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REVIEW

The evolution of thyroid function tests

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Thyroid gland disturbances are the second most common endocrine disorder after diabetes mellitus. Given that approximately 200 million people in the world have some form of thyroid disease, it is no wonder that thyroid function tests, i.e. thyrotropin and free thyroxine (T_4), currently account for a significant portion of the routine workload of clinical chemistry laboratories. This was not the case more than 100 years ago.

In the past, anterior swellings of the neck of different sizes were observed to be more common in certain regions of the world. Pliny and Juvenal called it "tumid guttur", meaning a swollen throat, which eventually became goitre or goiter.[1] The earliest mention of treatment for goitre was the use of a burnt sponge and seaweed in 1600 BC by the Chinese. And much later, there is mention of Chinese physicians of the Tang Dynasty (AD 618-907) who successfully treated patients with goitre with the thyroid glands of animals, such as sheep and pigs, in raw, pill or powdered form.[1] In 15 AD, Pliny referred to epidemics of goitre in the Alps, and also mentioned the use of burnt seaweed in the treatment thereof. Ali-ibn-Abbas was the first to discuss surgery as a treatment for goitre in 990 AD. From 1110 AD, there is the first written mention of the association of exophthalmus with goitre in Jurjani's *Treasure of medicine*. Wharton, in 1656, was responsible for naming the gland the thyroid, meaning "shield" in Greek.[2,3] Platter published the first clinical description of cretins found in the Valais region of Switzerland in 1602. In 1789, Fodere suggests that there is an association between goitre and cretinism.[1,2,4] Clinicians only became collectively aware that the thyroid gland was more complex than initially thought. He named it thyroxine (T4).

The isolation by Courtois of iodine by oxidising the burnt ashes of seaweed, i.e. kelp, with sulphuric acid, which set free solid iodine characterised by the giving of a violet vapour was a major discovery in the elucidation of thyroid function. This was published in 1811. Gay-Lussac subsequently named it "iodine", derived from the Greek word for violet.[1] It took some time for the realisation that this was the active ingredient in the treatments that were prescribed for goitre. Ten years later, Prout was the first to recommend iodine in the treatment of goitre after the careful follow-up of clinical cases. At the same time, Coindet suggested that iodine deficiency caused goitre, and began treating goitre with iodine.[1]

In 1834, Graves, an Irish doctor, described a syndrome of palpitations, goitre and exophthalmus in three women. At roughly the same time, Basedow provided a classical description of the Merseburg triad, i.e. exophthalmos associated with goitre and tachycardia. The original descriptions were purely clinical. Both Graves and Basedow believed the cause to be of cardiac origin. It was only in 1886 that Moebius suggested that Graves-Basedow disease might be due to a hyper-functioning thyroid gland.[1,2,4,5] Following the description in 1882 by Ord, of middle-aged women with cretinoid features and excessive mucin in their skin, for which he coined the term "myxoedema", Horsley, using experiments on monkeys, showed that myxoedema, cretinism and post-thyroidectomy cachexia were all due to a deficiency of thyroid function. He concluded from his observations that the thyroid gland is important in metabolism. In reviewing the possible function of the thyroid gland, he said that[6] "cretinism, congenital or acquired myxoedema, and cachexia strumipriva, are merely phases of the same state, and are due to the same cause, namely, arrest of the function of the thyroid gland".

The clinical picture of Graves-Basedow disease is fully described in the first edition (1892) of Osler's famous textbook, *The principles and practice of medicine: designed for the use of practitioners and students of medicine*.[7] The treatment given was a combination of digitalis and iron. The classical diagnostic features of myxoedema and cretinism are also described, for which no satisfactory treatment was known unfortunately. It is important to note that mention was not made of any available diagnostic tests for these conditions because there were none. Diagnosis was made on clinical grounds only. Of the pathophysiology of these conditions, Osler said: "It is evident that the thyroid gland supplies some essential secretion of first importance to normal metabolism. What it is, or how it acts, is at present beyond our knowledge".

