The History: From Ivar Sandström to the Sequence of Parathyroid Hormone

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1.1 Introduction

The history of the discovery of the parathyroid glands (Table 1.1) and the evolution of knowledge about parathyroid hormone (PTH) are rewarding for the insights it provides into the basic physiology and pathophysiology of bone and mineral metabolism and the diseases hypoparathyroidism and hyperparathyroidism. The early history illustrates the surprising contradictions and confusion that can occur in scientific investigations along the pathway to a clearer understanding of biology and disease. This chapter will focus on hypoparathyroidism consistent with the theme of this volume.

1.2 Discovery of the Glands

Credit for the discovery of the parathyroid glands clearly belongs to Ivar Sandström (1852–1889), who found this new organ in the dog in 1877 when he was a medical student in Uppsala, Sweden. Later, as a temporary research assistant in the Department of Anatomy, he systematically and thoroughly investigated these glands further and identified this novel organ also in cats, oxen, horses, rabbits, and finally humans. He examined 50 corpses and found all four glands in most of them. Although the relationship to the thyroid gland was unsolved, it was Sandström who gave them the name "glandulae parathyreoideae," parathyroid glands.

> Although both of the aforementioned kinds of glands [the parathyroids versus accessory thyroid glands] could with equal reasons claim the name of accessory thyroid glands, a special name seems to be required for those which are the subject of this paper [the parathyroids], both with regard to the essentially different structure and on account of the fact that this kind of gland [the parathyroids] is constant in its occurrence [referring to his careful work in dogs, cats, horses, and rabbits as well as 50 human postmortem subjects] while the other one [accessory thyroids] is extremely variable. I therefore suggest the use of the name *Glandulae parathyreoideae*; a name in which the characteristic of being bye-glands to the thyroid is expressed. [1, 2]

He described in great detail the variable size, form, and color of the four glands found in humans, their vascular supply, and their microscopic appearance. He had, of course, no knowledge as to the function of this new organ. In a style that is somewhat different from today's rigid scientific language, he writes

> Concerning the physiological importance of these glands for the organism, we are not able, from reasons that are quite apparent, to allow ourselves even to make a guess. [1, 2]

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M.L. Brandi, E.M. Brown (eds.), *Hypoparathyroidism*,
DOI 10.1007/978-88-470-5376-2_1, © Springer-Verlag Italia 2015

Table 1.1 Short history of parathyroid hormone

1880	*Sandström* identifies parathyroids in humans
1900	Parathyroids first recognized as functionally distinct from thyroid (*Vassale and Generali*) Acceptance of evidence mixed
1910–1925	Vital role established: removal causes tetany Function debated over control of calcium vs. detoxifying function (guanidine toxicity)
1925	Endocrine function established by *Collip* Parathyroid extracts reverse tetany
1929–1952	*Albright* describes idiopathic hypoparathyroidism, pseudohypoparathyroidism (PHP), and pseudo-PHP
1959	*Era of chemical biology begins* *Aurbach* isolates and purifies PTH intact by use of organic solvents
1971–1975	Structure and synthesis of PTH Definitive research and clinical uses
1987	Clinically useful immunoassays of PTH Laboratory diagnosis of hypoparathyroidism possible
1990	*Full impact of molecular biology unfolds* Receptor cloned Genetic defects in hypoparathyroidism defined Genetic manipulations define PTH function in vivo in rodents

The inability to publish his work in a major medical journal was a great disappointment to him and prevented his international recognition. In 1880, he published his discovery "On a New Gland in Man and Several Mammals" in a local Swedish journal [1, 2]. It turned out to be his only scientific publication. Although Sandström received two local Swedish prizes for his discovery, international recognition was not achieved before his death and not until decades later. His personal life story was a tragic one. He had apparently inherited a propensity to depression and although he continued his studies and even finished his medical degree in 1887, he was hospitalized several times during that period. He frequently expressed disappointment at his lack of recognition and failure to be permitted further opportunity to work as an investigator. He shot and killed himself in 1889 at the age of 37.

