Chapter 3

The Search for Physiological Functions

It was around 300 years ago that people first noticed that this small organ, firmly attached to the top of the kidney and yet apparently unrelated to it, had functions that are directly and intimately connected to the processes of life. There were rapid advances in research into this mystery, particularly in France, where many of the greatest minds in physiology of the time were to be found. The topic struck a chord with physicians in Britain as well, and a succession of significant discoveries was reported.

1. Submissions to Montesquieu's Essay Competition

In November 2005, the Royal College of Physicians in London hosted an exhibition of the major achievements in the history of hormones, showing a timeline of the history of hormone research (3-1). The starting point was the first comprehensive description of human glands, made in 1656 by Thomas Wharton, who appeared in Chapter 2. He described the endocrine glands at the front of the neck region, which he named the thyroid glands.

In 1716, 60 years after Wharton's achievement, an academic society was involved in an activity of great interest. This was a competition organized by the Bordeaux Académie des Sciences, in the region of France known worldwide for its red wine, which collected papers on the topic "*Quel est l'usage des glandes surrénales?*" A great many papers were submitted to Montesquieu, who presided over the competition.

Montesquieu, whose real name was Charles-Louis de Secondat, had graduated from the law department of the University of Bordeaux, and had been a judge at the High Court in Bordeaux for two years at the young age of 27. He was selected to join the *Académie française*, which is limited to just 40 members, in 1728, at the age of 39 (3-2).

According to a record of a lecture by Professor Schäfer of the University of London, in which he spoke about the competition, it appears that the planning of the competition was most likely carried out by Montesquieu (3-3). He is known throughout the world as one of France's leading philosophers, political thinkers, and writers, and he submitted a number of scientific reports on physics, botany, and zoology to the Bordeaux Académie around the time he wrote his masterpiece *Lettres persanes* (Persian Letters) in 1721 at the age of 32.

When not in the robes of a lawyer he was a remarkable natural scientist, and a highly cultivated aristocrat. His works include "Discours sur la cause de l'echo (Discourse on the Cause of the Echo) (1718)", "Essai d'observations sur l'histoire naturelle (Observation Essay on Natural History) (1719 et 1721)", "Discours sur la cause de la pesanteur des corps (Discourse on the Cause of the Gravity of Bodies) (1720)", "Dissertation sur le ressort (Dissertation on the Elasticity) (1723)", and "Dissertation sur le mouvement relatif (Dissertation on Relative Movement) (1723)" (3-4) [Note 3-1]. Montesquieu carefully examined the papers that were submitted, and his evaluations of some of them still remain.

Note 3-1.
Montesquieu's *Lettres persanes*, written at the age of 32, is a collection of essays on a wide range of topics, including society, law, civilization, culture, and even sex. These take the form of 161 letters written by two Persians travelling through Europe, particularly Paris. Reading this work is enough to convince most people that Montesquieu was a genius philosopher of experimental methods, and also gives weight to the supposition that Montesquieu may have been behind the planning for the competition of papers relating to the function of the adrenal glands. It is known that he also has a work "Discours sur l'usage des glandes rénales (Discourse on the use of renal glands) (1718)" (3-4).

In Chapter 2, we met the Danish anatomist Casper Bartholin, who named the adrenal glands the "*capsulae atrabiliare*" as he believed they were full of black bile.

Bile was one of the four humors (*quatre humeurs*: sanguine, choleric, melancholic and phlegmatic) of the ancient physician Galen, and it appears from the reports of Montesquieu that the physicians and medical scientists of Bordeaux saw a strong connection between the adrenal glands and the mood or humor known as the bilious temperament (3-4).

Unfortunately, none of the papers that were submitted were considered worthy of the prize and so this intriguing project of the Academy ended with no award being made. However, two years later, on August 25, 1718, Montesquieu himself gave a review lecture (3-3) based on the papers that had been entered into the competition. This suggests that the shift from anatomical research to physiological consideration of the adrenal glands started at a very early stage (3-3, 3-5).

Montesquieu is world famous for his immortal classic *De l'esprit des lois* (The Spirit of Laws), which was published in 1745. This work was introduced to Japan through the translation of Reishi Ga around the time of the Meiji Restoration [Note 3-2].

Note 3-2.
Montesquieu worked on *De l'esprit des lois* for 20 years, and this was translated into Japanese as *Banpo Seiri* by Reishi Ga in 1875. *Banpo Seiri* was in fact a secondhand translation of the English translation by Thomas Nugent, titled *The Spirit of Laws* (1750), and it is said to have contributed to the development of thought on freedom and civil rights in Japan at the time.

Reishi Ga opened a private English school in Nagasaki in around 1865, where Jokichi Takamine studied. Nagasaki, a port in the far western tip of Japan, was the only port open to the West during Japan's perod of national isolation, which lasted from around 1633 until 1853, and Jokichi Takamine studied here under Ga.

With lofty ambitions of becoming a doctor, little Jokichi made a dangerous journey by boat from his home in Kanazawa to Nagasaki. Finding that the age of Dutch studies had already ended [Note 3-3], he set about learning English at Reishi Ga's private school.

Unfortunately, Ga was an extremely busy man, and the teaching Jokichi received was far from satisfactory. He therefore began studyng under the Dutch missionary Guido Verbeck at the Chienkan, a domain school, established by the local government of Saga Domain. Here he acquired a good grounding in English (3-6, 3-7).

Note 3-3.
From the early 15th century, merchants from Portugal, Spain and Italy had been arriving at Nagasaki Harbor to trade. Although Japan had sealed itself off from the outside world at that time, it adopted some western culture and knowledge through these countries. Later, Dutch merchants were allowed to use "*Dejima*", a fan-shaped artificial island constructed off the port of Nagasaki, to conduct their business, which became an important source of foreign information for Japanese academics of the time.

For about 200 years, Japanese academics learned western science and technology from the Dutch merchants in Dutch. However, in 1853, the cutting-edge American paddle steamer "Susquehanna" and its captain Matthew C. Perry arrived at Uraga channel in Yokosuka and changed the country's closed-door policy for good, and English subsequently and rapidly became the most important foreign language.

In 1775, half a century after papers were solicited for the competition, the medical scientist Théophile de Bordeu put forward the concept of "internal secretion." This was the idea that all organs, tissues, and cells in the body released substances into the blood to act on other parts of the body. His work was eventually carried on by the genius Marie François Xavier Bichat, two generations his junior (3-1).

Théophile de Bordeu was born to a doctor's family and worked at the Royal Hospital in Versailles, where his studies included pulse rate, mucous membrane tissue, chronic disease, and medical history. He also put forward anatomical remarks and research reports on the functions of gland tissues (3-8). De Bordeu passed away in November 1776, just one year after he suggested the momentous concept of internal secretions.

Scientific advances invariably involve masterful intuition, and in 1785 the German Johannes Schmidt put forward his theory—which we have already seen in Chapter 2—that secretions were formed within the adrenal glands and released into the blood, where they circulated and helped the functioning of the heart. Schmidt did not back his theory up with any evidence, but his intuition foresaw the later discovery of the active inotropic effect of adrenaline on heart muscle (3-5).

2. The dawn of physiology

It is worth devoting a little space here to the state of research at the forefront of medical science and physiology in the 19th century as a background to the entry into our story of Vulpian, the discoverer of adrenaline.

