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John T. Potts Jr.

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John T. Potts, Jr, MD*

Ed. Note - This overview was originally presented at the International Symposium on Clinical Disorders of Bone and Mineral Metabolism, May 9-13, 1983. The following list indicates the presentations given in this session at the Symposium and the contents of the corresponding chapter in the Proceedings of the Symposium published by Excerpta Medica. The numbers in parentheses refer to pages in this volume. Complete information about the contents of the Proceedings can be found at the back of this issue.

Chemical bioassay for parathyroid hormone: Validation and clinical applications. D. Goltzman, H. Gomolin, M. Wexler and J.L. Meakins (6)

Clinical utility of 'mid' and 'carboxyl' region specific assays of parathyroid hormone. C.G. Arnaud and L.A. Zitzner (10)

Amino-terminal radioimmunoassays for human parathyroid hormone. G.V. Segre (14)

The contribution of synthetic human parathyroid hormone of its fragments to parathyroid hormone measurements. F.A. Tschopp, M.A. Dambacher, and J.A. Fischer (18)

Comparison of amino- and carboxyl-terminal assay methods. E. Slatopolsky (20)

Problems seen today in interpretation of the radioimmunoassay for parathyroid hormone were not understood when Berson, Yalow, Aurbach, and I developed the first radioimmunoassay for the hormone in 1963, using bovine parathyroid hormone which Aurbach had purified. In the last two decades, intensive basic research has been carried out on parathyroid hormone, including structural analysis and chemical synthesis of the hormone from human and other mammalian species. It has become apparent that the biosynthesis and the metabolism of the hormone are more complex than was evident in 1963. Multiple fragments and possibly even precursors of the hormone are present in blood of humans and animals. As a result of these discoveries, attempts have increased to produce immunoassays that measure specific forms of the hormone of greatest clinical significance.

In 1970, Arnaud and his colleagues first introduced C-terminal assays, based on the bovine sequence, when the large concentrations of circulating carboxyl fragments were recognized. Since then there has been a gradual movement toward the use of human hormone. Mallette, Marx, and their colleagues recently showed the value of antisera that recognize the middle region of the human molecule. In 1983, Segre and his colleagues introduced a very sensitive amino-terminal immunoassay developed by immunization with the synthetic human 1-34 fragment.

Many questions about parathyroid hormone remain to be answered.

- What assays should be used in different diseases associated with abnormal function of the parathyroid?
- What is the biological and pathophysiological meaning of the heterogeneity of circulating hormone that accounts for the multiple circulating fragments?
- How can we use the assays in the most cost-effective manner when we evaluate patients with hypercalcemia and hypocalcemia?
- What assays will be most useful in the future?

*Department of Medicine, Harvard Medical School and Massachusetts General Hospital, Boston

Address reprint requests to Dr. Potts, Department of Medicine, Massachusetts General Hospital, 32 Fruit Street, Boston, MA 02114.

Parathyroid hormone is composed of 84 amino acids in human, porcine, bovine and rat species; the structures are nonidentical but closely homologous. Biological activity resides in the amino-terminal portion of the hormone (residue 2-25). Once it was understood that fragmentation of the hormone occurs both within the gland and in the periphery, due to several as yet uncharacterized enzymes at several sites, obvious questions were raised about what constitutes the active molecular species. It is clear that if an amino-terminal fragment was formed, it could be biologically active.

We do not know whether the fragments entering the blood from the gland and those produced by peripheral metabolism are the same, or whether the same enzymes are responsible in each site. Some groups (our own, for example) have concentrated on peripheral metabolism, and others have concentrated on fragmentation within the gland. These studies all have methodological difficulties, including problems with artifacts. There is need to develop special techniques to examine the products produced by glandular or peripheral metabolism. Although progress has been slow, the issues raised are intriguing.

The entire molecule of 84 amino acids is still, by conservative estimates, the most likely candidate to interact with receptors in bone and kidney, the two known principal target organs. It is also possible, however, that a biologically active amino-terminal fragment either leaves the site of cleavage and enters the blood, or is produced by cleavage at target sites to serve as an alternate or even sole active molecular species, as Slatopolsky's work suggested (pp. 20 ff.). We have to understand the metabolism of the hormone not only to know what we are measuring, but also to define (and measure) all molecules that are candidates for significant biological activity.

Fragments produced include a large fragment of 40 to 50 amino acids, extending from region 30 to 40 to the carboxyl-terminus (usually referred to as C-fragments) and an amino-terminal fragment. Carboxyl fragments disappear from blood more slowly than the intact hormone (which is taken up by tissues and cleaved); hence there is much more carboxyl fragment than intact molecule in blood. Although several groups have detected an amino-terminal fragment(s) in the blood, there is general agreement that, if present, its concentration is very low compared to other hormonal forms. The clearance half-time of such N-fragments must be very rapid. At steady state, at least three basic molecular species are present: the intact hormone, carboxyl fragment(s), and possibly amino-terminal fragment(s).

Fragments arising from the gland may differ from those that reenter the blood from peripheral sites. Also, smaller fragments may be formed from the initial cleavage products. No one today knows how many circulating fragments exist. As knowledge increases, various assays can be used more effectively to help discriminate between primary hyperparathyroidism and tumor hypercalcemia and other related clinical issues.

Arnaud and Zitzner (pp. 10 ff.) summarized their experience with assays that measure the biologically inactive molecules (carboxyl and/or mid-region fragments). Because of the high concentration of these fragments, the inert fragments have been measurable with many more antisera. The contributions by Tschopp, et al (pp. 18 ff.) and by Slatapolsky summarized the issue of the relative sensitivity and detection limits required for assays that measure only the amino-terminal region of the molecule. Arnaud and Slatapolsky discussed the utility of assays that use antisera based on the middle region (M) or carboxyl region (C) of the molecule. They emphasized their clinical utility as an "integrative" index of normal or abnormal glandular activity both in primary hyperparathyroidism and the secondary hyperparathyroidism of renal failure. The limitation of such M-assays or C-assays to measure rapid changes in glandular secretory activity was also discussed.

It is not yet clear that any single assay, however desirable, can be used effectively in all clinical disease states where parathyroid status is to be assessed. Segre (pp. 14 ff.) presented evidence for the possible wide utility of amino-terminal assays using an antiserum with the very high affinity constant needed to detect only the intact molecule (by an exclusive amino-terminal recognition site that does not detect inert fragments).

In most instances, endocrinologists apply suppression tests when overactivity is suspected and stimulation tests when underactivity is suspected. Until recently, such approaches have not been practical with parathyroid hormone because of problems with the heterogeneity of circulating hormone and varying clearance half-times of the material produced peripherally.

Segre suggested that stimulation and suppression tests are now practical. His reports of the stimulation and suppression effect of varying serum calcium concentration in normal subjects and those with primary hyperparathyroidism confirmed in vivo (excised glands) the findings observed in vitro (primary cell cultures) by Habener and Brown that a set point defect in primary hyperparathyroidism exists.