The glands were also found by others before him, but had not been carefully and systematically examined. Sir Richard Owen probably first recognized them around 1852 in the Indian rhinoceros [3]. His publication, which was in all likelihood not accessible to Ivar Sandström, describes the glands in one single sentence:

> A small compact yellow glandular body was attached to the thyroid at the point where the veins emerge. [3]

The systematic description of the parathyroids in humans and in several other species and therefore their potential significance for human physiology and disease clearly begin with Sandström. The entertaining and highly readable monograph by Jörgen Nordenström [4] recalls the early history and explains the unusual circumstances that led to a postmortem examination of the rhinoceros in the nineteenth century. More tellingly, it chronicles the tragic story of Sandström who never received full credit for the significance of his painstaking work.

1.3 Clarification of the Separate Anatomy of the Parathyroids

Two lines of observation, one based on clinical experience and the other on animal experiments, led, but surprisingly quite slowly, to the identification of the key role played by the parathyroids. Clinical reports discussed the poorly understood complex set of symptoms that we now recognize as hypocalcemic tetany in patients operated on

for thyroid disease after extensive (probably often complete) thyroidectomy. Mortality from thyroidectomy was so high (as great as 40 %) that the famous Austrian surgical pioneer Theodor Billroth (1829–1894) stopped thyroid surgery for several years. In some cases, tetany was the cause of death. Nordenström in his monograph provides some of these dramatic examples of patients who developed tetany and sometimes death after thyroidectomy [4]. The physicians were at a loss to understand, let alone treat, patients in whom tetany developed. He notes that, because of the typical spasm of the hands that can be seen in shoemakers, the condition came to be called Schusterkrampf (shoemaker's cramp).

There were many false steps, and constant controversy, on the way to a full appreciation of the role of the parathyroids, but not until the early part of the twentieth century was it recognized that it was inadvertent removal of the parathyroids and not the thyroid that caused tetany in patients undergoing thyroidectomy (see below). Even after others eventually duly noted the work of Sandström, it was not clear that the parathyroids were a separate organ system rather than embryonic thyroid glands and/or an accessory part of the thyroid. This confusion in animal experiments in part arises from the presence of multiple glands as well as their variable location particularly what we now term the inferior parathyroids, which may be intrathyroidal.

In a comprehensive and scholarly review of the experimental studies, Boothby [5] observes (but in retrospect not entirely correctly) that Sandström believed that the glands were likely accessory thyroid tissue. Baber [6] in 1881 clearly described these glands under the name "undeveloped portions of the thyroid." (Baber was apparently not aware of Sandström's earlier paper). Horsley [7] in 1886 correctly deduced as a result of careful experimentation that these tissues recognized by Sandström and Baber were not undeveloped tissue of the thyroid, but separate organs. Horsley demonstrated that after partial thyroidectomy, while the "undeveloped tissue" (the parathyroids) did not show any enlargement or conversion to thyroid tissue, the remaining thyroid tissue did immediately hypertrophy.

In 1891, Gley reported his findings that experimental thyroidectomy in animals often resulted in tetany [8, 9]. However, he incorrectly deduced that the external parathyroids were indeed embryonic thyroid tissue. He arrived at this deduction because he correctly noted that tetany did not develop if these external parathyroids were spared, but he noted that these glands doubled in size after removal of the thyroid. Therefore, he promulgated the view that they were then taking on the function of the thyroid without realizing, of course, that the internal parathyroids had been removed with the bulk of the thyroid tissue, and this led to hyperplasia of the remaining parathyroids. He persisted in this view despite the observations of Horsley.

It was the work of Vassale and Generali published in 1896 that disagreed with Gley's contention that the glands were embryonic thyroid rests and established that they were special organs distinct from the thyroid. In a series of papers resulting from careful work [10, 11], they demonstrated that removal of all four parathyroid glands caused tetany even if significant amounts of thyroid tissue were preserved, whereas total thyroidectomy did not cause tetany if at least one parathyroid gland was spared.

