Eugen Steinach: The First Neuroendocrinologist

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In 1936, Eugen Steinach and colleagues published a work that brought steroid biochemistry to the study of sexual behavior and, using synthetic androgens and estrogens, foreshadowed by an astonishing 4 decades the discovery of the central role of estrogen in the sexual behavior of male rats. We offer an English translation of that paper, accompanied by historical commentary that presents Steinach as a pioneer in reproductive neuroendocrinology. His work 1) established the interstitial cells as the main source of mammalian gonadal hormones; 2) launched the hypothesis that steroid hormones act on the brain to induce sexual behavior and that chronic gonadal transplants produce sexual reversals in physiology and behavior; 3) demonstrated the influence of sensory stimulation on testicular function; and finally 4) spearheaded the development of synthetic commercial hormones for clinical use in humans. Although its applications were controversial, Steinach's research was confirmed by many, and his concepts were applied to fields such as oncology and vascular disease. His contemporaries lauded his research, as indicated by his 7 Nobel Prize nominations. But Steinach's basic research was rarely acknowledged as the field flourished after 1950. The translation and our commentary attempt to reverse that neglect among behavioral neuroendocrinologists and clarify his central role as a founder of the neuroendocrinology of sexual behavior and reproduction. **(*Endocrinology* 155: 688–702, 2014)**

In 1936, Eugen Steinach, Heinrich Kun, and Oskar Peczenik published a paper (1) describing the role of estrogen in androgen-activated sexual behavior in male rats. That role of estrogen was rediscovered 36 years later by investigators who were unaware of Steinach et al's paper (2–4). His seminal discovery remained unnoticed as reproductive and behavioral endocrinology flourished in the 1950s, and it was found only recently (5). By contrast, in Steinach's time, its significance was immediately recognized by biochemists (6–8), oncologists (9), circulatory physiologist (10, 11), and by the Nobel Prize Committee (12); Steinach was nominated for the Nobel Prize 7 times. The international acclaim afforded Steinach did not extend to the United States, where physiological and behavioral research was, like the country as a whole, provincial and isolationist. Only Frank Beach (13) acknowledged the significance of Steinach's work:

"Steinach's animal experiments were carefully conducted and his theoretical concepts were highly original some of them so much so that their final test was delayed for half a century until necessary technical advances had taken place."

We offer here an English translation of the paper announcing this finding accompanied by our historical commentary, which presents Steinach as a pioneer in reproductive neuroendocrinology (Figure 1). Although his work was sometimes controversial, well before the chemical structures of steroid hormones had been uncovered, Steinach used chronic same-sex, cross-sex, and dual-sex gonadal transplants in mammals to explore the action of the putative hormones on the development and maintenance of sexual anatomy, gonadal physiology, and sexual behavior. We summarize these accomplishments as the

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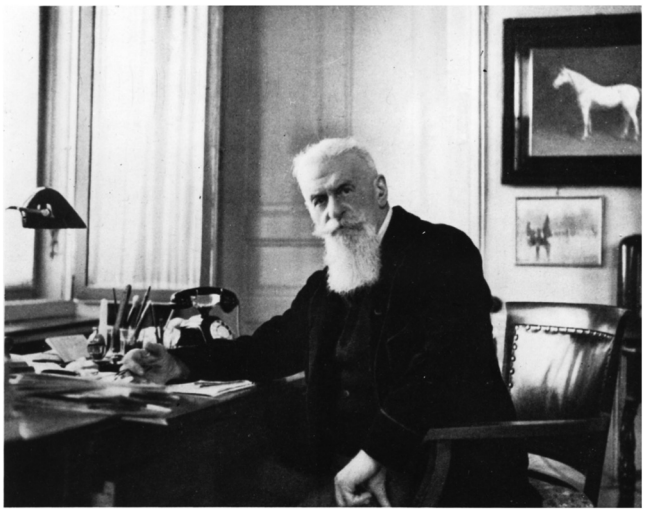

Figure 1. Eugen Steinach (1861–1944). Reproduced by permission of the Library of the New York Academy of Medicine.

scientific foundation of the discovery of estrogen's role in male mammals. By publishing the translation of Steinach et al's paper (1), *Endocrinology* takes a major step in recognizing a pioneer in the history of endocrinology.

Biographic Sketches

Eugen Steinach was born in 1861 to prominent Jewish parents in the city of Hohenems, in the Austrian province of Vorarlberg. Both his grandfather and father were physicians; his father a student of Ernst von Brücke, who advocated a physiology based solely on physico-chemical processes. After studying in Geneva, Vienna, and Innsbruck, Steinach became assistant to Ewald Hering at the German University in Prague, where he directed one of the first comparative physiology institutes. In 1912, he was appointed head of the Department of Physiology at the Institute for Experimental Biology of the Imperial Academy of Science, the so-called Vivarium. Steinach was well known in Vienna; Karl Kraus mentioned him in *Die Fackel*; with his wife Antonia (nee Thumim), he was acquainted with Arthur Schnitzler, and they were guests at the influential Salon of Bertha Zuckerkandl. They even interacted with Sigmund Freud, although probably skeptically, as a Freudian analysis of homosexuality elicited the response: "Freud ist ein Trottel" (Freud is a Meshugana) from one of the participants (14). In 1922, the Austrian Federal Film Agency supported the production of "The Steinach Film," a documentary on Steinach's research and the first sex education film (Supplemental Movie 1 published on The Endocrine Society's Journals Online web site at http://end.endojournals.org). When the Nazis seized power in Austria in March 1938, Steinach and his wife, who was also of Jewish descent, were on a lecture tour in Switzerland. The new regime destroyed Steinach's library and his research material and prevented him and his wife from returning to Vienna. Steinach's wife committed suicide in Zurich in September 1938, and Steinach died lonely and disillusioned near Montreux in May 1944 (14, 15).