Many great 19th-century scientists came from the Paris medical school founded by Philippe Pinel. Among them was Marie François Xavier Bichat, who turned his prodigious talents to physiology before dying at an early age. Bichat carried out profound investigations of form and function, as a result of which he developed the concept of tissues (from the French "*tissue"*) as distinct units that started with membranes (also French). He explained the significance of these, which was a monumental achievement in the annals of biology and medicine.

The first person to garner major scientific results from the fertile ground of this basic science was François Magendie, who is known in France as the father of experimental physiology and a pioneer of experimental pharmacology. After discovering morphine in 1805, over the course of 20 odd years he discovered quinine, cinchonine, strychnine, brucine, caffeine, codeine, and atropine, which are all important physiologically active natural substances. Magendie compiled a wealth of methods for using these substances properly as medicines, and his textbook, *Formulaire pour la préparation et l'emploi de plusieurs nouveaux médicaments* (Formulary for the Preparation and Usage of Many New Medicines) was a ground-breaking work (3-9).

François Magendie worked as the medical director of the *Collège de France* for 25 years. The *Collège de France* was established by King Francis I of France in 1530 as an alternative to the *Collège de Sorbonne*, with the main subjects of Hebrew, ancient Greek, and mathematics. Like John Hunter, whom we encountered in the previous chapter, Magendie was a notorious vivisectionist, but he is now highly regarded for his achievements in opening up the way forward to modern physiology and pharmacology.

Around this time, in 1834 a young man from a winegrowing family in the *Rhône department* of France moved to Paris; he had graduated from University of Lyon and was working at a pharmacy in the city, but he had dreams of establishing himself as a playwright. At age 21, he had written a manuscript of an historical tragedy, titled *Arthur de Bretagne*, and a letter of introduction to the prominent literary critic Saint-Marc Girardin. The critic read his manuscript, but was unimpressed; "You would do better to find a profession other than writing," he advised the young man. The man heeded this advice—already familiar with pharmaceuticals, he entered the medical faculty of the University of Paris. This man was Claude Bernard, and along with Louis Pasteur, he was to become a giant of science and a source of eternal pride for France [Note 3-4].

Note 3-4.
Other examples of aspiring French writers who went on to become prominent scientists are Brown-Séquard, four years Bernard's junior, who was mentioned in Chapter 2, and a great French man, Antoine Lavoisier (1743–1794), who was older than both Brown-Séquard and Bernard. Lavoisier discovered through careful measurement of weight increases that combustion is caused by a reaction with oxygen. He was a talented playwright, and after leaving the Université de Paris he worked as a lawyer. He subsequently carried out his combustion experiments after being excited by the British scientist Joseph Priestly's research into oxygen. At the time, no one could have thought that something that burns and disappears before their very eyes was actually undergoing a chemical reaction that resulted in an increase of weight.

Bernard took up an apprenticeship with Magendie after graduating. Magendie had built up a reputation as a professor at the Collège de France and as a physician at the Hôtel-Dieu de Paris hospital. Describing himself as a mere "rag picker of facts," he excelled as a scientist and was the founder of experimental physiology.

While working under Magendie, Bernard published a number of groundbreaking papers. He was appointed assistant professor in 1845, and from then on he devoted himself to experimental research in physiology. One of his achievements worthy of special note was to demonstrate that the body is capable of both decomposition and synthesis of complex chemical substances, and he proposed the term "*sécrétion intérieure*." Along with the concept of *homéostasie* (homeostasis) of the living body that he later proposed, this was a huge step forward for experimental physiology and biochemistry, as it viewed the living body from a perspective of chemistry.

Among the works Bernard produced during his life, the best-known is *Introduction à l'étude de la médecine expérimentale* (An Introduction to the Study of Experimental Medicine). Published in 1865, this has become obligatory reading for scientists and researchers. The book was later translated into Japanese (3-10). Even today the Bernard's book is considered one of the most influential along with Darwin's "Origin of the Species" in Japan (3-11). It taught the people in the early Meiji era (1868–1912) the importance of experiments in confirming the truth rather than relying on the traditional Chinese theories like *yin* and *yang* and the five elements that were so widely spread at that time.

The freshness of Bernard's idea made a definite mark on the outstanding young Japanese researchers emerging immediately after Japan opened itself to the world, and some reviewers have even suggested that it helped bring about the remarkable advances of Japan during the Meiji era (3-11).

Around the same time, a scientist in Germany became the first person to detect the action of a hormone. This was Arnold Berthold, Professor of Physiology at the University of Göttingen, who published the results of his elegant experiments using cocks in 1849. It was well known that if roosters were castrated when young, the crest and the wattle (the red protuberances hanging down from the neck), which are sexual characteristics of the rooster, recede. Berthold repeatedly implanted gonads removed from other roosters into areas other than the testicular area (such as the back and the abdomen) of castrated roosters. The roosters from which the gonads were removed became hoarse and were no longer aggressive, while those that had received the implants showed normal behavior and developed secondary sexual characteristics (3-12).

Through microscopic observation, Berthold found that while there were no nerves around the gonads, there were a great many living sperm cells. This proved that the gonads had the effect of causing the development of secondary sexual characteristics in some way that did not involve nerves. He conjectured that the change was brought about by chemical substances—this was the first demonstration of the existence of hormones (3-13).

3. Vulpian's keen insight

Finally, the man who discovered adrenaline, the French scientist Alfred Vulpian, makes his long-awaited appearance in the story (3-14).

The German physiologist Carl Friedrich Wilhelm Krukenberg of Jena University, who was engaged in research at about the same time as Vulpian, stated that it was Vulpian who discovered the color-developing compound in the adrenal glands, and he described Vulpian as the foremost expert (3-15).

Vulpian was intrigued by impactful news coming from Britain, France's neighbor on the other side of the Straits of Dover, of the discovery of Addison's disease. He set about directly addressing the change of skin color that characterizes Addison's disease by searching for the changes in pigmentation that occur. In 1856, he published two reports about Addison's disease in the journal of the French Biological Society. One of these was in the field of pathological anatomy, titled "Examen microscopique de la peau d'un malade mort a la suite de la maladie bronzée (maladie d'Addison)" (Microscopic examination of the skin of a patient died from rusty color disease or Addison's disease). In this, he starts with a description from microscopic observation of the change in coloration of the Malpighian layer of the skin to a brown or rusty color. He notes that it is the color seen in cases of pulmonary tuberculosis, like the color of Europeans exposed to the burning African sun (3-16). Straight after this report, he published another in the field of pathology, in which he described in detail his pathological anatomy observations of a man of about 45 who had died of tuberculosis. Here, he mentioned the kidney tubules (3-17).

At the same time as these studies, Vulpian was also working on another piece of research, which would ensure his name would live on. This was the earliest and most important experimental result in relation to the physiologically active principle of the adrenal glands. His work was published in 1856 in the weekly bulletin of the same influential French scientific journal as the previous two papers (Figure 3-1).

CHIMIE APPLIQUÉE. — *Note sur quelques réactions propres à la substance des capsules surrénales; par* **M. Vulpian** (1).

(Commissaires, MM. Dumas, Pelouze, Cl. Bernard.)

