REVIEW OF LITERATURE

2.1 HORMONAL CONTROL OF BREAST AT VARIOUS STAGES IN WOMEN'S LIFE

The major endocrine changes in girls occur at the time of puberty which begins at the age of 10 to 12 years (Styne and Grumbach, 1978). The microarchitecture pattern of breast tissue changes in response to hormonal changes throughout the menstrual cycle and during the life of the women (Vogel et al., 1981). The changes in LH pulsing mechanism or the ‘firing rate of pituitary hormones’ are believed to be responsible for the onset of puberty (Boyar et al., 1972, Wildt et al., 1980) but whether such changes are responsible for breast stromal development is unknown.

As menarche occurs, initially, the great majority of cycles are anovulatory, resulting in estrogen excess period. These estrogens induce the growth and maturation of breast and genital organs. The physiological effects of estrogens on the maturing breast is to stimulate longitudinal ductal growth of ductal epithelium. Later, progesterone produced by corpus luteum during the ovulatory cycles stimulates lobuloalveolar proliferation of the breast.

During the menstrual cycles, periodic changes take place in mammary tissue under the influence of sex steroid hormone. The complex interplay of estrogen and progesterone, secreted during the follicular phase and the luteal phase under the influence of FSH and LH takes place on breast tissue to bring
about a differential growth of its various components.

An extensive ductulo-lobular alveolar growth takes place during pregnancy under the influence of estrogen, progesterone, PRL and gonadotropin. The level of PRL in blood goes on increasing during pregnancy and becomes almost 3 to 5 times the normal value.

The action of PRL on cell multiplication and the synthesis of milk protein, lactose and milk fats is affected by a number of other hormones either directly or indirectly (Fig. 2.1). As lactation initiates, reduction in placental lactogen and sex steroids hormone occurs. The secretion of PRL inhibiting factor (PIF) from the hypothalamus into the hypothalamo-adeno-hypophyseal portal system decreases as a result of which PRL secretion by pituitary lactotrophs further increases and with the help of growth hormone (GH), insulin, cortisol, PRL converts mammary epithelial cells from a pre-secretory to a secretory state of lactation. The release of PRL is maintained by suckling. Following removal of the infant from the breast feeding, the gland returns to an inactive state and as milk is no longer synthesised and removed, PRL and oxytocin release is not stimulated, thereafter the secretory activity of the lactogenic epithelium decreases.

After menopause, the decrease in ovarian secretion of estrogen and progesterone causes progressive involution of the ductular and glandular components of the breast. There is quantitative decrease in the number and size of the glandular elements of the breast, with the epithelium of the lobule and duct becoming hypoplastic or atrophic. Apocrine change and microcysts are also frequently seen at this stage. This results in an increased proportion of adipose tissue and connective tissue.
Fig. 2.1: Mammary gland is a target organ for a number of hormones. PRL and in some species PL (Placental lactogen) controls mammary activity directly. PL also stimulates SM (Somatomedian production by liver). $E_2$ (Estrogen) and Pg (Progesterone) are responsible for the differentiation of the mammary gland. $E_2$ also have a role in producing growth factors (GF). Pg also inhibits lactation. Adrenal glucocorticoids synergised all the actions of PRL. $T_3$ and $T_4$ (Thyroid hormone) are both inhibitory and stimulatory. OXY (Oxytocin) affects lactation. SM (Somatomedian) and INS (Insulin) are involved in cell multiplication.
Fig. 2.1: Mammary Gland: A Target Organ for various Hormones
2.2 ROLE OF HORMONES IN MAMMARY TUMORIGENESIS

The breast or mammary glands of mammals are important for the survival of the new born and thus of the species. As described previously, breast undergoes changes throughout the life of women and thus is affected by various hormones like estrogen, progesterone, androgen, GH, PRL, insulin, corticosteroids, glucosteroids and thyroxine. The reproductive endocrine system which changes during the menarche, menopause and child birth may contribute to the aetiology and pathogenesis of breast cancer (Mac Mohan et al., 1973). Further, diet (Aslyworth et al., 1984), obesity (Henderson et al., 1984) and radiation induction (Baverstock et al., 1981) are also associated with the risk of development of breast disease or breast cancer. The UK National case control study group reported an increased risk of breast cancer associated with oral contraceptive (UK National Case Control Study Group, 1990).

Considerable evidence has now accumulated from animal models, epidemiological studies and direct hormone measurement studies in population at risk which has led to the hypothesis that reproductive hormones contribute mainly to the aetiology and pathogenesis of breast cancer. It was reported by Korenman (1980) that in an appropriate hormonal environment the mammary carcinoma was induced by a single exposure of the carcinogenic agent, dimethyl benzanthracene (DMBA). This appropriate hormonal environment was thought to have been provided by estrogen unopposed by the presence of progesterone and sufficient amount of anterior pituitary hormones, particularly PRL.
The first study which developed the concept that hormones can cause or increase the incidence of neoplasia was done by Bittner JJ (1948) on experimental animals while studying the relationship of estrogen and mammary cancer. Later on, in humans, it was observed that single and nulliparous women are at increased risk of breast cancer (Logan WPD, 1953). Moreover, women who had their ovaries removed before the age of 40 or 45 and thus had artificial menopause have a reduced risk of breast cancer (Tichopolous et al., 1972). Further reproductive disturbances in animals as a result of mastectomy indicates the influence of hormones in breast pathology (Diamond et al., 1982). Recent studies have shown that radical mastectomy is followed by hyperprolactinemia in most of the breast cancer patients (Wang et al., 1986; Barni et al., 1987). These results indicated that there may be a significant endocrine abnormality in women with breast cancer but studies have not convincingly demonstrated elevated production or serum levels of either estrogen (Shore et al., 1983) or PRL (Franks et al., 1974; Sheth et al., 1975) in breast cancer patients. The changes thus appear subtle.

Sherman & Koreman (1974) put forward a hypothesis attempting to provide a pathophysiological interpretation of the various endocrine related risk factors of breast cancer (Table 2.1).

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<thead>
<tr>
<th>Increased</th>
<th>Decreased</th>
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<tr>
<td>Late first pregnancy</td>
<td>Early first pregnancy</td>
<td>Oral contraceptives</td>
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<tr>
<td>Nulliparity</td>
<td>Castration</td>
<td>Lactation</td>
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<tr>
<td>Early menarche</td>
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<td>Late menopause</td>
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<td>Obesity</td>
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<td>Low androgen excretion</td>
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The most widely held view for the genesis of this hypothesis is that there is an imbalance between estrogen and progesterone in certain stages of life of women. Koreman S.G. (1980) in the ‘Oestrogen Window Hypothesis’ suggested that exposure of the breast to unopposed estrogen during the post menarchial or pre menopausal years increases the risk of breast cancer. Higher risk during late first pregnancy, nulliparity, obesity, low androgen secretion may also be due to the same reasons.

