1. INTRODUCTION

1.1. Osteoporosis:

Osteoporosis is defined as "a disease characterized by low bone mass and micro-
architectural deterioration of bone tissue, leading to enhanced bone fragility and a
consequent increase in fracture risk". It is often referred to as a "silent" disease, because
the first visible clinical sign of osteoporosis is often a fracture of the hip, spine or
forearm. Osteoporosis ranks as one of the 5 costliest diseases of aging after diabetes,
hyperlipidaemia, hypertension and heart diseases. Elderly people are the fastest growing
populations in the world and, as people age, bone mass declines and the risk of fractures
increase (Cummings, 2002). Osteoporosis is a major public health problem throughout
the world (Genant, 1999).

Osteoporosis can be primary which includes post-menopausal (Type I) and senile
osteoporosis (Type II) or secondary osteoporosis which is secondary to specific causes
such as endocrine abnormalities, steroid induced etc. The Type I variety occurs typically
between 55-75 years, affects mainly trabecular bone and is more common in women than
in men (6:1 ratio). Prior to menopause, bone loss occurs at the rate of 0.5% to 1.0% per
year. At menopause, bone loss accelerates at the rate of 2% to 5% per year due to decline
in estrogen levels and is greatest in the first 3-6 years post menopause. The Type II or age
– related osteoporosis occurs after the age of 70 years, affects both cortical and trabecular
bone and affects women twice as frequently as men.
1.1.1 Prevalence:

Osteoporosis is a global problem occurring in every geographic area and affecting 200 million men and women worldwide. Ethnicity and race are well known determinants of skeletal health and bone mineral density. In the USA, 10 million individuals already have osteoporosis and 18 million have osteopenia making it to a total of 28 million Americans affected by this condition. American women are four times more likely to develop osteoporosis than men. Osteoporosis affects 30% of postmenopausal white women in the USA and the proportion rises to 70% in women over the age of 80 years (Melton, 1995). The incidence in Europe is projected to double in the next 50 years, and the incidence in Latin America is also expected to rise significantly (www.osteo.org). Although data on the prevalence of osteoporotic fractures are limited, hip fractures are extremely serious and are responsible for substantial mortality. Each year, osteoporosis causes more than 1.5 million fractures, resulting in permanent disability, loss of independence, and death. It is predicted that one out of every two women and one in eight men over 50 will have an osteoporosis-related fracture in her or his lifetime. Fifteen to 30% of those with a hip fracture will die of complications related to the fracture, of those who survive 50% are unable to walk again independently and 1/3 becomes totally dependent functionally (Brunner, 2003).

Globally, osteoporosis is highest in Whites and Asians, and lowest among Blacks. Blacks have more bone density than other racial groups, lowering their risk of osteoporosis. Hispanic-American women have somewhat greater bone density than do non-Hispanic whites (Wehren, 2003). Of all the varieties, postmenopausal osteoporosis is the commonest and most preventable. Postmenopausal osteoporosis today is recognized
to be a major public health problem and is a common cause of morbidity and mortality in women. According to World Bank report, the worldwide population of postmenopausal women which was 470 million in 1990 is expected to increase to 1.2 billion by the year 2030 and 76% of these women would be living in developing countries. In India, it is projected that by the year 2030, the population of postmenopausal women will be 2nd highest in the world, second to that in China. Thus, the burden of osteoporosis in the Indian scenario will also be immense.

1.1.2 The Indian scenario

Osteoporosis is highly prevalent in India (Gupta, 1998, Goswami, 2000, Mithal 1999, Gandhi 2005). An estimated 61 million people in India are reported to be affected by it (Joshi, 1998). It is reported that osteoporotic fractures occur 10-20 years earlier in Indians as compared to Caucasians (Gupta, 1998).