« La substance des capsules surrénales, mise dans certaines conditions que je déterminerai tout à l'heure, présente des réactions spéciales et toujours les mêmes. Je prends, comme exemples, les capsules surrénales du mouton, les plus faciles à se procurer, et celles qui m'ont offert les phénomènes les plus marqués.

» Les capsules surrénales du mouton se composent, comme celles des autres Mammifères, de deux parties : une partie corticale, à déchirure fibreuse, d'une couleur analogue à celle des reins, et une partie médullaire plus homogène, d'une teinte grisâtre, un peu perlée. C'est cette dernière substance qui produit surtout, et presque exclusivement, les réactions.

Figure 3-1. The beginning of the paper by the French chemical physiologist Alfred Vulpian, in which he announced the discovery of the color reaction of adrenaline (3-14).

That year in Japan, Townsend Harris, the first United States Consul General to Japan, established the US Consulate at Gyokusenji Temple in Shimoda, Izu; Jokichi Takamine was a three-year-old boy in Kanazawa, Kaga Province, with nothing more on his mind than playing. Keizo Wooyenaka had yet to be born.

The gist of Vulpian's historic paper (3-14) is given in an abridged translation in Chapter 1, Column 1-2, and it shows that his research was very substantial indeed. The key areas are listed below.

1. The medulla, which is more homogeneous, presents a grayish color slightly tinged with pearl. It is this latter tissue alone that causes the color reactions.
2. If ferric chloride or ferric oxide is added to extract of the medulla, a slightly blackish sea green slightly tinged with blue or green is produced.
3. Staining with iodine aqueous solution produces a highly distinctive rose-carmine color.
4. The same color reactions were found using adrenal medulla extract from 14 animal species, including humans.
5. Fourteen different organs other than the adrenal glands were tested, but none of them produced a color reaction.

6. Is the principle present in the adrenal medulla fated to be broken down at the surface of this tissue, or does it permeate into the blood, to be taken into the circulatory system? I [Vulpian] strongly support the latter theory.
7. I reason that as this phenomenon occurs constantly after death, the same thing must also take place when the animal is alive. This will be determined by future experimentation. Similarly, the hypothesis that the adrenal glands may be regarded as gland tissue normally known as blood gland—in other words, the hypothesis that these glands directly shed their secretion products into the blood—will surely be proved definitively for the first time in the future.

Vulpian's report of his experiments clearly shows that he was an extremely precise researcher. He very carefully separated and removed the cortex of sheep adrenal glands from the medulla, and then collected the squeezed liquid of the medulla. He filtered this liquid and added various different types of experimental reagent to observe the changes, verifying the presence of unique constituents in minute quantities.

In order to use the color reactions, he prepared small pieces of filter paper soaked in reagent and then dried them, much like the pH test paper used to test acidity or alkalinity. He placed samples from a variety of organs, and in particular different parts from dissected samples of adrenal glands, under a microscope, and using fine forceps he gently touched his test paper against the samples to see if the color changed.

One cannot help but be touched by an image of Vulpian [Figure 3-2] peering into his microscope with bated breath, consumed with curiosity.

Figure 3-2. A statue of Vulpian in the *Rue de l'École de Médicine*, Paris. (Photo by Luca Borghi, courtesy of Himetop - The History of Medicine Topographical Database)

In the last years of his life, Keizo Wooyenaka, who first crystallized adrenaline spoke about Vulpian in an interview. "How did you feel when you discovered the white crystals?" he was asked. "It just seemed strange to me that something like that had been overlooked," Wooyenaka replied, laughing. "Vulpian had written everything down properly, so it was already clear. So really, if someone had followed Vulpian's experimental method, they must have been able to isolate adrenaline before me" (3-18).

Vulpian became Dean of the Faculty of Medicine at the University of Paris in 1875, and the following year he was nominated for membership of the *Académie des Sciences* of France, eventually becoming lifelong secretary of the *Académie*. He is certainly one of the people to have the greatest impact on medical science in France, and he was awarded the National Order of the Legion of Honor (Chevalier), France's highest honor. During his lifetime, he wrote some 225 scientific papers (3-17, 3-19).

4. Meanwhile, in other countries

In 1856, the year that Vulpian announced his historic discovery, a concerted increase in research was observed in the use of histochemistry to study the adrenal medulla. In France, Gabriel Constant Colin discovered that if the surface of the adrenal medulla is treated with ferric sulfate, it turns blue.

In Germany at this time, science was enjoying great advances and research reports were being continually produced. The best known histochemical reaction of the adrenal medulla is the chromaffin reaction, which takes its name from a relatively specific reaction with chromic acid, it was evidently Bertholdus Werner who first discovered this in 1857 as the brown precipitates that appear when the medulla is fixed with chromate or dichromate. Gregor Joesten made the same observations in 1864 (3-5).

In 1865, the giant of medical science, Friedrich Gustav Jacob Henle [Note 3-5], conducted the first detailed histological research into the adrenal glands using a potassium hydroxide solution of chromic acid. He published the results as "Ueber das Gewebe der Nebenniere und der Hypophyse (On the Tissue of the Renal Gland and the Pituitary Gland)" (3-20).

That same year, M. Rudneff, who examined cellular staining of various tissues, reported with his co-worker, M. Schultze, that the cortex and the medulla of the adrenal gland gave different colors when stained with osmium (3-21).

Note 3-5.
Henle proposed a set of postulates regarding disease-causing microbes, which are well known in the field of pathogenic microbiology. His student, Robert Koch, continued Henle's work, and using the postulates as a guiding rule he discovered such important bacteria as *Bacillus anthracis*, the anthrax bacillus, and *Mycobacterium tuberculosis*, the tuberculosis bacillus. Koch was a pioneering figure of pathogenic microbiology, who cannot be omitted from any history of medicine.

The terms "chromaffin reaction" and "chromaffin cell" did not appear until the next century, when they were coined by the German Alfred Kohn in 1902, after the crystallization of adrenalin. Kohn was a Jew and was interned in the Nazi concentration camp at Theresienstadt in the present-day Czech Republic in World War II, although he somehow managed to survive this terrible experience (3-5). Chromaffin cells have now been shown to have extremely important functions for digestion.

Vulpian was 30 years old when he published his report of the color reactions, and that same year in London, research was announced that was to take a permanent place in the history of organic chemistry. This was the discovery of mauve (mauveine, aniline purple), the first synthetic dye by William Henry Perkin. Perkin was an 18-year old student at the time of this major discovery, which he made over the weekend in a humble laboratory in his home.

However, this discovery was the trigger for the development of the vast area of chemical industry along the Rhine in Germany. Japan at this time was a minor island country, but there were outstanding young people exploring the paths that would lead to the country's period of enlightenment during the Meiji period.

5. Why was Vulpian doing this research?

Edmé Félix Alfred Vulpian was born to an aristocratic family, but at an early age he lost his father, who was both a lawyer and a playwright, to smallpox. He grew up in great poverty and failed to enter the *École normale*, which was an elite institution of higher education.

To support himself, he found work as an assistant at the *Muséum national d'histoire naturelle* (the French National Museum of Natural History) in Paris. At the laboratory there he had the good fortune to receive the guidance and patronage of the well-known physiologist Marie Jean Pierre Flourens, allowing him to unleash his talents.

