unaltered gonadotropins with elevated estrogen may suggest the non existence of feedback regulation of gonadotropins by estrogens in these subjects. However, these subjects had regular menstrual cycle.

Interest in prolactin as a potential hormone involved in the genesis of breast cancer has been strengthened by laboratory and clinical observations. The role of prolactin in experimental mammary tumorigenesis is fairly established (see Nagasawa 1984). Attempts to establish such a critical role for prolactin in human mammary neoplasia have not been considerably successful due to inconsistent results, but there are several lines of evidence which suggest that prolactin play a role in the induction and/or growth of human breast cancer (Nagasawa 1979).

In the present study significantly increased prolactin was observed in 32% of the postmenopausal women with breast cancer. However, 21% of premenopausal women with breast
cancer also exhibited increased prolactin level, but due to the individual variation this was not found to be statistically significant.

In women with established breast cancer, most studies have shown normal levels of serum prolactin (Franks et al 1974; Kwa et al 1974; Mittra et al 1974). However, some studies have reported increased level of this hormone in breast cancer subjects (Aldinger et al 1978; Tarquini et al 1978; Rose and Pruit 1981). There is evidence that estrogens augment prolactin release by acting at the level of hypothalamus (delPozo and Brownell 1979). It has also been shown that estradiol induces the synthesis of prolactin and prolactin stimulates the production of estradiol receptors as well as its own receptors in mammary gland (see Korenman 1981).

From the results of this study it is evident that many of the cases with carcinoma of breast were with elevated level of both estradiol and prolactin suggesting their interrelationship in the genesis of mammary carcinoma. It may be evident from this study that estradiol which stimulates prolactin release (delPozo and Brownell 1979) may be the causative factor for increased level of prolactin in these subjects.

Nagasawa (1981) have claimed that one of the roles of prolactin in mammary tumorigenesis is to create mammary gland conditions favourable to the action of carcinogens.
Prolactin stimulates the rate of mammary gland DNA synthesis, a primary factor for mammary tumorigenesis. Prolactin also acts as a promoter of tumor growth (Furth 1973). It has been proposed that one of the stimulating effects of prolactin on mammary tumorigenesis is accumulation of estrogen receptor, which results in the local hypersensitivity of mammary glands to estrogen (see Nagasawa 1984).

The data obtained in the present study on serum hormonal profiles indicate that all subjects with carcinoma breast do not have hormonal involvement. Increased level of estrogen and prolactin may form a conducive environment for neoplastic growth. Peripheral conversion of androgenic steroids appear to be the main source of estrogen in cancerous subjects and is independent of pituitary-ovarian negative feedback control.

**Glycolytic Enzymes:**

Glucose plays a central role in mammalian metabolism. Some cells have a specific requirements for glucose e.g. nerve cells, erythrocytes and mammary cells. Aerobic glycolysis and respiration are two important metabolic processes that supply energy for cell viability, differentiation and growth. Since the original observation of Warburg that animal tumor cells have an elevated rate of glycolysis and this biochemical trait is unique requirement
for neoplastic growth (Warburg 1931), intensive studies related to this biochemical change and malignant transformation have been developed.

Acceleration of glycolysis, in general, is characteristic of neoplasia. The carbohydrate metabolism of 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, via hexose mono phosphate shunt, for increased DNA synthesis (Webber 1977 a, b). Most of the systematic studies on tumor glycolysis were performed on rat hepatomas, because they provide a spectrum of well defined tumors ranging from slowly growing, undifferentiated tumors.

From the present study on human mammary cancer, it is clear that the specific activities of all the glycolytic enzymes studied, except G-3-PDH, were enhanced in the carcinoma breast tissue, indicating the high rate of glycolysis in neoplastic tissues. Since these enzymes are unaffected in fibroadenoma tissues, they may provide a good tool to differentiate fibroadenoma from carcinoma.

The elevated activity of hexokinase observed in the present study may be associated with the growth rate in breast carcinoma. Hexokinase activity in tumors, in general, has been correlated with tumor growth and differentiation (Shatton et al 1969; Bustamante and Pederson 1977;
Bustamante et al. 1981). The high rate of glycolysis in tumor cells was shown to be due, at least in part, to an elevated activity of hexokinase in the mitochondrial fraction (Bustamante and Pederson 1977; Bustamante et al. 1981). Since the tumor mitochondrial bound hexokinase was coupled to oxidative phosphorylation, the observed elevated activity of hexokinase in the present study may suggest increased rate of phosphorylation in carcinomatous breast tissue. The increased activity of hexokinase in the carcinomatous breast tissue was also reported by some early workers (Hilf et al. 1970; Isben et al. 1982; Balinsky et al. 1984).