Heinrich Kun, also of Jewish decent, was born in 1906, received his PhD with Steinach in 1931, and participated in some of the pioneering discoveries including the 1936 paper. Sadly, Kun fared even worse than Steinach, after the Nazi take over he was deported to an unknown concentration camp in Yugoslavia and his fate is not known, but presumably he died there (16).

Oskar Peczenik, born in 1898 and a student of Alois Kreidl in the Department of General and Comparative Physiology of the University of Vienna, became a member of Steinach's group in 1935. He was also forced to emigrate, working in the Department of Zoology at the University of Glasgow and in the Research Department at the Boots Pure Drug Co. Ltd. in Nottingham. Peczenik went to Israel in 1954, working in the Central Laboratories of the Ministry of Health, Joha, Jerusalem, on local anesthetics, the adrenocorticotropic hormone, and the toxicology of nitrogen mustard.

These were the people. What was the science of their times?

Prevailing Concepts of Bisexuality

A major idea of Steinach's is that each sex houses the potential to develop as the opposite sex: "Between a real man and a real woman there are innumerable others, some of which are significantly characterized as belonging to the 'intermediate sex'" (17). Steinach noted that the gonads of both sexes produce the hormones of the opposite sex and suggested that individual differences in physical characteristics and behavior, as exemplified by the quote, were the result of differences in endocrine balance (17).

The concept of bisexuality, which Steinach entertained, has a long history. The ancient Greeks believed that human life began as a single being created by the gods as a plaything. Concerned their creation was becoming too rambunctious and might challenge their power, Jupiter divided them using a hair, creating Halflings that would spend their lives constantly seeking their opposite so as to reunite and become whole (18). Subsequent myth and scripture regarded humans and animals as inherently bisexual such that each individual could be placed on a continuum between masculinity and femininity; that is, each individual contained the essence of maleness and femaleness, with the balance of the 2 properties specific to the

individual. This carried over into belief systems regarding how to predetermine and diagnose the sex of offspring (19). Further, many recognized a complementarity of these states that was essential for successful reproduction and a happy family life. It is important to emphasize that the brain was thought to be inherently bisexual, and philosophers emphasized the similarities between the sexes.

The realization that reproduction (union of gametes and their result) and sexuality (as sets of individual attributes and behavior) were different in origin was relatively recent, beginning in the late 1800s to early 1900s. Two Viennese psychiatrists, Richard von Krafft-Ebing and Sigmund Freud, and a Berlin otolaryngologist, Wilhelm Fliess, posited that the mechanisms controlling sexual behaviors were equally represented in "centers" in the brain and that a dynamic tension between these centers accounted for the degree of masculinity vs femininity an individual exhibited (20). This view was based on the then recent discovery that during embryogenesis, the ovaries and testes developed from a common anlagen, whereas the accessory sex structures developed from dual anlaga (21, 22). It was during this period that "bisexual" came to indicate "bipotential," meaning that the same anlagen would give rise to 1 of 2 states, rather than the same structure housing 2 distinct states. According to Freud, "the conception which we gather from this long known anatomical fact is the original predisposition to bisexuality, which in the course of development has changed to monosexuality, leaving slight remnants of the stunted sex" (23).

By the early1940s, it was well accepted that: 1) hormones change the individual's sensitivity to specific stimuli (eg, tactile, visual, and odor cues) and 2) although males and females exhibit characteristic behaviors, they have the capacity to display the behavior of the opposite sex. Indeed, Frank Beach in his compendium *Hormones and Behavior* (24) devoted the second chapter ("Reversal or Bisexuality of Mating Behavior") to this common observation. Beach stressed that heterotypical behaviors were never exhibited coincidentally, but alternately, and were elicited by the stimulus context and not by specific hormones. Hence, the roles of internal context (hormones) and external context (behavioral stimuli) were given equal weight. Subsequent research, particularly after 1959, tended to drop the latter in favor for the former (25).

Hormones, Development, and the Brain

Already in 1894, while still in Prague, Steinach made the pioneering observation that sexual behavior of male rats persists very long after castration (26), replicated only 30 years later (27). And as early as 1910, Steinach showed that injection of extracts of testes and of brain taken from male frogs in reproductive condition, but not those from nonreproducing males, restored sexual clasping in castrated frogs (28). Unfortunately, there is no information on how the extracts were prepared, the records may have been lost when the Nazis destroyed Steinach's laboratory materials. These findings allowed him to challenge those who believed that the clasping reflex was controlled by nerve impulses from the gonads, arguing instead for the specific effect of chemicals produced in the testes on the brain. Interestingly, the only other control "organ juice" that showed any effect was an ovarian extract. This suggested to Steinach that female gonads produce a "relative" of the substance secreted by the testes (28).

In the same paper, Steinach (28) inferred that normal copulatory behavior also required the action of hormones on the developing brain. Three- to 6-week-old rats were castrated, and both testes were repositioned either in their stomach musculature or pelvis. The animals showed full masculine somatic development, and as adults, they behaved like intact breeding males. Steinach concluded that the development of masculinity resulted from the action of hormones on the central nervous system, enabling males to respond to signals from females. Because in frogs the behavioral effects occurred more rapidly than those affecting peripheral tissues, Steinach asserted that the first effect of the secretions was in the brain, not in peripheral tissues. He termed the process the "erotization (Erotisierung) of the central nervous system." Steinach's 1910 work offers perhaps the most precise early experimental evidence that hormones shape the development of brain and behavior in frogs and mammals.

The Importance of Sensory Stimulation

In the late 19th century, as Steinach began his research, the nervous system was thought to control the body. Steinach changed that paradigm by demonstrating the autonomous effect of endocrine secretions, while maintaining the concept of bisexuality (29). Although Steinach's early work falsified the established hypothesis that nerve impulses from the gonads release ready-made sexual reflexes, he worried that others might oversimplify his alternative hypothesis: that behavioral development was guided by secretions. Stressing the effect of secretions did not mean that the nervous system played no role. In part to explore neural control of the secretions, Steinach began isolation/stimulation experiments in 1924 to show that the supposed "dethroning" of the nervous system was an erroneous oversimplification. Indeed, the pattern of questions addressed by his research program (including work on the

pituitary begun in 1914) shows that Steinach believed that in mammals, hormones, brain, and behavior functioned in dynamic interplay with one another and with the environment.