Still, others as cited by Boothby contributed to the work that finally established the glands as essential to prevent tetany. Especially notable was the work of the great Viennese pathologist Jakob Erdheim (1874–1937) in 1904–1906. Through postmortem observations in patients who died of tetany after thyroidectomy, Erdheim established with painstaking care that the parathyroids were totally absent. Erdheim also undertook experimental studies in rats, which normally have only two parathyroid glands, which are readily visible. Using cautery, Erdheim was able to destroy various portions of these glands without damaging the thyroid. Complete removal of all parathyroid tissue with preservation of the thyroid resulted in tetany similar to the earlier work of Vassale and Generali.

Erdheim even provided what could have been an early clue to the role of the glands in calcium metabolism through his observations in his

parathyroidectomized animals. He demonstrated that the tooth discoloration that developed in some of the surviving rats (teeth constantly grow in all rats) was due to the sudden cessation of calcium deposition coincident with their loss of the parathyroid glands (hypoparathyroid state) and subsequent low blood calcium levels [12].

1.4 Physiological Role of the Glands

Intense debate, surprising in retrospect, centered on the cause of the tetany and the role of these vital glands. Although the true explanation, severe hypocalcemia, was carefully documented by a number of investigators, others concluded that the principal function of the glands was detoxification. One of the main reasons the detoxification theory could survive so long was because attempts to treat animals with extracts of the parathyroid glands was not effective in reversing the tetany. We can now appreciate that obtaining parathyroid hormone from parathyroid extracts was unsuccessful at the time.

William MacCallum and his coworkers beginning in 1908 were strong proponents of the view that the parathyroid glands were somehow involved in control of blood calcium. In 1909, MacCallum and his colleague Carl Voegtlin were able to demonstrate that infusions of calcium completely reversed the symptoms of cramps that dogs suffered after removal of their parathyroids [13]. They also measured blood calcium levels and reported that they were lower than normal in parathyroidectomized dogs. They concluded

> Tetany occurs spontaneously in many forms and may also be produced by the destruction of the parathyroid glands… The injection of a solution of a salt of calcium into the circulation of an animal in tetany promptly checks all the symptoms and restores he animal to an apparently normal condition. [13]

However, they were unable to reverse the tetany by administration of extract of the glands. Others also demonstrated that calcium would reverse the tetany in experimental animals after parathyroidectomy [14]. Confusion developed, however, when Koch stated in 1912 that there were high levels of methyl guanidine found in the urine of animals with tetany after parathyroidectomy [15, 16]. A few years later, Paton demonstrated that administration of guanidine or methyl guanidine could apparently cause symptoms characteristic of tetany in rats [17].

In closing his very scholarly review summarizing the field as of 1921, Boothby concluded:

- Removal of all parathyroid tissue in animals causes tetany and death; the younger the animals, the worse the problem. (Some noted that herbivores were more resistant.)
- Preservation of small amounts of parathyroid tissue prevents or greatly minimizes the tetany.
- The parathyroids have a function separate from that of the thyroid – their only relationship is an anatomic proximity.
- Their function remains unclear. It seems to be concerned with calcium metabolism or guanidine metabolism or both. Nonetheless, administration of large amounts of calcium is usually of benefit in lessening the symptoms in patients suffering tetany after thyroid surgery.
- Reported cases of idiopathic tetany are not necessarily related to the parathyroids, and the association of tetany with the function of the parathyroids is only firm in humans after extensive thyroid surgery.

It is evident that these early workers were imaginative investigators who learned much with what today might be considered rudimentary tools. Sometimes progress was stalled for years. Investigators, as well as clinicians, were not often aware of some innovative findings lacking the rapid access to medical information available today. Sometimes, innovative findings that did not fit into the paradigms of the day were rejected or ignored.

1.5 The Parathyroids Are Endocrine Glands

A definitive series of experiments by James Collip (1892–1965) in 1925 resolved the controversy about the function of the glands [18]. Collip prepared hot hydrochloric acid extracts of the

parathyroid glands; an approach that he correctly hypothesized was needed to free the active substance from other stromal components of the gland and to render it soluble. He showed that these acid extracts of the parathyroid gland would completely relieve the tetany that followed parathyroidectomy in experimental animals and in humans [19]. Thereby, he established that the parathyroids are endocrine glands that secreted a hormone, PTH. Another author, Adolph Hansen, reported a similar acid extraction procedure in 1924 [20, 21] and claimed priority for the discovery although his efforts to demonstrate biological actions with his extract were at best inconsistent, so the bulk of the credit belongs to Collip in the opinion of the present authors.

