Historical Note: Many Steps Led to the ‘Discovery’ of Thyroid-Stimulating Hormone

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Key Words

Thyroid-stimulating hormone · Discovery · History of science

Abstract

Finding thyroid-stimulating hormone was a process rather than a circumscribed event, and many talented persons participated over many years. Key early participants were Bennett M. Allen and Philip E. Smith who had the misfortune just prior to World War I of independently and simultaneously starting very similar experiments with tadpoles. This led to a series of back and forth publications attempting to establish priority for finding evidence of a thyrotropic factor in the anterior pituitary. Decades of work by others would be required before sophisticated biochemical techniques would bring us to our modern understanding.

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Introduction

Who discovered insulin? Penicillin? These questions have answers although they involve caveats and stories rather than one-word responses. So, who discovered thy-

roid-stimulating hormone (TSH)? In fact, the answer includes many stories and many names. This short paper is intended simply to present some little-known facts about the early discernment that there existed a thyrotropic factor in the pituitary, with emphasis on the activities of two biologists in the early 20th century. This is not intended as a comprehensive review but instead highlights that our thyroid field has stories of human interest and illustrates that finding TSH was a process rather than a circumscribed event.

Development of Knowledge of TSH: Period 1

Table 1 lists some key events that gradually led to a fuller understanding that there existed a pituitary factor that stimulated thyroid tissue, followed by the isolation and characterization of that factor. The development of knowledge of TSH occurred in three periods. First, there was general recognition that the pituitary contained a thyrotropic substance. Two young biologists (Bennett M. Allen of the University of Kansas, and Philip E. Smith of Cornell University and the University of California, Berkeley) independently used tadpoles as inexpensive model systems since it was known (in large part due to Allen) that the thyroid was necessary for the tadpole to go

Table 1. Some names and events in TSH history

Niépcé [21] (1851): observed at autopsy that the pituitary of a cretin was grossly enlarged.
Cushing's group [22] (1910): noted thyroid atrophy after some pituitary surgeries.
Adler [23] (1914): preliminary studies of hypophysectomy of mammals and tadpoles causing thyroid atrophy.
Allen [1] (1916) and Smith [2] (1916): careful experiments using tadpoles proved that a thyrotropic substance existed and was present in the anterior portion of the pituitary.
Aron [24] (1929) and Loeb and Bassett [25] (1929): developed bioassay of pituitary gland extracts in guinea pigs.
Jansen and Loesser [26] (1931): first partially purified a preparation of TSH (still with critical contaminants, such as LH).
Greep [27] (1935), White and Ciereszko [28] (1941), and Bates et al. [29] (1959): many methods used to purify TSH activity by 50–300 fold.
Hoskins [30] (1949): concept of 'feedback' in the 'thyroid-pituitary axis'.
Heideman [31] (1953) and Crigler and Waugh [32] (1955): improved purification of TSH by ion-exchange chromatography. In the 1950s and 1960s, attempts to sequence the peptide of TSH provided confusing data.
Condliffe and Bates [33, 34] (1957): much improved ion exchange chromatography. TSH was found to be retained on diethylaminoethyl cellulose (DEAE cellulose) only at high pH and low ionic strength, while LH was not absorbed.
Papkoff and Samy [35] (1967): recognized that ovine LH had two nonidentical subunits.
Liao and Pierce [36] (1970): recognized the subunit nature of TSH and described a common subunit in bTSH and bLH.
Liao and Pierce [37] (1971): determined the peptide sequences of bTSH α - and β -subunits.
Fiddes and Goodman [38] (1981): cloned the common α -subunit of the human glycoprotein hormones, while Wondisford et al. [11] (1988) and (independently) Guidon et al. [12] (1988) and Tatsumi et al. [13] (1988) cloned the β -subunit of hTSH.
Chin et al. [39], Ridgway, Shupnick (1980s), and others studied the transcriptional regulation of the subunits.
Weintraub, Magner [40] and Szkudlinski and others [41] (1980s and 1990s) studied the oligosaccharides of TSH and structure-function correlates.

through metamorphosis into a frog. Larval amphibians survived 'small cut and needle scoop surgeries' (my term) better than did young mammals. In addition, using a microscope, anterior pituitaries of larval amphibians could be removed relatively precisely during a certain anatomically favorable stage of larval development; then one could watch for a subsequent biological effect on the thyroid follicle structure. Both Allen and Smith were doing such experiments. In July 1916, Allen was traveling through Berkeley on his way to a scientific meeting to be held in San Diego, and he met Smith. To their mutual consternation, they were both scheduled to present similar data at the Western Society of Naturalists on August 9–12, 1916! They both wrote summaries of their presentations at that August meeting which only appeared in print several months later in the *Anatomical Record* [1, 2]. Both had noted that many of the hypophysectomized tadpoles developed thyroid follicular changes with less colloid, the tadpoles grew slowly, the legs did not enlarge,

and the dark pigment granules in the skin shrank so that the tadpoles took on a creamy silver color that was quite striking (fig. 1) – we now know that the color change was probably due to the absence of MSH. While the creative and insightful Bennet Allen was taking the long, slow train ride back to Lawrence, Kansas, the ambitious and talented young Smith [3], not wanting to be scooped, was able to return rapidly to Berkeley and wrote a 3-page article that was quickly published in the August 25, 1916, issue of *Science*, only 13 days after the close of the San Diego conference. In that paper, Smith inserted a sentence stating that he had first attempted such experiments in 1914. Allen [4] was necessarily later in submitting his results to *Science*, and his article appeared in the November 24, 1916, issue.