Flourens was an expert in brain physiology with a distinguished career that included the discovery of the respiratory center of the medulla oblongata. He was made a member of the *Académie des sciences*, and received the National Order of the Legion of Honor (*Commandeur*) for his achievements (3-22). He was a student of Cuvier, who, as we saw before, had early on identified the importance of researching the physiological functions of the suprarenal glands.

As a physiologist, Flourens most likely shared the enthusiasm of many of the leading researchers in Europe at that time to study the suprarenal active principles. This was a difficult area of research, but one that was worth the challenge, and we might suppose that Flourens passed on this research theme to Vulpian, his young but outstanding student.

To get a better insight into the scientific world of France at the time, from which Vulpian was learning, let us look briefly at the three reviewers whose names appear at the head of his historic paper on the color reactions (3-14) (see Chapter 1, Column 1-2).

First is the chemist Jean-Baptist Dumas. He is famous for Dumas' method for the quantitative analysis of nitrogen, the halogenation of hydrogen in organic compounds, and the measurement of atomic weights for many elements. It is said that 21-year-old Louis Pasteur, who was studying to enroll at the *École normale*, was inspired to pursue a chemistry career by Dumas' lecture; Dumas was 42 years old and was Professor at the Université de Paris at that time (3-23).

The second reviewer was Claude Bernard, the founder of physiology. We have already seen how he was a world leader in the field closest to Vulpian's area of specialization.

The third reviewer was Theophile-Baptiste Pelouze, a professor at the *École polytechnique* and the *Collège de France* who was conducting research into a wide range of areas. Pelouze was an influential scientist, and his students included Ascanio Sobrero, who discovered nitroglycerin, the main component of dynamite. Vulpian's paper was at a high enough level for these three world-class authorities to consent to its publication.

Vulpian did not describe Addison's disease in his report of the color reaction, nor did he mention it in the follow-up report, which was a similar experiment using reptiles (3-24). Nonetheless, it was in France that the importance of this disease was recognized, and although the disease is named after Addison it was not an Englishman but a Frenchman, Armand Trousseau, that gave the disease its name—*la maladie d'Addison*.

Perhaps it was because of this that Vulpian was so interested in the suprarenal glands, from both a physiological and a pathological point of view. As we have already seen in the chronological order of research into the adrenal glands, the interest of the scientific world in Europe moved from anatomical research to embryological research, and then to the function of the glands, particularly their action on the nervous system. At that point in time, the adrenal glands were undoubtedly a very attractive topic for study.

Around the age of 40, Vulpian announced a novel theory on the mode of action of curare, an herbal poison. This was a field that Bernard had pioneered. Curare is a powerful toxin that Native Americans use to tip their arrows when they hunt.

Vulpian's theory was that curare affected the point of action at which nerves send signals to the muscles by chemical substances (3-25). Figure 3-3 shows that this was a voluminous work, and if we look, for example, at the record of the regular meeting of the *Societé de Biologie* in France on January 4, 1873, we can see that in the discussion on sensory nerve physiology, Bernard praised Vulpian's research report as an enormously interesting work. By this time, Bernard was a respected elder at the age of 60, while at 47, Vulpian was still in his prime—nonetheless, we can see the close friendship that remained between these two men (3-26).

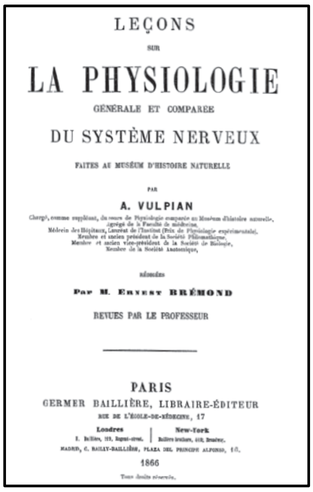
LEÇONS
SUR
LA PHYSIOLOGIE
GÉNÉRALE ET COMPARÉE
DU SYSTÈME NERVEUX
FAITES AU MUSÉUM D'HISTOIRE NATURELLE
PAR
A. VULPIAN
Chargé, comme suppléant, du cours de Physiologie comparée au Muséum d'histoire naturelle,
Agrégé de la Faculté de médecine,
Médecin des Hôpitaux, Lauréat de l'Institut (Prix de Physiologie expérimentale),
Membre et ancien président de la Société Philomathique,
Membre et ancien vice-président de la Société de Biologie,
Membre de la Société Anatomique,
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Figure 3-3. Vulpian's textbook on nerve system physiology (*Leçons sur la physiologie générale et comparée du système nerveux*. G. Baillière, Paris (1866))

Nerve physiology research was passed from Cuvier to Flourens, while Magendie's successor in experimental physiology was Bernard. It was at the confluence of these two great streams that Vulpian was given the space to work, and perhaps it was the inevitability of history that destined him to attain the honor of discovering the first hormone. Vulpian continued the traditions of French medicine, in particular physiology and biochemistry, of that time, and it is likely that he thus had ample motive to investigate life phenomena from a chemical perspective.

6. Vulpian's many friendships

In his biographies, Vulpian is usually referred to as a physiologist and neurologist, but he left a record of pioneering achievement in an extremely wide range of fields.

For example, at the regular meeting of the *Societé* on January 4, 1873, the French medical scientist Casimir Davaine, who discovered the pathogen for anthrax in the blood of goats and in human pus, put forward the topic of *septicémie*; Vulpian argued that this should be called *bactériémie*, explaining his idea that it was a disease caused by infectious organisms (3-26, 3-27).

In the regular meeting of the *Societé* one month previously, Vulpian joined an argument with Jean-Martin Charcot during a lively debate over bacteria-infected blood (3-28).

Vulpian and Charcot had worked together from 1861 to 1862 on adding a great many more clinical records on the syndrome in the records left by James Parkinson, and they proposed the name "Parkinson's disease." Charcot left behind a considerable body of work on hypnosis and hysteria (3-29) [Note 3-6].

Note 3-6.
In 1862, Vulpian and his close friend Jean-Martin Charcot, a neurophysiologist and a professor at the *Université de Paris*, jointly took over a welfare facility for patients with chronic diseases that was in a state of disarray. This facility was known as the *Salpêtrière*, because it was on the site of a former saltpeter factory. In 1872, Vulpian took up a post as chair of experimental and comparative anatomy, and at the same time he was also working for the Paris *Charité*. He was a member of the Academy of Medicine for three years from 1867 (3-19).

Vulpian studied harder than Charcot, but was more reserved and did not stand out as much as his friend. Vulpian reconfirmed the observations of his teacher, Flourens, on the function of the semicircular canal and the cerebellum, and established the principles of nerve regeneration and vasomotor function. He discovered the chromaffin system of the adrenal gland using chromium salts, and proved that the herbal poison curare induced paralysis by acting on the point between the nerves and the muscles.

He was an extremely hard worker, starting from four o'clock every morning, and was held in great respect by both teachers and students. He had an unprecedented good sense for experiments, and he carefully verified and controlled his experiments by repeating them again and again until his results were confirmed. He is said to have had an inestimably good influence on his students.