Phosphofructokinase plays a critical role in the energy metabolism of organs which largely or entirely dependent upon glycolysis i.e. mature red cells, exercising muscle and neoplastic cells (Oskam et al. 1985). The increased activity of phosphofructokinase observed in carcinoma breast tissues may be correlated with the rate of replication of malignant cells. The activity of phosphofructokinase may be a limiting factor in the glycolytic pathway of mammary carcinoma as it has been demonstrated that in HeLa cells and Ascites tumor cells, phosphofructokinase acts as a limiting factor (Wu 1959; Wu and Racker 1959). Probably an increase in such a limiting enzyme may further facilitate the increased availability of glucose to meet the demand of increased mitotic activity in carcinoma breast. Increased activity of phosphofructokinase in human breast cancer has been reported by few early workers (Deshpande et al. 1977; Bezwoda et al. 1977).
Hexokinase and phosphofructokinase are estrogen dependent enzymes in mammary carcinoma. The activities of both the enzymes were found to be increased in the carcinoma breast tissues of women with elevated estradiol. Further increase in the enzyme activities were observed in women with elevated level of both estradiol and prolactin. It appears that estradiol has a positive role to play on the induction of these enzymes, apart from their neoplastic onset in breast tissue. Even though estradiol alone couldn’t bring about any significant change, it may facilitate the action of estradiol on these enzymes and thus on the mammary gland tumor growth.

G-3-PDH is one of the key enzymes of carbohydrate metabolism involved in the transfer of substrates into the pathways of fat deposition, thus regulating energy generation by substrate availability. The decreased activity of G-3-PDH observed in the carcinoma breast tissues of this study indicate that lipids in neoplastic cells are channelled into pathways of carbohydrate metabolism rather than fat deposition. The data on this enzyme may suggest a block in the supply of precursors for lipid metabolism in carcinoma breast tissues. This block in lipid metabolism was associated with enhanced rate of glucose utilization for glycolysis. A recent study from our laboratory (Krishnamoorthy 1987) showed decreased lipid content in the carcinoma breast tissues.
The measurement of LDH as biological markers has been employed by many investigators to differentiate the normal tissue from its malignant counterpart (Goldmen et al 1964; Hoch-Ligeti et al 1965; Grayhack et al 1977). The elevated activity of LDH in the carcinoma breast tissues indicate the high metabolic rate and increased glycolysis. Tissue LDH activities can be correlated with the growth of the tumor in human mammary tissue. Such a correlation has been shown in the mammary tissues of Sprague-Dawley rats (Rees and Huggins 1960).

It has been shown that lactate utilization by the neoplastic tissues is lower than that of the normal tissues (Abraham and Chaikoff 1965). The decreased lactate utilization resulting in lactate accumulation in the carcinomatous tissues could be due to a deficiency in the mechanisms responsible for pyruvate decarboxylation or condensation of the resulting acetyl CoA with oxaloacetate to form citrate (Abraham and Chaikoff 1965).

LDH composition depends on the metabolic pattern of the tissue. Although it is unclear whether a change in LDH isoenzymes represents cause or effect in the development of the malignant cells, the shift toward predominance of the M type of LDH in the tumor tissue (Richards and Hilf 1972) is consistent with and supportive of its altered metabolism to sustain the high rate of glycolytic activity (Rees and Huggins 1960; Goodfriend and Kaplan 1965; Schroeder et al
Unlike hexokinase and Phosphofructokinase, LDH was found to be influenced more by prolactin than estradiol. It is obvious from this study that LDH in carcinoma breast tissues was increased in women with selective increase in serum prolactin alone. These impact of prolactin was potentiated by estradiol as the enzyme activity was found to be further increased if estradiol was elevated along with prolactin. Nevertheless, estradiol alone could not bring about any obvious change in the enzyme activity. Increased activity of LDH in human breast carcinoma was also reported by Goldman et al (1964), Hilf et al (1970) and Balinsky et al (1984).