The first major development of thyroid function testing was the measurement of the basal metabolic rate (BMR) in the 1890s by Magnus-Levy, who described the elevation of the BMR in a patient with a toxic goitre. He followed this up in 1895, describing the influence of the thyroid on the BMR.[4] These classical studies by Magnus-Levy were later expanded by Aub, Dubois, Benedict and Harris, who introduced the measurement of BMR as a routine clinical method in 1918. Until the development of radioimmunoassays in the 1960s for the measurement of blood thyroid hormone concentrations, the BMR was the only important clinical measurement used to assess the thyroid status of patients. In hindsight, it is important to note that none of the tests that have supplanted BMR measurement directly assess the metabolic impact of thyroid hormones on peripheral tissue because it is this function of thyroid hormones which determines the clinical thyroid status.[8-10]

It took a long time for the isolation and purification of the active principle, following Baumann's finding in 1895 of the presence of iodine in a saline extract of thyroid tissue with significant biological activity.[11] This was accomplished by Kendall in 1914.[3]

He named it T_4. Soon afterwards, T_4 became commercially available in the USA at $350 per gram. Given that three tons of bovine thyroids only yielded 33 g of T_4, it is no wonder that it was quite expensive. In 1926, Harrington determined the correct structure of T_4, and together with Barger, synthesised it, thus making it available to physicians at a cheaper rate.[3]

In 1931, Loeb and Bassett extracted and purified thyrotropin, i.e. thyroid-stimulating hormone (TSH), from bovine pituitaries, and soon afterwards, the first quantitative bioassay for TSH was devised by Junkmann and Schoeller. They used the histological changes in the thyroids of guinea pigs injected with pituitary extracts as a measurement of extract potency. They defined the Junkmann-Schoeller unit "as the smallest daily dose of TSH extract required to produce definite signs of histological stimulation in the guinea pig thyroid. The histological response was graded as 0-3+ after the animals had been injected with TSH extract for three days".[12] Soon, other investigators were developing their own in vivo bioassays using various animals, even goldfish, each with their own unit of bioactivity. As animals were very expensive, the in vivo measurement changed to in vitro bioassay methods, using cultured follicular cells derived from the human thyroid gland. The rate of intracellular cyclic adenosine monophosphate accumulation was found to be proportional to the TSH activity, and led to the definition and acceptance of the international unit of TSH activity.[12,13] Before the 1950s, these bioassays were all in-house methods, and were generally unsuitable for routine plasma TSH determination. All of them were insensitive to very low TSH levels, and were laborious, requiring several days to complete.

In the 1920s, routine quantitative blood chemistry tests were not available because microanalytical technology was a development of the future, and was only being advanced then by physiological chemists in a few academic hospital laboratories. The knowledge that each T_4 molecule contained four iodine atoms per molecule led them to focus on developing methods to measure iodine concentration in the blood. To give an indication of the concentrations involved, it is necessary to know that the amount of iodine in the human body is less than one part per million, of which 70-80% is in the thyroid. The rest is mostly in the blood as inorganic iodide and protein-bound iodine (PBI), with a total concentration of 35-70 µg/l. PBI is mostly T_4 coupled with binding proteins. These were later identified as thyroxine-binding globulin (TBG), albumin and transthyretin, i.e. prealbumin. Realising that serum PBI might be a useful marker of thyroid gland activity, attempts were made to develop methods to determine the serum PBI concentration. They were unsuccessful until Sandell and Kolthoff published their description in 1937 of the chemical reaction in which iodine catalyses the reduction of ceric ion by arsenite; the change easily quantifiable by photometry. A workable method was developed by Chaney in 1940. Although it could measure PBI reliably, it was deemed unsuitable for routine analyses because of the use of strong corrosive acid.[14]

