CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE
Cellular development is a complex process consisting of various intra- and intercellular events involving the induction of specific sequential changes in the cell. Cell differentiation can be regarded as the acquisition by individual cells of various cytological and biochemical features of the differentiated state.

During the last few decades, a large amount of evidence has accumulated implicating the polyamines in the regulation of cell proliferation and differentiation. The polyamines spermine and spermidine and their precursor putrescine occur in most living organisms (Bachrach, 1973). They play an important role in proliferation, are useful markers of cellular growth (Morris, 1974) and neoplasia (Nishioka et al., 1978), and are thought to be associated with several diseases. Cellular polyamine levels and their respective biosynthetic enzymes increase substantially during the early phase of growth and development.

The important characteristic of polyamines is their basic nature, which gives them a high affinity for acidic constituents, and this may be important in determining their physiological actions. It is therefore natural that nucleic acids should figure prominently as targets for polyamines. There are a wide variety of effects exerted by polyamines, including the following:

a) **Requirement as growth factors:** Polyamines are required for optimal growth in all cells tested and this requirement seems to be absolute. It has been shown that cellular polyamine levels increase progressively as cells traverse the cell cycle from the
G₁ phase to mitosis (Heby et al., 1982).

b). Embryogenesis: During pregnancy, growth of fetus and maternal reproductive tissues is accompanied by increased polyamine levels (Fozard et al., 1980a; Huber and Brown, 1982). Difluoromethylornithine (DFMO), a potent inhibitor of ornithine decarboxylase (and thereby of polyamine biosynthesis) has been shown to have contragestational effects in mice (Fozard et al., 1980a), rat (Fozard et al., 1980b; Reddy and Rukmini, 1981; Slotkin et al., 1983), rabbit (Fozard et al., 1980b) and hamster (Galliani et al., 1983). Polyamines were also shown to play an essential role in the early stage of Xenopus oocyte maturation (Lowkvist et al., 1983).

c). Organ development: Polyamines are essential for organ development in vivo. In humans, long term DFMO administration was found to inhibit the differentiation of erythropoietic cells (Abeloff et al., 1984). Polyamine deficiency has been shown to produce adverse effects on the secretory activity of the ventral prostate gland (Danzin et al., 1979; Kapyaho et al., 1984; Danzin et al., 1982).

d). Maintenance of chromosomal integrity: It has been shown that polyamine deprivation causes major chromosomal aberrations in a polyamine dependent Chinese hamster ovary (CHO) cell line (Pohjanpelto and Knuutila, 1982; 1984).

e) Regulation of protein synthesis: It has been postulated by
Panagiotidis et al. (1989) that interaction between polyamines, polyamine-synthesising enzymes and S20/L26 and L34 ribosomal proteins may participate in regulation of protein synthesis.

Ornithine decarboxylase (ODC), a key enzyme in polyamine biosynthesis, is one of the most highly regulated enzymes in eukaryotic organisms. ODC is somewhat unique by virtue of its rapid and dramatic inducibility by a variety of growth stimuli, based on its extremely rapid turnover rate. Another special feature of this enzyme is its regulation by a unique feedback mechanism by its products, involving a specific inhibitory protein known as antizyme (Heller et al., 1976). ODC is present in very minute quantities in cells, but can be induced severalfold by various growth factors, hormones, mitogens and other stimuli. As a result, ODC is one of the most intensively studied enzymes and a complete review is beyond the scope of this thesis. Therefore only some of the salient aspects will be covered.

ODC is a homodimer of 52 to 55 kDa subunits and is quite well conserved in sequence from fungi to humans (Coffino, 1989). The amino-acid sequences of the mouse, human, hamster and rat ODC obtained from the DNA sequences indicate that the protein consists of 461 amino acid residues (Pegg, 1989). All known forms of ODC use pyridoxal phosphate as a cofactor. Mammalian ODCs are strongly dependent on the presence of dithiothreitol or other thiol reducing agents for maximal activity. The enzyme has a pH optimum of 7.0 and the K for L-ornithine is roughly 0.1 mM.
There have been many reports of multiple forms of ODC in mammalian cells, E. Coli, Physarum polycephalum, and T. pyriformis. ODC belongs to a multigene family and it is possible that there are multiple functional genes which could give rise to different forms. Two forms of ODC differing in isoelectric points have been reported in mouse kidney; it is possible that these represent forms having different extents of post-translational modification.

Accumulating evidence indicates that ODC is regulated at the levels of transcription, translation and enzyme degradation. In addition, overproduction of the enzyme occurs rather easily by gene amplification when cells become resistant to an ODC inhibitor, and rapid activation of the enzyme takes place to minor degrees under some conditions.

