INTRODUCTION

The maintenance of normal cell function and tissue homeostasis is dependent on the precise regulation of multiple signaling pathways that must accurately control cellular decisions to either proliferate, differentiate, arrest cell growth, or initiate programmed cell death (apoptosis). Cancer arises when clones of mutated cells escape this balance and proliferate inappropriately without compensatory apoptosis. Many studies have revealed that the disruption of multiple pathways is required for the development of cancer. Thus, not only is it critical to understand the normal function of specific cellular pathways, but also equally important is an understanding of how they interconnect to synchronously regulate cell growth versus apoptosis.

The liver plays a primary role in body homeostasis. It regulates levels of circulating nutrients, excretes waste products into the bile, reduces circulating ammonia through production of urea, produces important serum proteins, bile acids required in digestion of lipids and acts as the primary site of a metabolic defense against carcinogens that enter through the gastrointestinal tract. Under normal physiological conditions, liver size is tightly controlled in almost all species. When baboon livers are transplanted into humans, they grow to the mass of a human liver. The liver has a remarkable capacity to regenerate after cellular damage or tissue removal. Liver regeneration is mostly the result of increased mitosis of hepatocytes. Removal of as much as 80-90% of the liver can be restored in the absence of liver disease. Livers from rats have been subjected to partial hepatectomy (removal of 2/3rd of the liver) 12 times in a row. Each time, the liver is restored to its normal size within a few weeks. It has been estimated that one rat hepatocyte has the capacity to generate at least 50 livers (Michalopoulos.G.K & DeFrancis.M.C, 1997).

The liver has the unique capacity to regulate its growth and mass both in humans and animals. The mammalian liver is one of the few adult organs capable of completely regenerating itself in response to injury through the release of growth factors that stimulate reentry of terminally differentiated hepatocytes into the cell cycle (Michalopoulos.G.K & DeFrancis.M.C, 1997, Fausto.N et al., 1995 &., Taub.R, 1996). This property is particularly remarkable because hepatocytes are cells, which in their
normal state rarely divide. However, their proliferative capacity and the ability of the liver to adapt to variable metabolic demands are not lost. These properties are quickly displayed when a deficit or excess of hepatic mass occurs (Fausto.N & Webber.E.M, 1994). The surgical removal of 70% of the liver induces a partially synchronised growth response that leads to the rapid restoration of organ mass.

Lead nitrate (LN) is a direct mitogen, which specifically induces liver hyperplasia. A single intravenous injection of LN caused hepatic cell proliferation, which peaked at 48 hours followed by hepatic apoptosis. Apoptosis was found to be markedly increased at 5 days after LN treatment (Shinozuka.H et al., 1996 & Columbano.A et al., 1985). Apoptotic cells die a stereotypical death, regardless of the initiating death signal (Martin.S.J et al., 1994). During apoptosis the cytoplasm shrinks, the plasma membrane blebs and vesiculates, and phosphatidylserine redistributes to the cell surface. Simultaneously, the nucleus shrinks, chromatin condenses and DNA fragments into high molecular weight oligonucleosomal pieces. Insufficient apoptosis because of caspase inactivation may promote oncogenesis by allowing cell accumulation (Martin.S.J & Green.D.R, 1995). Recent evidence supports this hypothesis, and careful study of the caspase knockout animals should provide more definitive answers to this question (Resnicoff.M et al., 1998 & Green.D.R, 1998).

Globally, primary liver cancer was ranked seventh in men in 1975 and by 1990 it reached the fifth position (Perin.N.N, 2001). It is estimated that approximately 1.25 million people die from hepatocellular carcinoma each year. Tumourigenesis is a complex process involving at least three steps, initiation, promotion and progression (Farber.E, 1976). Many oncogenes and protooncogenes are now known and some of these are closely associated with cell growth (Weinberg.RA, 1989, Goustin.A.S et al., 1986 & Bishop.J.M, 1987). The activation of protooncogenes by a variety of mechanisms that affect their expression is believed to play an important role in formation and progression of tumours. N-nitrosodiethylamine (NDEA) is a well known carcinogenic agent causing hepatocellular carcinoma (Narurkar.L.M. & Narurkar.M.V, 1989). NDEA induces liver tumours in guinea pigs, rabbits, dogs, and rats and nasal cavity tumours in rats (Nakae.D et al., 1997).
Identification of mutations in tumours that lead to decreased apoptosis is not only of academic interest but rather an important goal in the light of cancer therapy. Clearly, mutations in cell death control do affect sensitivity of tumour cells to anti-cancer therapy which in most cases functions by inducing apoptosis (Zhang L et al., 2000 & Finkel E, 1999). It has become clear that, together with deregulated growth, inhibition of programmed cell death (PCD) plays a pivotal role in tumourigenesis.

*In vivo* and *in vitro*, many substances have been shown to influence the entry of hepatocytes into DNA synthesis. Serum proteins, peptide growth factors, androgens, estrogens, glucocorticoids, thyroid hormones and adrenergic agents all have been implicated in the regulation of growth. Growth factors and growth factor receptors play an important physiological role in the normal process of growth and differentiation. The binding of the growth factor to its receptor leads to receptor dimerisation and cross phosphorylation, activating the receptors. The activated receptors phosphorylate a series of cytoplasmic proteins which in turn sets off a cascade of events leading to the activation of transcription factors in the nucleus resulting in increased mRNA synthesis. The translation of the mRNA results in increased protein synthesis finally leading to either growth or differentiation (Fantl WJ et al., 1993). Aberrations of growth factor signaling pathways can lead to abnormal growth and development. Cancer is now recognised to be the result of a multistep process. Among the events that can lead to malignant transformation is the unregulated expression of growth factors or components of their signaling pathways. Apoptosis or programmed cell death is an important physiological phenomenon playing crucial role in growth and development of an organism. It also plays an important protective role in DNA damaged cells which fail to have their DNA damage repaired but attempting to enter the cell cycle. By triggering apoptosis, these abnormal cells are destroyed, thereby preventing tumour induction. In the absence or inhibition of apoptosis, these cells survive, cumulate more DNA damage and tend to acquire an altered phenotype.