Vulpian and Charcot together launched an academic journal, titled *Archives de Physiologie Normale et Pathologique*. In his later years, Vulpian became a leading figure in the French Academy of Sciences, and he gave warnings to researchers in France that they were not making sufficient use of the microscope. His remarks probably came from a sense of crisis, as German researchers at that time were attracting international attention through successive discoveries with the microscope. Be that as it may, he believed that before all else, researchers needed to make detailed observations.

Vulpian was also close friend with Louis Pasteur, who was four years older and his senior in the French Academy of Sciences. The story of their meeting, when Pasteur was building a new age in medicine with his therapeutic serum for rabies, is inspiring. It happened on July 6, 1885, when a mother had come rushing to see Pasteur with her nine-year-old son, Joseph, who had rabies. The town doctor had told the mother that he could do no more to help Joseph, and the only remaining option was an injection of the therapeutic serum that Pasteur was still working on. Fortunately for Joseph, this happened to coincide with a regular meeting of the French Academy of Sciences that same day. At the meeting, Pasteur immediately conferred with Vulpian about Joseph. Vulpian at once requested the Professor of the Medical Department, Jacques Joseph Grancher (1843–1897) to visit Joseph at home. Grancher saw that the boy had been bitten by a dog in more than 14 places, and the wounds were getting worse; he told Pasteur that Joseph could not be saved. Pasteur explained his latest findings on the rabies therapeutic serum in detail to Vulpian and Grancher, and listening to Pasteur, the two were convinced that his results were beyond any doubt.

Pasteur did not have a physician's license, but his two colleagues encouraged him to treat the boy. Pasteur believed in his research results, but was nonetheless very anxious. With both Vulpian and Grancher present at his laboratory, at eight o'clock that very night he started to inject Joseph unsparingly with the spinal fluids he was still preparing. Over the course of 10 days, Joseph received 13 such injections. Three months and three weeks later, Joseph had recovered and was able to return to his mother in Alsace. The French authorities overlooked

Pasteur's breach of the medical practitioner's law (3-30) [Note 3-7].

Note 3-7.
In an address at the regular meeting of the French Academy of Sciences on October 26, 1885, Vulpian said the whole medical profession should share the sentiment of wonder for Pasteur, who had saved young Joseph's life, and he pleaded for all people afflicted with rabies to be able to receive the benefit of this great discovery. Three years later, at the opening ceremony for the Institut Pasteur, Louis Pasteur recalled past times, emotionally saying, "[I am] deprived of my masters, Dumas, Bouley, Paul Bert, and lastly Vulpian, who, after having been with you, my dear Grancher, my counselor at the very first, became the most energetic, the most convinced champions of [our research] method" [underlines added]. It is said that Pasteur was unable to hold back his emotions, and his son had to read the manuscript of his speech (3-31).

There is a legendary epilogue to Joseph's story. Some 55 years after his miraculous recovery, Paris was captured by the invading Nazi German army during World War II. One day, a German army doctor came to the cemetery where Pasteur was buried, and ordered the elderly caretaker to show him Pasteur's grave. The elderly man refused, and ultimately committed suicide rather than reveal the location. This man was Joseph Meister, who had worked as a porter at the Institut Pasteur. This was the same Joseph whose life Pasteur had saved many years earlier; he had dedicated his life to repaying his debt of gratitude to Pasteur (3-32).

7. Courageous human experiments

(1) The elderly venerable scientist who experimented on himself

Now we come to a discovery in which the effects of "hormones" were actually felt. This discovery was announced on June 1, 1889, at a meeting of the French Society of Biology in Paris. The room was packed with normally staid scientists, but when the speaker began to present the results of his bizarre experiment, an uproar broke out (3-33). Standing at the podium was an elderly scholar of 72 years—given the average life span of the time, he must have been the oldest person. This scholar was Brown-Séquard, the energetic researcher who, as we saw in Chapter 2, had demonstrated 33 years earlier that removal of the suprarenal glands was fatal, and that these glands were therefore essential to maintaining life.

Brown-Séquard was a highly respected member of the society, but his report was startling: by giving himself subcutaneous injections of aqueous extracts of the testes of marmots and dogs, he had undergone rejuvenation. The other members were greatly shocked.

The content of the lecture was duly published two weeks later in the weekly bulletin of the society, *Comptes rendus*, with the title "Des effets produits chez l'homme par injections sous-cutanées d'un liquide retire des testicules frais de cobaye et de chien (The Effects of hypodermic injection of extracts from fresh testicles of guinea pig and dog on a man)" (3-34).

The lecture makes gripping reading—it seems incredible that 120 years ago researchers went to such lengths, and it is impossible to read to the end without being moved.

Very briefly summarized, the content of Brown-Séquard's lecture was as follows: "It is well known that castration of a human during childhood or adolescence brings about profound, lasting changes to the physiology and the mental state of the individual. In particular, it is known that eunuchs [the castrated officials of the ancient Chinese court] were remarkably weak and suffered both physical and mental deficiencies. We also know that castration can have similar effects on individuals who engage in excessive sexual conduct or masturbation. This demonstrates that a component imparting energy to the nervous and muscle systems is secreted from the sperm into the blood. I believe that the fact that the elderly normally grow weak is due, in part, to reduced function of the testicles."

He went on to report the results of his research on marmots, dogs, and rabbits at a laboratory in France and also in the outskirts of Boston in the United States including many negative results. "I resolved to use myself as a laboratory animal," he said at last. "This was my duty, and would be far more conclusive in every aspect than experimenting on laboratory animals."

Brown-Séquard went on to speak in great detail about his experimental method, before finally getting to the bit that the assembled scholars were waiting with bated breath to hear, anxious not to miss anything—this was the results. He explained what happened: "I turned 72 on the eighth of last month. For the last 11 or 12 years, I have felt my vitality gradually decline; I can no longer endure experiments in which I have to stay standing for long periods, and after just 30 minutes I need to sit and rest."

He also spoke about sleep and other areas of the aging process. "Today is the third day since I started to inject myself," he said. "The symptoms of aging have ameliorated, and I have regained the strength I had at least a few years ago. The tiredness I felt when working in the laboratory has almost disappeared, and my assistants are surprised to see me standing for long periods without the need to sit down. It has been my custom for the past 20 years or so to write down everything that has happened, and I can now complete this task after dinner. Even my friends have noticed this. I am now able to run up the stairs with ease, something I have not been able to do since I was 60. Measurements made with a dynamometer have shown improved, and the strength of my forearm has increased by an average of about 6–7 kg since starting the injections." He also said that he had regained greater force when urinating, improved bowel movements, and more energy for intellectual tasks.

He closed his lecture by saying, "I wish for other physiologists to repeat this experiment, in order to verify whether or not the results I have obtained from my own body are due to my own individual characteristics. In response to the question put to me that this is the result of some sort of autosuggestion, I am unable now to offer any evidence to the contrary."

Brown-Séquard's audience of scholars must have left the meeting greatly impressed, but at the same time perhaps feeling that something was missing. While there would undoubtedly have been great expectations for the results with regard to sex, this had not been mentioned at all.

Brown-Séquard's trial was perhaps an extension of the work of Berthold, who 40 years earlier had implanted gonads into roosters, but the impact was completely different because this time the experimental animal was a human.