1.1.3 Causes and risk factors

Low bone mass results due to insufficient bone deposited in the skeleton during growth or due to a subsequent loss of bone tissue at an excessive rate. Several forms of osteoporosis have been identified. Osteoporosis can be classified as primary (Type I or Type II) or secondary. Secondary osteoporosis can occur due to specific causes such as endocrine disease (eg. hyperparathyroidism, hyperthyroidism, glucocorticoid excess etc.), drug induced (eg. glucocorticosteroids, barbiturates, heparin, ethanol etc.) and miscellaneous conditions (eg. prolonged immobilization, rheumatoid arthritis, chronic liver failure etc).
The risk of developing osteoporosis is increased in women with slender built, inactive lifestyle, extensive bed rest, a lifetime diet low in calcium and vitamin D, history of excessive alcohol intake, cigarette smoking/tobacco use, premature or surgical menopause, or with use of medications which affect bone turnover. The risk of osteoporosis also increases as age advances, if there is a family history of osteoporosis/atraumatic fractures and due to racial and genetic factors. Risk factors may help explain contributing causes of osteoporosis or help guide therapeutic recommendations, but they cannot be used to diagnose osteoporosis. Although many risk factors for osteoporosis and fractures have been identified, yet one cannot determine why some individuals show a marked reduction in bone mass and are prone to multiple fractures, whereas others with similar risk factors do not exhibit these characteristics (Lawrence, 1997).

1.1.4 Diagnosis:

Assessment of existing bone mass, determining the fracture risk based on this clinical assessment, and making decisions regarding the appropriate therapeutic intervention are the ultimate goals when evaluating patients for osteoporosis. The WHO established diagnostic criteria for osteoporosis on the basis of Bone Mineral Density (BMD) T-scores (WHO study group). The T-score describes the patient’s BMD in terms of the number of standard deviation (SDs) by which it differs from the mean peak value in young, healthy persons of the same sex (Brunader, 2002). The WHO uses a threshold of 2.5 SDs below the mean of young adult women as the criterion for a diagnosis of osteoporosis. The criterion for a diagnosis of osteopenia (low bone mass) is more than
1.0 SD but less than 2.5 SDs below the reference mean. However, T-scores were developed for the estimation of the prevalence of osteoporosis across populations not for the assessment of osteoporosis in specific patients. Moreover, although T-scores originally were based on the BMD of the hip measured by dual-energy x-ray absorptiometry (DXA), the scores are now applied to BMD at other skeletal sites and/or measured by different methods. Currently, the National Osteoporosis Foundation and the International Society for Clinical Densitometry consider central DXA of the hip and/or spine as the preferred measurement for a diagnosis of osteoporosis (Leib, 2004).

1.1.5 Prevention and Treatment:

Although there is no cure for osteoporosis, there are steps you can take to prevent it or to slow or stop its progress.

Adequate calcium, vitamin D, appropriate exercise and, in some cases, medication are important for maintaining bone health. Currently, bisphosphonates (alendronate, ibandronate and risedronate), calcitonin, estrogens, parathyroid hormone and raloxifene are approved by the US Food and Drug Administration (FDA) for the prevention and/or treatment of osteoporosis.

The bisphosphonates (alendronate, ibandronate and risedronate), calcitonin, estrogens and raloxifene affect the bone remodeling cycle and are classified as anti-resorptive medications. Bone remodeling consists of two distinct stages: bone resorption and bone formation. During resorption, special cells on the bone’s surface dissolve bone tissue and create small cavities. During formation, other cells fill the cavities with new bone tissue. Usually, bone resorption and bone formation are linked so that they occur in
close sequence and remain balanced. An imbalance in the bone remodeling cycle causes bone loss that eventually leads to osteoporosis and fracture risk. Anti-resorptive medications slow or stop the bone-resorbing portion of the bone-remodeling cycle but do not slow the bone-forming portion of the cycle. As a result, new formation continues at a greater rate than bone resorption, and bone density may increase over time.

Teriparatide, a form of parathyroid hormone, is a newly approved osteoporosis medication. It is the first osteoporosis medication to increase the rate of bone formation in the bone remodeling cycle (www.nof.org). Though there are numerous products in the market that are designed to prevent the loss of bone in persons with osteoporosis or at risk of osteoporosis, only “Parathyroid hormone” (PTH) stimulates the bone formation. hPTH is a potential drug for therapy of osteoporosis.