Transcription: Feinstein et al. (1985) showed that rapid induction of ODC activity by nerve growth factor in cultured PC12 pheochromocytoma cells is accompanied by a roughly parallel change in the amount of hybridisable ODC mRNA. It was later shown using nuclear run-on transcription assays that both NGF and epidermal growth factor enhance transcription of the ODC gene (Greenberg et al., 1985). Similarly, androgens induce ODC gene expression and activity in murine kidney, and the phorbol ester TPA also induces ODC transcription.

Translation: A number of stimuli affect ODC at the level of translation. It has been shown by Kanamoto et al. (1987) that the
synergistic induction of ODC activity in primary cultured rat hepatocytes caused by glucagon and asparagine was due to a threefold induction of ODC mRNA by glucagon and at least a six-fold stimulation of translation rate caused by asparagine. Stimulation of translational initiation has also been suggested by White et al. (1987) for ODC induction in mitogen-activated lymphocytes.

In the case of mouse kidney, androgens cause a 25-fold increase in the rate of ODC synthesis (Persson et al., 1984), whereas there was only an 8-20 fold increase in ODC mRNA (Berger et al., 1984). This strongly suggests an involvement of translational stimulation. In rat liver, a large induction of ODC activity both by thioacetamide treatment (Kameji et al., 1984) and by feeding (Kameji et al., 1987) is preceded by only severalfold increases in the activity of polysome-associated ODC mRNA, again suggesting the involvement of a translational mechanism.

In human osteosarcoma cells, Goto et al. (1991) suggested that parathyroid hormone regulates ODC activity at the level of translation. In lymphocytes, concanavalin A causes a shift of the ODC message from its untranslated pool into polysomes, suggesting that the increase in ODC activity is in fact due to the stimulation of translational initiation (White et al., 1987). EIF-4E, the least abundant of the translation initiation factors, plays a regulatory role in translation and it has been suggested that cell transformation brought about by eIF-4E overexpression may be caused by relief of suppression of translation of mRNAs.
encoding proteins that regulate cell growth. It has been recently suggested that ODC levels are controlled by eIF-4E and that ODC is an essential component of the transformation of 3T3 cells by this factor (Schantz and Pegg, 1994).

ODC undergoes negative feedback control by polyamines, which induce an increase in the rate of ODC degradation as well as a decrease in the rate of ODC synthesis. It has been observed that the cellular content of ODC mRNA does not change significantly during the rapid ODC decay induced by polyamines (Hayashi, 1989). It is likely therefore, that polyamines inhibit ODC synthesis at the translational level.

Degradation: An extremely rapid turnover rate is one of the fundamental characteristics of eukaryotic ODC and makes the regulation of its synthesis a very effective means of bringing about rapid changes in amount. Also, the turnover rate of ODC itself changes considerably under various conditions, indicating that the process of ODC degradation is also subject to regulation.

Important progress has been made recently with respect to ODC degradation by polyamines. In 1976, Heller et al. discovered a protein inhibitor of ODC which is induced by polyamines, is bound to the enzyme, and non-competitively inhibits its activity. This protein was named antizyme. Subsequently, there have been reports of the presence of antizyme in various mammalian tissues, avian tissues, and E. coli, either after treatment with exogenous
polyamines or under physiological conditions. The specificity of action of antizyme suggests that its primary role is to bind to ODC and neutralise its activity. Antizyme seems to be primarily involved in ODC degradation, at least in mammalian cells.

Recently, Li and Coffino (1993) have proposed a model to explain the facilitation of ODC degradation by antizyme. They propose that ODC degradation is a two-step process, wherein ODC and antizyme associate in the first step, stabilising an enzymatically active conformation, with the C-terminus of ODC exposed. The second step is likely to be ATP-dependent, and proteolysis of ODC occurs. Thus, according to this model, the role of antizyme seems to be to render ODC susceptible to proteolysis.

**HORMONAL REGULATION OF ORNITHINE DECARBOXYLASE:**

A number of hormones enhance ODC activity in their respective target organs, as well as in mammalian cells in culture.

Epidermal growth factor induces a marked but transient increase in ODC activity in cultures of chick embryo epidermis, as well as in vivo in mice (Stastny and Cohen, 1970). Studies on the effects of EGF on ODC activity and DNA synthesis in rats during the perinatal period have suggested that EGF has mitogenic effects on neonatal and maternal rat liver (Yamamoto et al., 1993).
The effect of androgens on ODC in various target tissues has been widely studied. In fact, androgen-treated mouse kidney is one of the richest sources of ODC. Testosterone elicits a several hundredfold increase in the ODC level that is generated by changes in both ODC synthesis and turnover (Berger et al., 1984). In the rat, renal ODC mRNA levels were largely unchanged by testosterone deprivation by castration, and subsequent repletion. In contrast to the kidney, ODC expression in the rat ventral prostrate and seminal vesicles is testosterone dependent (Blackshear et al., 1989). Epididymal ODC activity in the rat is also androgen-dependent (de las Heras and Calendra, 1987).