The mass media got hold of this information, and reported it in sensational style. Brown-Séquard had finished his lecture with the highly significant comment that his results needed to be verified separately, but this was completely ignored—in fact, anyone who tried to verify Brown-Séquard's work was threatened or ostracized. Unscrupulous people sought to use the announcement for their own good, and started offering injection therapy purporting to bring about rejuvenation. Quack doctors made vast sums of money by peddling this miraculous rejuvenation to rich people seeking a cure for impotence. However, in the 1930s, when it became possible to obtain male hormone with a far greater level of purity, the results of Brown-Séquard's experiment on himself were proved to be correct (3-33). Some 95% of the body's male hormones are said to be synthesized and secreted by the testicles (testes), and the other 5% by the adrenal glands.

(2) Verification with patients

In his astonishing presentation to the French Society of Biology, Brown-Séquard had made a step toward hormone therapy which was discovered two years later. This was a treatment developed by a scientist in Newcastle upon Tyne, England, who did not follow the instructions of his superior.

Sir Victor Alexander Haden Horsley, professor of pathology at University College London, directed his young coworker George Redmayne Murray to treat a patient suffering from hypothyroidism (myxedema) with a transplant of sheep thyroid. Murray, however, treated the patient through a different approach. He did not mishear the instructions, but he was harboring an idea for treatment that he longed to try.

He first extracted fresh sheep thyroid, and using glass utensils that he had sterilized with either heat or phenol he prepared an extract of the thyroid in glycerin. He wrapped this in a handkerchief sterilized with boiling water, and by squeezing the handkerchief, he produced a slightly cloudy, pinkish solution.

The patient was known as S, a 46-year-old woman. Six years previously she had had a miscarriage, and her periods had not returned, in addition to which her arms and legs had swollen greatly and she no longer perspired; these were the classic symptoms of myxedema. The patient herself and her friends had started to notice that over the previous few years her speech and behavior had grown sluggish, and she had become lazy over her domestic chores. With the consent of the patient, from April 13, 1891, Murray started to administer consecutive subcutaneous injections of the liquid he had prepared, gradually increasing the dose. The symptoms steadily disappeared. On July 13, Murray recorded the patient's joy that her periods had started six weeks previously and were regular, she perspired when she walked, and she was no longer sensitive to cold. The following year, another doctor discovered that oral administration was also effective, and from then on Murray continued with oral therapy. The woman went on to live in good health for 28 years (3-35, 3-36).

Administration of thyroid extracts became the standard therapy for hypothyroidism until the 1950s, when synthetic thyroxin could be obtained in large quantities (3-33).

Murray's teacher, Horsley, was a close friend of Edward Sharpey Schäfer, who later became professor of physiology at University College, London. Horsley and Schäfer collaborated on research to determine the effects of stimulating or removing specific areas of the cerebral cortex of primates (3-37). Murray achieved considerable success as a physician and teacher, and became the first President of the Endocrine Section of the Royal Society of Medicine (3-38, 3-39).

(3) The doctor who experimented on his son

With the stage now set for some new development to occur at any time, a decisive breakthrough was made. The leading part was played by a doctor in private practice who had a fondness for machines.

This was George Oliver, a doctor who provided routine treatments near the hot spring health resort of Harrogate in northern England. During the summer he was kept busy looking after the wealthy patients who came to take the waters of the spa, but when the weather turned colder he would have more time on his hands. As a scientist, he enjoyed using this time to conduct physiological experiments with devices that he designed gauge, and in Autumn of 1893 he came up with a device that was fitted to the wrist and could detect minute changes in the diameter of the radial artery.

Using this device, he measured the diameter of the radial artery of his 20-year-old son following a subcutaneous injection of glycerin extract of calf adrenal glands. The data he obtained showed clear constriction of the artery in that area. Oliver immediately took his sample of adrenal extract to London, where he went to his old university, University College, to see the professor of physiology, Edward S. Schäfer. He had to wait patiently until Schäfer finished a lecture he was giving, and he asked Schäfer to inject a dog that still remained on the laboratory table.

8. Professor Schäfer's astonishment

At that time, people often came to Schäfer with make-believe stories, and he assumed this would be no different—in fact, the whole thing was something of a burden. However, he could scarcely refuse the request of an acquaintance from the same university who had studied under the same teacher, so, somewhat unwillingly, he injected the dog. His expectations had been utterly wrong: the mercury on the blood pressure gauge to which the dog was connected rose rapidly, soon overshooting the scale. Schäfer was astonished. The two scientists threw themselves into vigorous joint research, compiling results to show that the adrenal medulla contained a blood-pressure raising principle. Their work caused a sensation when it was announced at the Physiological Society the following spring.

As an interesting aside, the story of the experiment was recounted by Henry H. Dale, professor at the University of London, who witnessed the event. Dale, whom we will meet again in Chapter 7, was an 18-year-old student at the time, but he subsequently went on to win a Nobel Prize in this field. He recalled the emotion of the moment in subsequent lectures in 1938 and 1948 (3-40, 3-41).

In another twist to the story, the details of the study were investigated in a paper published in 1968 by Henry Barcroft and J. F. Talbot of the Sherrington School of Physiology, St Thomas's Hospital Medical School, London (3-42). This is an enormously interesting paper, but to keep the present story brief it has been consigned to Column 3-1 at the end of this chapter.

Soon, the day came that marked the beginning of a new age of physiology. At a regular meeting of the Physiological Society held on March 10, 1894 at University College, London, Oliver and Schäfer gave a lecture titled, "On the Physiological Action of Extract of the Suprarenal Capsules." The details of the lecture are recorded in the well-known journal, *Journal of Physiology* as preliminary communication (3-43) [Note 3-8].

Note 3-8.
This is a brief summary of the paper by Oliver and Schäfer in the Journal of Physiological Society (*Journal of Physiology*):

1. Sample were extracted from calf, sheep, and dog adrenal glands with water, alcohol, or glycerin, and then dried. The action on dogs, cats, rabbits, and frogs was studied.
2. Extreme contraction of the arteries was observed. The effect was peripheral.
3. Arterial blood pressure shows a marked, rapid increase. This occurs despite the powerful inhibitory

action on the heart; the effect is further increased by cutting the vagus nerves.
4. The vagi nerve center is stimulated. This is extremely powerful, and the auricles come to a complete standstill momentarily. However, the venticles continue to beat with a slow, independent rhythm.
5. After the vagi are cut, contraction of the auricles and the venticles is greatly accelerated and augmented. This augmentation is particularly marked with the auricles.
6. There is a slight effect on respiration, which becomes shallower.

The lecture meeting was held on a Saturday, and as the members of the Physiological Society were gathered together, a demonstration of the experiment was given. Charles Scott Sherrington has left an excellent account of the emotion he felt watching the experiment (3-44). At 37 he was Schäfer's junior by seven years, but he was an up-and-coming physiologist. Personally witnessing the breathtaking and dramatic results of a blood-pressure raising principle in the adrenal glands made a great impression, which he expressed succinctly: "Maupassant, prince of romances, said he never let his fiction be so strange as life itself, lest it appear too incredible." To express it more prosaically, when Sherrington saw the sudden rise of the mercury, the shock of truth that was stranger than fiction must have been seared into his memory. Thirty-eight years after this, Sherrington shared the Nobel Prize at the age of 75 with another Briton, Edgar Douglas Adrian, for their discoveries regarding the functions of neurons.