Increased activities of G-6-PDH and 6-PGDH, enzymes of hexosemonophosphate shunt (HMP), observed in the carcinoma breast tissues strongly suggests the predominance of glycolysis in breast carcinoma. These enzymes may be stimulated for the production of NADPH, required for the synthesis of cholestrol. This is quite evident from the study of Krishnamoorthy (1987), from our Laboratory who observed elevated level of cholesterol in carcinoma breast tissues in human. He also found increased activity of HMG CoA reductase in carcinoma breast tissues. The increase in the HMP shunt pathway enzymes may also facilitate the production of ribose-5-phosphate for increased DNA synthesis in this highly proliferating tissues to meet the increased energy requirements in the carcinomatous conditions. This
suggestion is supported by the finding of Krishnamoorthy (1987) that DNA concentration is elevated in the carcinoma breast tissues. Increased activities of G-6-PDH and 6-PGDH have been demonstrated in human breast carcinoma tissues by some early works as well. (Hilf et al 1976; Bezwoda et al 1985).

Between G-6-PDH and 6-PGDH, G-6-PDH appears to be estrogen dependent breast carcinoma. This conclusion was derived from the finding of markedly increased G-6-PDH activity in women with increased serum estradiol. As in the case hexokinase and phosphofructokinase, prolactin appears to potentiate the action of estradiol with a further increase in the enzyme activity. Such a response was not shown by 6-PGDH.

Estradiol was reported to increase the activities of G-6-PDH and LDH in normal and carcinomatous mammary tissues of rodents (Richards and Hilf 1972). These authors also showed reversion of the enzyme activity to normal level when the estradiol administration was withdrawn. Therefore it becomes clear that estradiol has a specific stimulatory effect on G-6-PDH in carcinoma breast tissues. Nevertheless, LDH activity in human carcinoma breast seems to be specifically depending on prolactin rather than estradiol, even though it showed positive response to estradiol in rodents.
The hormone induced changes in these enzymes activities may reflect protein synthesis de novo, as it has been demonstrated by Hilf et al, (1967) that the estrogen induced elevations in enzyme activities were prevented by actinomycin.D. Although a number of authors have reported that estrogen receptor positive tumors carry a more favourable prognosis than estrogen receptor negative tumors, this finding has not been universal. The mechanism by which estrogen receptor positivity confers its effects has not been defined clearly. Recently Bezwoda et al (1985) reported that estrogen receptor concentration failed to predict the development of metastatic disease, while G-6-PDH activity predicted the same.

Since the changes in G-6-PDH activity has been shown to correlate with lipogenesis and with secretory activity in several endocrine target organs (Richards and Hilf 1972), the regulations of this enzyme by endocrine alterations may be partially responsible for changes in tumor growth.

It has been recognised that high glucose metabolism is not restricted to malignant tissues but is also a characteristic of highly proliferating cells (Franchi et al (1981). Very slowly growing tumors were shown to have low glycolytic activity; therefore it was suggested that this metabolic anomaly is not due to a fundamental difference between normal and tumor cells, but merely reflects the growth rate of tumor (Burk et al (1967). Possible reasons
to explain the high rate of glycolysis in carcinoma usually involve defective mitochondria (Warburg 1931; Cosalvez 1974), aberrant ATPase activities (Racker 1976) or an elevated form of mitochondrial hexokinase (Bustamante and Pederson 1977). Any minor decrease in the synthesis of ATP or increase in ATP breakdown will result in an amplification of glycolysis (Franchi et al 1981).

The increased rate of glycolysis in carcinoma breast tissues obtained in the present study is probably due to the increased energy requirements of the malignant tumor tissues. Since the activities of all the glycolytic enzymes in fibroadenoma breast were similar to that of normal breast tissues it may be inferred that fibroadenoma tissues may not require additional energy supply. Moreover, fibroadenoma tissues have slow growth rate. This may also be responsible for the unaltered glycolytic activity in fibroadenoma breast tissues. since the carcinoma breast tissues have very fast growth rates, the energy requirements are also speeded up resulting in the increased glycolytic activity.

Plasma Membrane Enzymes

A number of cellular functions, such as cellular adhesiveness, contact inhibition of growth and movement, and antigenecity are regulated by the cell plasma membrane. Transformed or malignant cells aberrant in these biologic characteristics, differ from their normal counterparts with respect to structure and composition of their plasma membranes. The assay of membrane specific enzymes will be of
great importance in assessing the changes associated with malignancy.