Other steroid hormones also affect ODC activity in various target tissues. Estradiol stimulates uterine ODC activity in immature rats (Lavia et al., 1983). Estrogen stimulates renal ODC activity in male rats and hamsters, but has little effect in male mice (Wing, 1990). Progesterone is also thought to play a significant role in the rise of uterine ODC activity in the pregnant hamster (Luzzani et al., 1982).

Single, pharmacological doses of parathyroid hormone, calcitonin, vasopressin, d-aldosterone or L-triiodothyronine were shown to produce a significant increase in ODC activity of rat kidney in hypophysectomised rats (Scalabrino and Ferioli, 1976). A single dose of synthetic salmon calcitonin stimulated ODC activity in brain, liver, kidney, testis and ovary (Nakhla, 1987).
It was shown by Wing and Rillema (1983) that prostaglandins PGE_1, E_2 and I_C elicited a concentration-dependent stimulation of ODC activity in mammary gland explants from mid-pregnant mice.

PROLACTIN AND ITS ACTION:

The anterior pituitary hormones are included among the major hormones that influence ODC activity. Prolactin (PRL) is known to exist in all vertebrates and more than 85 diverse and distinct functions have been attributed to this hormone. These actions can be broadly divided as follows: i) reproduction and lactation, ii) water and salt balance, iii) growth and morphogenesis, iv) metabolism, v) behavior, vi) immunoregulation, vii) effects on skin and ectoderm. In addition, prolactin is known to have a mitogenic action and has been implicated as a tumor promoter in rat liver. In mammals, PRL is primarily responsible for the development of the mammary gland and lactogenesis. It acts in association with insulin and glucocorticoids to stimulate milk protein gene expression at both the transcriptional and post-transcriptional levels (Guyette et al., 1979).

The biological roles of the high levels of PRL receptors in rat liver are not well understood. Administration of PRL to rats causes hepatic hypertrophy and increases ODC activity (Buckley et al., 1985; Richards 1975). PRL has been shown to stimulate the expression of mRNA for cytosolic PEP carboxykinase in liver of
lactating rats and in primary cultures of hepatocytes (Zabala and Garcia-Ruiz, 1989). Also, PRL induces a factor called synlactin in the liver of pigeons and rats that acts synergistically with PRL to promote the growth of the crop sac or the mammary gland (Nicoll et al., 1985).

Prolactin is also known to regulate the expression of a large number of receptors, including the interleukin-2 receptors on rat splenic lymphocytes (Mukherjee et al., 1990) and nerve growth factor receptors in the β-cell line INS-1 (Scharffmann et al., 1994). PRL and GH have been shown to control β-cell proliferation and insulin production and secretion.

In humans, PRL regulates the gene for a protein known as PRL-inducible protein (PIP). PIP is a secreted glycoprotein whose transcription is regulated by androgens, while PRL has an essentially post-transcriptional (mRNA stabilisation) effect (Murphy et al., 1987b). PIP is present in several human breast cancer cell lines and in benign and malignant tumor biopsies (Murphy et al., 1987a).

Recent evidence has shown that GH and PRL are immunostimulatory factors. Hormone-replacement studies have shown that either of these hormones was able to restore immune function in animal models such as hypophysectomy models or genetic dwarfs. Effects of PRL on lymphocytes have been reported. Russell et al. (1984) reported that PRL could increase ornithine decarboxylase activity in peripheral lymphocytes. Antibodies to PRL inhibit lymphocyte proliferation (Hartmann et al., 1989).
Hyperprolactinemia results in a marked suppression of antibody production, delayed type hypersensitivity, or development of adjuvant-induced arthritis. PRL also regulates lymphocyte growth in hypophysectomised rats and stimulates c-myc expression and DNA synthesis in lymphoid tissues. In the PRL-dependent Nb2 node T-lymphoma cell line, the immunosuppressive compounds cyclosporine and didemnin B inhibit PRL-stimulated ODC activity and subsequent proliferation (Russell et al., 1987). Similarly, cyclosporine significantly inhibited PRL-stimulated ODC activity in spleen, kidney and adrenal glands in intact rats, and kidney, liver, thymus and adrenal glands in hypophysectomised rats. The major side-effects of the drug cyclosporine being kidney toxicity and depressed renal function, Russell et al. (1984) have suggested that kidney failure in response to cyclosporin A may involve the ability to suppress the normal level of protein and RNA synthesis maintained by circulating prolactin.