To demonstrate this, mature male rats were raised for weeks in isolation from females. When at 4–5 months of age males were exposed for a few minutes to receptive females, all responded with strong sexual pursuit. With longer periods of isolation, however, the intensity of males' reactions to receptive females became weaker; eventually they lost their "libidos," their prostates and seminal vesicles atrophied, and spermatogenesis ceased. Steinach then modified their cages, adding small compartments that gave the males olfactory exposure to, but no direct interaction with, receptive females. Some males were even blinded to rule out visual stimulation. After 2 or 3 weeks of exposure, the barriers were raised, and the males actively pursued the females, which responded with lordosis and mating. Histological analysis of the males' gonads showed both live sperm and numerous interstitial cells filled with secretory granules. The males' normal drive states had been restored through sustained exposure to the odor of receptive females. To Steinach, this meant that the nervous system played a critical role in the integrity of the sex drive. The libidoless males had undergone a "new erotization (Neuerotisierung)" (30). Steinach concluded: "The nervous, that is to say, psychic processes exercise a powerful controlling influence on the inner-secretory activity of the gonads through which bodily and psychological maturity can be automatically protected from regression or possibly from persistent depression" (30). He speculated that the mechanisms involved increases in blood flow mediated by the autonomic nervous system's effect on the gonads. He also noted the possible role of the anterior pituitary, which, in 1928, he and Kun had shown could cause similar changes in the sex drive (31).

In all of his work, Steinach repeatedly used the word "psychic" to refer to both brain and behavior. Plus, because neither the cellular origins of the chemicals nor their chemical structures were known before 1930, Steinach correlated behavioral changes with changes in gonadal cellular morphology using behavior to infer the impact of secretions on sexual development, brain, and behavior. But in the 1920s, behavior was not yet considered a reliable indicator of physiological processes. As a result, some skeptical scientists disregarded or challenged his research. That work nonetheless had a pioneering effect on behavioral neuroendocrinology well before many scientists accepted behavior as bioassay of brain processes.

Transplanted Gonads, Behavioral Hermaphrodites, and the Woman Question

Steinach pioneered castration plus gonadal transplants and published extensively using this method (eg, Refs.32–34). He completed heterologous transplants, as well as crossed-sex, and dual transplants in males and in females. All procedures produced clear changes in masculine or feminine behavioral development that matched the character of the implanted gonad. Importantly, in all experiments, histological analysis of the cellular structure of the gonads showed large increases in interstitial cells coincident with the changes in behavior. And when both ovaries and testes were positioned in the stomach musculature of the same animal, male vs female behavioral attributes alternated periodically in a single animal. The alternating phases began with a variety of male behaviors and shifted to a phase of female behaviors, which lasted for 2–4 weeks. Steinach expressed surprise that the nervous system responded so markedly to the fluctuations in hormone levels and that the neurobehavioral changes recurred throughout the rest of the animals' lives. In the third edition of Arthur Biedl's ground-breaking textbook, *Innere Sekretion* (35), the author noted that Steinach's transplants had established a sexually specific effect of gonadal secretions on the development of masculine and feminine behavior. Steinach had created masculine females, feminine males, and, sometimes, behavioral hermaphrodites at just the time when many people resisted the great changes then occurring in Western sexual mores. His findings must have instilled the fear of social chaos in many, particularly as regards the "women question," the possibility of "physiological hermaphroditism," and nature of homosexuality. For a time the German sexual reformer Magnus Hirschfeld used Steinach's experiments to argue for gay rights, stating in a pioneering film on the topic: "His experiments prove that sexual intermediates who seemingly differ only on a psychological level are in fact physically determined" (36).

The Interstitial Cell Question

In the 1920s, the vasoligature procedure became associated by many in the United States with Steinach's advocacy of "rejuvenation," a forerunner of hormone replacement therapy promoted in the United States by the New York physician Harry Benjamin (37). But in the preceding decade, Steinach's research program was devoted to what in Central Europe was called the "interstitial cell question." This was the scientific debate surrounding the func-

tion of gonadal interstitial cells, which were discovered in the testes by Franz Leydig in 1850. At issue in the early years was which tissues in the testes produced the putative hormones. Were the interstitial cells secretory cells or merely connective tissue that might absorb the metabolic products of other processes? Two camps emerged that divided physiologists who were interested in sexual function, from cellular morphologists more interested in morphogenetic tissue origins. The debates usually pitted those who argued that in males sperm were the source of the sexual chemicals (they viewed the interstitial cells as connective tissue) against those who, following the French anatomists Paul Ancel and Pol Bouin, who proposed the idea, and Steinach, who confirmed it experimentally in mammals, instead accepted the interstitial cells as secretory cells that produced the gonadal hormones shaping sexual development.

Although the interstitial cell question was still in debate and many cytologists supported the connective tissue hypothesis, by the 1920s Steinach had become the leader of a Central European school of reproductive endocrinology advocating the interstitial cells as the source of the hormones involved in shaping masculinity and femininity in birds and mammals. His histological analyses regularly showed that the behavioral changes produced by transplants were accompanied by the proliferation of interstitial cells in the repositioned gonad (sometimes with few or no spermatozoa present). But Steinach's science was embedded in social and medical disputes that added to the controversy surrounding his work. He was devoted to clinical application as well as basic science, and his strong advocacy of rejuvenation, a surgical procedure that he developed to increase sex hormone secretion and thereby invigorate the elderly (37, 38), was linked to the interstitial cell question. The procedure, which was sometimes termed "being Steinached," was overly popularized in both literature and in advertisement in America, a practice that Steinach himself abhorred. In addition, Hirschfeld used Steinach's experimental and clinical evidence to argue for the decriminalization and emancipation of male homosexuality (39). At the time, much European psychiatry still characterized homosexuality as a degenerative neural disease. Hirschfeld and Steinach instead presented homosexuality as an unusual developmental state originating in normal endocrine processes, a highly controversial view.