Increased activities of membrane enzymes, alkaline phosphatase, 5' nucleotidase and gamma glutamyltransferase observed in the carcinoma breast tissues may suggest the absence of tight junctions (Emmelot and Benedetti 1967). Structural contacts between normal cells in a tissue are maintained mainly by three types of intercellular junction: desmosomes, tight junctions and gap junctions, each of which has a particular function (see Emmelot et al. 1981). An almost complete loss of gap junctions and tight junctions have been observed in human breast tumors (Inove and Skoryna 1979). A deficiency of tight junctions, which have an isolating function by sealing off the cell facing the environment on all sides and to random uptake and disappearance of normal gradients, is involved in the local expression of membrane enzymes (Emmelot and Benedetti 1967).

In tumors, the plasma membranes of adjacent cells are often separated from each other over much of their surfaces (Weinstein et al. 1976). This may have an effect on cell proliferation, since the latter process can be modulated by adjacent cells either by cell to cell contact or through specific secretory products (see Emmelot et al. 1981).

The ectopic production of alkaline phosphatase is very well known in neoplastic tissues. The increased level of alkaline phosphatase observed in the present study in
carcinoma breast tissues may be of placental type. In human breast cancer, placental type of alkaline phosphatase have been reported by early workers. (Cadeau et al 1974; Wada et al 1979; Mc Dicken et al 1983). The increased level of alkaline phosphatase observed in the present study may suggest the increased tumor growth and their metastatic potential.

Membrane bound enzymes are useful in predicting the extent of metastases. Among this 5' nucleotidase and gamma glutamyl transferase serve to predict the involvement of liver. The possible function of 5' nucleotidase is currently a matter of debate while gamma glutamyl transferase has been implicated in amino acid transport (Griffith et al 1979) and ammonia production in the kidney (Tate and Rose 1977). Increase in level of gamma glutamyl transferase observed in the present study may reflect the de-differentiation in the breast, which has been suggested earlier in the hepatoma (see Dawson et al 1979). Gamma glutamyl transferase in the tumor may also be considered as an oncofetal protein since the immunological and kinetic properties of the enzyme in hepatoma are similar to those of the foetus (see Dawson et al 1979).

Bridges and Maister (1985) suggested that the transport of gamma glutamyl amino acids is dependent on intracellular glutathione levels. Osuji (1980) has shown that gamma glutamyl transferase has two amino acid transporting sites.
From the results of gamma glutamyl transferase obtained in the present study, it can be suggested that activity in normal and fibroaderoma tissues may be a reflection of normal gamma glutamyl amino acid transport; whereas in carcinoma it may be a reflection of impairment in intracellular glutathione metabolism which has also been reported in some transformed cells (Meister and Anderson 1983).

The increased activities of membrane enzymes observed in the carcinoma breast tissues may be due to the increased proliferation and increased cellular metabolism. During the transformation of normal cells into malignant cells the plasma membrane components including the enzymes are liable to exhibit increased activities. The normal activities of enzymes observed in the fibroadenoma breast implies the normal differentiation and metabolism which may not reflect the altered cellular function, as seen in carcinoma.

All the three membrane enzymes studied were found to have increased activities in the carcinoma breast tissues of women with elevated level of prolactin and they were further increased in women with elevated level of both estradiol and prolactin. This may be due to the increased proliferation and transformation under the influence of prolactin.

It has already been shown that proliferative signals are mediated by polypeptides present in the serum (Holley 1975; Gospoderawicz and Moran 1976). Elevated level of circulating prolactin in these cases might have been
amplified the proliferating signals thereby resulting in the 
increased level of these enzymes when compared to the 
subjects with normal level of serum prolactin. It has been 
reported by Puente et al (1979) and Pocius et al (1980) that 
prolactin and estrogen modulate the membrane enzymes 
particularly gamma glutamyl transferase activity in the 
mammary gland.

The data obtained in the present study delineates the 
influence of prolactin from estrogen. It appears that in the 
absence of prolactin estrogen may not be effective in 
stimulating plasma membrane enzymes. Probably prolactin may 
bring about membrane changes by binding to its receptors, 
which may not be possible for estrogens which has receptors 
only in the cytosol and nucleus. Since these plasma membrane 
enzymes show specific response to increased prolactin, this 
may be considered as a marker to distinguish prolactin 
dependent mammary carcinoma.

Lysosomal Enzymes:

Lysosomal enzymes are able to degrade cell organelles 
and digest cell material. Allison (1969) has demonstrated 
the participation of lysosomes in mitosis and mutagenesis.