The unopposed estrogen may be due to short luteal phase or abnormal ovarian follicular maturation resulting in irregular or deficient progesterone production by corpus luteum, short luteal phase cycles (Sherman and Koreman, 1974a) that are common cause of endocrine infertility in young women, are often accompanied with an excess PRL secretion (Askel S., 1980). Long follicular phase cycle though producing full ovulation also demonstrate prolonged unopposed estrogen stimulation, post menarchial cycles and premenopausal cycles are irregular in length of luteal or follicular phase, thus during these periods women are exposed for longer time to excess estrogen production and deficient or no progesterone secretion. Early first pregnancy on the other hand, may be considered to be protective as normal hormonal environment is established during this period.

Estrogen which stimulates ductal proliferation and increases PRL receptors in mammary gland are not by themselves mutagenic but they promote breast cellular susceptibility to neoplastic transformation, progesterone on the other hand opposes estrogen action and thus is considered to have a protective role in developing breast cancer.
MENARCHE AND BREAST CANCER

A significantly higher risk of developing breast cancer has been found among women with an early age at menarche in case control study, the risk ratio being of the order of 1.5 (Stazzewski J., 1971), later other studies also supported this view (Mac Mohan et al., 1973; and Grey et al., 1979) though the difference between the average age at menarche of breast cancer cases and that of controls was found to be of only few months yet this difference at early age may result in two fold increase of risk of breast cancer (Pike et al., 1983) but it was observed that it was not only the age at onset of menstruation that determines the risk but also the age when 'regular' menstruation was first established is important as regular ovulatory cycles increases women's risk of breast cancer (Henderson et al., 1985). The risk of breast cancer was more than double in women who had their regular menses within one year of menarche than in women having menarche at the same year but had a delay of 5 years for their menses to become regularised i.e. women with early menarche and rapid establishment of regular cycle had four fold increased risk than women with late menarche and longer period of establishment of regular cycles (Henderson et al., 1981). The women with later menarche were more likely to have anovular cycles than women with early menarche, given the same number of elapsed years since menarche (MacMohan et al., 1982). Apter and Vihko in 1983 confirmed this finding while studying the relation between menarche, ovulatory cycle and breast cancer in 200 school going girls. Thus contrary to the belief of Korenman (1980), greater number of ovulatory cycles increases a women’s risk of breast cancer as in these normal luteal phase there are greater levels of cumulative estrogen which in turn is
risk determinant factor (Venturoli et al, 1986; Henderson et al, 1985). No relationship had been found between PRL level and age at menarche (Yu et al., 1981).

MENOPAUSE AND BREAST CANCER

Late menopause having irregular ovulatory cycles and excess estrogen is also a breast cancer risk (Treolar et al., 1967). The women whose normal menopause or artificial menopause occurred before the age 45 years had only one half the breast cancer risk of those whose menopause occurred after the age of 55 (Trichopoulous et al., 1972). This was explained, as before menopause in women the menstrual cycle becomes irregular resulting in a period of unopposed estrogenic stimulation of up to 8 years. If the menopause is delayed the women is subjected to extended exposure to cumulative estrogen and thus to the risk of breast cancer (Sherman et al, 1976). The characteristics that predict risk of this cancer diagnosed before and after the menopause are more or less the same except few like weight and height (Paffenbarer et al., 1980).

PREGNANCY AND BREAST CANCER

There is a complex relationship between breast cancer risk and age at first birth. Some workers (Choi et al., 1978) have shown no association between them but others have strongly associated the two. There is a striking relationship between the age at first birth and breast cancer risk, late age at first birth being associated with greater cancer risk (MacMohan et al., 1970). Women having their first full term pregnancy (FFTP) at the early age had about one third risk of breast cancer than the women delaying their first
pregnancy until the age of 30 or later. A reanalysis of the same data with multivariate method showed that, even though age at first birth was the most important factor, age at subsequent birth had additional, independent effect (Trichopoulous et al., 1983).

Inverse relationship between breast cancer and parity had also been stated (Tulinuis et al., 1978) but the incomplete pregnancies before the FFTP did not have any protective effect (MacMohan et al., 1970). Even the first trimester abortion or a miscarriage before the FFTP doubles the risk of breast cancer (Pike et al., 1981). The reverse of this i.e. abortions after FFTP did not carry any increased risk (Hadjimichael et al., 1986).

The long term protective effect could arise through FFTP causing either structural changes in breast (Russo et al., 1982) or/and long term changes in the hormone level and their receptors. It is the duration of time between menarche and first complete pregnancy that is important and critical (MacMohan and Cole, 1972). The longer this period, the longer will be the time for which the stem cells (which later mutate to cancer cells) that are increased in number and then undergo a certain fluctuation in number with each ovarian cycle until the first pregnancy, will be exposed to promoters (Cairns J., 1975).

Drife J. (1981, 1986) had proposed that it occurs because of differential responsiveness of breast tissue before and after the FFTP to the protective effects of progesterone. Before a FFTP the breast has few progesterone receptors and therefore, during the normal menstrual cycles the breast is stimulated largely by estrogens. FFTP somehow results in the increased development of progesterone receptors so that after the first birth, normal
cycles results in adequate stimulation of the mammary ductal cells by progesterone, cellular differentiation and a consequent reduction in risk of breast cancer.

The observation that the early part of a women's first pregnancy differs endocrinologically from the second pregnancy was also stated by Bernstein et al. (1986). The level of estrogens differs in nulliparous and parous women (Bernstein et al, 1985). The level of PRL was also found to be lower in parous as compared to nulliparous women (Yu et al., 1981). In other words increase parity is significantly associated with decrease amount of PRL (Burning et al., 1983; Wang et al., 1987).

Obesity increases the risk of breast cancer in post menopausal women (Henderson et al., 1984; Boyle and Leake, 1988), the opposite may be true for breast cancer occurring in premenopausal women (Willett et al., 1985; Kampert et al., 1988; Trelit S, 1989). In the former, this could be either due to effect exerted by estrogen aromatized from androgenic prohormones in adipose tissue (Siisteri PK, 1987) or due to energy rich diet (Dewaard and Trichopolous, 1988). Also high fat diet causes elevation of plasma PRL concentration (Hill et al, 1980) the suggestion has been made that the tumourigenic effects of high fat diets are mediated through actions of PRL.
2.3 BREAST CANCER AND STEROIDS

2.3.1 ESTROGENS

As breast cancer was considered to be hormone dependent from the beginning of the last century (Boyd S., 1900), it was assumed theoretically, in such a case, mammary gland will progress from its normal growth to hyperplasia to neoplasia as a consequence to the proliferative effects of the particular hormone. As a result, a major role of estrogen and other hormones in the etiology of breast cancer was emphasised.