Brain plays an important regulatory role in hepatic functions (Lautt W.W, 1983). The liver is richly innervated (Rogers R.C & Hermann G.E, 1983) and autonomic nervous system has an important role in the process of hepatic cell proliferation (Tanaka K et al., 1987). Lateral lesions of hypothalamus cause an increase in DNA
synthesis during liver regeneration while sympathectomy and vagotomy block this effect (Kiba T et al., 1994). There are several reports regarding the brain regulation of hepatic proliferation but the role of central nervous system, neurotransmitters and their receptors in mediating these effects are not well characterised.

Neuronal stimulation of rat liver could result in changes in cellular metabolism and liver regeneration through a combination of direct innervation and intercellular communication via gap junctions (Seseke F.G et al., 1992). Vagotomy was shown to cause a marked depression of cell proliferation following hepatectomy (Maros T, 1970 & Ohtake M et al., 1993). Extirpation of the brain cortex was shown to increase the rate of cell proliferation implying that the cortex exerts a normal inhibitory function on liver cell division and growth. It was reported that transection of the spinal cord above the area innervating the liver resulted in decreased DNA synthesis (Vaptzarova K.I et al., 1973). Norepinephrine (NE) was shown to induce DNA synthesis in a dose-dependent manner in hepatocyte cultures, acting through the $\alpha_1$ adrenergic receptor (Cruise J.L & Michalopoulos G, 1985).

Neurotransmitters stimulate or inhibit cell proliferation in non-neuronal cells by activating receptors coupled to various second messenger pathways (Kluess C et al., 1991). 5-hydroxytryptamine (5-HT) has been recognised to cause proliferation of a variety of cells in culture, including those of vascular smooth muscle cells and hepatocytes. The proliferative effect is synergistic with that of more conventional growth producing polypeptides. Most evidences indicate that cellular cyclic nucleotides play an important role in the intracellular signaling process for growth regulation by 5-HT, and newer studies point to protein phosphorylation pathways as being important in the mitogenic response (Fanburg B.L & Lee S.L, 1997). Using a pancreatic cell line, Ishizuka et al., (Ishizuka I et al., 1992) proposed that activation of cellular proliferation occurs through a pertussis toxin-sensitive 5-HT$_{1A/1B}$ receptor activated through PLC and PKC that resulted in downregulation of cellular cAMP. Mene et al., (Mene P et al., 1991) suggested activation of a 5-HT$_2$ receptor in rat renal mesangial cells to account for 5-HT induced cell proliferation. The involvement of 5-HT and its receptor subtype in the induction of hepatocyte DNA synthesis was investigated in primary cultures of adult rat hepatocytes.
5-HT caused a dose-dependent increase in DNA synthesis in primary cultures of rat hepatocytes in the presence of epidermal growth factor (EGF) and insulin. 5-HT can act as a potent hepatocyte co-mitogen and induce DNA synthesis in primary cultures of rat hepatocytes, which is suggested to be mediated through the 5-HT$_2$ receptors of hepatocytes (Sudha.B & Paulose.C.S, 1997). 5-HT$_{1A}$ receptor is known to mediate cell differentiation and cessation of proliferation in neuronal cells (Azmitia.E.C, 2001).

The work that is presented here is an attempt to understand the role of 5-HT, 5-HT$_{1A}$ and 5-HT$_{2C}$ receptors in the regulation of liver cell proliferation using *in vivo* and *in vitro* models. The work also focuses on the brain serotonergic changes associated with hepatocyte proliferation and apoptosis to delineate its regulatory function. The investigation of mechanisms involving different models of hepatocyte proliferation may contribute to our knowledge about serotonergic regulation of cell growth, apoptosis and carcinogenesis of liver.
OBJECTIVES OF THE PRESENT STUDY ARE:

1. To induce controlled liver cell proliferation by partial hepatectomy and lead nitrate treatment, hepatic apoptosis by lead nitrate treatment and hepatocellular carcinoma by N-nitrosodiethylamine treatment in male Wistar rats.

2. To study DNA synthesis by [3H]thymidine incorporation/thymidine kinase assay in regenerating, lead nitrate induced hepatic hyperplasia and apoptosis and NDEA induced hepatic neoplasia in rats.

3. To study the 5-hydroxytryptamine (5-HT) content in brain stem, cerebral cortex, hypothalamus and liver during controlled cell proliferation, controlled cell death and uncontrolled cell proliferation in rat liver using High Performance Liquid Chromatography (HPLC) integrated with an electrochemical detector.

4. To study the plasma norepinephrine levels in the experimental animals using HPLC.

5. To study 5-HT1A and 5-HT2C receptor status in brain stem, cerebral cortex, hypothalamus and liver during active hepatocyte proliferation and apoptosis in male Wistar rats.

6. To study the alteration of 5-HT1A and 5-HT2C receptor mRNA in brain stem, cerebral cortex, hypothalamus and liver of experimental rats using reverse transcription polymerase chain reaction (RT-PCR).

7. To study the effect of 5-HT1A and 5-HT2C receptor ligands in DNA synthesis in primary hepatocyte culture in combination with epidermal growth factor and/or transforming growth factor β1.