ENDOCRINE HISTORY

The history of discovery, synthesis and development of testosterone for clinical use

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Abstract

As the most important male hormone, testosterone has an impact on almost all organs and body functions. The biological effects of testosterone and the testes have been known since antiquity, long before testosterone was identified as the active agent. Practical applications of this knowledge were castration of males to produce obedient servants, for punishment, for preservation of the prepubertal soprano voice and even for treatment of diseases. Testes were used in organotherapy and transplanted as treatment for symptoms of hypogonadism on a large scale, although these practices had only placebo effects. In reaction to such malpractice in the first half of the 20th century science and the young pharmaceutical industry initiated the search for the male hormone. After several detours together with their teams in 1935, Ernst Laqueur (Amsterdam) isolated and Adolf Butenandt (Gdansk) as well as Leopold Ruzicka (Zürich) synthesized testosterone. Since then testosterone has been available for clinical use. However, when given orally, testosterone is inactivated in the liver, so that parenteral forms of administration or modifications of the molecule had to be found. Over 85 years the testosterone preparations have been slowly improved so that now physiological serum levels can be achieved.

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Introduction

Extirpation and transplantation of endocrine glands are among the earliest tools in experimental and applied endocrinology. The testes, in their exposed position, are vulnerable and easily accessible to manipulation including both accidental trauma and forceful removal. Loss of virility and fertility are easily recognizable, not only by physicians but also by laymen, so that the results of loss of the testes or testicular function were known since antiquity and long before the discovery of sperm and their function in the 17th and 18th century, and

Invited Author's profile

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long before testosterone, as the active agent, was isolated and synthesized in the 20th century (1). Although even present-day endocrinologists often assume that the discovery and synthesis of this important hormone must have been honoured by a Nobel Prize; this assumption is not completely correct. This article deals with the history of testosterone and tries to explain this misunderstanding. In order to understand why the road to testosterone was so long and convoluted, we will briefly describe what was known about the physiological role of the testes and how this knowledge was translated into practical applications over centuries, before, at the beginning of the 20th century, the hype surrounding testis transplantation and organotherapy challenged academic research and industrial enterprise to isolate and synthesize testosterone in successful cooperation; an account of the development of testosterone preparations for clinical use follows.

Consequences of castration

Aristotle (384–322 B.C.), the universal genius and philosopher of the Hellenistic era, observed and wrote *The Generation of Animals* in which he described the generation of visible organs in fertilized chicken eggs. He knew the effects of castration in men as well as in animals and described its consequences in animal husbandry. However, even before this written documentation, castration was used to produce obedient slaves, loyal to their masters and rulers. As documented since the Ming dynasty (1368–1644), at the Chinese imperial court, eunuchs obtained high-ranking positions, as exemplified by Admiral Zhèng Hé (1371–1435), leader of seven large expeditions into countries around the Indian and Pacific oceans, or Lin Yin (1451–1510), who is still counted among the richest persons in history. The last imperial eunuch, Sun Yaoting, died in 1996 at the age of 94 years. In Islamic societies over the centuries castrated slaves formed elite troops deployed in wars of conquest.

Castration was also applied as lawful punishment. In Scandinavia high treason was not subject to capital punishment, but to castration combined with blinding, which was adopted by the Normans who introduced this legislation wherever they ruled for example in Sicily and France. After 1066, William the Conqueror abolished the Anglo-Saxon death penalty and replaced it by castration and blinding: 'I also forbid that anyone shall be slain or hanged for any fault, but let his eyes be put out and let him be castrated'.

Deliberate mutilation also served other purposes. It was known that prepubertal castration maintains the high voice of boys so that soprano and alto voices with the acoustic volume of an adult male result and were featured in operas in the 17th and 18th centuries. Some of these castrates became famous soloists, such as Carlo Farinelli (1705–1782) or Domenico Annibaldi (1705–1779). In the Vatican choirs these voices could be heard until the early 20th century. Alessandro Moreschi (1858–1922), who was castrated at the age of 9 years under the pretence of protection against cholera, became the last castrato to sing in the Sistine Chapel and his performance was preserved by gramophone recordings in 1902 and 1904 so that his voice can still be heard today (2). The medical school in the middle Italian cities of Norcia and Preci were specialized in surgery on young boys, going back to the 13th century, and the 30 family dynasties monopolizing the trade there guaranteed utmost secrecy concerning this illegal operation, forbidden since 1587 by Pope Sixtus V.

Castration was also recommended for therapeutic purposes during Greek-Roman times and the Middle Ages for the treatment of leprosy, epilepsy, gout, priapism, excessive masturbation and insanity (3), reflecting the knowledge or rather the lack of knowledge of the respective period.