Earlier reproductive stage of a woman was considered to predict course of hormonal therapy to treat a women with this malignancy but only one third of the patients responded to endocrine therapy, thus till the end of seventies there had been no reliable way to predict the type of tumours that were hormone dependent without the actual trial.

Estrogen receptors were reported in human breast carcinomas by using radioactive estrogen for binding to these specimens (Jenson et al., 1967). The binding of estrogens were found to be present in higher concentration in cytosolic fraction of human mammary cancer cells (Korenman and Dukes, 1970) and was considered to be prerequisite for hormonal stimulability of breast cancer. With these findings, studies were performed to understand the mechanism of hormones interaction with the target tissue (Jenson and DeSombre, 1972). The initial step in hormone actions was found to be the binding of the hormone to highly specialized receptor protein that initiates a series of biochemical steps and eventually leads to a alteration in cellular metabolism or growth.
The importance of receptor studies in recommending hormonal therapy to breast cancer patients was emphasised as these were the main biological factors which were responsible for the intracellular accumulation of estrogen in normal estrogen target tissues and certain breast cancers. Presence of estrogen receptor (ER) gave an idea that tumor was hormone dependent while absence of this receptor would make the cell devoid of estrogenic activity thus making it hormone independent. Since then estrogen receptor assay has become important in the clinical assessment of breast cancer (Wittliff, J.L. 1984).

The clinical parameters such as age and menopausal status was correlated with the presence of ER. Olden women or post menopausal women were more likely to be ER positive and contain a higher ER concentration (Clark et al., 1984). Post menopausal women at high risk for recurrent disease demonstrated that the natural course of the disease is significantly better for ER positive then ER negative patients. (Thorpe et al, 1986). Primary tumours were more hormone dependent than the secondary tumors i.e. histological features which differentiate into poor pathological conditions were estrogen receptor negative (Vihko et al, 1980). However, in other study no such relation could be observed (Brdai et al., 1988). The pregnant women with ER negative tumours had poor prognosis (Holdaway et al., 1984) whereas lactating women usually have ER positive tumours that are responsive to hormonal therapy.

Data from study of Crows et al. (1986) suggested that the relationship between ER content of a tumour and obesity to predict the prognosis appears to be related to the breast cancer stage and patient race. Black patients with ER negative tumours during postmenopausal stage have poor prognosis.
2.3.2. PROGESTERONE

It is reported that not all patients with ER positive tumours respond to hormonal therapy. One possibility for this failure may be the defect in the intracellular cascade of events that normally controls the biological activity in response to endocrine stimulus. The determination of progesterone receptor (PgR) in breast tumours might assess intactness of the estrogen response mechanism as estrogen exerts its biological effect through the formation of PgR both in normal as well as in neoplastic cells. It was hypothesised that the presence of PgR in human breast tumour might be a sensitive marker for predicting the response to endocrine therapy as it will indicate both the presence of ER and that the biochemical steps after hormone receptor interaction are functional (Horwitz et al., 1975).

A widely held view is that progesterone reduces the carcinogenic effects of estrogen. This view coincides with the earlier epidemiologic data that breast cancer risk is increased with the occurrence of early menarche and late menopause when the menstrual cycles may be anovulatory and estrogens are unopposed by progesterone.

Progesterone inhibits estrogen action by suppressing its receptors in uterus (Hsueh et al., 1975). Another evidence in favour of progesterone having a protective role in breast cancer is that when exogenous estrogens are given to oophorectomised women, their breast cancer rates increased from those having ovaries (Hoover et al., 1976).

Specific progesterone receptors in human breast cancer were reported (Horwitz and McGuire, 1975). Since PgR may be a better marker for tumour
endocrine dependence than ER, it was suspected that presence of PgR may be an important prediction of time to recurrence for patients who received adjuvant endocrine therapy for primary breast cancer. Breast cancer patients who having PgR comprise a group with a better prognosis (Pichon et al., 1980). Frequency of metastasis and disease free survival were inversely proportional to these receptors. In the study of Manson et al. (1983) though both ER and PgR status predicted overall survival in patients with or without nodal involvement, only PgR predicted prolonged disease free survival. Inspite of these and other studies, there were some findings which opposed the importance of progesterone as prognostic factor (Allegra et al., 1979; Stewart et al., 1983).

Progesterone is also known to stimulate growth in breast tissue. However no abnormality in progesterone level was found in women with breast cancer (Malarkey et al., 1977a; Read et al., 1985) but late menarche and early menopause, which are associated with decreased risk also mean a net reduction in progesterone stimulation, implying that progesterone is co-carcinogen, enhancing the adverse effect of estrogens.

2.3.3 ANDROGENS

Androgen receptors (AR) may also act as marker for tumour differentiation, though these receptors have been found in upto 35% to 50% of human breast cancer assayed (Ochi et al., 1978; Bryan et al., 1984), there is some evidence that their presence may correlate with response to endocrine therapy and with patient survival. The data also suggests that the response to endocrine treatment of mammary tumours that are AR and ER positive is
higher compared with the mammary tumors that are positive for only one receptor (McGuire WI, 1977).
2.4 BREAST CANCER AND PROLACTIN

In the past two decades there had been a large body of investigation in the PRL as a potentially important hormone in human breast tumorigenesis. Ben David et al. (1981) in his studies on binding site for PRL reported a higher dependency of breast cancer on PRL than on steroid receptors. Much of the evidence for demonstration of its important role in developing mammary carcinoma had come from experimental data. Daily administration of PRL (Boot et al., 1962), induction of hypothalamic lesions which sharply increases pituitary PRL release (Burni and Montemurro., 1971) and grafting pituitaries in kidney capsule (Boot et al., 1962) led to higher incidence of mammary cancer in mice. In contrast, inhibiting the PRL level to less than normal, in ovarectomized mice by Bromoergocryptine, a drug, which inhibits PRL secretion prevented the development of mammary cancer in mice (Welsch and Nagasawa, 1977). Pregnancy which elevates PRL levels enhances the growth of hormone dependent mammary tumours in rats (McCormick and Moon., 1965).

The exact role of PRL in human mammary tumorigenesis is unknown although clinical studies have provided evidence suggesting responsiveness to suppression of this hormone in some cases of metastatic carcinoma of the breast (Murray et al., 1972; Minton JP, 1974). The beneficial effects of hypophysectomy in stage IV breast cancer patients who were treated with antiestrogens suggested the role of pituitary hormones in maintaining the growth of some human breast cancers (Pearson et al., 1978). These studies showed tumours dependency on PRL. However only a very small fraction of mammary tumour explants derived from 26 biopsy specimens of human breast tumours responded with increased DNA synthesis when PRL was added to insulin and
hydro-cortisone-enriched medium, as judged by increased in cooperation of $^{3}H$-thymidine into DNA (Welsch et al., 1976), which suggested that a very small fraction of human breast malignancies may respond to the growth stimulating effect of PRL. It may be stated that an interrelationship between high levels of PRL and the incidence of breast cancer could be established in certain circumstances (Simon et al., 1984) where mammary carcinoma cells are stimulated by PRL but not in others (L'Hermite-Baleriaux et al., 1984).