The observed elevated level of lysosomal enzymes in the 
carcinoma breast tissues may be due to the lack of 
differentiation. Poole (1973) has observed the correlation 
between the increased activities of lysosomal enzymes and 
the lack of differentiation in hepatomas. It may also be due
to the increased rate of endocytosis and phagocytosis. Leighton and Moore (1982) has demonstrated that tumor cells have a higher rate of endocytosis than normal cells.

The extra cellular fluid of tumor has a high content of lysosomal enzymes that could be derived from either cell necrosis or secretion (see Poole 1973). Lysosomal enzymes, in general, are more concentrated in tumor cells when compared to their normal counterparts. Allison (1969) has shown the ability of lysosomal deoxyribonuclease to break both strands of DNA double helix. This leads to the potential labilization of the lysosomal membrane by mutagenic factors. He has also suggested that lysosomal deoxyribonuclease from selectively damaged lysosomes enters the nucleus of living cells and is responsible for chromosomal breaks.

The increased activity of arylsulphatase observed in breast carcinoma may be due to the increased metabolic rate of the tissue, which is quite evident in these cases. It may be possible that the increased proliferations might have also caused the increased activity of arylsulphatase (Farooqui and Mandel 1977). Increased activity of beta glucuronidase parallels the amount of cellular infiltrate in breast carcinoma tissues. (Orinda et al 1977). The activity of beta glucuronidase may also be related to the host resistance (Orinda et al 1977).
Previous studies using histochemical techniques demonstrated increased activities of acid phosphatase in malignant mammary tumor tissue compared to normal and benign tissues. (Koudstaal et al. 1975; Machinami 1976). Recently Filmus et al. 1984) and Podhajcer et al. (1986) observed the increased activity of acid phosphatase in human breast carcinoma suggesting the increased cellular degradation and digestion.

Among the lysosomal enzymes studied, ribonuclease alone was stimulated by the elevated level of estradiol plus prolactin. However, this enzyme activity was not influenced by estradiol alone or prolactin alone.

Lysosomal enzymes studied suggest the involvement of breast tissue in tumorigenesis and the resultant damage in the breast cells. In such circumstances, cellular and connective tissue damages might have caused an increase in the lysosomal enzymes. These enzymes may reflect the extent of damage in the breast cells.

Tumor Markers

There has been general agreement that elevated CEA levels in breast cancer patients are found mainly in patients, with metastatic disease. The greatest incidence of CEA positivity, and the highest levels, are seen in patients with liver and bone involvement (Tormey et al. 1977).
The observed increase in CEA, in more than 40% of the carcinoma subjects indicate that all these subjects may have liver metastases. Some direct evidences for the presence of CEA in breast cancer cells have been provided by immunofluorescent (Border et al 1973) and immunoperoxidase studies (Heyderman and Neville 1977) of biopsy specimens and by measurement of CEA level in tumor extracts (Pusztaszeri and Mach 1973). 30-50% of patients with elevated level of CEA including 70% of patients with advanced disease, have been reported by Laurence et al (1972), Myers et al (1978), Rochman (1978), Beatty et al (1979). Some studies have failed to detect CEA in most breast cancers examined (Denk et al 1972; Goldenberg et al 1978) but this failure may be related to differences in the specificity of the antisera used and the sensitivity of the methods employed.

Serial assessment of CEA levels during therapy for metastatic breast cancer have shown a rise with disease progression and a stabilisation or decline with response (Tormey et al 1977; Falkson et al 1982; Caffier and Brandon 1983) often preceding clinical changes (Coombes et al 1982).

From the results of CEA obtained in the present study it may be suggested that CEA can definitely serve as a marker to know the extent of metastases, possibly the involvement of liver.
Alphafetoprotein, like CEA, is another oncofetal antigen produced by the cells of the developing human liver and yolk sac (see Hirai 1982). Previous studies have shown that AFP was never positive in human breast cancer (Franchimont 1977; 1980). In the present study only 12% of the women with breast cancer exhibited slightly elevated level of AFP. Elevated serum levels of AFP have been reported in benign conditions such as acute viral hepatitis, liver cirrhosis and obstructive jaundice (Staab 1985). Therefore, elevated serum concentrations of AFP may not be considered as a reliable diagnostic indicator of malignancy.