At least 59 methods that dealt with blood iodine determinations were published up to 1950. They were unsuitable for routine determination until Barker et al published the first practical, reproducible method that was appropriate for measuring serum PBI routinely in 1951. This method, and the measurement of BMR, were the only two routine available thyroid function investigations for more than a decade. They were considered to be the best automated in vitro routinely available test of thyroid function even in the late 1970s.[14]

In the 1930s, nuclear physicists produced radioactive isotopes. The short half-life radioactive iodine isotopes, ^{131}I and ^{125}I, were among these, and soon became available to medical researchers. Hertz and Roberts used this opportunity and started using ^{131}I (with a half-life of eight days) to study thyroid physiology and treat patients with hyperthyroidism. Tracer doses of ^{131}I are metabolised by the body in the same way as the natural element, ^{127}I. The radioactive iodine uptake test was developed as an in vivo test of thyroid function, and was usually performed in the medical physics department of an academic medical centre. Following the oral administration of a fixed dose of ^{131}I, radioactivity over the thyroid was measured at 2, 6, and 24 hours after intake. Alternatively, the percentage ^{131}I excreted in 48-hour urine collections was determined. The degree of uptake of exogenously administered radioactive iodine versus time reflects the activity of the thyroid gland. The radioactive iodine is taken up more rapidly and at a greater amount in hyperthyroidism compared to normal, and less rapidly than normal in hypothyroidism.[15-17] This test has steadily been replaced by the measurement of circulating hormones because of the lack of diagnostic discrimination, a large overlap between the normal and abnormal reference ranges, worrisome exposure to ionising radiation, time and inconvenience to patients and laboratory staff, and radioactive waste disposal.

Up until 1948, laboratory medicine was very different from what it is today.[18] The contribution of clinical chemistry, compared to that of bacteriology and haematology, was minimal, and mainly consisted of urinalysis. Thyroid status was evaluated by determining the BMR. The few hormones that could be

measured, e.g. to determine pregnancy, were bioassayed using frogs, rabbits and mice. Chemical analytical precision was satisfied by analysis in duplicate. For those who are interested, Means provides a comprehensive overview of the state of thyroid function testing in the late 1940s. A very good description of the BMR is included.[8]

The 1950s was the start of the golden age of clinical chemistry.[19] Early highlights were the isolation and synthesis of the second, more bioactive thyroid hormone, 3,5,3′-triiodothyronine (T_3) and its inactive isomer, 3,3′,5′-triiodothyronine, i.e. reverse T3, by Gross and Pitt-Rivers, in 1954.[2] This was followed shortly afterwards by the identification of the long-acting thyroid stimulator in the serum of thyrotoxic patients independently by Adams, Purves and MacKenzie, which coincided with the report of the identification of autoantibodies in Hashimoto's disease by Roitt, Doniach, Campbell and Hudson.[20] However, the introduction of three major innovations, i.e. automation with "kit" methodology, indicator-labelled immunoassay and proficiency testing, had a major impact on modernising operations in the clinical chemistry laboratory.

Recant and Riggs were the first to recognise from clinical evidence that radioactive iodine uptake by the thyroid was normal or greater than normal, and did not correlate with the low serum PBI concentrations in patients with nephrosis in 1952.[21] These patients also responded to injected TSH, with a definite rise in PBI. This was followed-up by the free hormone hypothesis devised by Robbins and Rall,[22] namely that T_4 circulates partly in the free form, and that this fraction is physiologically active. Experimental evidence was presented by Sterling and Hegedus in 1962, when they reported on the first experimental demonstration of "free" or diffusible T_4 in human serum, as well as a method to directly determine the "free" T_4 concentration of serum, which proved to be unsuitable for routine use.