The studies designed to determine whether or not patients with breast cancer or benign disease have higher mean serum PRL levels have been conflicting and confusing, some workers have reported higher plasma PRL levels in breast cancer patients than in healthy controls (Murray et al., 1972; Rolandi et al., 1974) but others failed to find any correlation (Dicky and Minton, 1972; Boyns et al., 1973; Franks et al., 1974).

The conflicting results obtained with serum PRL in epidemiological study might be due to the fact that its secretion varies according to age (Vekemans and Robyns, 1975), menstrual stage (Ehara et al., 1973; McNeilly and Chard, 1974), drug usage, time of day (Bulbrook et al., 1981) and emotional conditions. Also there is a problem in relating plasma PRL level with the risk factors in the pulsatile fashion of secretion exhibited by the hormone. It is thus difficult or almost impossible to exclude in clinical studies all or even part of these factors influencing human PRL secretion and plasma levels, thus rendering an obstacle for the proper evaluation of the role of this hormone in breast cancer.
To overcome this in part preoperatively hourly serum PRL levels over a 24 hrs. period in women with breast cancer were determined and nocturnal PRL concentration was found to be significantly decreased in post menopausal breast cancer patients and significantly elevated in premenopausal women with breast cancer when compared to age and weight match control subjects (Malarkey et al., 1977) but other workers have reported increased PRL levels in association with increased breast cancer in post-menopausal women (Kwa et al., 1981; Bonneterre et al., 1986). This increase in PRL level was found in subjects even 5 years prior to their clinical diagnosis of breast cancer was made.

In the case of benign breast disease patient's basal PRL concentrations do not differ significantly from that of the controls (Boyns et al., 1973; Sitruk-Ware et al., 1979; Mansel et al., 1980). However when PRL level in serum samples taken daily throughout the menstrual cycle of patients with benign breast disease was estimated it was found that plasma PRL levels was abnormally elevated, especially in women over the age of 30, indicating a possibility of involvement of PRL in this condition (Cole et al., 1977). The failure to get any difference in PRL level in patients and controls in the former studies might be due to the fact that these studies were based on single samples taken at random times in the menstrual cycle. Also basal serum PRL levels have also been reported marginally but significantly elevated in patients with cyclical mastalgia (Watt-Boolsen et al., 1981). It might be due to altered central regulation of PRL secretion and not caused by altered dopaminergic tone (Peters et al., 1981; Kumar et al., 1984). In other words, hypothalamic pituitary axis is not primarily disturbed in patients with cyclical mastalgia and
fibrocystic disease so as to cause PRL elevation. However, there is no evidence to suggest that patients with cyclical mastalgia have increased risk for breast cancer except when there is proliferative type of hyperplasia in the breast (Dupont and Page 1985).

There are also some circumstantial evidence to implicate PRL in the aetiology of human breast cancer. A full term pregnancy lowers the PRL concentration and this may explain the epidemiologic observation that early FFTP lowers breast cancer risk (MacMohan et al., 1973). Other workers also related the age of the woman at her first as well as last delivery with the cancer risk (Hunt et al., 1980; Kwa et al., 1981; Wang et al., 1985). Earlier is the event of last birth, greater is the risk e.g. five birth spread over 20 years would have more protective effect than if those births were spread over 5 years. This could be due to the fact that protective environment of lowered PRL level caused by deliveries in premenopausal women with increasing parity is transitory. After the last delivery PRL level rises as the time elapses.

Musey et al. (1987) concluded in their study that (1) a first pregnancy causes a significant decrease in basal levels of serum PRL and a significant decrease in pituitary secretory capacity for PRL, (2) this effect occurs after both an early and late first pregnancy, (3) these are long term changes lasting at least 19 months for the depression of PRL secretory capacity and at least 12 to 13 years for the depression of basal PRL levels, (4) these changes are not related to age, (5) there are no corresponding changes in basal levels of LH or FSH or in the pituitary secretory capacity for LH & FSH.

The level of PRL is also affected if a patient has a family history of breast cancer. Kwa et al. (1974) were the first to demonstrate elevated PRL levels in
patients with a family history of the disease. The differences in PRL levels between daughters of cancer patients and that of control were significant only when other hormones, estrone plus estradiol, were considered together with PRL (Henderson et al., 1975; Trichopoulous et al., 1981) and not otherwise (Fishman et al., 1978). At the luteal phase when both estradiol and progesterone levels were higher, an abnormal evening peak of PRL levels were found in women with a history of cancer (Kwa and Wang, 1977). It was also stated that other risk factors of breast cancer such as nulliparity may be linked with higher evening PRL level (Kwa et al., 1978).

Radical mastectomy in patients with breast cancer was followed by hyperprolactinemia which was normalised at 3-12 months postoperatively (Barni et al., 1987). Authors related the elevated PRL level with the metastasis and estrogen receptors positivity. According to them, the percentage of cases with hyperprolactinemia induced by mastectomy was significantly higher in patients without node involvement compared with those with axillary node involvement (12/17 Vs 6/17; p). Also, the percentage of hyperprolactinemia was higher in negative estrogen receptor patients than in those with positive receptors (10/13 Vs. 8/21; p).

The elevated PRL either preoperative and/or post operative were significantly correlated to survival, in all cases, the least favourable prognosis was associated with highest PRL levels (Wang et al., 1986). Similar findings were observed by Dewsett et al (1983); and by Holtkamp et al (1984). The higher the PRL level prior to treatment, the less probability of response to endocrine treatment or even chemotherapy. It was also the observation of
Holtkamp et al (1984) that hyperprolactinaemic patients experiencing remission after chemotherapy exhibited a return of their PRL levels to normal. In spite of the poor prognosis of patients with hyperprolactinaemia, remission were achieved with chemo or hormonal therapy in some patients, but it remains to be established in which patients PRL inhibitors are of therapeutic value.