Endogenous prolactin has been shown to participate in some of the early immunologic events leading to the development of autoimmune diseases (Buskila et al., 1991), such as experimental lupus in B/W mice (McMurray et al., 1991), induced experimental allergic encephalitis (Riskind et al., 1991) and adjuvant arthritis (Jara et al., 1991). It has also been recently reported by Dardenne et al. (1994) that cells of the human immune system, and hematopoietic tissues including thymus, bone marrow and peripheral blood express high affinity receptors for PRL. These data, along with those showing that the PRL gene is specifically expressed in human T-cells, suggest that lymphocyte PRL may act
in an autocrine or paracrine fashion in both central and peripheral lymphoid organs.

In 1980, Horrobin reviewed the evidence that prolactin is a regulator of fluid and electrolyte metabolism in mammals. He came to the conclusion that this action of prolactin mainly involves modulation of the actions of other agents. Prolactin receptors are found in mammalian kidneys. PRL modulates renal cyclic AMP formation and that of polyamines, and leads to demonstrable histological changes in the proximal tubules. PRL also seems to be able to cause a prolonged reduction in water, sodium and potassium excretion.

The most important role played by PRL is its control of casein synthesis and regulation of the mammary gland and also its effect in mammary carcinogenesis. When PRL secretion is chronically elevated, as in old female rats and some strains of mice, many pituitary and mammary tumors are present. However, only normal levels of PRL are necessary for development of carcinogen-induced mammary tumors in rats and mice. During reproductive life, PRL secretion is elevated during postpartum lactation, galactorrhea, and during chronic administration of neuroleptic drugs. This may result in suppression of gonadotropic hormone secretion and cessation of estrous and menstrual cycles (Meites, 1988). In general, PRL is essential for initiation and maintenance of lactation.

Prolactin-secreting pituitary tumors (prolactinomas) are the
most common type of pituitary tumor in rats, mice and humans, and are associated with an increase in PRL secretion. Chronic administration of estrogens induces pituitary tumors in rats. The principal role of PRL in mammary tumorigenesis is to sensitise the glands to carcinogenic agents by constantly stimulating mammary mitosis.

In 1975, Richards reported an increase in ODC activity in various organs of female rat upon treatment with prolactin. In young male rats an optimal dose of 3 mg per rat induced renal ODC activity which peaked at 2.5 hours after injection. In females, the induction varied with age, being highest in unweaned rats.

In 1980, Levine et al. showed that renal ODC activity is located primarily in the medulla, and ODC in both renal medulla and cortex is sensitive to GH and ACTH-stimulation. Hurley et al. (1980) injected fetuses in utero with 100 μg of various prolactin, GH and placental lactogen (PL) preparations and monitored ODC in liver, heart and brain, at various time intervals. Their results indicated that ovine PL has somatotropic effects in the fetus, and that rat liver ODC becomes responsive to GH and PRL in the perinatal period.

Russell et al. (1984) in their studies on one month old female rats, showed that $10^{-7}$ M PRL resulted in a two-fold elevation of ODC activity within 2h of injection, with peak activity at 6h post-injection. The activity then declined by 8h. $10^{-6}$ M PRL caused maximal stimulation of ODC activity at 6h in
liver and kidney. They concluded that the ability of PRL to induce ODC activity in a variety of tissues in hypophysectomised rats suggests that it may be a direct induction effect and not merely a secondary effect of PRL action. Later, Buckley et al. (1986) showed that even a low dose (5.5 mg/kg body weight) was able to stimulate hepatic DNA synthesis in both male and female rats, adult and weanling.

Gonzalez et al. (1991) carried out some experiments in PRL-deficient Ames dwarf mice (df/df) to assess the effect of hyperprolactinemia on various functions. The mice were implanted with two pituitaries each, some were castrated, given testosterone propionate (TP) and implanted, some were sham-operated and the last group was castrated and given TP. It was found that hyperprolactinemia produced an increase in ODC activity in seminal vesicle and liver, and also polyamine levels. There was also an enhancement of plasma FSH levels in both intact and castrated, TP-treated mice implanted with pituitaries.

The Nb2 node lymphoma cell line is a good system in which to study the role of PRL in cell proliferation as it requires PRL for growth. Russell et al. (1987) found that PRL to be present in the culture medium for a minimum of 3 to 6 hours to invoke a maximal effect on mitogenesis. They also found that products of the lipoxygenase pathway may contribute to the mechanism of PRL-stimulated mitogenesis. Inhibitors of protein kinase C (PKC) and phospholipase A and C activities blocked the PRL-stimulation of ODC and mitogenesis. This suggested a role for PKC in the
coupling of PRL receptors to the stimulation of ODC activity and mitogenesis in Nb2 lymphoma cells.