Proof of endocrine function of the testes

While removal of endocrine glands is one basic tool of experimental endocrinology, replacing glands is the other. As physician and physiologist at the University of Göttingen, Arnold Adolph Berthold (1803–1861) observed that testes transplanted from roosters to capons restored androgenic functions: 'They crowed quite considerably, often fought among themselves and with other young roosters and showed a normal inclination to hens'. Berthold concluded that these effects 'must be caused by the productive relationship of the testes, that is to say, through their action on the blood, and then through the suitable ensuing action of the blood on the organism as a whole' (4). He was thus the first to postulate a humoral effect of the testes on distant organs as a general principle and is therefore widely recognized as the 'Father of Endocrinology'. However, Berthold's rival at the University of Göttingen, Rudolf Wagner (1805–1864), was jealous, tried to repeat the experiments, but failed and declared them as rubbish (5). As he became the full professor of physiology, his opinion prevailed and Berthold's personality did not allow him to fight for recognition of his findings (6).

Incidentally, at the same time the anatomist Franz Leydig (1821–1908) at the University of Würzburg described the interstitial Leydig cells in the testes of many species, however, without understanding their function and importance (7).

Berthold and his research were rehabilitated and acknowledged only half a century later when Moritz Nussbaum (1850–1915), professor of anatomy in Bonn repeated Berthold's experiments and confirmed the results in frogs in 1909 (8), as did Eugen Steinach in Vienna in rats 1910 (9). Finally, A. Pézard in Paris confirmed Berthold's original results in cocks in 1911 (10). Earlier Ancel and Bouin (11) clearly attributed an endocrine function to the Leydig cells by summarizing their conclusions from extensive experimentation as follows: 'In numerous previous studies we have assembled a group of morphological, physiological and chemical facts that, taken together, allow us to formulate the following hypothesis: that the general action of the testes on the organism, ascribed in the past to the testes as a whole, is actually due to the interstitial gland (12)'.

Erroneous conclusions from Berthold's experiments

Probably prompted by the revival of Berthold's experiments (1), surgeons turned to testes transplantation as a means to treat hypogonadism and bring about rejuvenation and therapy for all sorts of disorders. George Frank Lydston (1858–1923) at Cook County Hospital in Chicago 1915 was one of the first to perform human testicular transplantation from accident victims to recipients (13). Also in Chicago Victor D. Lespinase (1878–1946) published in 1913 his experience with transplanting human testes from donors to patients for rejuvenation (14) and Leo Stanley (1886–1976), at the California State Prison San Quentin, reported in 1923 20 cases of transplantation of testes from executed prisoners to other inmates who reported signs of revitalization. Later he turned to rams as sources for his testicular grafts and reported satisfaction on the part of the patients, including 13 physicians (15).

John Romulus Brinkly (1885–1942) a half-educated medic, turned goat testis transplantation in his clinic in Milford, Kansas, into a booming business between 1918 and 1930. However, in 1939 a Texan judge found him guilty of acting as a charlatan and quack, thus unleashing a series of lawsuits demanding millions of dollars as compensation. Brinkly declared bankruptcy and died of heart attacks soon thereafter.

In Vienna, Eugen Steinach (1861–1944) convinced surgeons to perform unilateral vasoligation for rejuvenation (16). One of his followers, Serge Voronoff (1866–1951) turned to xenotransplantation and used monkey testes to be transplanted for rejuvenation (17). He first offered his surgery in Paris, but after some scandals continued his questionable operations in Algiers, where he was visited by patients from all over the world. In many countries, Voronoff's followers xenotransplanted animal testes and pieces thereof to patients demanding rejuvenation. When unrest among the medical profession grew concerning this quackery, in 1927 the Royal Society of Medicine (London) sent an international committee to Voronoff in Algiers. The committee concluded their investigations by declaring Voronoff's claims as poppycock.

These scandals and the hope that steroid biochemistry would ultimately lead to the discovery and synthesis of the male sex hormone, following that of female sex hormones, finally terminated the questionable business of testes transplantation. However, before the chapter of modern testosterone biochemistry and pharmacology can be opened, another century-long medical misapprehension needs to be discussed.

Testes for organotherapy

Since antiquity the knowledge of the powerful function of the testes in the normal male organism induced patients and healers to turn to the ingestion of these organs in various modalities. Early on in Rome Gaius Plinius Secundus (23–79) prescribed the consumption of animal testes for the treatment of symptoms of hypogonadism and impotence. For the same purpose the Arabic physician Mesue the Elder (777–837) in Baghdad recommended testis extracts. Also in Chinese medicine – at least since 1132 – Hsue Shu-Wei prescribed raw and desiccated animal testes. The 'Universal Doctor' and founder of the University of Cologne, Albertus Magnus (1192–1280), concerned with the taste of his prescription, recommended powdered hog testes in wine as a vehicle (1, 18).