The availability of biologically active extracts of parathyroid hormone made available by pharmaceutical firms such as Lilly immediately attracted the interest of clinical investigators, who administered the preparations in clinical investigation in patients to better understand the etiology and pathophysiology of such conditions as idiopathic tetany. Prior to the availability of active preparations of PTH, the state of knowledge in the field was as summarized above by Boothby [5], namely, that it was unproven whether idiopathic tetany could be due to a failure of the parathyroid glands. Leading clinical investigators in several institutions, most notably Fuller Albright (1900–1969) and his colleagues in the endocrine group at Massachusetts General Hospital (MGH) used these clinically available preparations (termed parathormone) to reverse hypocalcemia in hypoparathyroidism. (Beyond the scope of this chapter is the use of these preparations that led clinical investigators to discover the first patient with overactivity of the parathyroids in the United States). Administration of these PTH preparations to patients with idiopathic hypoparathyroidism confirmed the diagnosis by the demonstration of prompt phosphaturia achieved with what was then termed the Ellsworth-Howard test [22]. The brilliant observation of Albright and colleagues led further to the identification of a form of hormone resistance to parathyroid hormone as the cause in some patients with apparent hypoparathyroidism. The investigators demonstrated a failure of the extracts to promote phosphaturia in certain patients with additional striking phenotypic features, later termed Albright's osteodystrophy, leading them to clarify the entity of pseudohypoparathyroidism (PHP) [23] and later (foreshadowing the delineation of the role of gene imprinting in hereditary disorders many years later) the entity of pseudo-pseudohypoparathyroidism [24]. Their remarkable foresight obtained on clinical grounds alone linked the two diseases with similar phenotypic features, the former PHP with hormone resistance and the latter pseudo-PHP devoid of hormone resistance per se.

The successful extraction of PTH from the glands created problems that blocked further progress toward fully characterizing the structure of parathyroid hormone. When techniques for protein structural analysis became available (following the seminal work of Sanger who determined the structure of insulin [25]), there was interest in applying the techniques to parathyroid hormone. The Collip hot acid extraction method had an undesired side effect (as we understand the issue in retrospect). The hormonal peptide was not only liberated and solubilized, but also cleaved at multiple sites (most likely at asparagine or aspartate acid sites within the sequence) giving a multiplicity of peptides of varying length with a low yield of any one. In a 1954 report, for example, Handler et al. [26] summarized their frustration at the inability to use the techniques then available for purification. They stated

> 1) the active material in the gland… may be a large protein which in the course of isolation is degraded into fractions of varying size each of which still has activity, or 2) the active material may not be a large molecule at all, but instead a small molecule which adheres to each one of the fractions. [26]

The problem was compounded because the method of monitoring purification, the bioassay, was itself difficult. The assay in use at that time involved injections of purified preparations into parathyroidectomized rats to raise the blood calcium concentration. The precision of the technique was much less than that of an enzymatic assay. The field remained stalled until a breakthrough development in 1959.

1.6 Era of Chemical Biology

In 1959, Aurbach reported a new technique that solved the problem and resulted in purification of the intact, native polypeptide [27]. By using organic solvents like hot acid, he liberated the peptide in an active form but without producing multiple cleavage products. Later Rasmussen and Craig confirmed his results using an analogous technique [28].

With continued advances in protein sequencing techniques, which became available in the late 1950s and early 1960s, two independent groups determined the structure of PTH, first of bovine hormone, in 1970 [29, 30]. Accumulation of sufficient amounts of parathyroid tissue was possible using cows and other large animal species used for meat consumption by scientists working with slaughterhouses and the meat production industry. Only several years later, after laborious accumulation of sufficient material from human parathyroid glands that were available as the byproduct of surgically removed parathyroid tumors, could the structure of human hormone itself be approached and ultimately completely solved by 1978 [31].