The inability to demonstrate elevated PRL levels in patients with breast cancer in some studies does not rule out the possible importance of PRL in the pathogenesis of breast cancer or the possible usefulness of its suppression in therapy. As PRL plasma level were of poor value to study the PRL sensitivity of human breast cancer and assuming that PRL stimulates human breast tumour, the tumoral tissue should contain PRL receptors. *In vitro* studies on human breast cancer cells suggest that specific PRL receptors are present in some lines (MCF-7), and increased synthesis of estrogen receptor content has also been reported in tumour cell line MCP-7 by PRL (Shafie & Brook 1977). Physiological concentration of PRL were showed to promote the growth of breast tumour cells in culture (Malarkey et al., 1983; Manni et al., 1985) by acting at the level of gene expression, synthesising three unique proteins in the PRL receptor positive human breast cancer cell line (Shiu and Iwasio, 1985). Since PRL facilitates the growth of human breast cancer cell lines, these results suggest that many human breast cancer may be PRL dependent.

In the last few years several reports (Turcot-Lemay et al., 1982; Di Carlo et al., 1983. Murphy et al., 1984; L. Hermite Baleriaux et al., 1987) stated the presence of low but measurable levels of PRL-R in both benign and malignant human tumours. Though the role of PRL in the development of
human breast cancer is less well established (Costlow and McGuire 1978), yet specific binding of human PRL of greater than 1% was seen in 20% of breast tumours. Binding capacity for PRL in pre-menopausal patients was greater than that of the postmenopausal patient (Partridge and Hahnel 1979) but this study has not discussed the presence of endogenous PRL. A certain portion of the PRL receptors may be occupied by the endogenous circulating PRL. Since the binding of PRL to its receptors is relatively stable, most of these receptors remain occupied after the homogenization and membrane fractionation procedure. Therefore these receptors remain undetected unless the bound PRL is removed. When the endogenous bound PRL was dissociated from its receptor by a short exposure to MgCl₂ (in vitro) or CBI₅₄ (in vivo) (Kelly et al., 1979), there is rise in percentage positivity of tumour from 49% to 71% (Peyrat et al., 1982). Bonneterre et al. (1982) examined PRL-R on tumour plasma membrane.

The physiological concentration of PRL could promote the growth as well as DNA synthesis of human breast cancer cells in 29% of the cases through these receptors. In the absence of PRL the stimulation of DNA synthesis did not occur (Peyrat et al., 1984). It was reported that women with breast cancer patients having tumours with high PRL-R have poor prognosis (Waseda et al., 1985) but this was later contradicted (Bonneterre et al., 1986).
2.5 HORMONAL MANIPULATION

Breast disease, as discussed previously is mostly hormone dependent and therefore its course could also be influenced favourably or unfavourably by hormonal manipulation. George Beatson (1896) introduced tumour regression in patients with breast cancer by Oophorectomy, but the failure of response to oophorectomy in some patients was due to the continued secretion of estrogens by the adrenal gland. Huggins and Bergenstal (1952) introduced adrenalectomy as a successful means of postmenopausal endocrine ablation in advanced breast cancer.

Metastatic breast carcinoma in one patient regressed when hypophysectomy and termination of pregnancy was done (Barett et al., 1975). Pituitary ablation may decrease tumour growth by removing PRL & GH. Adrenalectomy and hypophysectomy are thought to affect breast cancer by ablating all adrenal function, thus removing the last remaining source of androgens that can be converted to estrogen in post menopausal women. However, hypophysectomy was shown to be more effective than adrenalectomy. But as both procedures result in high morbidity and mortality, they are not used frequently.

Other approach to hormonal therapy was the use of antiestrogens that antagonize estrogen effects. Their main characteristic is blocking the uptake of estradiol in both experimental and human tumours. Tamoxifen, a triphenylethylene antiestrogen, enjoys wide clinical usage. As low levels of estrogen production and excretion remain even after oophorectomy, adrenalectomy and hypophysectomy, tamoxifen was used to antagonize this
estrogen effect. With this both early as well as advanced breast cancers could be treated and also survival was prolonged in some cases (Baum et al., 1983).
2.6 RELATIONSHIP BETWEEN ESTROGEN, PROGESTERONE AND PROLACTIN RECEPTOR

Relationship between PRL-R and steroid receptors are suggested in literature. Bonneterre et al. (1986) showed that a statistically correlation existed between ER and PgR, between ER and total PRL-R or free PRL-R, and between PgR and total or free PRL-R. This had been suggested by Holdaway and Friesen (1977) and confirmed by Murphy et al. (1984) for cell lines as well as for mammary cancer. Partridge and Hahnel (1979) found that the affinity of PRL-R was higher in ER positive tumours. Conversely neither Vihko et al. (1980) nor Waseda et al. (1985) found a relationship between PRL-R and steroid receptors. There is a complex "interplay" between the three hormones, viz. estrogen, progesterone and PRL (Table 2.2). All the three hormones induces the appearance of each other's receptors. It may therefore be inferred that no single hormone can be responsible for pathological lesions seen in the breast.
Table 2.2 Hormone Interaction in breast-estrogen, progesterone & prolactin

a. Estrogen
   — stimulate proliferation of ductal epithelium (Porter, 1974)
   — stimulate PRL secretion from anterior pituitary. (Miller et al., 1977)
   — increase PRL receptors in breast (Sheth et al., 1978)
   — induced progesterone receptors (Horwitz & McGuire, 1978)
   — suppress LH/FSH secretion (Yen et al., 1975).

b. Progesterone
   — stimulate proliferation of terminal duct lobular unit (Porter, 1974)
   — inhibits PRL secretion by increasing dopamine secretion.
   — inhibits lactation during pregnancy (Davis et al., 1972)
   — inhibits estrogen receptors in uterus (Hsueh et al., 1975)
   — facilitates estradiol inactivation (Tseng & Gupide, 1975).

c. Prolactin
   — stimulates lactation (Kandon et al., 1983)
   — stimulates mammary cell division (Malarkey et al., 1983)
   — stimulates hypothalamic dopamine production (Peter et al., 1982)
   — stimulates its own receptors (Posner et al., 1975)
   — stimulates estrogen receptors in breast (Shafie & Brooks, 1977)
   — inhibits granulosa and luteal cell E2 and progesterone secretion. (McNatty et al., 1974).
2.7 PROLACTIN

2.7.1 HISTORY

PRL was identified in 1928 as a substance in anterior pituitary extract capable of causing lactation in pseudopregnant rabbit (Stricker and Grueter 1928), though extensive physiologic and chemical studies was carried out on PRL from different animal species, its recognition as a separate hormone in man could not be achieved for the next 40 years. The reason was its close resemblance with GH which is about 100 times the amount of PRL in human pituitaries, and interfere with its separation procedure and obscured detection of the smaller amount of PRL. Later its independent identity was recognised in human blood and it was observed that despite their chemical and biological similarity, hPRL and hGH were quite distinct immunologically, with no overlap in immunological reactivity (Frantz and Kleinberg et al., 1970). With this finding more sensitive bioassays were developed and Guyda and Friesen (1971) succeeded in separating human and primate PRL from GH. In the following year Hwang et al. (1971) isolated small amount of primate PRL from pituitary extract and developed a radioimmunoassay for human PRL for measuring its concentration in sera at different age and at different stages. They also came out with an unexpected finding that amniotic fluid concentrations of PRL were about 5-10 fold higher than the mother serum concentration.