Similar preparations, but now in tablet form, continued to be manufactured, prescribed and consumed up into the 20th century. In the 1920s Testifortan® became a financially successful drug for treatment of impotence (19). Its main constituents were testis extracts and yohimbin. Another famous preparation from the 1920s and marketed until today is Okasa® which, among other components, also contains *testis sicca* and thereby minute amounts of testosterone, as we could determine

in the 1970s (unpublished). However the testes synthesize testosterone but, unlike other endocrine glands such as thyroid or pancreas, do not store its products. The daily production of an adult man of about 6–8 mg is contained in roughly 1 kg of (bull) testes; even if this amount of testosterone were to be consumed, the testosterone taken orally would be inactivated by the first-pass effect in the liver (20). Therefore, all testicular organ therapy administered orally can only be considered as a placebo medication which, however, may not be without its own effects. Nevertheless, many companies worldwide continued to manufacture extracts and pills well into times when genuine testosterone was already long on the market. For example, Ciba (Switzerland) withdrew their Androstin® ('biologically titrated full extract from male gonads' for oral and parenteral use) only in 1961, after three decades of successful sales for the treatment of 'male gonadal insufficiency, impotence, infantilism, premature ageing and endocrine obesity' (21).

Organotherapy literally exploded at the end of the late 19th and early 20th century when, at age 72 Charles E Brown-Séquard (1847–1894), who until then was a well-reputed scientist and member of several scientific academies, published the results of his famous self-experimentation in the *Lancet* 1889 (22). He gave himself 1-mL injections of a mixture of one part testicular vein blood, one part semen and one part juice extracted from dog or guinea-pig testes daily, and after 20 days made astonishing observations on himself: 'A radical change took place in me … I had regained at least all the strength I possessed a good many years ago. I was able to make experiments for several hours. After dinner I was able to write a paper on a difficult subject. My limbs, tested with a dynamometer, gained 6 to 7 kg in strength. The jet of urine and the power of defecation became stronger'. Certainly all these were placebo effects, as confirmed by replication of the experiment a century later (23), but at that time the world had obviously been waiting for such quackery, because in no time the 'extracts of animal organs by the Brown-Séquard method' were sold all over the (Western) world and factories sprung forth in Europe as well as in America, for example next to Central Park in New York (24).

Isolation and synthesis of testosterone

The craze for such products caused concern about the image of the young field of endocrinology. The famous neurosurgeon, Harvey W Cushing (1869–1939), went so far as to talk about 'endocri*mi*nology' in the context of this organotherapy. Finally reacting to the hype generated by testicular transplantation and organotherapy, the pharmaceutical industry and academic research cooperated in order to rehabilitate endocrinology and replace organotherapy by proper hormone substitution.

As steroid biochemistry progressed, the great breakthroughs were the discovery of the ring structure of steroids and bile acids at the National Institute of Medical Research in London in 1932 (25) and at the Bavarian Academy of Sciences in Munich also in 1932 (26). A heated discussion ensued whether there were three of four rings in the steroid structure and, if four rings, whether the fourth had five or six C-atoms. Under the sponsorship of the Health Organisation of the League of Nations (the predecessor of WHO) highly recognized chemists including Edmund A Doisy, Adolf Butenandt and Guy Marrian assembled at University College London and reached a consensus that steroids had four rings and the fourth ring had five C-atoms (26) (Fig. 1). Shortly before, these eminent researchers, including Ernest Laqueur, had isolated pregnandiol and estrone from pregnant mare urine provided by various drug companies co-operating with scientists. In 1934 Butenandt as well as three further teams added progesterone to the array of sex steroids (28). Their purpose was to replace the miscredited organotherapy and to make proper steroid substitution available to patients (Table 1) (29).

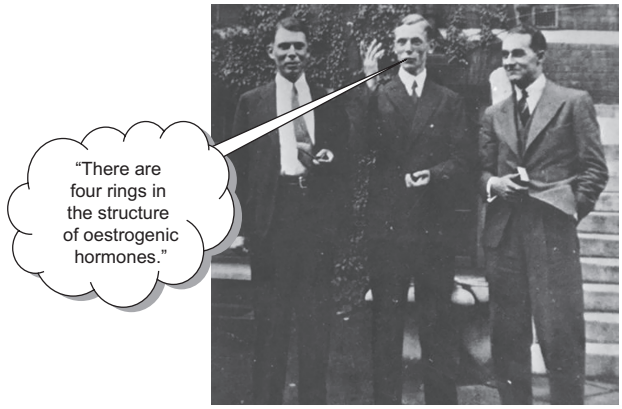

Figure 1
EA Doisy, A Butenandt and GF Marrian at the First International Conference on the Standardisation of Sex Hormones in London 1932, sponsored by the Health Organization of the League of Nations (89).

Table 1 Early isolation of sex steroids.