1.2 Parathyroid hormone:
Parathyroid hormone (PTH) is a naturally occurring peptide hormone involved in bone morphogenesis and remodeling. Calcium ion homeostasis in vertebrates is regulated primarily through the action of parathyroid hormone. In humans, PTH is synthesized as a 115-amino acid precursor polypeptide, which is processed by the endoplasmic reticulum, golgi apparatus and secreted as an 84-amino acid peptide (Potts et al. 1980). Interestingly, the fragment consisting of the 1-34 amino acid sequence appears to contain all of the information necessary for full biological activity. In the original chemical synthesis of PTH, the phenylalanine residue at position 34 was chosen as the amino acid to couple to the solid support resin (Potts et al., 1971), virtually all studies for the past three decades have used this synthetic fragment or analog of it.
Discovery of the parathyroid glands and their biological role evolved later than that of the thyroid gland. Four distinctive phases of study were described over the last hundred years. These include determination of the physiological function of the parathyroids, the pathophysiology due to hormone excess or deficiency, chemical characterization and synthesis of PTH and study of its molecular and cellular biology and finally, the pharmacological use of PTH as an effective treatment for osteoporosis – a use that seems paradoxical in light of the hormone’s physiological role and pathophysiological effects (See Table 1) (Potts 2005).

1.2.1. Chemical characterization and synthesis of PTH:

Molecular and cellular biology of PTH action

Although the field of PTH research, particularly the understanding of its clinical implications in disease, was greatly aided by Collip’s breakthrough in preparing active extracts, progress was slow in determining its chemical structure. This occurred, in part, as an undesired side effect of the successful hot acid procedure used by Collip (Collip, 1925). As we understand at present, the hormonal polypeptide was not only liberated and solubilized by hot acid extraction but also cleaved at multiple sites, particularly at sites within the molecule where aspartic acid or asparagine is found. The cleavage induced by dilute acid gives a multiplicity of biologically active products of varying chain length.

Handler et al (1951) summarized that it seemed impossible to purify the hormone if it kept subdividing into multiple fractions which still has activity. Aurbach (1959) and then Rasmussen and Craig (1959), solved the problem 35 years following the original Collip
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<tr>
<td>1850–1900</td>
<td>Parathyroid glands discovered as separate entities from thyroid</td>
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<td>Function unknown</td>
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<td>1900–1925</td>
<td>Parathyroid gland function debated</td>
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<td></td>
<td>Tetany after parathyroidectomy: cause—hypocalcemia vs methyl guanidine</td>
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<td>1925</td>
<td>Active gland extract purified</td>
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<td>Calcium regulation established</td>
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<td>1927–1950s</td>
<td>Pathophysiology of hormone excess and deficiency defined</td>
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<td>Hyper- and hypoparathyroidism</td>
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<td>1929</td>
<td>Bone mass increase in rats</td>
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<td>Paradox (largely ignored)</td>
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<td>1970s</td>
<td>Hormone structure and synthesis</td>
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<td>Bone anabolic effects in animals confirmed</td>
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<td></td>
<td>Human clinical trials in osteoporosis start</td>
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<td>1990s</td>
<td>Parathyroid hormone receptor cloned</td>
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<td>Rapid advances in understanding hormone action at the cellular and</td>
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<td>PTHrP gene knockout—abnormal bone development</td>
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<td>2001</td>
<td>Striking clinical benefit in osteoporosis established</td>
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<td>Era of skeletal ‘anabolic’ agents begins</td>
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observation. Considering the unwanted side-effects of hot acid extraction, they turned to extraction with organic solvents which accomplished the same task—namely, liberating the active principle (polypeptide) from the other cellular constituents without fragmenting it into several pieces. These breakthrough observations signaled the beginning of rapid chemical characterization and synthesis. Two independent groups determined the structure of bovine and human PTH (and one of the groups that of porcine PTH) by conventional techniques of protein sequence analysis after laborious accumulation of sufficient material, particularly difficult with the human hormone available only from surgically removed tumors (Brewer & Ronan 1970, Niall et al. 1970, 1978, Brewer et al. 1972, Sauer et al. 1974, Keutmann et al. 1978). Based on the deduced amino acid sequence and the knowledge that hot acid produced active fragments, it was deduced that the first 34 amino-terminal amino acids should be sufficient for biological activity. The PTH (1–34) regions of first bovine and then human PTH were synthesized (Potts et al. 1971, Tregear et al. 1973, 1974). These fragments are analogous to the natural peptide fragments in the Collip preparation, but the latter were heterogenous.