PRL is secreted by mamnotrophs cells that belong to acidophili cells series of pituitary (Halmi et al., 1975). It was found that two forms of PRL electrophoretically separable and having different bioassay and radioimm-
munon assay activities, were secreted by rat adeno-hypophysis (Asawaroengchai et al., 1978). Different forms of PRL were present in the circulation at different times which differ from each another in biological and immunological potencies (Owens et al., 1985).

Structural changes in PRL occur also in women during pregnancy and the menstrual cycle which are probably influenced by the hormonal environment (Larrea et al., 1989). Four PRL related monomers were characterised in humans pituitary homogenate having molecular weight 25,000 D, 29,000 D, 45,000 D and 16000 D (Meuris et al., 1983). Lewis UL (1984) studied these variant and their post translational modifications. Frawley et al. (1986) demonstrated that not only the hormone differ from each another in biological and immunological potencies but also their releaser cells. Some cells secrete PRL with extremely high biopotency and very low immunoreactivity whereas the converse is true for other cells.

2.7.2 STRUCTURE

Human PRL having molecular weight of 22,000-23,000 D is generally a single polypeptide chain consisting of 198 amino acid residues. It has stable three dimensional structure which is important for its biological activities including its interaction with the receptors. PRL was crystallised by Bell et al. (1985) for analysing its structure by X-ray diffraction.

Human PRL has more marked amino acid homology with bovine PRL than the ovine PRL. Its complete amino acid sequence was examined by Shome and Parlow (1977). The end group analysis of human PRL showed a single amino acid terminal residue, leucine in high yield confirming its homogenicity.
Review of Literature

(Hwang *et al.*, 1972). It contains two tryptophan residues and six half cystine comprising three disulphide bridges. It has double the number of methonine residues as compared to rabbit PRL, but nearly half as that of ovine's. It is somewhat less negatively charged than either ovine PRL or human growth hormone with an isoelectric point of 6.5 or slightly less (Hummel *et al.*, 1975).

### 2.7.3 SITES OF DETECTION

PRL can be detected in high levels in plasma, amniotic fluid and milk but in low levels in cerebrospinal fluid, semen and urine. Substantial quantities of PRL are found in human amniotic fluid ranging from 2-100 times the concentration in maternal serum (Fang and Kim 1975). PRL can also be easily detected from all samples of human milk whether from nursing mothers or patients with galactorrhea. PRL detected in urine in very low amounts are not useful from a clinical or diagnostic stand point as it undergoes considerable degradation before excretion into urine.

### 2.7.4 ROLE

PRL is a potent stimulator of lobuloalveolar proliferation of breast and of lactation. In rats it was found to be essential for the normal development and differentiation of the virgin female breast (Lyons *et al.*, 1958). The growth of mammary glands in the hypophysectomized gonadectomized and adrenalectomized rats, in the presence of estrogens or estrogens in combination with other steroids were ineffective in inducing any significant degree of breast growth unless PRL was coadministered. Similar importance of PRL in human is unclear as the deficiency of PRL in human is very rare. Also rarely there is any sign of enlargement of breasts in cases of hyperprolactinemia conditions caused due to pituitary tumors in humans. Gynaecomastia patient
Review of Literature

(Men with enlarged breasts) do not have elevated level of serum PRL (Frantz et al., 1972). These findings, however, do not rule out an essential role of PRL in the development of human breast cancer.

PRL is an essential hormone both for the initiation and maintenance of lactation. Though other hormones are also required, the production of milk proteins is critically dependent on PRL (Topper YJ, 1980). There is increase in PRL level in blood steadily from early gestation till term. During the period of gestation PRL together with estrogens and progesterone further develops the lobulo-alveolar system of breast. At the time of parturition, levels of estrogen and progesterone are decreased due to expulsion of placenta. This decrease initiates the onset of lactation which is maintained by the suckling stimulus. The level of PRL remains high, but as time ceases it returns to the normal level. During lactation even when PRL levels are high, ovulation occurs (Hall et al., 1980). Oral administration of estrogen antagonises PRL for the lactation to take place. In many species including man, the excess secretion of PRL influences, stimulating and suppressing, the production of steroid hormones (Veldhuis et al., 1980). It stimulates the production of estrogen receptors in the breast (Shafie and Brooks, 1977) but on the other hand it inhibits estrogen and progesterone secretion from the ovary (Mc Natty et al., 1974).

PRL together with insulin and hydrocrotisone stimulates fatty acid synthesis in mammary glands (Wang et al., 1972), in its presence short chain (C) fatty acid while in its absence long chain fatty acids are synthesised. PRL also controls lipoprotein lipase activity in the mammary gland (Zinder
et al., 1974), as PRL can retain its activity in hypophysectomised lactating animals (Falconer and Fiddler 1970). It also promotes the active transport of ions out of the gland (Falconer and Rowe 1977).

2.7.5 CONTROL OF SECRETION

Data from various studies on animals and humans show that PRL secretion is under the control of hypothalamus and this action is rather inhibitory in nature via the secretion of PIF into the portal circulation. The variation of this factor in the portal blood causes the short term fluctuations in the secretion of the hormone. PIF is considered to be a neurotransmitter, dopamine or its constituents, as the latter also has an inhibitory effect on the hormone by acting directly on the specific dopaminergic receptors present in the pituitaries (Shaar and Clemens et al., 1974; Takahara et al., 1974; Peters et al., 1982). Drugs that block dopaminergic receptors raise serum PRL in humans (Foord et al., 1983).

Like the other peptide hormones, the PRL itself may inhibit its own secretion via a short feedback loop as it has no target organ inhibitory feedback system (Mac Leod and Lehninger, 1974). The stimulatory action of hypothalamus on PRL release is due to the presence of at least two factors TRH and non TRH (PRF). Though TRH can directly stimulate pituitary PRL secretion in female and male subjects (Yuen et al., 1973), the response being significantly greater in the former than in the latter (Jacobs et al., 1973), yet it is not a physiological releaser of the hormone (Reichlin, S., 1966, Chen et al., 1970). TRH induced PRL release is not altered by pretreatment with estrogens or androgens (Yuen et al., 1973) or during pregnancy (Tyson and
Friesen, 1973). In addition to its direct action on pituitary cells, TRH also acts at the hypothalamic level to inhibit PIF secretion (Diefenbach et al., 1976).