Even in the face of controversy, however, by the mid 1920s, Steinach's experimental conclusions on the interstitial cell question had been elaborated by several others, including Marianne Stein, Alexander Lipschütz, and Knud Sand. Some scientists, nonetheless, rejected the endocrine function of the interstitial cells well into the 1930s and 1940s (40). And resistance to the idea prevented Steinach from getting the Nobel Prize in 1921 (see below).

Synthetic Sex Steroids and the Contraceptive Pill

Steinach's research on the effects of transplantation of the gonads fuelled the interest of the pharmaceutical industry in preparing ovarian and testicular steroids for clinical use. Steinach and Walter Hohlweg started developing synthetic gonadal steroids with the Schering research laboratory in Berlin in 1923, and in 1928, when Hohlweg moved to Berlin, the first oral estrogen, Progynon, was launched on the market (Figure 2) (41). In the same year, Steinach and Kun proposed pituitary-ovarian feedback (31), and simultaneously, Ludwig Haberlandt described the principles of hormonal contraception (41). These were times of rapid development; in 1932, Hohlweg and Junk-

Figure 2. The ad used for the launch of Progynon by Schering in 1928. Reproduced with permission of Bayer AG.

man (42) demonstrated the role of the brain in the pituitary-ovarian feedback that Steinach and Kun (31) had suggested, a major step ahead in the history of neuroendocrinology (43).

Very quickly, Adolf Butenandt elucidated the structure of the first steroidal sex hormone (44), Hohlweg and Hans Herloff Inhoffen synthesized the powerful oral estrogen, ethinylestradiol (45), and subsequently, Inhoffen developed ethisterone (Proluton C), the first oral gestagen (46). By 1939, the principles of contraception were understood, the structures of the decisive hormones had been analyzed, a relatively economical synthesis based on cholesterol was available, and an oral estrogen, as well as a progestin preparation, had been developed (47). Thus, all prerequisites for a contraceptive "pill" were fulfilled. However, the project was prevented by the Nazis, who rejected birth control.

The translated paper shows the power of these newly available synthetic sex hormones. Steinach, who had moved the field forward with pioneering mammalian transplants, also helped pioneer the methodological shift from transplants to chemical extracts and synthetic hormones. And, in the process, he discovered the role of estrogen in androgen-activated sexual behavior in male rats.

Steinach's Nobel Prize Nominations

Steinach was nominated for the Nobel Prize for Physiology or Medicine 7 times between 1920 and 1938 (12), and he was a leading candidate on 4 occasions, competing with 12, 18, 25, and 26 other nominees, including Edgar Adrian, Walter Cannon, Otto Loewi, Ivan Pavlov, Charles Sherrington, and Otto Warburg.

Already in 1921, the nominator pointed out that Steinach had demonstrated the behavioral effects of gonadal transplantation and suggested that the interstitial cells mediate those effects. With the 1927 nomination, it was noted that the role of the interstitial cells had been confirmed and that the gonads exerted sex specific and antagonistic effects, which also had been confirmed by several laboratories. Additionally, the clinical implications of the work were mentioned. In 1930, Steinach's contribution to the development of the synthetic ovarian hormone, Progynon, was mentioned, and in 1938, the nominators pointed out that Steinach's research was so well known around the world that only a short presentation was necessary. This prompted an evaluation by Göran Liljestrand, professor of pharmacology at the Karolinska Institute and secretary of the Nobel Prize Committee for 42 years (12), longer than anyone else has served as secretary and a period over which he met quite a few prize winners. Liljestrand pointed out that Steinach had been nominated several times, that he clearly deserved to be awarded the prize, and that he barely missed it in 1921 because 2 members of the Nobel Committee expressed skepticism concerning the role of the interstitial cells and the clinical applications. Liljestrand outlined Steinach's main findings. Thus, Steinach had managed to "malemake" females and "femalemake" males and "numerous replications have shown that these observations are correct." He further wrote, "it appears obvious to me" that the "lability" of sex and the existence of "*bipotentiality . . . is a fact of fundamental importance*" (italics added). In fact, Liljestrand considered the discovery of bipotentiality to be Steinach's most important finding. Liljestrand commended Steinach for developing "a lively scientific activity" despite his advanced age, including studies of the "*sensory control of sexual behavior and the role of estradiol in potentiating the effect of androgens in the control of sexual behavior*" (italics added). Liljestrand wrote, "*addition of a minor amount of female hormone reduced the dose of male hormone that was otherwise necessary*" (italics added), and he concluded that Steinach deserved the Nobel Prize long ago but that he was now too old (Steinach was 77) and "the value of his contribution has not undergone a substantial increase" since 1930.

Concluding Remarks

The birth of the new "glandular science," ie, endocrinology, instilled optimism to the extent that the hope for the solution of virtually all clinical problems was created (29). Interestingly, this hope is remarkably similar to today's hope that the new science of "personalized medicine" will be similarly effective (eg, 48). Unsurprisingly, Steinach's advocacy of rejuvenation and his promotion of clinical treatments with synthetic hormones did not quite live up to expectations. This, however, should not subtract from the fact that Eugen Steinach made discoveries of fundamental importance in the endocrinology of reproduction and that he is clearly a founder of the neuroendocrinology of sexual behavior. He may even be the first neuroendocrinologist.