Both Smith and Allen were smart, skilled, and ambitious, and this collision of careers was unfortunate. It is painful today to read a paragraph inserted by Allen into his November 1916 article, as follows:

This phase of my work was duplicated by Dr. P.E. Smith, who published a preliminary account of his work in the August 25, 1916, number of *Science*. During the month of July prior to this time I had the pleasure of discussing my work with Dr. Smith at Berkeley. Previous to this time I had no knowledge of his work nor of his plans and he assures me that he was equally ignorant of my work. We both presented papers upon our experiments at the meeting of the Western Society of Naturalists at San Diego, August 9 to 12. On June 7, before starting west, I demonstrated specimens and explained my results to a number of scientists, including Dr. Frank R. Lillie, Dr. Emil Goetsch, Dr. Chas H. Swift and a number of others whom I met in Chicago at that time. It is thus clear that these experiments were independently conceived by Dr. Smith and myself and that we worked contemporaneously upon them each without knowledge of the other's work until July, 1916, two months after the experiments had been performed. [4]

Although Smith had published first in *Science* with amazing rapidity, and Smith also included interesting photographs of specimens in his 1916 *Anatomical Record* report, one can tell by the tone of Allen's 1916 *Science* publication that he was not about to fade into the night. Allen [5, 6] published a detailed 14-page summary of his data in the *Biological Bulletin* in 1917, followed by another paper in *Science* in 1920, this time describing how pituitaries from adult *Rana pipiens* could be transplanted under the skin of hypophysectomized tadpoles to partially restore their thyroid follicles. These papers were impressive counterpunches. Smith responded in a 1922 paper that showed that administration of bovine anterior pituitary to hypophysectomized tadpoles could repair and activate their thyroids, and Smith published other papers in the mid-1920s extending the work to mammals. Allen then responded by publishing a book chapter in January 1927 summarizing data about the influence of the hypophysis upon the thyroid gland in amphibian larvae. The passing of years would eventually prove both gentlemen to be superb biologists, keen intellects, and skilled researchers who accidentally started simultaneously on the same path.

Smith was invited to participate in the Harvey Lectures Series of 1930. In the 1931 publication of his lecture, Smith [7] illustrated how extracts from ox pituitaries partially restored the thyroid follicular structure in hypophysectomized tadpoles and how rat pituitaries when administered daily to hypophysectomized rats could partially restore their thyroid follicular structure.

Smith (1884–1970) (fig. 2) was born in De Smet, South Dakota, and earned a BS from Pomona College and a PhD from Cornell. He worked briefly at Berkeley and Stanford before moving to the Department of Anatomy at Columbia University in 1927, where he remained until his retirement in 1952 [8]. He became an expert at performing hypophysectomy in the rat and played a role in the elucidation of several important pituitary hormones. Smith served as president of the Endocrine Society in 1939–1940.

Fig. 1. Tadpoles as published in the *Anatomical Record* by Smith [2] in 1916.

Bennet Allen (1877–1963) (fig. 3) was born in Indiana, attended college at DePauw University, and received a doctorate in zoology from the University of Chicago in 1903. After teaching at the University of Wisconsin, he became professor of zoology at the University of Kansas in 1913 and headed the department. He moved to the nascent Southern Branch of the University of California at Los Angeles in 1922. During his long career there he served as chairman of the Department of Zoology and as a dean. He served as officer in several scientific societies and was a respected research investigator. Even before good research facilities were available, he mentored many students in research projects and developed a loyal following of trainees. Over the years, he pushed for funding better facilities, with huge influence. He 'retired' at age 70 in 1947 but then began a new career in the Atomic Energy Project at UCLA, and he published 26 additional scientific papers, many about amphibians, reptiles, and aspects of radiation exposure (details courtesy of UCLA).

Development of Knowledge of TSH: Periods 2 and 3

The second period of increased understanding about TSH began in 1953 when ion exchange chromatography was applied to prepare much more pure preparations of

Fig. 2. Philip E. Smith, undated.

Fig. 3. Bennet M. Allen, 1899.

Fig. 4. John G. Pierce, undated.

TSH, although LH was still a confounding contaminant [9]. Attempts to perform peptide sequencing were unsuccessful. In the late 1950s the third period of understanding was entered when a much improved ion exchange chromatography technique led to purer preparations of TSH along with recognition that TSH consisted of 2 peptide chains (that were heavily glycosylated).