Steroid	Investigators	Source of material	Reference
1928 Pregandiol	Guy Frederick Marrian, Dept. Physiology and Biochemistry, University College London	Crystalline material from pregnant mare urine provided by BRITISH DRUG HOUSES	(84)
1929 Oestron	Edward Albert Doisy, Dept. Biological Chemistry, St. Louis	From urine of pregnant women	(85)
1929 Oestron	Adolf Butenandt, Chemical Laboratory Univ. Göttingen	Raw extracts and crystals from pregnant mare urine provided by SCHERING	(86)
1929 Oestron	Ernst Laqueur, Pharmacotherapeutic Laboratory, University Amsterdam	Benzol extracts from pregnant mare urine provided by ORGANON	(87)
1931 Androsterone	Adolf Butenandt, Chemical Laboratory Univ. Göttingen	Extracted from 15,000 L urine provided by the Prussian Police Academy in Berlin and processed by SCHERING	(31)
1934 Progesterone	Adolf Butenandt, Chemical Institute, Technical University, Danzig	Isolated from 50,000 sow ovaries, extracts provided by SCHERING	(27)
1935 Testosterone	Ernst Laqueur, Pharmacotherapeutic Laboratory, Univ. Amsterdam	Isolated from 100 kg bull testes provided by ORGANON	(32)

From observation of the growth of a cock's comb under the influence of transplanted testes, Moore *et al*. (30) in 1929 established the standardized capon comb's test measuring androgenic activity in square centimeters of comb surface. This first bioassay facilitated determination of androgenic activity in body products as well as in chemical solutions. In 1930 Loewe and Voss (31) used the biological effects of androgens on the accessory sex organs and developed the 'cytological regeneration test' which was based on regrowth of the seminal vesicle epithelium under the influence of androgenic substances (Loewe-Voss-Test). The then still hypothetical male hormone was called 'Androkinin'. This biological test helped to resolve the question whether *only one* or *several* androgenic steroids existed and, if more than one – which one might be the more potent.

In the dawn of testosterone emerging as a biochemical and marketable entity, and after lengthy negotiation in 1935, Ciba (Switzerland) and Schering-Kahlbaum (Germany), pharmaceutical companies active in the field, started a cooperative effort to inform each other about progress and thus forced their academic protagonists Leopold Ruzicka (1887–1976) at the Technical University of Zürich and Adolf Butenandt (1903–1995) at the then Technical University of Gdansk, to exchange their respective advances, to which the former rivals reluctantly agreed. In 1937 the Ciba-Schering cooperation was extended to include Boehringer (Germany), Chimio Roussel (France) and Organon (The Netherlands) to form a real cartel/syndicate to share knowledge, to stake market claims worldwide and to agree on pricing of the products (1, 21). While economically profitable, this syndicate is also an early example of successful academic-industrial cooperation.

In 1931 Adolf Butenandt, then at the University of Göttingen, isolated the androgenic steroid androsterone (androstan-3α-ol-17-one) from 15,000 L of urine provided by young policemen from Berlin and which were processed by Schering to obtain 15 mg of this substance. Butenandt considered androsterone the principal androgen (32). Ernst Laqueur (1866–1947) and his group at the University of Amsterdam and at Organon were specialized in extracting hormones from animal glands. From 100 kg of bull testes they extracted and isolated 10 mg of another androgen, 17β-hydroxy-4-androstene-3one, which they found to be more active than androsterone in biological tests in 1935 (33). They baptised this hormone 'testosterone'. Although Butenandt liked neither this 'dreadful' name nor the competition, in the same year Butenandt and Hanisch (34) at that time at the University of Gdansk, (Fig. 2) as well as Ruzicka and Wettstein (35) in Zurich/Basel (Fig. 3), published the chemical synthesis of testosterone, thus marking the beginning of modern clinical pharmacology of testosterone and male reproductive physiology. For the synthesis of testosterone Butenandt had started from dehydroandrosterone, while Ruzicka used a degradative approach which he also used for 17alpha-methyltestosterone, the first orally effective testosterone preparation.

The close cooperation among the researchers, reinforced by the pharmaceutical companies, may explain why these discoveries were published more or less at the same time. Marius Tausk, one of the former heads of research at Organon who knew the competing protagonists personally, describes the race to testosterone isolation and synthesis very vividly and how the key respective papers were submitted for publication in short sequence in 1935 (36).