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Contributions to the Analysis of the Effects of Sex Hormones. Animal experiments and clinical investigations[1]

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Contents:

1. Increased cerebral blood flow. The differential effect of male and female sex hormones.

Progress in the chemistry of sex hormones presents physiology with the task of comparing the effect of the isolated and synthetically derived products with that of the physiological testicular secretion. The overall effect of the physiological hormone is revealed by the results of experiments with ligation of the vas deferens, which causes an increase in the level of the hormone. In a recent review, Steinach (1) pointed out and confirmed that in addition to having a sex specific effect, the physiological testicular hormone also plays an important role in non-specifically increasing blood flow. Steinach found that about two weeks after vasoligation, cerebral blood flow increased as revealed by staining experiments. Thus, after intravenous injection of Alizarin blue, the brain of vasoligated senile or castrated rats was markedly stained in comparison with the brain of rats that were not vasoligated because a reduction of vascular tone facilitated the capillary penetration of the stain. This was not merely a matter of increase permeability, but rather the consequence of enhanced blood flow as demonstrated by the following experiments. If, for example, cerebral blood flow is enhanced by the intravenous injection of adrenaline,[3] more dye passes through the blood-brain barrier and so the brain gets more markedly stained. If, however, one generates vasoconstriction, by stimulating the sciatic nerve, that is to say producing cerebral ischemia, no staining is observed. The degree of cerebral staining is thus related to the functional state of the blood vessels of the brain and therefore cerebral blood flow.

The next task was to compare the results produced by vasoligation with those obtained with the male or female sex hormones.

The experiments were performed on at least 8–10 months old castrated rats. Some animals were castrated several months previous to and others were castrated immediately before the experiment. It has already been found that the post castration period plays no role for the outcome, instead the age of the animal is crucial. Younger animals show strong staining of the brain after injection of Alizarin blue without hormone treatment with no difference from control animals.

[1]Beiträge zur Analyse der Sexualhormonwirkungen. Tierexperimentelle und klinische Untersuchungen. *Wien klin Wschr.* 1936;49:899–903. Permission to translate this paper into English was kindly granted by Ms Nel van der Werf of Springer Verlag. There are instances where we have been unable to trace or contact the copyright holder. If notified, the publisher will be pleased to rectify any errors or omissions at the earliest opportunity.

[2]"Functionsprüfung" or "test" in the original text refers to tests of sexual behavior.

[3]As opposed to other vascular territories, adrenaline causes brain vessels to dilate.

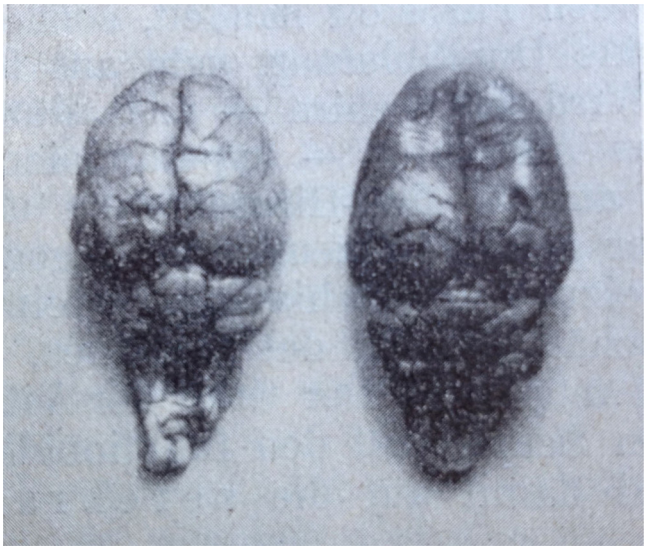

Figure 1. Effect of chemically produced sex hormone on cerebral blood flow (Alizarin blue stain). Brain of animal treated with androsterone (left), no staining. Brain of animal treated with Progynon (right), strong staining.

Both common carotid arteries were simultaneously ligated before the infusion of the dye, thereby obstructing hemispheric blood from this source and producing a uniform circulation from the vertebral artery, which can replace the carotid system in young animals but is insufficient to stain the brain of old rats. The simultaneous ligation of the carotid arteries has the purpose of avoiding irregularities and differences in the staining of the hemispheres. One milliliter of a freshly prepared 5% solution of Alizarin blue (0.3 g Alazarin blue obtained from Dr. G. Grübler & Co was dissolved in 6 ml distilled water with addition of 0.03 ml 1 mol/L NaOH to adjust pH) was infused into the vena cava inferior immediately after the ligation in both hormone-treated and control castrates. The animals were killed 5–10 min later and their brains were finally exposed.

As a start, we used male hormone prepared from urine extract; in subsequent experiments we used synthetic androsterone, androstandiol and testosterone. Daily injections were given for 9 days, in some cases for 20 days and the injection of Arizarin blue was given the day after the last injection. The total injection of urine extract was 225–325 H.E.,[4] 45 mg of androsterone, 50 mg of androstandiol and 25 mg of testosterone.

For the parallel experiments with female hormone we used Progynon benzoate, injected daily in total doses ranging from 1000 to 15000 M.E.[5] over the same period of time as above.

Every injected animal had a control matched for age and body weight.

Injection of male hormone free of estrogenic substance had no effect on the staining of the brain, both treated animals and their controls were weakly stained. Only urine extract, which has a small amount of estrogenic substance, had a somewhat clearer staining effect which, however, was not comparable to that of vasoligation.

The results were consistent in all experiments in which the techniques worked. We must therefore conclude that male sex hormones have no effect on cerebral blood flow.

However, administration of female hormone (Progynon benzoate) had the same effect as that of physiological testis hormone in the experiments using vasoligation (compare to ref 1, Figure 8). The brains of the injected animals were stained dark blue to violet blue, in stark contrast to the weakly colored brains of the control animals. Even the lowest dose of 1000 M.E. had a positive effect after 6 days of treatment. Figure 1 shows the difference between the effect of androsterone and Progynon.