It was hypothesized that a molecule comprising the first 34 residues might be sufficiently long to be biologically active. This somewhat arbitrarily chosen peptide length was based on the deduced amino acid sequence of PTH, on the reports that hot acid produced active fragments, and on considerations of peptide synthesis techniques then available. Successful reports of full biological activity of PTH(1–34), first for the bovine hormone in 1971 [32] and then later the human in 1974 [33], confirmed that the structure of the compound had been accurately deduced and even more importantly provided a material for definitive animal and clinical use.

Availability of highly purified parathyroid hormone and active synthetic fragments made it also possible to develop improved immunoassays based on the principles clarified by Ekins in 1980 [34]. He championed the use of double antibody methods or so-called sandwich assays. Much of the circulating parathyroid hormones are fragments, most of them biologically inactive [35]. These fragments were often detected in the earlier radioimmunoassay techniques. Overall, as noted in earlier reviews [36], this caused a lack of precision in the results with these earlier assay techniques (see also Chap. 4). The introduction of an effective double antibody assay in 1987 [37] greatly improved the detection capacity of the assays such that the low levels of PTH seen in patients with hypoparathyroidism could be readily distinguished from normal levels making it possible to accurately confirm by laboratory techniques the presence of hypoparathyroidism. The even greater advance in this instance (but beyond the scope of this chapter) was to greatly improve the capacity of the assays to discriminate between the diagnosis of primary hyperparathyroidism (elevated levels of PTH) and hypercalcemia of malignancy (low levels of PTH) (see also chapter 4).

1.7 Era of Molecular Biology

The wide availability of the powerful techniques of molecular biology accelerated progress leading to the successful cloning of the receptor for the hormone in 1991 [38] (see Chap. 9). Parallel advances in cell biology from many fields provided improved techniques that permitted a much clearer delineation of critical steps in hormone action in target cells (especially in bone and kidney) using the cloned receptor and synthesized fragments of PTH and introduced the current era of the molecular biology of parathyroid hormone [39].

As will be reviewed in Chaps. 16, 17, 18, 19, 20, and 21, the powerful techniques of molecular biology have aided in characterizing the many genetic defects responsible for hypoparathyroidism [40]. They include, but are not limited to, the rare loss-of-function mutations in the PTH gene itself or in transcriptions factors key to the development of the parathyroid glands (such as GCM2 and GATA3) and mutations in the AIRE gene leading to inherited forms of autoimmune hypoparathyroidism (APECED). The importance of the molecular diagnosis for patient care is illustrated by the autosomal-dominant hypocalcemia

(ADH), common among the inherited forms of hypoparathyroidism, which is caused by activating mutations in the calcium-sensing receptor. Patients with ADH are particularly prone to hypercalciuria and nephrocalcinosis, therefore rendering the molecular diagnosis important for the treating physician. Mutations in the gene encoding the guanine-binding protein G11 have recently been identified as a cause of hypoparathyroidism [41, 42], demonstrating the power of genetics in shedding light on important signaling pathways in the parathyroid glands. Molecular biology also clarified the mechanisms of resistance to PTH in pseudohypoparathyroidism. Mutations in *GNAS*, the gene encoding the alpha subunit of Gs, or methylation changes at the GNAS locus are responsible for this imprinted disorder (see Chaps. 10, 32, 33, 34, and 35). In addition, the greater understanding of the molecular actions of parathyroid hormone has led to such advances as a long-acting form of parathyroid hormone termed LA-PTH [43] which has potential as a hormone replacement therapy for hypoparathyroidism, one of the few endocrine deficiency states heretofore not treated by replacement with the missing hormone. Clinical investigators have successfully demonstrated that treatment with PTH(1–34) and PTH(1–84) is a possible therapy for patients with hypoparathyroidism (see Chaps. 30 and 31). Recently, the first randomized, placebo-controlled phase 3 clinical trial using human recombinant PTH(1–84) was successfully completed [44]. PTH replacement therapy for hypoparathyroidism, which addresses the underlying defect, could therefore become a practical reality in the not too distant future.

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