After World War II, the test repertoire and tests performed per capita increased every year. Up until 1960, tests were performed manually on the bench. This had a negative effect on the turnaround time of the test results to unacceptable levels. A solution to the problem of laboratory overload was supplied by Skeggs Jr with the introduction of the AutoAnalyzer®, a continuous flow analyser, in 1957.[23] This was the introduction of automation into the clinical laboratory, whereby an analytical instrument performed many tests per day with the minimal involvement of a technologist. A series of engineering marvels was developed over the years, culminating in a variety of automated multi-test random access analysers linked to a hospital information system, as seen in clinical chemistry laboratories today. Interestingly, serum PBI determination was among the first tests to be automated in the early 1960s.

The discovery and isolation of the third thyroid hormone started in 1959, with the description by Hazard, Hawk and Crile of medullary thyroid cancer as a distinct clinical entity. This led Copp, Davidson and Cheney to isolate and purify calcitonin, the third thyroid hormone involved in calcium metabolism.[24] It was later identified as the secretion of the C cells of the thyroid gland. Hypercalcaemia stimulates the release of calcitonin, which acts by inhibiting osteolysis, thus lowering plasma calcium. Today, the measurement of plasma calcitonin is used as a screening test for medullary carcinoma of the thyroid.

The very versatile analytical technology, indicator-labelled immunoassays, was introduced by Yalow and Berson when they published their radioimmunoassay (RIA) method for the measurement of insulin in blood in 1959.[25,26] Yalow received the Nobel Prize for this work in 1977. Berson died in 1972, and thus could not be nominated. It was the first use of antibodies as a quantitative analytical tool. This new analytical method was based on competitive interaction with added radioisotope-labelled insulin and native insulin in a serum sample for insulin-specific antibody binding. Followed by the separation of the bound and free fractions, and measurement of the bound radioactivity.Bound radioactivity was inversely proportional to the insulin concentration of the sample. This permitted rapid and accurate quantification of biologically important hormones, polypeptides and drugs which were previously measured by time-consuming chemical procedures or bioassay, if measured at all.

Because of its specificity and sensitivity to nanogram per ml range, or even lower and technical simplicity, RIAs, the precursors of the generally very versatile quantitative indicator-labelled immunoassay technique, were used increasingly in clinical laboratories. In-house RIA methods for the determination of TSH, T_4 and T_3 in blood were soon developed. Serum-containing antibodies to TSH, and T_4 and T_3, i.e. haptens, coupled with an inert protein to make them antigenic, were obtained by immunising mice, pigs or sheep. In 1965, Odell, Wilber and Utiger published their specific in-house developed RIA for TSH, with only a brief report of the results in normal subjects and patients with various thyroid diseases.[27] This was a competitive antigen antibody-binding immunoassay using TSH labelled with ^{131}I (^{131}I-TSH). A fixed amount of ^{131}I-TSH competed with TSH from patient serum samples for a fixed and limited number of TSH antibody binding sites. The radioactivity of bound ^{131}I-TSH was inversely related to unlabelled TSH that was present in the sample. The sensitivity of a RIA is ultimately determined by the affinity of the antibody for the antigen; TSH in this case. This is an intrinsic property of the antibody and cannot be manipulated.

Standardisation of TSH assays was a problem. Firstly, this was because available TSH standards were either extracts of human or bovine pituitaries calibrated by different bioassays and expressed in international units. Secondly, it is important to remember that immunoassay tests measure the immunological activity expressed as milli-international units (mIU) of biological activity, whereas bioassays measure biological activity. Thus, it is possible for the TSH molecule to lose its biological activity without losing its immunological activity. The *US Pharmacopeia* (1951) made a reference substance for TSH available. Twenty milligrams of this material was equivalent to one *US Pharmacopeia* unit. Then, in 1954, a committee of the World Health Organization developed the first international standard for TSH, defined as the activity present in 13.5 mg of the reference standard [Mussett and Perry (1955)]. Both these standards were derived from bovine pituitary tissue and were approximately equivalent. The Department

of Biological Standards in London made available another standard in 1964, i.e. ampoules of Human Thyrotrophin Research Standard A, extracted from human pituitaries. The content of each ampoule was assigned an arbitrary potency of 50 mIU of TSH in 1964.