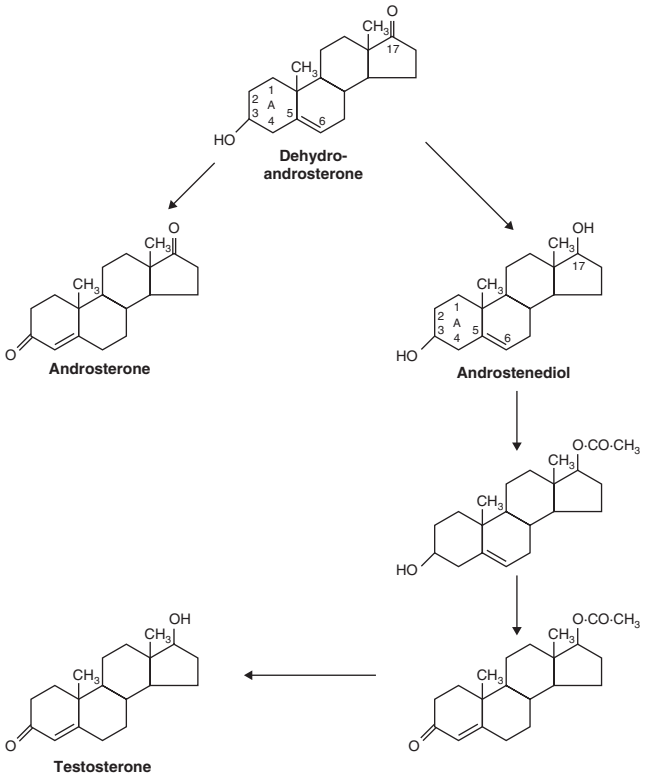

Figure 2
Synthesis of testosterone from dehydroandrosterone as described by Butenandt and Hanisch in 1935 (34).

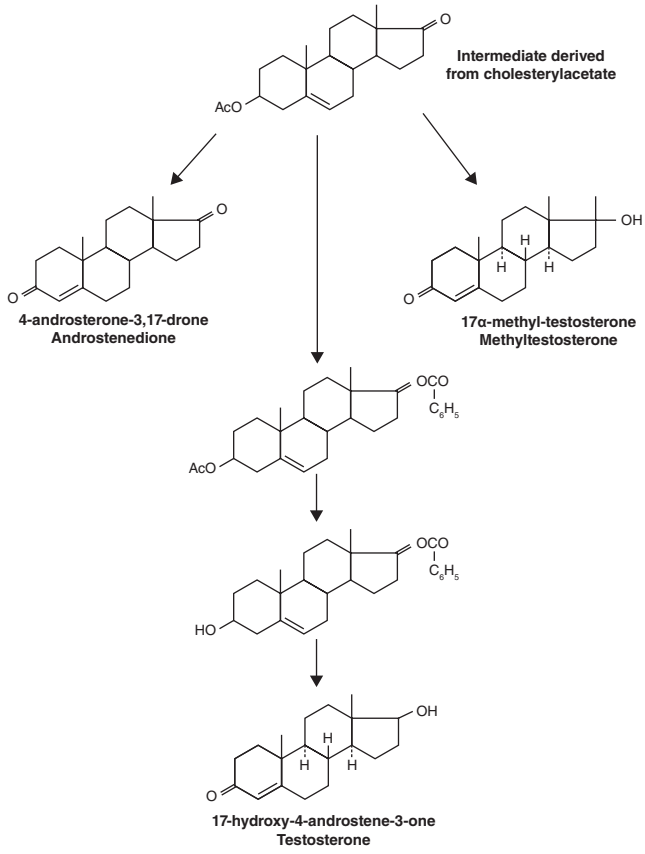

Figure 3
Degradative synthesis of testosterone and 17alpha-methyltestosterone as described by Ruzicka and Wettstein in 1935 (35).

In 1939 Butenandt and Ruzicka received the Nobel Prize for chemistry jointly, Butenandt 'for his work on sex hormones' (oestrogen, progesterone and androsterone – but not for testosterone!) and Ruzicka 'for his work on polymethylenes and higher terpens' and also not explicitly for testosterone!

Nevertheless, neither laureate could attend the prize ceremony in Stockholm. Butenandt had become director of the Institute for Biochemistry of the Kaiser-Wilhelm-Society in Berlin in 1936; he was prevented by the Nazi regime from accepting the prize since Hitler had forbidden Germans to accept the Nobel Prize after, in 1937, the Nobel Prize for Peace had been awarded to Carl von Ossietzky, a journalist and strong opponent of the regime. In 1949, at the Swedish Consulate in Frankfurt, Butenandt received the Nobel Prize diploma and the medal, but not the money, since according to the statutes, the prize money must be collected within a year.

Incidentally, in 1939 the same indignity was suffered by the Laureate for the Nobel Prize for Medicine, Gerhard Domagk (1894–1964) who worked at the Bayer Company and was honoured for his invention of the first clinically used sulfonamide Prontosil®. Domagk was even imprisoned for a few days for accepting the honour and only in 1947 received the Nobel Prize documents from the Swedish King in Stockholm, but – for the same reason as Butenandt – no money. (By the way, Domagk had started his academic career between 1925 and 1929 at the University Hospitals in Münster and therefore the author's institutional street address is named after him.) (37). Back to Butenandt, it should be noted that after the war, his research concentrated on insect hormones and he became president of the Max-Planck-Society (the former Kaiser-Wilhelm-Society), serving from 1960 to 1972.