In females, administration of high doses of estrogen can increase PRL release (Yen et al., 1974) but apparently the amounts of estrogens secreted during the menstrual cycle are not sufficient to raise serum PRL level. However, the difference in estrogen among both the sexes in human accounts for the low level of basal PRL and no response to stimulation in men. Estrogen or estrogen contraceptives stimulate PRL-RNA synthesis by an independent mechanism than that of pituitary protein synthesis (Shull & Gorski, 1984). Indirectly, estrogens also increase the response of lactotrophs to TRH (Harper MJK, 1977). The interrelation between the two hormones is very complex as they at times synergise the action of each other (during breast development), while at other times, they oppose each other (during lactation).

Progesterone influence on PRL secretion is not fully defined in humans. However, it is the main inhibitor of lactation (Davis, et al., 1972). Androgens inhibit PRL binding as shown in rat livers (Kelly et al., 1977).

2.7.6 PRL LEVEL AT DIFFERENT STAGES

Secretion of PRL rises from the 25th week of gestation in the foetus till birth. After birth till puberty in girls there is no difference in PRL level between the two sexes. The rise in PRL levels in girls may be due to increase in estrogen level at puberty. The PRL level in older women gradually declines till menopause and after the age of 65 its level is decreased due to reduction in ovarian stereogenesis. In post menopausal women, basal PRL levels are significantly lower than in premenopausal females. In contrast PRL levels in
men tend to increase after 45 years of age (Sarfaty et al., 1976; Vekemans and Robyns, 1975).

PRL shows pulsatile secretion like the other pituitary hormones. Its level was found to be highest when the subject is asleep but not when it is awake (Sassin et al., 1972; Nokin et al., 1972). The night increase in PRL level is absent in pituitary tumours. In hyperprolactinemia it may be present or absent due to other causes. Stresses of all kinds, either physical or surgical increase the PRL level.

During the menstrual cycle, the PRL levels are irregular and inconsistent (McNeilly and Chard, 1974) and most of the workers did not observe a significant variation in circulating PRL levels. However, PRL levels in the plasma were elevated during the luteal phase than during the follicular phase (Cole et al., 1977). Menstrual cycle disorders like amenorrhea, oligomenorrhea and luteal insufficiency are associated with excess of PRL secretion. Hyperprolactinemia interfere with gonadal function, thus causing infertility in humans. This is done by binding to the specific sites in the ovarian tissue (Ben David and Schenker, 1982).

In humans, there is a more or less steady rise of maternal serum PRL throughout gestation (Tyson et al., 1972). This progressive rise in pituitary PRL release is related to the relatively high estrogen secretion during this period. An intact mature hypothalamus is not necessary for attainment of high plasma PRL levels at term since encephalic infants show plasma PRL level similar to healthy newborn. This is in contrast to the very low levels of GH, FSH, LH, TSH, ACTH in the umbilical cord blood of an encephalic new born (Aubert et al., 1977).
The suckling effect by the newly born in the post partum mothers causes the maximum rise in the serum PRL levels (Noel et al., 1974; Schmidt-Gollwitzer and Saxena 1975). This effect could also cause rise in PRL levels in non lactating women (Kolodny et al., 1972). The rise in PRL level is maintained even after this period till the mother is nursing as higher PRL concentrations have been reported in lactating mothers even later than 13 week post partum (Rolland et al., 1975; Bonnar et al., 1975).

Pharmacologic factors, e.g. dopaminergic antagonists, phenothiazines, butyrophenones, calecholamines depletors, synthesis inhibitors, increase PRL secretion whereas dopaminergic agonists like L-dopapomorphine and dopamine and bromocriptine suppress PRL secretion.
2.8 GLYCOPROTEIN HORMONE

FSH and LH are secreted by anterior pituitary. They are termed as gonadotropins as they regulate gonadal functions. They control target cell function by modulating adenylate cyclase activity in their respective target cells (Crooke et al., 1976).

Molecular weight of these vary from 28,000-43,000 D. They are composed of two non identical peptide chains alpha and beta, which are linked by hydrogen bonding and Vander Waal's forces. The alpha chains of both these hormones are identical in amino acid sequence, the beta chain of each is unique. This alpha chain possesses 96 amino acids and two complex moieties at amino acid positions 56 and 82. The carbohydrate moieties are linked via asparagine and contain in order galactose, N-acetyl glucoamine, mannose and sialic acid. The beta chain of human FSH contains 115 amino acids and two asparagine linked carbohydrate moieties at position 7 and 24 whereas beta chain of LH contains complex carbohydrate moieties at different positions i.e. 13 and 30. Complex feedback mechanism which involves participation of the target organs. The beta sub-chain of both the hormones are specific and bring about the biological function in conjunction with the alpha-subunit (Hall et al., 1980).

A decapeptide known as gonadotropin releasing hormone (GnRH) stimulates the pituitary to release these hormones. This GnRH is in turn controlled by a complex feedback mechanism mediated by estrogen and progesterone (ovarian steroids) tissue via the blood stream. LH/FSH secretion is low during the luteal phase of the cycle when the secretion of ovarian
estrogen and progesterone is maximal and increases again in the early follicular phase when ovarian hormones are low, thus demonstrating the negative control of GnRH and also of LH & FSH. At mid-cycle there is a sharp rise in the ovarian estrogen secretion due to the maturing follicle, there is a positive feedback stimulus to the hypothalamus and pituitary resulting in a surge of GnRH and LH, with some FSH, which leads to rupture of the follicle and ovum release.

These hormones reach their target tissue via the blood stream in the free form. Administration of GnRH results in an increase serum LH levels within few mins., the peak level reaching in 20-30 mins. Baseline levels are restored 6 hours after a dose of 100 ug of GnRH. The FSH response is similar but of lessor magnitude (Yen et al., 1975).

The reports on the role of other neurotransmitters in regulating the gonadotropin secretion and secretion of GnRH from hypothalamus are controversial (Krulich, L 1979). The evidence for the dopaminergic inhibition of LH is also clearcut but the action of several DA receptor agonists on LH secretion may not be similar (Martin et al., 1981).

Pituitary and gonadal hormones have been evaluated in blood and urine respectively of women with benign cystic breast disease. In the cyst fluid, the level of LH and FSH were significantly lower as compared to PRL levels (Bradlow et al., 1983).
2.9 PEPTIDE HORMONE RECEPTOR

PRL being a peptide hormone appears to initiate its action by interacting with a specific receptor on the cell surface of the mammary epithelial cell. Since the PRL receptors (PRL-R) are obligatory for mediating some, if not all of the actions of the hormone, the receptors may be important in determining the sensitivity of the tissue to the hormone. The mode of action was first suggested by Turkington (1970) who showed that PRL covalently linked to Sepharose beads, is biologically active in mouse mammary epithelial cells and initiates its effect by an action on the cell membrane, as PRL Sepharose complexes presumably cannot enter the cell. Subsequent studies, however, suggested that the result might have been due to a slow release of PRL from the Sepharose beads. These findings were confirmed by Birkinshaw and Falconer (1972) by means of autoradiographic methods. Since then much information on PRL receptors in the rabbit and rat mammary gland and now recently in human mammary gland has accumulated.