The biological activities of the purified natural peptide and the synthetic amino terminal sequence of 34 residues were shown to be similar in vitro and in vivo. Availability of highly purified parathyroid polypeptide and active synthetic fragments made it possible to develop radioimmunoassay and to open the era of molecular and cell biology in which the PTH actions at target sites could be properly evaluated and defined with purified hormone preparations. Of course, great advances in cell biology in other fields provided techniques that helped accelerate advances in PTH research. In addition, in the early 1970s, breakthroughs in molecular biology culminated in the development of
recombinant DNA technology, which made it possible to deduce polypeptide sequence from nucleotide sequence of the responsible gene experimentally through analysis of cDNA – that is, a reverse copy of the mRNA for the protein. It became possible to deduce the amino acid sequence of the hormone in species in which the active hormonal polypeptide principle had never been isolated; the active peptide could then be synthesized.

1.2.2. Structure/activity studies of the PTH ligand:

Nucleotide sequence analysis of cDNA for human and bovine PTH was used to confirm the amino acid sequences that had originally been determined by conventional peptide sequence analysis (Kronenberg et al. 1979, Hendy et al. 1981). The amino acid sequences of rat, chicken and dog PTH were determined exclusively by molecular cloning techniques without isolation of the protein (Heinrich et al. 1984, Khosla et al. 1988, Russell & Sherwood 1989, Jüppner et al. 2000).

Structures of mammalian forms of PTH and chicken PTH, shown in Fig. 1, also include the structures of PTH-related peptide (PTHrP). The two molecules share structural homology and some overlap in function (using the same G-protein-linked receptor, discussed below). An extensive evaluation of PTHrP (Martin et al. 2005) was triggered by the search for the cause of hypercalcemia of malignancy. There is clear evidence of a common ancestry for PTH and PTHrP that can be inferred from the similarity in their amino terminal sequence regions, the intron–exon organization of the genes encoding the two molecules, and the structure of the exons that encode part of the precursor peptide (propeptide sequence). Both molecules have bone as a principal target tissue: PTHrP is vital during embryogenesis in regulating bone formation while PTH, possibly the later
evolutionary arrival, has its principal physiological function, the mobilization of calcium from bone in the adult as part of its protection of calcium homeostasis (Jüppner et al. 2000). Extensive sequence homology is present in the known mammalian PTH species (Fig. 1A, left panel). PTHrP structures are shown for comparison (Fig. 1A, right panel). All mammalian PTH molecules consist of a single chain polypeptide with 84 amino acids and a molecular weight of approximately 9400 Daltons.