At the same time, RIA methods for total T_4 and total T_3 were initially developed in house. However, the demand for routine availability of these tests was such that the laboratories that had initially developed these could not deliver. Because the production of antigen, antibody and radioisotopic labels for analysis was beyond the facilities and acceptable expense of the clinical laboratory, this new technique gave rise to a new industry, i.e. the production of packaged kits containing all the necessary components for a successful RIA, including calibrators and controls for > 100 tests. Support industries that fed this new technology with accessories for the assay were also established. These companies were the original suppliers of reagents and analysers, and were joined by the pharmaceutical manufactures who grasped the opportunity because they had the means and capacity to generate great quantities of specific antibodies and radioactive-labelled antibodies or antigens, and package them in kit form. Soon, these companies started their own research and development units, staffed with biochemists and engineers who tinkered with the general method, and made useful modifications to the general principle. Skelley, Brown and Besch remarked in 1973: "The RIA technique is revolutionising all areas of investigational and clinical sciences".[28] For example, the use of different labels, such as enzymes and chemiluminescent compounds, eliminated the need to count radioactivity, thus making immunoassays more suited to automation. This led to the development of different types of indicator-labelled immunoassays, classified briefly as competitive or noncompetitive. The analyte or ligand is labelled in the competitive type, while a labelled antibody is used in the noncompetitive type.

In the 1970s, a number of discoveries that were important to the field of thyroidology were made. Guillemin and Schally identified thyrotropin-releasing hormone, i.e. thyroliberin (TRH) from the hypothalamic extracts of tens of millions of sheep and pigs to obtain less than 1 mg of purified TRH. They received the Noble Prize for this work in 1977. TRH is a tripeptide which can easily be synthesised, making it available for medical use. It was used in the TRH stimulation test to help in the diagnosis of hyperthyroidism or secondary hypothyroidism because the available TSH assays at that time were not sensitive enough at concentrations < 1.0 mIU/l. This was followed by the discovery by Braverman, Ingbar and Sterling of the conversion of T_4 to T_3 and reverse T_3.[2] This was a major finding which helped to prove that T_3 was the more active thyroid hormone. Then, in 1979, Liao and Pierce obtained the first ultrapure TSH preparation, used it to elucidate its structure and established that TSH shares a common alpha subunit with luteinising hormone and follicle-stimulating hormone and a unique ß chain that specifies the hormone's biological activity.[29]

Most techniques measuring plasma T_4 in common use in the late 1970s assessed the levels of protein-bound plus free T_4, i.e. serum or plasma total T_4. More than 99% of circulating T_4 is protein bound. It was frequently observed that quite a few patients with high total T_4 or high total T_3 with high levels of TBG were not hyperthyroid. It was generally accepted that the clinical effects of changes in T_4 secretion by the thyroid gland were due to changes in the free T_4 fraction. The number of free binding sites on TBG varies inversely with the T_4 concentration in hyperthyroidism and hypothyroidism. Several methods were used in an attempt to assess or correct changes owing to altered T_4-binding protein levels. Whichever method was used, "correction" was often incomplete when TBG levels were very abnormal. Methods used to correct this centered on the estimation of the unoccupied binding sites on TBG. Assessment was based on the in vitro addition of radioactive T_3 to the patient's plasma in amounts which exceeded the capacity of TBG to bind it. A resin was added after incubation, which took up the unbound hormone. The more unoccupied binding sites there were on the protein, the less radioactive hormone would be left to bind to the resin. The resin was separated from the plasma, and either the amount of radioactivity left on TBG or that bound to the resin, was determined. This was usually expressed as a percentage of the normal. Being able to assess all T_4 binding proteins, not only TBG, was a theoretical advantage. The ^{125}I-T_3 resin uptake test was routinely available from the reagent suppliers. The result obtained from this test was combined with the total T_4 or total T_3 concentration in various formulae to calculate a free-T_4-index, which was a useful measure of the freeT_4 concentration.[30]