Involvement of lysine residues in the binding of ovine PRL and human GH to lactogenic receptor was shown (de la Llousa et al., 1985). Tyrosyl residue(s) present on the receptor molecule is also important for the receptor ligand interaction as the receptor in rabbit mammary gland was inactivated when treated with N-Acetylimidazole (Mahajan and Ebner, 1986).

The binding of the hormone to its specific receptor causes the formation of an intracellular messenger molecule which then stimulates (or depresses) some characteristic biochemical activity of the target tissue. For the water soluble hormones, for example, peptide hormones, the cyclic nucleotides and Ca$^{2+}$ (and other ions) have emerged as the two main types of secondary
messenger molecules for communicating between the cell surface and the intracellular metabolic machinery. Cyclic AMP is formed from MgATP$^{2-}$ via the membrane localised enzyme adenylate cyclase. The cyclic AMP breakdown is regulated by membranal and cytoplasmic phosphodiesterases. In keeping with its ubiquitous and important nature, the ligand receptor cyclic AMP system may be regulated at a variety of levels including stimulation and inhibition of both adenylate cyclase and phosphodiesterase.

2.9.1 PRL-RECEPTOR MECHANISM

As discussed previously PRL receptors (Mol wt. 41-49 KDa) are enriched in plasma membrane fraction derived from pregnant and lactating rabbit mammary glands (Shiu and Friesen, 1974). The highest levels occur during the lactational period in the rabbit (Djiane et al., 1977) and rat (Holcomb et al., 1976). Self induction of PRL receptors by PRL is indicated by the observation that receptor content increases after parturition. This increase is abolished when suckling is prevented or with administration of ergocornine, an inhibitor of PRL secretion (Bohnet et al., 1977). The two major hormones which modulate the actions of PRL are glucocorticoids which are synergistic to most actions of PRL and progesterone which is the major inhibitor of PRL's action (Fig. 2.2).

In the differentiated mammary gland that are being primed by insulin and cortisol, PRL can stimulate the synthesis of milk proteins, the first or the essential step being the binding of the hormone to its receptors.

Unlike that of most polypeptide hormones, PRL's binding to the cell membrane receptor does not lead to the activation of the membrane bound
Fig 2.2: Schematic representation showing the events that takes place as PRL enters the mammary cell. As PRL enters the cell through PRL receptor.

i) Stimulation of mitotic activity takes place.

ii) An activation of transcription of milk protein gene occurs.

iii) The transcriptional products (in RNA) are stabilized.

iv) Translation of messenger RNA is stimulated.
Fig. 2.2: Schematic representation showing the events that take place as PRL enters the mammary cells.
enzyme adenylate cyclase. Furthermore, neither exogenous adenosine 3'-5'-cyclic monophosphate (cAMP) nor its analog N\textsuperscript{6}-2'-O-dibutyryl cAMP mimics the action of PRL in the mammary gland explants maintained in organ culture (Majumdar \textit{et al.}, 1971). Rillema and Wild (1977) showed that PRL at smaller concentrations, for example $\mu g \text{ ml}^{-1}$ concentration range was able to stimulate membrane associated phospholipase A to release $[^3H]$-arachidonic acid from phosphatidyl choline. They thus associated the binding of PRL to its receptor and the activation of this membrane associated enzyme with the milk protein synthesis (Rellima JA, 1975).

Indomethacin, an inhibitor of prostaglandins synthesis blocks the stimulating effect of PRL. Studies on explants of mouse mammary gland in organ culture suggest that PRL stimulates the transcription of ribosomal RNA and the accumulation of messenger RNA for casein and alpha lactalbumin synthesis (Turkington \textit{et al.}, 1973). The action of PRL on milk protein synthesis (casein) may in part be mediated by polyamines. This is because PRL stimulates the activity of ornithine decarboxylase - an enzyme involved in the pathway of polyamines biosynthesis (Oka and Perry, 1976). PRL may also stimulate the lactose synthetase system, the enzyme responsible for the formation of lactose, in the mammary gland (Turkington \textit{et al.}, 1968).

The PRL receptor molecule has been purified (Haeuptle \textit{et al.}, 1983; Sakai \textit{et al.}, 1986) and antibodies have been raised to the antigen. Antireceptor antibodies were able to block PRL action on milk protein synthesis (Djilalic \textit{et al.}, 1985). Antibodies raised in goats, of sow PRL receptor inhibit PRL binding to its receptors in several organs of various species. This suggests that
PRL receptors shared numerous antigenic similarities between species located on the part of the molecule that is chiefly involved in recognition of the hormone. Monoclonal antibodies to the PRL receptor have also been prepared (Djiane et al., 1985).

2.9.2 LH AND FSH RECEPTORS

The endocrine control exerted by glycoprotein hormone upon the respective target cells is made possible by specific cellular receptors located on the cell surface. When bound to hormone, the receptor-complex activates the enzyme adenyl cyclase (Rodbell M, 1980) leading to the production of the 'second messenger', adenosine 3'5' cyclic monophosphate (cAMP), and stimulation of intracellular metabolic events.

FSH receptors from seminiferous epithelial cells of immature male rats have been reported to bind FSH with high affinity. Rat granulosa cells also contain specific FSH receptors (Means AR, 1975). A protein component and phospholipid present on the receptor, are important for receptor activity. Its molecular weight is 146,000 D. Binding was found to be temperature dependent, optimal binding was observed at 24°C (Abou-Issa and Reichart, 1977).

The LH receptors are obtained from testicular or ovarian homogenates by extraction with non-ionic detergents, Triton x - 100 (Dufau ML and Catt KL, 1976). Maximal binding was obtained at 24°C (pH 7.4). Molecular weight of the receptor by SDS PAGE was found to be 90,000 D but the naturally existing receptor is in dimeric form with a molecular weight of 200,000 D. Similar in case of FSH receptor, a protein component and phospholipids are essential for normal receptor activity suggesting it to be a glycoprotein (Dufau et al., 1973).
GnRH or LH-RH that controls the secretion of LH and FSH from anterior pituitary has its receptors on the membranes of target cell such as luteotrophic cells. A number of studies have reported that analogues of LH-RH are useful for the treatment of hormone-dependent breast cancer (Klign et al., 1985; Miller et al., 1985; Butzow et al., 1987), both in pre and post menopausal women. Although the antitumour effect of LH-RH analogues is thought to be primarily a result of estrogen deficiency, a detailed correlation of objective response by breast cancer patients treated with LH-RH analogues has not been conducted (Plowman et al., 1986).