1.2.3. PTH receptors and hormone action:
An important breakthrough in understanding the physiological role of PTH and testing its molecular and cellular actions occurred when the receptor was successfully cloned in 1991 (Jüppner et al. 1991, Abou-Samra et al. 1992). Since that time, receptors for PTH from many additional species have been cloned (reviewed in Jüppner et al. 2000). Because of the diverse actions of PTH in multiple target tissues and due to in vitro evidence for multiple second messengers of hormone action from studies with cellular membrane fractions enriched in the PTH receptor (prior to its cloning), it was initially thought that several different receptors would be found to mediate some of these pleotropic actions of this peptide hormone. It was therefore somewhat surprising when the initial cloning approaches in several species led to detection of only a single G protein-coupled receptor, now referred to as the common PTH/PTHrP receptor, or PTHR1 (Fig. 2). This receptor mediates most of the traditional actions of PTH in mineral ion homeostasis and is critical to its actions on bone and kidney. There are a variety of biological actions ascribed to them, usually involving interaction with portions of either PTH or PTHrP beyond the amino terminal 34 residues (Jüppner et al. 2000). One of these
Figure 1 (A) Alignment of the amino acid sequences of known PTH (left panel) and PTH-related peptide (right panel) species. Conserved residues are shaded; numbers indicate the positions of amino acids in the mammalian peptide sequences. The figure does not include all recent PTH molecules such as zebrafish PTH (Potts 2005).
Figure 2. Schematic representation of the human PTH/PTHrP receptor and its gene organization. Amino acids are shown in single-letter code; the amino terminus of the receptor is at the top; bars indicate boundaries between each of 14 coding exons; exon 5 encodes the putative signal peptide. (Reproduced with permission from Elsevier (Lipuner et al. 2000)).
additional receptors of particular interest is the carboxyl terminal PTH receptor that responds to C-terminal fragments of PTH (which are both generated by peripheral metabolism of the secreted intact hormone and by release from the parathyroid gland itself). PTHR1, belongs to a distinct family of G protein-coupled receptors. After the cloning of the original receptors from several animal species, cDNAs encoding human PTHR1 as well as mouse, rat, chicken, pig, dog, frog and several PTH/PTHrP receptors from fish were isolated. The gene encoding the PTHR1 is located on chromosome 3 in humans. The gene involved in its synthesis has a total of 14 exons, the contributions of each of which are shown in Fig. 2. Family B receptors have a long amino terminal extracellular domain which is critical for binding peptide ligands such as PTH.

The cloning of the receptor and the intense studies of structure–activity relations with the PTH ligand and receptor have provided further tools with which to examine the cellular biology of PTH action (Bergwitz et al. 1996, Iida-Klein et al. 1997, Jüppner et al. 2000, Shimizu et al. 2000, 2001, 2002). These insights from cellular and molecular biology complement a variety of careful in vivo studies to help provide a more complete picture of the physiological role of the hormone in vivo. PTH acts in the kidney to increase the synthesis of 1,25(OH)2D and thus indirectly increase intestinal calcium absorption. The hormone regulates renal calcium and phosphate transport, the latter action being important to support the overall homeostatic role of PTH (Jüppner et al. 2000). When calcium is needed (as with calcium-deficient diets or vitamin D insufficiency) calcium is mobilized from bone by increased PTH. The phosphate is not needed typically, since its dietary lack is infrequent, so PTH promotes phosphate excretion by blocking its reabsorption. PTH also works at distal tubular sites in the kidney to lower the amount of
urinary calcium excreted; with calcium deficiency, less calcium is lost because PTH increases renal calcium reabsorption. PTH affects a wide variety of specialized bone cells, including osteoblasts and stromal cells. It also has important actions on osteoclasts, but those are indirect and are mediated through osteoblasts. A number of cell lines of osteoblasts and stromal cells and specialized tissue culture systems have evolved to study the interaction between the different cell types and their role in bone formation and bone resorption. Through its abundant receptors on osteoblasts, PTH has a variety of actions that are directly involved in promoting bone formation but physiologically, most importantly, to stimulate osteoclast differentiation and development and ultimately increased bone resorption. It is the latter action which has been traditionally associated with PTH – i.e. bone resorption. As noted below, however, the action to promote osteoblast activity directly has been exploited pharmacologically in the paradoxical but effective use of PTH in osteoporosis.