In 1990, Yu-Lee reported that in the Nb2 lymphoma cell line, the growth-related genes c-myc, ODC, β-actin and a hsp 70 homologue, with rapid but different kinetics, primarily at the transcriptional level. ODC stimulation was maximal 1h after treatment and remained elevated at 4h. ODC mRNA accumulation lagged behind ODC gene transcriptional induction by 1h and showed a 5-fold induction at 8h. This was the first report that ODC mRNA levels are regulated by PRL.

Crowe et al. (1991) later showed that PRL activates PKC and stimulates growth-related gene expression in rat liver, in a dose-dependent manner. In male rats, ODC mRNA expression was essentially low in liver from control animals, but rapidly increased with time on PRL treatment. ODC mRNA levels also responded in a dose-dependent manner to PRL. PRL administration caused an elevation in liver diacylglycerol levels, which paralleled an increase in particulate-associated PKC activity. Prolactin was shown to play a significant role in the regulation of DNA methylation in the liver and kidney of adult and immature rats (Reddy and Reddy, 1990).

Despite a large amount of work having been carried out on prolactin's effects, its regulation and its mechanism of action, no single mechanism has been pinpointed as yet. It was suggested in 1980 by Rillem that once PRL binds to its plasma membrane
receptor sites, its subsequent actions may involve one or more of the following: increased intracellular concentration of potassium and decrease of sodium, increased cGMP level, enhanced rate of prostaglandin biosynthesis mediated by a stimulation of phospholipase A_2 activity, and a stimulation of polyamine synthesis. It has also been shown that the actions of prolactin require the presence of calcium ions in the extracellular environment.

In studies on the induction of milk protein gene expression by prolactin, Bayat-Sarmadi and Houdebine (1993) used various protein kinase inhibitors and concluded that a Serine/Threonine kinase which is not protein kinase C, and possibly a tyrosine kinase is involved in transduction of the prolactin message from the receptor to the milk protein gene. Rui et al. (1992) showed evidence for rapid tyrosine kinase activation on PRL receptor triggering, and Rillema et al. (1992) showed that there is a rapid stimulation of tyrosine kinase activity by PRL in Nb2 lymphoma cells. It was recently reported by David et al. (1994) that treatment of Nb2 rat lymphoma cells with PRL activates a latent protein factor, causing it to bind to an enhancer in the interferon regulatory factor 1 gene. This enhancer is required for IFN-γ-activated expression of the gene. In addition, PRL also induced tyrosine phosphorylation of Jak2, a tyrosine kinase required for IFN-γ-activated gene expression.

Many reports have linked various actions of PRL with the activation of protein kinase C. In 1988 Buckley et al. found that
PRL activated PKC several hundredfold within minutes of addition to rat liver nuclei. It was also found that hepatic PKC was rapidly translocated to the hepatic membrane, and it was suggested that rapid activation of PKC may be an intermediate step in the hepatotrophic responses stimulated by PRL (Buckley et al., 1987). Subsequently Russell et al. (1987) found that in Nb node lymphoma cells, PKC plays a role in the coupling of PRL receptors to the stimulation of ODC activity and mitogenesis. In rat aortic smooth muscle, PRL activated PKC in a dose-dependent manner and this action was blocked by H-7, a PKC inhibitor. The authors suggested that PKC has a role in the signal transduction pathway for PRL action, and this activation may be involved in vascular smooth muscle function.

It was recently found by Meyer et al. (1992) that the cell proliferation-linked protein stathmin in Nb lymphoma cells is phosphorylated in response to PRL-stimulation, but PKC does not mediate this response. They have suggested that phosphorylation of stathmin and stathmin-like proteins may mediate some actions of prolactin in these cells.

In 1993, Rao et al. found that in Nb lymphoma cells, the PRL receptor is constitutively expressed in both nucleus and membrane/cytosol compartments. Addition of PRL stimulated rapid internalization, and translocation of the hormone to the nucleus; this was ATP-dependent and reversible. Their results indicate that an early event coupled to the mitogenic action of PRL in Nb cells is transport of the hormone to the nucleus during the G

and S phases and they suggest that in the nucleus, PRL bound to its receptor may directly influence gene transcription. Recent studies by the same group showed that PRL stimulated rapid tyrosine phosphorylation of mitogen-activated protein (MAP) kinase. The phosphorylated MAP kinase translocated to the nucleus in a manner identical to that found for PRL. They have suggested a possible interactive mechanism whereby PRL, acting via the nuclear PRL receptor, together with MAP kinase, may regulate transcription of genes involved in the proliferative response (Buckley et al., 1994).