The measurement of "free hormone" is generally accepted for the diagnosis of thyroid disease, with exceptions,[31,32] as an appropriate measure of the thyroid's functional state. Thus, for diagnostic purposes, the focus should be on the measurement of free thyroid hormones, rather than on the total. However, ideally, valid assays measuring the free fraction of T_4 (free T_4) must perform without bias, despite large variations in the concentrations and affinities of serum T_4-binding proteins in the population. Several approaches have been adopted to overcome such bias. These are tedious direct methods of equilibrium dialysis or ultrafiltration. An early method was introduced by Ingbar et al in 1965, an example of a two-stage "double dialysis" procedure, in which the first stage involved equilibrium dialysis of a mixture of test serum and added ^{131}I-T_4 against a standard phosphate buffer. The second dialysis removed a radioiodide contaminant. This and other methods were not suitable for routine measurement, and with time were modified to serve as a reference method against which other measurements were standardised.

A useful method for the direct measurement of free T_4 and free T_3 suitable for routine purposes became available in the early 1980s. This was the analogue free T_4 RIA.[33] The method utilised as a radioactive tracer of high specific activity, a ^{125}I-T_4 derivative, which was chemically modified to inhibit its binding to the endogenous T_4-binding proteins in serum, but which binds normally to antibodies to T_4 - the analogue. When this radioactive tracer was mixed with a serum sample and a very small amount of a specific, high-affinity T_4 antibody, equilibrium was established in which the free T_4 and T_4 analogue competed for a limited number of binding sites on the antibody. As is common with conventional RIA techniques, the proportion of analogue bound to the T_4

antibody inversely related to the free T_4 concentration present in the serum. In the measurement of free T_4 it was essential that there was minimal disturbance to the normal equilibrium that existed between T_4, which is free in serum, and that which is bound to the serum proteins.

Nearly all modern TSH measurement methods are two-site "sandwich" heterogeneous immunoassays, whereby the second antibody has an enzyme or chemiluminescent label. Bioassays are available for the measurement of TSH activity and to calibrate TSH reference preparations. Migration to more sensitive TSH methods in the 1990s prompted a change in strategy for the laboratory investigation of thyroid dysfunction, establishing TSH measurement as its foundation. Sensitivity was the catalyst because TSH concentration may be decreased in nonthyroidal illness, and the lower limit of detection was necessary to distinguish between nonthyroidal illness causes of suppressed TSH and thyrotoxicosis, which is associated with a TSH concentration of < 0.01 mIU/l. In general, TSH methods are classified according to analytical sensitivities separated by roughly one log unit, i.e. a "generational" classification. Thus, first-, second- and third-generation methods have functional sensitivities of 1.0, 0.1, and 0.01 mIU/l, respectively. These definitions are not formal, but were loosely adopted by the manufacturers of TSH assay kits. Currently, functional sensitivity is defined as the lowest concentration of TSH at which an interassay coefficient of variation of 20% or less is achieved. The American Thyroid Association determined that only TSH assays with third-generation (< 0.01 mIU/l) functional sensitivity were sufficient for use as screening tests for hyperthyroidism. Their recommendation was consistent with the National Academy of Clinical Biochemistry laboratory medicine practice guideline for the assessment of thyroid function.[34]

There was poor agreement between various TSH immunoassays until an International Reference Preparation (IRP) isolated from pituitary tissue was made available in the 1980s. In 1999, a recombinant human TSH IRP was created, but significant differences were noted between the recombinant and isolates from pituitary tissue. TSH immunoassays are calibrated with reference preparation of human TSH (the World Health Organization Second International Reference Preparation, 2nd IRP 80/558).