1.2.4. Structure/activity relations in PTH and its receptor:

The work on defining the essential features of hormone/receptor interaction has become an area of intense interest both in basic studies by academic groups and, in largely unpublished studies, by several pharmaceutical firms looking for a peptidomimetic. Scores of synthetic peptide fragments and analogs have been used to determine the essential pharmacophore for the PTH ligand and/or the capacity of some PTH analogs to signal selectively (Takasu et al. 1999, Shimizu et al. 2000, 2001, 2002). The PTH receptor clearly has multiple signaling pathways, including the most prominent one, Gs-dependent, cAMP generation via adenyl cyclase, as well as inositol triphosphate
generation, a non phospholipase C-dependent protein kinase C action, and calcium transients that at least in certain cell types are not dependent on cAMP generation (Jüppner et al. 2000). In the process of these studies, mutagenized forms of the receptor have also been used to map regions of complementarity between ligand and receptor. The current state of this work was well reviewed recently (Gardella & Jüppner 2001). Gardella and colleagues have established a number of critical features of hormone/receptor interaction, which may apply to the entire class B family of peptide hormone G protein-coupled receptors. This work is of fundamental interest in understanding hormone/receptor interaction but also has great practical significance, since the possibility of developing a small molecule equivalent, a peptidomimetic for PTH, might emerge from such careful studies of the essential features of the critical step(s) in forming the active bimolecular complex of hormone and receptor. A working hypothesis has emerged concerning the interaction of different regions of the biologically active amino terminal 34-aminoacid region of the peptide with the receptor. The large extracellular domain of the receptor is referred to as the N-domain. The later, referred to as the J-domain, includes the portion of the receptor that contains the three extracellular loops that connect the seven transmembrane-spanning helices, the helices themselves, and three intracellular domains, with a large terminal intracellular domain (Bergwitz et al. 1996, Hoare et al. 2001), the J-domain is the functional portion of the receptor. There is evidence that the PTH peptide can interact with these two regions somewhat independently. The carboxyl-terminal portion of the parathyroid ligand binds to the N-domain of the receptor and provides critical docking interactions with the receptor that makes it possible for the otherwise weakly binding amino terminal domain of the ligand
to associate with the J-domain of the receptor (Fig. 3). The amino terminal domain of PTH, particularly the first several residues, has long been known to be critical for activation. The model, supported by a large amount of experimental data, indicates that this amino terminal portion of the PTH interacts with critical residues within the J-domain of the receptor, thereby inducing or selecting the active conformation that binds preferentially G proteins and thus begins the cascade of second messenger-mediated hormone-specific events within target cells.

1.2.5. PTH and the therapy of osteoporosis:

The most recent chapter in the history of PTH is its use as the most effective current therapy for osteoporosis. This fact is quite surprising and clearly paradoxical (See Table 2). Hyperparathyroidism is invariably associated with bone loss, not bone gain (even though the severe bone disorder, osteitis fibrosis cystica – or von Recklinghausen’s disease – is itself rare). How administration of PTH, which causes bone loss in hyperparathyroidism can be a cure for osteoporosis is the paradox. Strictly, of course, PTH therapy does not cure osteoporosis; rather, it simply greatly restores bone mass, especially trabecular bone, increases bone strength and dramatically reduces fracture incidence (Reeve et al. 1980, Tam et al. 1982, Lindsay et al. 1997, Lane et al. 1998, Dempster et al. 2001, Neer et al. 2001). In general, the therapy of osteoporosis has been a dramatic success story, particularly in the last several decades. There are multiple effective therapeutic agents (Rodan & Martin 2000). Prior to the latter part of the 20th century, osteoporosis was largely untreatable. The disease usually declared itself by one or more spontaneous or pathological fractures, often in the spine or in the hip, the latter
Figure 4: Model for modulation of ligand binding to the PTH1 receptor by C protein. The C-terminal portion of the ligand (C) interacts with the 1-domain of the receptor (A). Subsequently, the N-terminal portion of the ligand (D) binds to the 2-domain of the receptor (B). Receptor/G protein interaction (lower right) increases the affinity of the ligand/1-domain interaction (modified to a more closed receptor conformation). Therefore, the interaction of the bound with the 2-domain increases the affinity of receptor.
Table 2 Paradox of PTH biological action: therapeutic use vs physiological function

**Homeostatic role:** Sustained elevation of PTH can maintain blood calcium against challenge of prolonged calcium deficiency by withdrawal from bone ‘bank’, reduces bone mass.