Koduri and Rillema (1993) concluded from their studies using cholera and pertussis toxins to study the prolactin-stimulation of lactose synthesis and ODC activity in mouse mammary gland explants, that a G protein, but not G, may be involved in prolactin's mechanism of stimulation.

It has been shown that phospholipase C and arachidonic acid enhance the mitogenic effect of PRL on the Nb2 cell line, and the data obtained are compatible with a possible involvement of PKC in the PRL-stimulation of mitogenesis (Ofenstein and Rillema, 1987). It was also found by Manni et al., (1986) that the polyamine pathway seems to play an essential role in the expression of autocrine control of tumor growth by PRL in mammary tumor cells in culture.

**GROWTH HORMONE AND ITS ACTION:**
Growth hormone (GH) is another anterior pituitary hormone that influences ODC levels in various tissues. This hormone plays a central role in the regulation of post-natal mammalian growth. GH has been shown to directly stimulate in vitro erythroleukemia cell proliferation (Golde et al., 1978) and synthesis of nucleic acids and proteins (Desai et al., 1973). Many actions of GH, such as on skeletal growth, are indirect and are mediated by insulin-like growth factor (IGF). GH has been shown to directly regulate IGF-I production in liver cells as well as other cells in culture (Mathews et al., 1986). Other direct effects of GH include the regulation of cytochrome P-450\textsubscript{15β} by liver cells (Toilet et al., 1990), as well as glucose transport and glucose-metabolism in adipocytes.

In preadipocytes, GH is necessary for the initiation of the differentiation program for the cells to become responsive to IGF-I and for its mitogenic effect (Green et al., 1985). Rapid effects of GH in these cells include induction of c-fos and c-jun transcription (Gurland et al., 1990). A similar effect of GH on precursor cells exists in other tissues like adipose tissue, muscle and cartilage.

Prenatal somatic growth seems to be largely independent of GH. Binding of GH to liver membranes from calf, lamb and rat is minimal before birth and increases gradually during the first weeks of postnatal life (Maes et al., 1983; Gluckman et al., 1983).
Recent evidence has shown that GH, like PRL, is involved in immunoregulation. Removal of the pituitary leads to reduced antibody production, diminished skin sensitivity to toxic substances and adjuvant arthritis, and prolonged skin allograft survival (Nagy and Berczi, 1978). Genetic dwarf mice models lacking GH and PRL in their pituitary have atrophied thymus and lymphoid tissue, and a depletion of bone marrow (Baroni, 1967). GH stimulated erythropoiesis in lymphocytes (Golde and Bersche, 1977). It has also been shown to influence cytotoxic lymphocyte lysis (Snow et al., 1981). The direct production of GH by normal lymphocytes has also been noted (Hartmann et al., 1989).

GH appears to be required for the expression of late differentiation-specific genes, as demonstrated in Ob1771 preadipocytes (Doglio et al., 1986). In the same cells, GH has also been shown to trigger IGF-I gene expression, modulate the expression of the lipoprotein lipase gene, transiently increase c-fos gene expression, stimulate the formation of diacylglycerol and to be unable to affect intracellular Ca^{2+} levels (Catalioto et al., 1990).

Growth hormone was found to stimulate rat hepatic spermidine and putrescine synthesis (Jänne et al., 1968), and this was found to be due to GH-stimulation of ODC activity (Jänne et al., 1969). This stimulation was independent of the adrenal glands, and maximal stimulation occurred at 4h after injection. The stimulation in ODC activity was accompanied by an increase of
more than 100% in RNA polymerase activity.

Subsequently in 1971, Fausto showed that a single injection of GH caused an increase in liver ODC activity, and this effect could be blocked by puromycin and actinomycin D. Ovine GH or hydrocortisone were shown to rapidly increase ODC activity in rat kidney; this activity peaked at 3 to 4 h post-injection and returned to basal levels by 8h (Brandt et al., 1972). Similarly, in 1971, both Richman et al. and Russell and Lombardini reported GH-stimulation of hepatic ODC levels. The former group found that though adrenalectomy had no effect on ODC activity, hypophysectomy caused basal ODC activity to decline. Levine et al. (1973) showed that GH stimulates adrenal ODC activity, and when given along with ACTH, there is a synergistic stimulation. In neonatal rat brain and liver, bovine GH and PRL stimulated ODC activity. Of these, bPRL had only half the potency of bGH (Roger et al., 1974).