Although a routinely measured analyte, TSH is still prone to many endogenous interferences which plague all immunoassays, namely rheumatoid factor, heterophylic antibodies, paraproteins and exogenous T_4 ingestion. However, appropriate reference ranges for TSH and free T_4 are of greater importance. For example, free T_4 and TSH concentrations in young pregnant females differ substantially from those in older females. Medical decision levels for thyroid hormones necessarily depend on the population examined.[30] To compound the problem, it is now well recognised that the immunoassays used to measure TSH and thyroid hormones add another source of variability. This was recently addressed by the International Federation of Clinical Chemistry and Laboratory Medicine FCC Working Group for Standardization of Thyroid Function Tests.[35]

Although not markers of thyroid function, the introduction of tests for thyroglobulin, thyroid peroxidase antibodies and long-acting thyroid-stimulating antibodies greatly improved the diagnostic repertoire of thyroid laboratory investigations.

In conclusion, fantastic developments in the field of immunoassays had David Wild to wonder where they rank on the scale of human technological achievements as "they are based on a broad and deep scientific knowledge in physics, chemistry and biology. The engineering and biochemistry have become highly complex, and the pace of innovation is faster than that in many industries, including automobiles, construction, pharmaceuticals and protection of the environment. The dual application of cutting-edge biochemistry and engineering places immunoassay into a select group of highly advanced biotechnology products".[36] As an integral part of this, Yalow is sometimes asked whether or not she regretted not patenting the principle of immunoassay. Her response was: "Not only did I not regret it, but I felt very strongly that we, as scientists, should not seek to exploit our scientific discoveries for our own financial gain. We realised that our new technique could have widespread applications".[37]

References

1. Slater S. The discovery of thyroid replacement therapy. Part 1: In the beginning. J R Soc Med. 2011;104(1):15-18.
2. Temple R. The genius of China: 3,000 years of science, discovery and invention. New York: Simon and Schuster, 1986.
3. Hamdy RC. The thyroid gland: a brief historical perspective. South Med J. 2002;95(5):471-473.
4. Sawin CT. The heritage of the thyroid. Werner and Ingbar's the thyroid: a fundamental and clinical text. In: Braverman LE, Utiger RD, editors. 8th ed. Philadelphia: JB Lippincott Company, 2000.
5. Werner S. History of the thyroid. Werner and Ingbar's the thyroid: a fundamental and clinical text. In: Braverman LE, Utiger RD, editors. 6th ed. Philadelphia: JB Lippincott Company, 1992.
6. Lindholm J, Laurberg P. Hypothyroidism and thyroid substitution: historical aspects. J Thyroid Res. 2011;2011:809341.
7. Osler W. Diseases of the thyroid gland. The principles and practice of medicine. Internet: D Appleton and Company, 1892; p. 711-716.
8. Means JH. Methods of examination in thyroid cases. The thyroid and its diseases. 2nd ed. Philadelphia: JB Lippincott Company, 1948; p.145-172.
9. Harrison GA. Basal metabolism and metabolism experiments. Chemical methods in clinical medicine their application and interpretation with technique of simple tests. 2nd ed. London: J & A Churchill Ltd, 1943; p. 483-496.
10. Berger S, Quinn JL. Thyroid function. Fundamentals of clinical chemistry. 2nd ed. In: Tietz NW, editor. W B Saunders Company, 1976; p. 824-848.
11. Baumann E. *Ueber das normale vorkommen von jo dim thierkorkorper.* Hoppe-Seyler Z Physiol Chem. 1895;21:319.
12. DeGraw WA. An evaluation of a bioassay method for thyroid stimulating hormone and its application to Citellus Richardsoni [Thesis]. Fort Collins: Colorado State University,1965.
13. Henry CJK. Basal metabolic rate studies in humans: measurement and development of new equations. Public Health Nutr. 2005;8(7A):1133-1152.
14. Hoffenberg R. Thyroid function. Chemical diagnosis of disease. In: Brown SS, Mitchell FL, Young DS, editors. New York: Elsevier, 1979.
15. Henry JB, Krieg AF. Measurements of thyroid function. Todd Sandford: clinical diagnosis by laboratory methods. 14th ed. In: Davidson I, Henry JB, editors. Philadelphia: WB Saunders, 1969; p. 629.
16. Goodwin JF, MacGregor AG, Miller H, Wayne EJ. The use of radioactive iodine in the assessment of thyroid function. Q J Med. 1951;20(80):353-387.
17. Goolden AWG. Tests of thyroid function in vivo. J Clin Path. 1975;28(3):244-247.
18. Caraway WT. The scientific development of clinical chemistry to 1948. Clin Chem. 1978;19(4):373-383.
19. Rosenfeld L. A golden age of clinical chemistry: 1948-1960. Clin Chem. 2000;46(10):1705-1714.
20. Bürgi, Labhart A. The thyroid gland. Clinical endocrinology: theory and practice. In: Labhart A, editor. Berlin: Springer-Verlag, 1974; p. 135.