The difference between the effect of male and female hormone on blood flow can also be clearly demonstrated in other tissues, for example in albino rats. The influence of the female hormone on blood flow can be shown without staining in living animals and indeed in the blood vessels of the eye without using a microscope. Figure 2 and 3 clearly show the difference between the ciliary blood vessels of an untreated animal and an animal injected with female hormone.

[4]Method according to Scholler-Butenandt. H.E. = "Hahenkammeinheit" (cockscomb unit), a measure of hormon-activity of androgenic preparations at the time.

[5]M.E. = "Mäuseeinheit" (mouse unit).

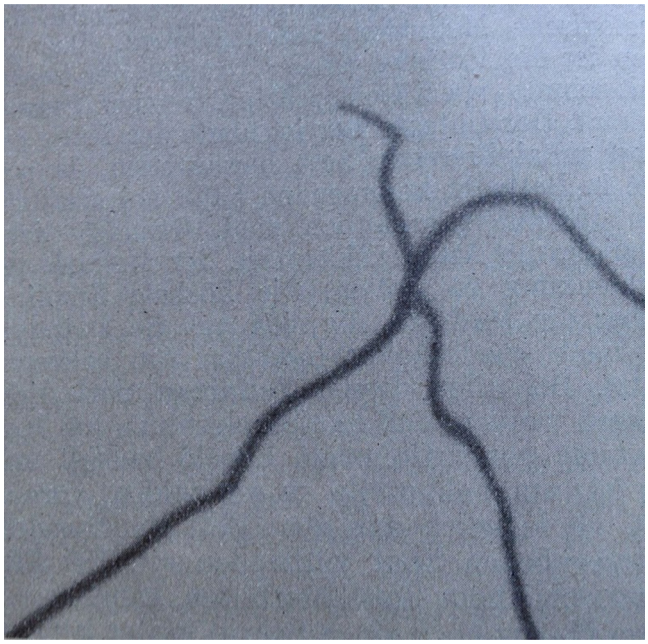

Figure 2. Ciliar stain of untreated castrated male (Albino rat). Drawn after photograph taken from living animal.

These experiments show that the physiological testicular hormone (experiments with vasoligation) and the isolated female hormone have similar effects on cerebral blood flow, as opposed to the chemically produced male sex hormones (androsterone, androstandiol, testosterone) which have no effect in the doses used.

2. Behavioral testing of the male hormone and the combination of male and female hormone. Superiority of the combination. (Behavioral assay in the rat.)

In the experiments described above we have started a physiological analysis of the testicular secretion with the statement that the isolated female hormone carries a factor that increases blood flow, which strengthens and generalizes

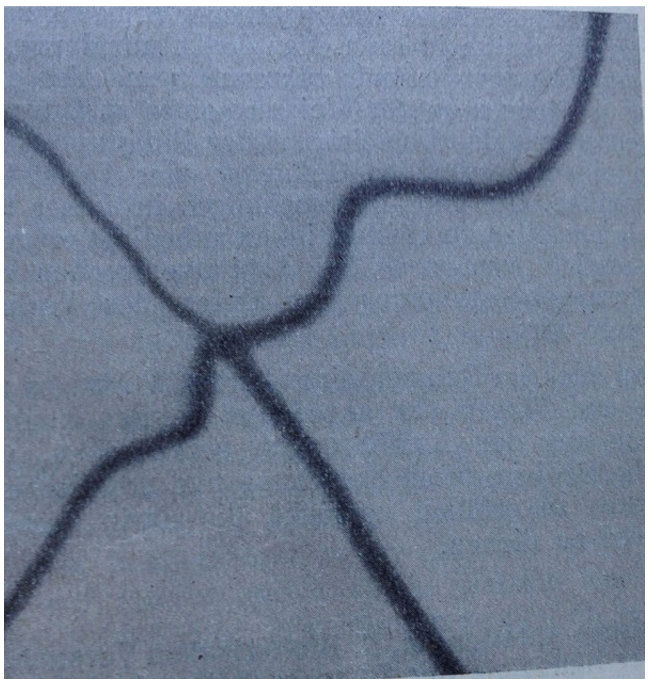

Figure 3. Ciliar stain (site of iris corresponding to that of Figure 2 at the same magnification) of castrated male (Albino rat) treated with female hormone. The vessels appear more clearly than in Figure 2. Drawn after photograph taken from living animal.

Table 1. Behavioral Assay of Male Hormones

Hormone	Injections	Total Dose (mg) Urine Extract (H.E.)	+ Ejaculation − No Ejaculation
Urine extract 5	50	+	
		30	−
Androsterone 5	10	+	
		5	−
Androsterone acetate	2	10	+
		5	−
Androsterone benzoate	2	5	+
		3	−
Dehydroandrosterone	5	25	+
		20	−
cis Androstanol-methylcarbinol acetate	2	3	+
		1	−
Androstanediol benzoate	2	3	+
		1	−
Testosterone	5	1	+
		0.5	−

the hormonal effect of the testicular secretion. Therefore the question arises if this factor also facilitates a specific psychological function. The possibility that the female hormone is involved is supported by the observations by Freud (2), who noted that the growth promoting effect of male hormone on the seminal vesicle in the rat was enhanced with Menformon[6] and that therefore the female hormone assumes a "pacemaker effect". However, our case does not deal with growth or a morphological effect but with a specific psychological function.

To study this problem, we have developed an easily controllable and manageable method of a psychosexual function based on the effectiveness of the male hormone in eliciting sexual behavior in castrated male rats.

Steinach and Kun (3) have already shown that chemically produced male sex hormone restores the typical somatic sex characteristics and the sexual behavior to normal levels as effectively as testicular transplantation in castrated rats as well as in eunuchoidal and senile rats. Nevertheless, the otherwise useful late castrate is not suitable for the present purpose as the effect of the male sex hormone on the brain persists many months after the loss of the gonads,[7] as has already been noted by Steinach (4). However, decades of work on these animals have shown that this is not the case when the rats are castrated immediately after functional maturity (ie, at 3–4 months of age) and not exposed to estrous females. In this case, sexual behavior declines in weekly tests already in 3–6 weeks. Castrates, so tested, are a suitable, highly sensitive model for testing the effects of hormones.