As Ruzicka was also not able to travel to Stockholm because of turmoil caused by the beginning of World War II, on January 16, 1940 – well within a year – at a special ceremony in Zűrich, the prize was handed over to Ruzicka by the Swedish ambassador in Switzerland, Baron H G Beck-Friis (Fig. 4). After the war had ended, Ruzicka delivered his Nobel Prize lecture on

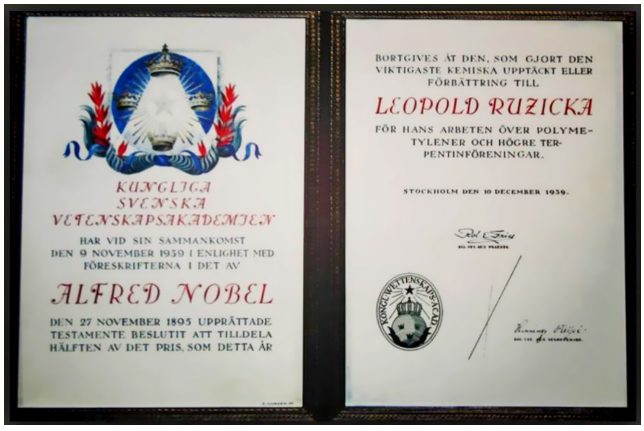

Figure 4
The Nobel Prize Diploma for Leopold Ruzicka.

December 12, 1945. In this lecture he explained how research on 'multimembered rings and higher terpens' guided him to steroids and to testosterone as 'cholesterol could be regarded as a triterpenoid' (38). In the 1920s he had worked in the perfume industry in Geneva (Switzerland) and Holzminden (Germany) to elucidate the chemical formulas and synthesis of fragrances. His major achievement was the structural analysis and synthesis of the polycyclical ketones such as muscone, the scent in musk pods (preputial glands of the musk deer), civetone from civetts and santanol from sandalwood, important ingredients in perfumes which before chemical synthesis had to be extracted from their natural sources. In 1929 he became professor of organic chemistry at the ETH in Zűrich and teamed up with researchers at Ciba in Basel. Ciba was interested in his knowledge of cyclical structures and cooperated with him in the field of triterpenes and steroids, another class of cyclical substances. For the synthesis of testosterone Ruzicka started from cholesterol and used the same degradative approach as for the synthesis of fragrances.

As Ruzicka remarked in autobiographical notes, beyond scientific recognition his research work was also financially rewarding: 'The patents for the degradative synthesis of testosterone and methyltestosterone earned me during subsequent years an enormous (compared with my professorial standard) amount of money as royalties from Ciba in Basel and Ciba in the USA'. (39). In the year 1939 alone Ciba transferred 56,744 Swiss Francs to Ruzicka as royalties (21). Part of this money was invested in a collection of 17th century Flemish and Dutch paintings which Ruzicka donated to the Kunsthalle Zürich in 1947 (39) where they form a substantial part of the museum's inventory.

Why Laqueur's identification of testosterone rather than androsterone as the most important male sex hormone was not recognized by the Nobel Committee in 1939 remains unclear. Laqueur was born in 1880 and grew up near Breslau (Silesia), at that time still in Germany and received his medical education and degrees in Germany. In 1920 he became professor of pharmacology at the University of Amsterdam. In 1923 he co-founded Organon located next to the slaughterhouses in Oss (The Netherlands) to extract insulin from pig pancreas. Organon became the first European producer of insulin for the treatment of diabetes. In 1930 Laqueur acquired Dutch nationality. When the Germans invaded The Netherlands in 1940, Laqueur lost his position because of his Jewish background and had to surrender his shares in Organon, but was not further persecuted in contrast to some members of his family.

New syndromes of hypogonadism

It may not have been a coincidence, but a sign of heightened interest in the clinics of hypogonadism that at the same period when testosterone became available for clinical use, that is, when rational treatment became possible, that major syndromes of primary and secondary hypogonadism were first described, that is the Klinefelter syndrome in 1942 (40) and the Kallmann syndrome in 1944 (41). Pasqualini and Bur (42) described the fertile-eunuch syndrome, characterized by all symptoms of lack of testosterone, but with active spermatogenesis. Also at that time the symptoms of the ageing male were first described systematically, but unfortunately incorrectly termed as 'male climacteric' (43), starting a controversial discussion that continues until today. Del Castillo *et al.* (44) published in1947 the first five cases suffering from Sertoli-cell-only syndrome. Reifenstein (45) first described a syndrome with partial androgen insensitivity (PAIS) that carries his name to date and Morris (46) published the first cases of complete androgen insensitivity (CAIS) as 'testicular feminisation in male pseudo-hermaphroditism' – of course without knowing anything about the androgen receptor or androgen receptor mutations.