Dr. John Pierce (fig. 4) and his team at UCLA separated the α - from the β -subunits of bovine TSH, which allowed proper amino acid sequencing. Pierce was known as a brilliant and kind man who shared reagents generously. He was born in 1920 and received his BA, masters, and PhD (1944) degrees from Stanford University in California [10]. He served in the navy during World War II, was a postdoctoral fellow at Stanford and Cornell, served as a faculty member at Cornell for 4 years, and then moved to UCLA in 1952. Pierce served as chairman of biological chemistry from 1979–1984, and at the end of his career he served as associate dean of the School of Medicine. Pierce died in 2006 and is fondly remembered. It was my privilege to have met him in the 1980s.

The work by Pierce and his colleagues led others to find the subunit genes in several species. Analogous to the contemporaneous work of Dr. Smith and Dr. Allen, the sequence of the human TSH β -subunit gene was reported by three groups working independently, all published in 1988 [11–13]. The human TSH subunit gene sequences were essential for the preparation of recombinant human TSH, which is made in large bioreactors using Chinese hamster ovary cells, since the posttranslational glycosylation achieved by eukaryotic cells is important in human TSH.

The Phenomenon of Simultaneous Discovery

I was struck by the example of simultaneous discovery illustrated by Smith and Allen. For context, it is interesting to reflect on other instances. Science is conducted by curious humans who can also be ambitious and competitive. Oftentimes great advances in science have been spurred by this competition, although there can be a darker side. Scientists have long known this, but the general public was not as aware until the publication of the remarkable book *The Double Helix*, by James Watson [14], in 1968. Watson [14] and Crick were substantially aided by helpful colleagues who pointed out the correct molecular configurations (under physiological conditions) of the purine and pyrimidine bases – a lack of this precise chemical knowledge would have severely impaired their model building. Watson [14] and Crick also had a helpful sneak peek at Rosalind Franklin's unpublished X-ray crystallography films, and they were also aware via a draft manuscript that Linus Pauling was about to publish an incorrect 3-chain model of DNA structure, yet they did not contact Pauling to stop him from making this error. Competition probably also spurred progress in the Human Genome Project after Craig Venter decided to attempt to outcompete the huge government program. After the two groups essentially ended in a tie, it took efforts by the president of the USA and scheduling of a White House ceremony in June 2000 to bring the situation to a reasonably peaceful resolution. The president of the USA and the prime minister of France also had to become involved to settle an acrimonious dispute between

Robert Gallo and Luc Montagnier regarding priority of the discovery of the human immunodeficiency virus (HIV). The Nobel Prize in Physiology or Medicine then was awarded to Montagnier and Françoise Barré-Sinoussi in 2008 for the discovery of that virus (and also to Harald zur Hausen for work on the papilloma viruses and cervical cancer). As has been widely discussed by historians interested in these events, Gallo was not recognized by the Nobel committee.

Numerous entertaining and fascinating examples of simultaneous work as well as competitive (mis)adventures abound in the history of science, and some are succinctly summarized in *Great Feuds in Science*, by Hal Hellman [15], and *Prize Fight: the Race and the Rivalry to be the First in Science*, by Morton A. Meyers [16], which has a good discussion of the Gallo-Montagnier controversy as well as other examples.

Within endocrinology there have been several other notable examples of intense rivalry between investigators. Roger Guillemin (born in 1924) of the Salk Institute, La Jolla, California, competed for more than 20 years against Andrzej ‘Andrew’ Schally (born in 1926) of Tulane University, New Orleans, Louisiana, as both teams worked intensely on hypothalamic hormones. In 1977 both men were awarded the Nobel Prize, shared with Rosalind Yalow, for their work on neurohormones, most importantly TRH and GnRH. A New York Times science writer, Nicholas Wade, subsequently prepared a somewhat controversial book about this competition, *The Nobel Duel* [17]. Persons who worked in one of the laboratories report that the book captures well the intense activity required 7 days per week as the groups competed, and is basically factually true and is recommended, although some errors in fact are claimed. In another instance of rivalry, teams of researchers in Belgium and California competed to report the sequence of the TSH receptor. Gilbert Vassart and his colleagues at the Free University of Brussels competed with Basil Rapoport and others in

California, and both groups published key data in 1989 [18–20].

In this article I elaborated on the Allen versus Smith situation. As things turned out, subsequent advances made by many people produced a detailed molecular and physiological understanding of a very important hormone, TSH. One application of that knowledge was the production of recombinant human TSH, to the great benefit of our patients.

In sum, important advances in the knowledge of TSH, as is true in nearly all scientific fields, were the work both of a few talented, insightful individuals and a large number of hard-working, dedicated, and skilled people who over time compiled key information that contributed to the whole. We should all keep in mind these aspects of discovery as we seek to make our contributions. We must be stimulated by healthy competition, but avoid overly competitive or rancorous activities. We must enjoy the human stories as well as the science, and appreciate the privilege of participating in the enterprise of science.

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Disclosure Statement

James Magner is an employee of Genzyme, a Sanofi company, the manufacturer of recombinant human TSH. He is also a shareholder of Sanofi.

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