To study the resumption of sexual behavior in these animals it is obviously necessary to use female rats in full heat. This is easily achieved by a single injection of 3000 I.U.[8] Progynon benzoate, female rats so treated remain sexually receptive for three weeks (5). A female in heat is introduced to the test male for 10–15 minutes. The ordinary signs of the sex drive, ie, pursuing and attempts to mount, are not sufficient; the complete copulatory act is required. The animals can be retested for sexual behavior after a pause of 2–4 weeks.

The hormones to be tested were injected in their free (non-esterified) form (Table 1), a fifth of the total dose was injected daily over five days. Because the esterified forms are absorbed differently, these were injected in two part doses at three day intervals. The effect occurs between 2–7 days after the last injection. The threshold dose is defined as the lowest total dose that restores sexual behavior within this period of time.

We have tested a series of male sexual hormones using this behavioral test. The results are displayed in the tables; the threshold dose is always shown by the +sign, doses below threshold are shown by the -sign.

Thus, in addition to the cock comb test there is now a behavioral assay of the effect of hormones in male mammals.

We thank Prof. Dr. W. Schoeller (Headlaboratory Schering-Kahlbaum, Inc, Berlin) for kindly making these tests possible.

[6] Menformon = the original name of synthetic estrogen, subsequently re-named Progynon.

[7] "die Erotisierung der Hirnzellen" in the original text refers to the fact that sexual behavior had been previously shown to persist long after castration, even for a year in individual rats.

[8] I.U. = I.E. = international unit, eg, in the case of "Progestin Degewop" = 5 mg.

This behavioral test made possible an affirmative answer to the question of whether a female hormone is involved in the development of male psychological functions. This occurred with the combined administration of male and female hormone. The procedure was as follows: 500 I.U. of the female hormone (Progynon benzoate) were added to doses of male hormones which were found ineffective in the behavioral assay, the dose of the male hormone was then gradually reduced until the combination also became ineffective. The difference between the threshold dose of the male hormone and the threshold of the combination gives an index of the reinforcing influence of the female hormonal component, as clearly shown in Table 2.

The promoting effect of the female hormone was marked in all combination experiments. Addition of 500 I.U. of Progynon benzoate reduced the threshold dose 2.5–3 fold, and even 10 fold in the case of testosterone. It needs no special mention that Progynon given alone in control experiments was completely ineffective.

One will not be mistaken in ascribing the enhancement of the effect on the central nervous system to the action of the female hormone on blood flow, as demonstrated in the staining experiments described above. The increased circulation in the central nervous system might result in an increased excitability of the neural apparatus, making it sensitive and now responsive to the otherwise sub-threshold dose.

The report of Miescher et al. (6) should be also be mentioned in which they found that the effect of testosterone on the weight of the seminal vesicles and the prostate was enhanced by addition of carbon-acids, eg, palmitic acid, especially so when the hormone and the acid were injected in combination or at adjacent sites. If, however, the injections were given at widely separated sites, the effect is lost. From these results, the authors concluded that the carbon acid improved the absorption of testosterone. In the view of the authors, the X-factor (7, 8) should act in the same manner.

This raises the question if the increased effect of Progynon in our combination experiments could be interpreted in a similar manner. This possibility can be excluded, because we administered male and female hormone at distant sites from each other and on different days. Our findings thus speak against the assumption that Progynon merely increases the absorption of the male hormone, and argues instead strongly for the activation of the target organ (central nervous system).

3. Conversion of the male sex hormone in the male organism.

The comparative experiments above showed that whereas the female hormone increased cerebral blood flow, chemically produced male hormone did not. Because testicular extract, that is to say the physiological male hormone, is very potent in increasing and maintaining blood flow it must be assumed that it contains a factor that has a similar action as the chemically produced female hormone. Now, the question forces itself: **how does the male organism normally produce this "female" factor?**

From the work of Dohrn (9), Frank (10), Hirsch (11), Loewe (12), and others it is known that an estrogenic substance can be measured in the testes. Whether this substance is derived from dietary factors, ie, from an exogenous origin, seems still very controversial (13–15). We have, however, considered the possibility that there is a conversion process and indeed the "female" factor is produced from male hormone.

We have determined the excretion of female hormone in urine from normal and castrated male rats and compared the collected values with those obtained after administration of a excess amount of male hormone. In order to obtain a measurable amount of hormone, values from four animals were pooled. Urine was collected over 24 hours in metabolic cages to get such a pool. Apart from the ordinary grain, the animals were fed milk through an external tank for the duration of the experiment. The experiment can be extended over weeks in this way without harming the animals. The

Table 2. Behavioral Assay of Male and Female Hormones Combined

Hormone	Threshold Male Hormone (mg) Urine Extract (H.E.)	Threshold Combination Male and Female Hormone (mg) Urine Extract (H.E.)	Reinforcement of Effect by Female Component
Urine extract	50	20	2.5
Androsterone	10	4	2.5
Androsterone benzoate	5	2	2.5
Androstanediol benzoate	3	1	3
Testosterone	1	0.1	10

Table 3. Conversion of Male Hormone

Hormone	Total Dose	Series	Excretion			
			Days	Injection of Male Hormone R.U.	Untreated R.U.	Difference Injected-Untreated
Urine extract	200 H.E.	4 intact	11	23	11	12
Urine extract	200 H.E.	4 castrates	2	15	2	13
Androsterone	40 mg	4 intact	3	22	3	19
Androsterone	40 mg	4 castrates	5	32	5	27

collected urine, which was the result of the addition of 60 to 90 ml milk, was vacuum-evaporated to 4 ml and evaluated by examining vaginal cornification after injection of two equal parts in the morning and afternoon in castrated females.