Early warning for testosterone

Around the same time Huggins also posted his warning about testosterone influencing prostate carcinoma (47), which prevailed until quite recently and led to castration

as the major treatment of prostate carcinoma, when androgen deprivation therapy (ADT) by GnRH analogues or antiandrogens was introduced instead. Huggins' statement 'Cancer of the prostate is activated by androgen injections' induced a general fear of testosterone, especially among urologists, which prevented testosterone treatment in many patients who might have needed it. Only recently it became clear that neither endogenous serum testosterone levels (48) nor testosterone treatment (49) have an impact on prostate carcinogenesis. Nowadays under careful supervision testosterone treatment is even considered for patients suffering from testosterone deficiency after radical prostatectomy and castration (50).

Development of testosterone preparations for clinical use (1)

Soon after its synthesis it became clear that, in reasonable doses, testosterone was not effective orally or – as we know today – would require extremely high doses which were simply not available and/or too expensive. Today we know that the lack of oral effectiveness is due to the inactivation of testosterone by the first-pass effects in the liver. Three approaches were used to overcome this problem:

1. Chemical modification of the steroid molecule,
2. parenteral application and
3. esterification in position 17β of the testosterone molecule.

For a more complete description of the many testosterone preparations and routes of administration the reader is referred to a review by Behre and Nieschlag (51). A major goal of the efforts to produce new testosterone preparations for clinical use was to optimize treatment of hypogonadism. Later, when serum levels of testosterone could be determined, an additional goal was to mimic physiological serum testosterone levels as closely as possible (52). As shown in the ensuing text, it took quite long to reach these goals.

As mentioned above, in 1935 together with testosterone Ruzicka *et al.* (53) had synthesized 17α-methyl-testosterone and had demonstrated its oral effectiveness. Since the 17α-configuration protected against degradation in the liver, it soon was well accepted for clinical use (54). However, due to its 17α-structure it was liver toxic, especially under long-term use or at higher doses (55), a feature shared by all 17α substituted androgens. In error some physicians considered liver toxicity a general feature of testosterone preparations, thus making testosterone a dangerous drug. Eventually, at least in Europe, 17α-methyl-testosterone became obsolete for clinical use after introduction of orally effective testosterone undecanoate in the late 1970s (see below).

As native testosterone proved to be ineffective orally, parenteral routes were explored. Subdermal testosterone pellet implants were the first to be investigated (56) and pellets are still in use today. Their application requires a small operation and harbours the risk of infection and extrusion. However, if enough pellets are implanted, they may provide substitution for up to half a year and are sporadically still used today (51).

When injected, testosterone has an extremely short half-life of only 10 min and as such is not suited for substitution purposes. Therefore, the third possibility of making testosterone clinically effective is esterification at the 17β-hydroxy group of the molecule, making it suitable for intramuscular injection. Testosterone propionate was the first of these esters marketed by Ciba and by Schering in 1936. However, this ester has a short half-life so that effective serum levels are reached for only 1–2 days.

Following the propionate ester, testosterone enanthate was synthesized by Junkmann (57) at Schering and marketed as intramuscular Testoviron® Depot injection in 250 mg doses, providing substitution for 2–3 weeks (58). However, the pharmacokinetics are characterized by transient supraphysiological peaks for a few days, followed by slow decline to levels below the lower limit of normal. Although patients do not appreciate these uneven swings in mood, activity and libido between injections, this remained the major testosterone preparation for substitution of hypogonadism for half a century and is still used today as a cost-effective alternative to more modern preparations.

Other parenteral routes were tested in the course of the steroid's history during which testosterone suppositories were marketed by Ferring (59), but yielded rather unpredictable serum levels (58) and are no longer commercially available. A more recent development in this area are bioadhesive buccal testosterone tablets, placed on the gingiva and resulting in effective serum levels if applied twice daily (60). However, due to low patient compliance they have never penetrated the market. The nasal route has also been explored (61) and has recently been revived for treatment of hypogonadism (62).

In the 1950s to 1970s, the pharmaceutical industry tried to modify the chemical structure of the molecule in order to disentangle the various effects and produce predominantly erythropoietic, osteogenic or anabolic

steroids. Although more than a thousand of these androgens were synthesized (63), it proved impossible to produce androgens with only one desired effect out of the wide spectrum of testosterone activities. Nevertheless, while some anabolic androgenic steroids (AASs) were clinically used, they disappeared again in the wake of evidence-based medicine. However, they retained a shadow existence for doping in sports and bodybuilding, potentially causing considerable undesired effects (64).

In the 1970s the orally effective testosterone undecanoate, absorbed from the gut via the lymph to avoid the first-pass effect in the liver (65) had been added to the spectrum of testosterone preparations available for replacement therapy. Initial clinical testing revealed that oral testosterone undecanoate was best absorbed with a meal, but the testosterone peaks were short-lived so that 3–4 capsules had to be taken during the day in order to produce effective serum levels (66). Oral testosterone undecanoate was introduced to the market worldwide in the late 1970s – except in the USA – and is still in wide use despite rather variable serum testosterone profiles.