21. Recant L, Riggs DS. Thyroid function in nephrosis. J Clin Invest. 1952;51(8):789-797.
22. Robbins J, Rall JE. The interaction of thyroid hormones and protein in biological fluids. Recent Prog Horm Res. 1957;13:161-208.
23. Skeggs LT Jr. Persistence … and prayer: from the artificial kidney to the AutoAnalyzer. Clin Chem. 2000;46(9):1425-1436.
24. Copp DH, Cameron EC, Cheney BA, et al. Eviodence for calcitonin: new hormone from parathyroid that lowers blood calcium. Endocrinology. 1962;70:638.
25. Yalow RS, Berson SA. Immunoassay of endogenous plasma insulin in man. J Clin Invest. 1960;39:1157-1175.
26. Yalow RS, Berson SA. Assay of plasma insulin in human subjects by immunological methods. Nature. 1959;184(Suppl 21):1648-1649.
27. Odell WD, Wilber JF, Utiger RD. Studies of thyrotropin physiology by means of radioimmunoassay. Recent Progr Hormone Res. 1967;23:47.
28. Skelley DS, Brown LP, Besch PK. Radioimmunoassay. Clin Chem. 1973;19(2):146-186.
29. Lepage R, Albert C. Fifty years of development in the endocrinology laboratory. Clin Biochem. 2006;39(5):542-557.
30. Thienpont LM, van Uytfanghe K, Poppe K, Velkeniers B. Determination of free thyroid hormones. Best Pract Res Clin Endocrinol Metab. 2013;27(5):680-700.
31. Midgley JEM, Christofides ND. Point: legitimate and illegitimate tests of free-analyte assay function. Clin Chem. 2009;55(3):439-441.
32. Wilcox RB, Nelson JC. Counterpoint: legitimate and illegitimate tests of free-analyte assay function: we need to identify the factors that influence free-analyte assay results. Clin Chem. 2009;55(3):442-444.
33. Mardell RJ, Gamlen TR. Thyroid function tests in clinical practice. Bristol: John Wright & Sons Ltd, 1985.
34. Winter WE, Schatz D, Bertholf RL. The thyroid: pathophysiology and thyroid function testing. Tietz textbook of clinical chemistry and molecular diagnostics. 5th ed. In: Burtis CA, Ashwood ER, Burns DE, editors. New York: Elsevier, 2012.
35. Klee GG. Harmonization and standardization of thyroid function tests. Clin Chem. 2010;56(6):879-880.
36. Wild D. Preface. The immunoassay handbook. 4th ed. In: Wild D, editor. New York: Elsevier, 2013.
37. Yalow R. Foreword. The immunoassay handbook. 4th ed. In: Wild D, editor. New York: Elsevier, 2013.