As a start, we used the previously mentioned urine extract as male hormone and the androsterone, which is free of Follikulin.[9] Animals were injected with 12.5 H.E. of urine extract or 2.5 mg androsterone for four days, and so each pool of four animals received a total of 200 H.E. and 40 mg respectively.

The normal excretion of estrogenic substance from a pool of four untreated animals was at the most 1 R.U.[10] (rat unit), in this regard, one should note that these castrated untreated rats behaved in the same way as the intact males. The results of this experiment are put together in Table 3.

It was possible to detect precipitation of more estrogenic substance as a result of inclusion of male hormone in all four pools. This occurred within a shorter period of time in the castrated than in the intact males. The possible objection that the administered urine extract was not sufficiently clean and that the result was caused by presence of female hormone in the extract is excluded by the experiment with androsterone, which contains no measureable amount of female hormone.

It follows from these results that the male organism is capable of converting excess male hormone into material with estrogenic activity. It is not possible to decide the site of this conversion process. However, it is clear that the testes alone are not responsible, because the conversion occurs in the castrated males. The product of the conversion is also unclear, as the ability to induce vaginal cornification in the castrated rats does not provide compelling evidence for Follikulin. We do not consider ourselves to be justified, therefore, to say that the conversion product is "female hormone" or "Follikulin". But the experiments on the brain showed that female hormone (Progynon) has a specific estrogenic effect in increasing blood flow. The male organism can thus produce the necessary "female" factor though a conversion process.

4. Clinical trial of the use of the combination of male and female hormone.

As the experimental analysis demonstrated that the "female" factor is the main carrier of the effect of the physiological testicular secretion on blood flow, we went on to examine this knowledge in clinical experiments. For this purpose, we examined the effect of the combination of male and female hormone with that we have obtained with Proviron alone (16). The results should be viewed with reservation as the material apparently is too limited to allow a final judgment.

We have embarked on two paths to convince ourselves of the influence of the female hormone (Progynon B, 500 international benzoate units injected in oil solution). Firstly, cases were treated with Proviron with no success, then, after an interval of several months, they were treated with the combination of Proviron and Progynon. Secondly, we have treated some patients with combined injections after they had received 10 doses of Proviron. The patients should obviously not be aware of the change in therapy. Four injections were given daily over 4–6 weeks.

Observations to date have revealed the favorable influence of the addition of Progynon, especially in general and in particular in the psychological effects: sudden improvement of depressive mood and persistence of a happy mood; striking revival of the energy to work; mastering of difficult situations; increased resistance to stress; cessation of insomnia and headache. The increase in blood flow after Progynon was noticeable in the palms.

The combined treatment has also had an effect on impotence in a few patients who did not respond to Proviron. We will just briefly refer to a single case to provide an example of the combined effect. The patient was 27 years old with a two year history of erectile failure. 24 injections of Proviron caused an improvement only in that short lasting erections

[9]Follikulin = the hormone produced by the "follikulären Apparat der geschlechtsreifen Frau" = the hormone produced by the ovarian follicle of the sexually mature woman.

[10]R.U. = rat unit, eg, in the case of an estrogen = one tenth of a microgram.

occurred, which were insufficient for performing the act. About one year later, when this limited success was gone, a new treatment was started, at this time with the combination. After 19 injections the patient reported a normal full coitus; his potency remained unabated over the following three months.

It should be pointed out that in many cases Proviron alone had the desired effect, which we have indicated in the above-mentioned work. But in those patients who were selected for the combination treatment, prior treatment with Proviron had no effect. We can therefore ascribe the improvement to an effect of Progynon. We have the impression that the marked effect of Progynon on blood flow enhances the therapeutic effect of Proviron.

Summary

The effect of the isolated female sex hormone (Progynon benzoate) on blood flow can be demonstrated in males through staining of the blood vessels of the brain. The effect of the female hormone is in accord with that of the physiological testicular secretion (experiments with vasoligation). However, chemically produced male hormones (urine extract, androsterone, androstanediol, testosterone) have no effect.

We also examined whether female sex hormone can exert the same effect as the male hormone on a specific psychological function. To test this possibility a method was developed, which determines the efficiency of male hormones in eliciting sexual behavior in castrated male rats (a behavioral assay in mammals). Testing a number of male sex hormones demonstrated the usefulness of this behavioral assay (Table 1).

The activating effect of the female hormone could be determined by use of the behavioral assay. The combined administration of male and female hormone brought about a 2.5 to 10 fold decrease in the threshold dose of the male hormone, thus by adding female hormone, the dose of the male hormone necessary to elicit the psychosexual function can be reduced by 2.5–10 fold (Table 2). This stimulatory effect may be explained by an increased sensitivity of the nervous system induced by the female hormone.

As demonstrated in the staining experiments on the brain, the testicular secretions, ie, the physiological hormone, can generate sufficient increase in blood flow, in contrast to the male hormones tested. From this it can be concluded that the testicular secretion contains a factor that acts as chemically produced female hormone. The question as to how the male organism provides the "female" factor was the subject of the third series of experiments.

After determining the excretion of estrogenic substance in the urine of intact and castrated males these animals were injected with male hormone. This resulted in the precipitation of a considerable amount of estrogenic substance, suggesting that the "female" factor is produced by conversion of the male hormone. As the precipitation also occurred in castrated animals, it cannot be doubted that the conversion takes place in the organism without the testes.

Based on the evidence that the main carrier of the effect of testicular secretions on blood flow is the "female" factor, we used the combination of male and female hormones in clinical experiments. In view of the beneficial experiences with the combined treatment with respect to the general effects but in particular the psychological effects and the effect on sexual behavior, the end result is that the potent effect of Progynon on blood flow can intensify the therapeutic effect of Proviron.

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