Human chorionic gonadotropin, is a glycoprotein hormone consisting of two dissimilar non-covalently linked sub units. The alpha sub unit is common to the pituitary glycoprotein hormones, while the beta sub unit is unique to each of the glycoprotein hormones and is responsible for the biologic and immunologic specificity. Although hCG is not a tumor specific antigen, it is quantitatively increased in trophoblastic and nontrophoblastic tumors (Braunstein et al 1973). Some tumors produce free beta sub units instead of or in addition to native hCG (Weintranb and Rosen 1973).

In the present study 10% of the women with carcinoma of breast exhibited elevated level of serum beta hCG. Previously Braunstein et al (1973) and Sheth et al (1974) have reported increased level of beta hCG in 9% and 14% of carcinoma subjects, respectively. All the patients with elevated values had metastatic disease, in the above
studies. In contrast, none of the patients with benign breast disease had elevated levels of beta hCG.

Franchimon et al (1977) performed assays of both native hCG and beta sub unit. They found elevated levels of hCG in 15% of patient and beta hCG in just 2% of the patients. Horne et al (1976) in a study of tissue sections by the immunoperoxidase technique, found that 60% of breast cancers and none of the 12 benign breast tissues contained hCG. It would appear, therefore, that although ectopic production of hCG is frequent in breast cancer, release from primary tumors is infrequent and quite variable, and not necessarily related to tumor burden.

Over all, only a low incidence of elevated AFP and beta hCG levels have been found in breast cancer, and even when the values were elevated, there was usually only a small increment above the normal range. Hence, the measurement of AFP and beta hCG levels in human breast cancers, may not be of any substantial clinical value.

Plasma membrane constituents are shed into the surrounding media in vitro and vivo, as cells replicate (see Stefanini 1985). Enzymes present in the nucleus, cytoplasm and mitochondria are also released when cells are destroyed. As the proliferation and metabolic rates of the tumor cells are higher than most of the normal cells, the rate of shedding into the circulation of a tumor bearing host would also be expected to be higher (Chatterjee et al 1981).
The observed elevated alkaline phosphatase, 5'-nucleotidase and gamma glutamyl transferase activities in the serum of women with breast cancer indicate the accelerated breakdown and release of cell surface material. It has been suggested that increased serum levels of membrane bound enzymes in breast cancer patients originate from the tumor cells of the patient (see Stefanini 1985). Therefore patients with increased tumor burdens would have a source of increased membrane enzymes which would be manifested by increased serum levels.

Alternatively, the elevated gamma glutamyl transferase and 5'-nucleotidase values may be explained on the basis of liver involvement rather than on the basis of tumor burden (Sahm et al. 1983). Patients with cancer metastatic to the liver, resulting in eventual liver damage, often have pronounced elevations of these enzymes in their serum. It has been shown that serum gamma glutamyl transferase along with CEA and alkaline phosphatase measurements were very useful markers to screen for breast cancer metastases (Coombes et al. 1982).

Schwartz (1984) found that serum 5'-nucleotidase is almost as useful as gamma glutamyl transferase in indicating the carcinoma metastatic to liver, Kim et al. (1975) assessed the diagnostic value of serum alkaline phosphatase, gamma glutamyl transferase and 5'-nucleotidase as an aid to the detection of liver metastases and found 5'-nucleotidase to
have the greatest predictive value.

In the present study it has been observed that women with elevated level of CEA also exhibited increased level of 5′nucleotidase and gamma glutamyl transferase in the serum and all these women might have had liver metastases. From the results it can be suggested that circulating CEA along with 5′nucleotidase and gamma glutamyl transferase may serve as useful markers in screening the patients with liver metastases.

The possible factors contributing to the elevated level of acid phosphatase, ribonuclease, arylsulphatase, LDH and PHI may indicate the size of the carcinomatous organ, the concentration or activity in these enzymes in the organ and the possible differences in rates of synthesis, degradation or excretion of these enzymes (Munjal et al 1976).

Several investigators have reported that serum PHI is often elevated in cancer of the breast and it has been considered that PHI levels to be the best index of malignancy in cancer patients. (Mitchel et al 1986). The increase in serum LDH in these patients could be explained by assuming that malignant tumors release LDH into the circulation before an accumulation of the enzyme in the tumor tissue.
The basis for the elevations of these enzymes in patients and animals with neoplastic disease has been presumed to be leakage of the enzymes from neoplastic tissue whose metabolism is reflected in increased concentration of some enzymes. The observation of increased level of serum glycolytic activity and membrane functions characteristic of neoplastic tissues.