The year after the lecture, Oliver and Schäfer published a follow-up to their report, in which they wrote that the same active principle was present in the adrenal glands of healthy humans, but no activity was found in the adrenal glands of patients who had died from Addison's disease (3-45). Their second paper ran to 47 pages, and they summarized it for publication under the title "The physiological effects of extracts of the suprarenal capsules" in 1895, once again in the *Proceedings of the Physiological Society* (3-46).

The same year, Oliver gave a detailed report of clinical experiments using adrenal gland extracts to the pharmacology and therapeutics section of the annual meeting of the British Medical Association, in which he introduced in detail cases that he had treated by administering formulations of adrenal glands prepared in various different ways (3-47) [Note 3-9].

Note 3-9.
A very interesting article showing the importance of always looking back over history was recently published by Dr. S. W. Carmichael, clinical anatomist of the Mayo Clinic in the United States (3-5). In his article, Carmichael notes that Johann Carl Jacobj, a pharmacological assistant at the *Institut zu Straßburg*, released a noteworthy report with the title "Beiträge zur physiologischen und pharmakologischen Kenntnis der Darmbewegungen mit besonderer Berücksichtigung der Beziehung der Nebenniere zu denselben" (Contributions to the physiological and pharmacological knowledge of bowel movements with special reference to its relations to adrenal glands) in 1892 (3-48). This paper describes research carried out in relation to the action of morphine, which investigated the effects of the adrenal glands on intestinal tract contraction function, and included removal of the glands. It was published two years before Oliver and Schäfer's historic discovery. Carmichael notes that this discovery was largely ignored at the time, but that with hindsight it can be seen as a sophisticated demonstration of adrenal gland function.

Schäfer had risen to the post of physiology professor at the University of Edinburgh, and he received an invitation to speak at the historic Royal College of Physicians of London. He gave an honorable lecture titled, “Present condition of our knowledge regarding the functions of the suprarenal capsules, Lecture II” at the Oliver-Sharpey Memorial Lecture on April 7 and 9, 1908. According to the minutes, Schäfer gave a lecture that brought gasps of surprise as the top physiologist; cleverly combining facts and anecdotes, he wove together the tracks left by a great many scholars and researchers.

We have already seen how some of the scientists that featured in this history aspired to literary careers, but just like Conan Doyle, creator of the great “Sherlock Holmes” who was both a doctor and a detective novelist, Schäfer clearly seems to have had a talent for winning over his audience (3-3, 3-49).

Schäfer finished his lecture with his hope for the future: “Perhaps within the next few years a successor of mine in this lectureship will be able to put before you as much positive knowledge regarding the cortex as we now possess regarding the medulla, the function of which seemed, no more than fifteen years ago, as obscure as that of the cortex appears at present. And with the hope that this obscurity may speedily be removed, I cannot do better than terminate my lecture.” However, it was to be more than a few years. Cortisone, a corticosteroid hormone, was clearly shown to be secreted by the adrenal cortex in 1935 when Edward C. Kendall collected this hormone, which he knew as compound E. It had taken 27 years since Schäfer expressed his hope. The achievements of research into adrenal medulla hormone predated the establishment of the Nobel Prize in 1901, but Kendall, Philip Showater Hench, and Tadeus Reichstein were awarded the Nobel Prize in Physiology or Medicine in 1950 for their research into corticosteroid hormone.

Another man well known for straightaway administering a drug to humans in order to test the results was Edward Jenner, who worked on smallpox about a century before Oliver conducted his research. Jenner’s teacher, John Hunter, who was an anatomist and had a checkered career (3-50), drilled into him the maxim of William Harvey, the discoverer of the circulatory system: “Don’t think. Try!”

Jenner lived during a time when smallpox was an ever-present menace. As a doctor, he had treated smallpox cases for many years, and when he came up with his idea for preventing smallpox he tested it by injecting eight-year-old James Phipps, the son of his gardener, with cowpox on May 14, 1796. After James recovered, Jenner injected him with human smallpox; the boy showed no sign of disease. This was the great discovery of vaccination. If Oliver really did inject his son with suprarenal extracts, he was probably continuing the tradition of eccentrics that appear from time to time in Britain (see Column 3-1).

While this is something of a sidetrack, it is worth noting that Jenner and Oliver had something in common. This was shared by Koch, the father of pathogenic microbiology, and also by Ryuzo Yoshida and Saburo Mikami, the two Japanese physicians who discovered *Schistosoma japonicum*, and Frederick G. Banting from London, Canada, who discovered insulin: all these men were small town "medical practitioners."

Koch's genius was noticed by Dr. Ferdinand J. Cohn, a professor of plant pathology at the University of Breslau, which was then part of Germany, and this led to Koch moving to Berlin, where his talents flowered spectacularly. Yoshida and Mikami left their mark on medical history with the help of Akira Fujinami, professor of Kyoto University, and Fujiro Katsurada, professor of Okayama Medical College, respectively. Banting won the Nobel Prize for results he obtained in the laboratory of John J. R. Macleod of University of Toronto. Great discoveries are not limited to academics working at universities or research laboratories, but nonetheless cannot be achieved by someone working alone [Note 3-10].

Note 3-10.
Oliver and Schäfer will be forever remembered in the history of physiology for their momentous discovery. As researchers, their relationship was that Oliver observed clinical results, and Schäfer checked these results using experimental animals—the question of which of the two is the "discoverer" is one that has persisted to the present day. Brandon Reines discusses this in an essay titled "The Process of Medical Discovery" (3-51).

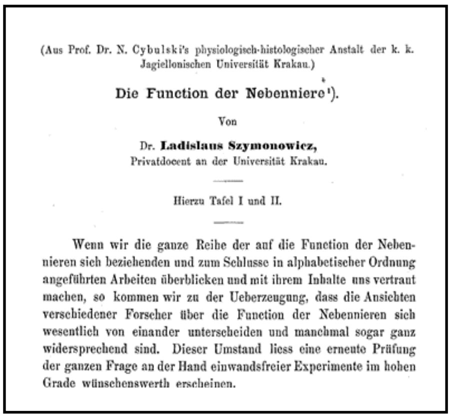
9. The anguish of a minor language

This is something that happens all the time, but the same research as Oliver and Schäfer did was being carried out with the same ideas in a country not so very far away.

This country was Poland, a country of high culture and civilization that has produced such geniuses as Copernicus, who devised the heliocentric theory (1473–1543), the composer Chopin (1810–1849), and Marie Curie (1867–1934), who won the Nobel Prize twice.

The cultural heart of Poland was Krakow (Cracow), and at the university in Krakow, two researchers, Napoleon Nikodam Cybulski and Ladislaus Szymonowicz worked not by proceeding straight to the stage of experimenting on humans as Oliver had done, but by starting with experimental animals, in a manner more befitting empirical scientists.

Although they were slightly later than Oliver and Schäfer, they independently produced exactly the same research results. Their paper begins on May 10, 1894 with "Experiment 1" and finishes on September 20, 1895 with "Experiment 14." The blood pressure and pulse rate data for all these experiments is shown, in some cases with the addition of respiratory rate (3-52, 3-53) [Figure 3-4].