Sogani et al. (1972) showed that GH treatment stimulates hepatic and renal ODC activity in hypophysectomised rats. ODC activity was also stimulated by GH in heart and thymus. Murphy and Brosnan (1976) demonstrated that the greatly increased liver ODC activity observed after GH administration was localised in the cytosol. The effect of stressful stimulation on tissue response to GH was studied in rat liver during forced exertion in rats (Martin et al., 1989). GH was found to cause a 15-fold increase in ODC activity in liver of resting rats; and forced walking of the rats enhanced this effect by about 60% more. The
authors have suggested that tissue hypersensitivity to GH stimulation may be a consequence of forced exertion.

Very little is known about the early events that occur after the binding of GH to its receptor. So far, no second messenger of GH has been positively identified. It has been suggested that a novel tyrosine kinase or kinase activity may be associated with the GH receptor in several cell types, including GH-treated 3T3F442A fibroblasts, human IM9 lymphocytes, rat H-35 hepatoma cells, and freshly isolated rat adipocytes (Anderson, 1992).

It has also been suggested that protein kinase C could be involved in the mechanism of action of GH. GH is able to stimulate phospholipase C activity in vitro with the production of inositol triphosphate and diacylglycerol in basolateral membranes of canine kidney (Rogers and Hammerman, 1989).

The stimulation of somatic growth by GH, including growth of long bones, seems to be mediated via stimulation of IGF-I (Behringer et al., 1990). GH appears to be necessary for attainment of normal liver size.

In OB1771 mouse preadipocyte cells, GH has also been shown to stimulate the production of diacylglycerol by means of phosphatidylcholine breakdown, involving a phospholipase C coupled to the GH receptor (Catalioto et al., 1990). In isolated hepatocytes, GH and PRL are able to rapidly stimulate the production of diacylglycerol (Johnson et al., 1990).
Further substantiation of the involvement of PKC is given by Doglio et al. (1989) who showed that in OB1771 cells, GH stimulates c-fos gene expression by means of protein kinase C activation. This activation was thought by them to be due to a phospholipase C-mediated hydrolysis of glycerophospholipids other than inositol phospholipids. GH is also known to cause acute down-regulation of rat liver somatogenic receptors, in contrast with its long-term stimulatory effects (Maiter et al., 1988).

It has been recently shown that in OB1771 cells, c-fos protein is involved in the GH-mediated regulation of transcription of the lipoprotein lipase gene in response to GH (Barcellini-Couget et al., 1993). Anderson (1992) showed that in 3T3-F442A preadipocytes, physiological concentrations of GH induced a rapid and transient activation of mitogen-activated protein kinase (MAP kinase) and S6 kinase. Protein kinase C seems to be involved in the mechanism of action.

**HORMONAL REGULATION IN THE TESTIS:**

The developing mammalian testis is characterised by a high rate of cellular growth, mainly engaging precursors of somatic cells and early spermatogonia at different time intervals during testicular ontogeny. In comparison to other organs, the testis is unique in that it is the only one in which meiotic divisions occur, appearing first during early puberty. The adult testis is also a site of intense mitotic cell proliferation, among the most
rapid known in mammalian tissues. Although testosterone and
gonadotropic hormones are required for initiation of spermatogenesis, there are a number of local paracrine factors
which also profoundly affect testis function.

While LH secreted by the pituitary is undoubtedly the principal regulator of Leydig cell steroidogenesis, a number of local growth factors are also involved. A testicular analog of GnRH appears to stimulate androgen production by Leydig cells (Hsueh et al., 1981; Sharpe et al., 1982). Specific receptors for insulin and insulin-like growth factors (Handelsman et al., 1985), prolactin, arginine vasotocin-like factor and epidermal growth factor, glucocorticoids and catecholamines (Cooke et al., 1982) have been identified on the surface of Leydig cells. While PRL stimulates testicular steroidogenesis (Catt et al., 1980), most of the latter factors appear to decrease steroidogenesis. The testis of several mammals contains large concentrations of the neurohypophyseal hormones oxytocin and arginine vasopressin (Nicholson et al., 1984; Kasson et al., 1985). β-endorphin (and other pro-opiomelanocortin-derived peptides) production in rat Leydig cells has been demonstrated and it may facilitate testosterone secretion either directly as an autocrine effect or via intermediates (Bardin, 1984). A number of reports also indicate that prostaglandins can directly inhibit LH-induced steroidogenesis in dispersed rat Leydig cells (Sairam, 1976). FSH and testosterone regulate spermatogenesis through the Sertoli cells (Steinberger, 1971).
Both high gonadotropin and estrogen levels are known to be inhibitory to adult testicular function including LH receptor down-regulation and blockade of steroidogenesis. However, both of these negative responses are missing in fetal and neonatal testis (Pakarinen et al., 1990).