**Therapeutic role:** Deliberate, short pulses of PTH dramatically build bone mass.
  a. Continuous elevation in PTH blood levels (>2 hrs): _ bone mass
  b. Intermittent elevation in PTH blood levels (<2 hrs/day): _ bone mass
being especially catastrophic with regard to medical consequences (Delmas & Chapurlat 2005). Multiple factors contributed to the improved diagnosis and therapy of this disease, including the development of reliable, non-invasive methods of measuring bone mass (Njeh et al. 2005). The second great advance has been the development of effective antiresorptive therapies, principally bisphosphonates (Delmas & Chapurlat 2005, Rodan & Martin 2000). PTH represents, however, much more than simply an additional agent for the therapy of osteoporosis.

The mechanism of action of PTH establishes it as the first in a class of anabolic agents for osteoporosis. An anabolic agent is one that directly stimulates bone formation and is therefore to be distinguished from antiresorptive agents which act by blocking bone resorption, allowing the endogenous rate of bone formation to build bone since it is now unopposed by resorption. The difference between the lowered bone resorption rate and the continuing intrinsic bone formation results in an eventual gain in bone mass, an effect that occurs more slowly than that seen with PTH. The first evidence that PTH increases bone mass occurred many decades ago at the time that the pathophysiological significance of excess PTH (hyperparathyroidism) was just being worked out, as outlined above. The first paper to report an increase in bone mass achievable by daily injections of PTH was published during studies of the use of newly available PTH in the therapy of lead intoxication (Bauer et al. 1929) (See Table 3). Because excess PTH in humans due to parathyroid tumors was known to cause severe bone loss, the results in rats appeared to be an anomaly not pertinent to human medicine. The observation made by Bauer et al. was, however, confirmed by Selye after several years (Selye 1932). After a lag of 40 years, interest in the topic resurfaced. The late pharmacologist, John Parsons, was one of
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<tr>
<td>1929</td>
<td>MGH: Bauer, Aub, Albright (Bauer et al. 1929) PTH _ bone mass in rats</td>
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<tr>
<td>1932</td>
<td>Confirmed by Selye (1932) After these reports, no further studies for 40 yrs</td>
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<tr>
<td>1965-72</td>
<td>NIH &amp; MGH: isolation, structure, synthesis of PTH provides pure material for clinical study</td>
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<tr>
<td>1975</td>
<td>Rapid improvement in techniques for accurate assessment of bone mass Resumption of clinical interest</td>
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<td>US, England, France: trials with PTH in osteoporotic patients begin</td>
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<td>2001</td>
<td>Striking efficacy found in bone mass and fracture prevention in controlled international study*</td>
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<td>2002</td>
<td>Approved by US FDA for therapy of osteoporosis</td>
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Table 3 History of PTH as an anabolic agent
the major proponents of the potential of PTH for the therapy of osteoporosis, arguing that
the paradox was merely the difference between results with continuous elevation of PTH
(i.e. bone loss) and intermittent, short elevations of PTH (i.e. bone mass increase).
Intermittent high PTH is anabolic for bone, while continuous elevation is catabolic
(Rodan & Martin 2000, Harada & Rodan 2003). The only receptor for PTH is on the
osteoblast; its activation by hormone may prolong osteoblast life and increase its activity,
leading to bone formation. The signaling by several intermediate messengers from
osteoblast to osteoclast to stimulate the latter and resorb bone occurs less rapidly than the
initial direct stimulation of the osteoblast. Hence, elevations that are transient may
stimulate the osteoblast anabolic activity while not yet triggering the coupled catabolic
response through osteoclasts. Timing is critical in the administration of PTH. An
important study by Dobnig and Turner (1997) in test animals with controlled rates of
administration of hormone showed that less than two hours of exposure to PTH elicited
the anabolic response and longer than two hours the catabolic response. The exact
cellular mechanisms remains unsettled by which the favorable anabolic response occurs
with short exposure to increased PTH levels.
The definitive clinical trial in humans involved over 1600 postmenopausal women with
prior vertebral fractures. PTH strikingly reduced 70% fracture incidence compared with
the placebo group (Neer et al. 2001). New nonvertebral fractures, fragility fractures, were
also reduced by approximately 50%. This therapeutic efficacy led the US Food and Drug
Administration to approve PTH for use in osteoporosis even though in extended
toxicology studies by the sponsor (lifelong exposure to PTH in a cancer-prone rat strain)
osteosarcomas developed near the end of the lifetime of the animals. Since the
therapeutic use of PTH is limited to two years, and with other reassuring evidence, the osteosarcomas in rats were deemed not relevant for PTH use in humans (Neer et al. 2001). This report was the culmination of several decades of investigator-initiated trials, the first major one was reported by Reeve et al. in 1980, which had shown striking benefits in bone mass increase. These trials were of insufficient size, however, to estimate fracture incidence (Reeve et al. 1980). The beneficial effects of PTH are primarily in trabecular bone, but this improvement in trabecular bone translates to improved bone strength, particularly in the vertebrae but also in the hip. There does not seem to be much improvement in cortical bone – for example, at the wrist – but in vertebral bodies cortical bone is increased, as well as trabecular bone itself (Dempster. 2001).