In the 1970s the WHO Human Reproduction Program as well as the Population Council of the Rockefeller Foundation had identified male contraception as an unmet need for family planning and as a means against global overpopulation.

Hormonal male contraception based on the combination of testosterone and a progestin was at that time – and remains so to date – the most likely candidate for general use. However, the existing testosterone preparations required too frequent applications (for review of clinical trials see (67)). To overcome this deficiency both organisations started programmes in search of long-acting testosterone preparations. Under the auspices of WHO, testosterone buciclate was synthesized (68) and identified as a long-acting preparation, well suited for male contraception – and by the same token, also for substitution (69). However, no pharmaceutical company could be persuaded to develop this promising preparation further (70), so that in its ensuing clinical trials for male contraception, WHO switched to intramuscular testosterone undecanoate as described below and is now widely used for the treatment of hypogonadism.

Meanwhile, the Population Council had turned to 7α-methyl-19-nortestosterone (MENT) as its preferred androgen for male contraception. This androgen might have the advantage of lacking conversion to DHT and thereby have little effect on the prostate. As MENT has a rather short half-life, it was administered via subdermal silastic implants, delivering the active substance for a year – or perhaps even longer – thus being well suited for contraception as well as for substitution (71, 72). However, the company interested in further clinical research with this androgen dropped its plans in the wake of being taken over by another company not interested in the male.

In the mid-1990s, transdermal testosterone films applied to the scrotal skin became the first transdermal testosterone preparation in clinical use. Invented by Virgil Place (1924–2012) at ALZA in Palo Alto (CA) and first tested in clinical trials in Münster (73, 74), they showed excellent pharmacokinetic and clinical results and, for the first time, physiological testosterone serum levels could be achieved. Patients were very satisfied with this physiological pharmacokinetic profile, as long-term substitution revealed. However, physicians were reluctant to prescribe a medication to be applied to the scrotum, preferring a subsequently developed non-scrotal testosterone system (75). This, however, caused unpleasant skin reactions as it required an alcoholic enhancer to drive testosterone through the skin (1).

For this reason the advent of the transdermal testosterone gels in 2000 was welcomed for substitution (76). These testosterone-containing gels are applied to the upper arms, shoulders or abdomen. Those applied in the morning result in physiological serum testosterone levels, almost mimicking the normal diurnal rhythm. Precautions against interpersonal transfer have to be taken, mostly by covering the application sites with a T-shirt. Of the various gels available today, the one with a high 2.5% testosterone concentration can be washed off the skin shortly after application, thereby reducing the danger of contaminating children or women. It has also been tested for scrotal application. Because of the high absorptive capacity of scrotal skin, only 20% of the gel needed for non-scrotal application is required, making this form of application economically and ecologically desirable (77), but has not yet been licensed for that purpose.

Finally, in 2004, the intramuscular testosterone undecaonate preparation entered the market and soon achieved great popularity as a real testosterone depot preparation. Testosterone undecanoate, originally provided in oral capsules (see above), had been turned into an injectable preparation by Chinese investigators using tea seed oil as a vehicle. When the author came across it at a meeting in Bejing in 1993 (78), samples were brought to Germany, injected into monkeys and showed a surprisingly long half-life (79), which could be confirmed in volunteering hypogonadal men who all showed serum levels in the normal range for several weeks (80). When finally a company could be interested in this fascinating

preparation, tea seed oil was replaced by castor oil as vehicle and the injection intervals could be extended to 12 weeks of physiological serum testosterone levels (81, 82). Since 2004, this preparation available in 1000 mg ampoules has been licensed in over 100 countries . The latest approval was issued by the FDA in 2014 – however in 750 mg doses.

Outlook

Although effects of testosterone were known for ages, the actual molecule itself was isolated and synthesized only within the last eight decades, that is, in 1935. Since then testosterone has been in clinical use for the substitution of hypogonadal patients. Substitution modalities have been improved slowly by the production of testosterone preparations resulting in physiological serum levels as requested (52). The most recent developments, transdermal testosterone gels and intramuscular testosterone undecanoate, are close to this goal, but have not yet reached perfection. Clinical trials for selective androgen receptor modulators (SARMs) have started, but as their goal is selected, testosterone actions such as required for treatment of sarcopenia or osteoporosis, they will not be suited for substitution of hypogonadism, where the full spectrum of testosterone actions is required (83). So far, they seem to be suffering the same fate as androgenic anabolic steroids, even before they have been licensed for clinical use by the drug authorities. As unlicensed substances they are applied as performance–enhancing substances and for doping. Ultimately, for ideal substitution hope rests on human stem Leydig cells transplanted to hypogonadal patients (84). The goal for these patients – at least those with an intact hypothalamo–pituitary system – would be that the testosterone production required would be provided by the transplanted Leydig cells and would be self-regulated by feedback to LH. These patients would then become independent of exogenous testosterone preparations.

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The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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