Since ODC activity and gene expression are markers of cell growth and proliferation, the testis is an ideal system in which to study its hormonal regulation. The ontogenesis of ODC mRNA and enzyme levels in testis has been extensively studied. Alcivar et al. (1989) found that in mouse testis, ODC mRNA levels increased substantially in enriched populations of pachytene spermatocytes, round spermatids and residual bodies isolated from mature testis, as opposed to low levels observed in prepubertal mouse testis. They also showed the distribution of ODC mRNA in both polysomal fractions prepared from total testis extract, suggesting that ODC is translationally regulated in the mouse testis.

Kaipia et al. (1990) showed increasing ODC mRNA levels during prophase of meiosis with highest levels in late pachytene spermatocytes and step 3-5 spermatids. They found three molecular sizes of ODC mRNA, and the relative abundance of these with respect to one another differed in rat and mouse testis, indicating a species difference in the usage of the polyadenylation signals within the ODC gene. They also concluded that the high levels of ODC mRNA in late pachytene spermatocytes and early round spermatids suggest that polyamines may play an important role during late meiosis and early spermatogenesis.
Weiner and Dias (1992) showed that rat testicular ODC mRNA levels began to rise at 21 days of age, reaching maximal levels by 40 days. In contrast, ODC activity decreased with age.

ODC activity in the rat testis is regulated by a number of various hormones and growth factors. FSH and LH have been shown to affect ODC activity, and it was proposed that this action was via cyclic AMP stimulation (Reddy and Villee, 1975). It has been shown that prostaglandins (Madhubala and Reddy, 1980a; 1980b), catecholamines (Madhubala and Reddy, 1981; 1983), gonadotropin releasing hormone (Madhubala and Reddy, 1982), and gonadotropic hormones (Madhubala and Reddy, 1985) stimulate ODC activity in immature rat testis. Similarly, arginine vasopressin (Reddy et al., 1986) was also shown to enhance testicular ODC activity.

Prolactin exerts a marked influence on the gonads, by modulating the effects of gonadotropins (Kelly et al., 1980). Some main actions have been documented: (i) stimulation of the number of LH receptors in the testis during puberty, (ii) stimulation of steroidogenesis, (iii) increase of HDL and LDL binding in the corpus luteum, and (iv) regulation of the growth of ovarian follicles. In humans, hyperprolactinemia caused mainly by pituitary tumors is considered clinically to be one of the major causes of sexual malfunctions such as impaired libido, impotence and male hypogonadism (Hoshino, 1988), leading to infertility.
Zipf et al. (1978) found that maintenance of testicular LH receptors is at least partially dependent on PRL and GH; these hormones seem to act at different sites and by different mechanisms. From their studies on the effects of PRL on testicular regression and recrudescence in the golden hamster, Bartke et al. (1980) concluded that in contrast to findings in rats and mice, chronic hyperprolactinemia in the male hamster does not inhibit gonadotropin release.

The presence of PRL receptors in the interstitial compartment of the testis shows that PRL can act directly on the testis and testicular steroidogenesis. In hypophysectomised rats, PRL potentiates the effects of LH treatment on testosterone production and spermatogenesis. PRL also regulates the growth and function of male accessory reproductive glands (Bartke et al., 1980). In addition to its effects on testicular LH receptors, PRL can stimulate accumulation of esterified cholesterol and the activities of 36- and 17β-hydroxysteroid dehydrogenases in the testis (Bartke, 1980).

Dombrowicz et al. (1992) suggested that during puberty, PRL stimulates testicular function by promoting multiplication and differentiation of Leydig cells (acting at various steps of steroidogenesis and on tissue responsiveness to LH) and germ cells.

Studies carried out by Park et al. (1993) indicate that the rise in FSH after the prolactin-induced suppression of FSH and LH
release after castration requires the involvement of stimulatory factors from the adrenal gland.
AIM AND SCOPE OF THIS STUDY

The vast amount of literature available on ornithine decarboxylase and the polyamines has established their importance in the processes of growth and differentiation. Ornithine decarboxylase is an important cellular marker of proliferation and expression of the ODC gene is one of the early events associated with stimulation by mitogens and growth factors like hormones. Recent reports indicate that expression of the ODC gene is necessary for transformation of some cell types (Auvinen et al., 1992), and it has been suggested that the ODC gene itself may act as an oncogene.

The hormones prolactin and growth hormone are known to exert a mitogenic action on certain tissues, and hyperprolactinemia can lead to the formation of some types of tumours. However, their exact mechanism of action in causing various effects have not been satisfactorily elucidated. It was therefore of interest to study the way in which these hormones affect ODC activity and gene expression in some of their target organs, namely the liver, testis and kidney of the rat.