Recent experimental investigations, including animal and human studies, showed that rhPTH (1—34) was significantly effective in the treatment of osteoporosis and prevention of bone fracture coupled with remarkably low toxicity. Daily injections of low dose rhPTH (1—34) can prevent bone loss and stimulate new bone formation in patients (Finkelstein et al. 1994, 1998). It was shown to preserve or increase bone mineral density (BMD), bone mass and strength, maintain bone quality and reduce the fracture risk in humans and various animal species, including osteoporotic women (Lindsay et al. 1997) men (Kurland, 2000; Orwoll, 2003), children (Koch, 2001), monkeys (Jerome, 2001; Turner, 2001), greyhounds (Podbesek, 1983), rabbits (Hirano, 1999; Mashiba, 2001), rats (Ejersted, 1995; Sato, 1997) and mice (Alexander, 2001; Zhou, 2003). Based on the results of preclinical and clinical trials, ForteoTM (Teriparatide), a recombinant human PTH (1—34) product, was approved by the U.S. Food and Drug Administration in December 2002 as the first anabolic agent that actually stimulates new bone formation.
(Quattrocchi and Kourlas, 2004). It is approved for the treatment of postmenopausal osteoporosis in women as well as primary or hypogonadal osteoporosis in men who are at high risk for fracture.

Major developments have occurred in bone biology in recent years. Research has defined the cellular lineages involved in bone formation and bone resorption (the osteoblasts and marrow stromal cells, the osteocytes, and osteoclasts with their precursor cell types), and the method of intercommunication between bone cells. Many new potential targets for stimulating bone formation are being recognized. The hope is that eventually all of the current agents, including the antiresorptives and even PTH, will be surpassed in convenience and effectiveness by other compounds, preferably orally acting, that are superior types of anabolic agents for bone. Such developments seem predictable, based on new advances in bone research and the intense interest of biotechnology and pharmaceutical firms in this field's potential.

The current therapeutic PTH for osteoporosis treatment is costly as the current cloning techniques involved in the preparation of PTH are laborious and yields low amount of PTH.

The true potential of drugs can only be realized if we are able to produce them in a cost saving strategies. This remains a cardinal principle in the scientific circles because the fruits of biotechnological revolution can only be completely realized when they are in the reach of common man.
The objective of my thesis work is to generate an efficient protocol to generate biologically active PTH in an economical way with high purity and functional activity. The final goal is to produce recombinant PTH in an industrial scale with high purity and quality so that its therapeutic touch will be in reach of everyone.

Aims and objectives:

The present work has been carried out with the following objectives.

- To construct a recombinant hPTH (1-34) plasmid with high levels of expression
- To optimize the purification conditions for the lab scale production of hPTH
- To optimize the fermentation and purification conditions for the pilot scale production of hPTH.
- Characterization of the purified recombinant hPTH.
- To check the biological activity of the purified hPTH
- Safety evaluation of recombinant human para thyroid hormone (1-34) [rhPTH (1-34)] in Rabbits and Rats.