(Aus Prof. Dr. N. Cybulski's physiologisch-histologischer Anstalt der k. k. Jagiellonischen Universität Krakau.)

Die Function der Nebenniere[1]).

Von

Dr. **Ladislaus Szymonowicz,**
Privatdocent an der Universität Krakau.

Hierzu Tafel I und II.

Wenn wir die ganze Reihe der auf die Function der Nebennieren sich beziehenden und zum Schlusse in alphabetischer Ordnung angeführten Arbeiten überblicken und mit ihrem Inhalte uns vertraut machen, so kommen wir zu der Ueberzeugung, dass die Ansichten verschiedener Forscher über die Function der Nebennieren sich wesentlich von einander unterscheiden und manchmal sogar ganz widersprechend sind. Dieser Umstand liess eine erneute Prüfung der ganzen Frage an der Hand einwandsfreier Experimente im hohen Grade wünschenswerth erscheinen.

Figure 3-4. Szymonowicz's paper on the principles of the adrenal glands. He discovered the blood-pressure raising activity of the adrenal medulla principle at Cybulski's laboratory (3-53).

It is worth pointing out that the results are from investigative research into active principles carried out by Cybulski. These results showed for the first time that the color-developing compound secreted by the medulla, which Vulpian discovered in 1856, had blood-pressure raising activity. The results were later confirmed by Abel and many other researchers (3-54). However, unfortunately for Cybulski and Szymonowicz, it took a long time for the results published in 1895 to become widely known, because they were presented in Cybulski's native language, Polish.

Cybulski's paper appeared in a journal with a French title, the content was in German (the title of the paper was in both German and Polish (3-55)), and when Cybulski co-authored with his student Szymonowicz (3-52) they wrote in German. It was a complicated situation.

In an academic journal article published by Szymonowicz in 1896, he writes in the footnote on the first page that the paper he wrote to earn his qualification as a university professor did not gain a wide circulation because it was in Polish; he therefore had to repeat the content, a huge work running to 67 pages, in German (3-53).

He must have felt the same anguish of a minor language as the scientists working in Japan during the Meiji period [Note 3-11]. Cybulski and Szymonowicz achieved posthumous fame for their many achievements, which were later widely recognized [Note 3-12].

Note 3-11.
This is something of a digression, but a very similar thing happened in a different field during the following century. This is the sad tale of the Polish-born economist Michal Kalecki. He discovered the principles described in the classic work on economy by John M. Keynes, *The General Theory of Employment, Interest, and Money*, before Keynes, and he published them in 1933 in his native Polish. The paper failed to attract much interest, so two years later he translated it into French, which he spoke well, and published it again. Unfortunately, Keynes published his *General Theory* in English the following year, becoming a hero of modern economics. Kalecki claimed antecedent rights in Polish, but due to the clout of the English language he ended up as the unsung hero of economics (3-63).

Note 3-12.
The well-known Polish physiologist Napoleon Cybulski was head of the Department of Physiology of the Universytet Jagielloński of Krakow for 35 years from 1885 until 1919. He is remembered for inventing the photohemotachometer, a device that measures blood flow velocity in blood vessels, and a device that measured the calorific value of heat produced by muscle contraction, as well as for his achievements in the field of the electrophysiology of brain sensory nerves (3-56).

Szymonowicz was an outstanding scholar, and this can be seen from his massive textbook, *Lehrbuch der Histologie und der mikroskopischen Anatomie*. This was published in German in 1901, with revised editions in 1909 and 1924, and was translated into English as *A Textbook of Histology and Microscopic Anatomy of the Human Body: Including Microscopic Technique* by John Bruce MacCallum in 2007, and is still available today.

Around this time, interest in the adrenal glands was increasing in academic circles throughout Europe. Slightly after the Polish group, and midway between Krakow and Straßburg, A. Spina and A. Velich were working on similar research at the Experimental Pathology Institute of Prague, in what is now the Czech Republic. In a rather complex study, they observed the effect of adrenal active principles on dogs that had been treated with piperidine, using an extract of adrenal glands (3-57, 3-58, 3-59).

Incidentally, the Dutch physician Christiaan Eijkman, who won the 1929 Nobel Prize for Physiology or Medicine for his demonstration of the relationship between beriberi and rice bran, first published his findings in 1895 in Dutch, but they did not cause much of a stir. His findings became widely known when he published them in German two years later in 1897, leading to his Nobel Prize for his work on vitamins. This was about the same time as the discovery of the physiological activity of adrenaline.

10. Looking back over research into the functions of the suprarenal glands

Let us take a chronological look back over the research so far, using the scientific literature cited by Szymonowicz in his paper (3-53). He cites a total of 111 papers over the 56-year period from 1840 to 1895.

If these citations are grouped by research theme, as in Table 3-1 (1), it can be seen that most of them are research into the adrenal glands themselves. Table 3-1 (2) groups them according to language: over half are French, and if German and Italian are included, the three languages make up 95%.

There was clearly vigorous research in these three languages, aiming to find out what the adrenal glands did as an organ.

Looking only at French, the language with the most activity in this area, Table 3-1 (3) shows when the research was conducted.

Finally, Table 3-1 (4) shows the distribution of all the studies over time, and it can be seen that there are three distinct periods with a high concentration of studies.

There was an extremely high concentration of studies over the three-year period from 1856, when Addison's discovery of Addison's disease coincided with Vulpian's separate discovery of a specific principle in the adrenal medulla.

After this, there was a period of groping in the dark during the 1870s, culminating in the final stage of the collaborative reports produced independently by Oliver and Schäfer and by Cybulski and Szymonowicz on the existence of a blood-pressure raising principle.

Table 3-1[(1)～(4)] An analysis of the scientific literature cited by Szymonowicz in his paper (111 studies during the period 1840–1895).

The tables are drawn up from the list of references cited by Szymonowicz in his paper "Die Function der Nebenniere (The Function of the Adrenal Glands)", published in 1896 (3-53).

(1) Research subject

Studies of the adrenal glands	101
Studies relevant to the adrenal glands	6
Studies relating to Addison's disease	4
Total	111

(2) Language of research papers

Language	Number of papers	%	Year of publication and circulation
French	57	51.4	See Table (3)
German	30	27.0	1880–1899: 23 papers
Italian	18	16.2	1880–1899: 14 papers
English	5	4.5	1858: 1 paper 1894: 1 paper 1895: 3 papers
Polish	1	0.9	1895: 1 paper
Total	111	100	

(3) Papers in French by period

Year	Number of papers	Period
1856	8	15 papers in 3 years
1857	5	
1858	2	
1884	1	5 papers in 5 years
1886	1	
1888	3	
1890	4	37 papers in 6 years
1891	6	
1892	14	
1893	7	
1894	3	
1895	3	

(4) Distribution of papers by chronological period

Year	Number of papers	Total for a 10-year period
1840	1	—
1856	8	**1850s:** 19 papers (17.1%) in a 3-year period 6.3 papers/year
1857	6	
1858	5	
1863	2	—
1873	1	**1870s:** 4 papers in a 7-year period 0.6 papers/year
1879	3	
1883	2	**1880s:** 25 papers (22.5%) in a 7-year period 3.6 papers/year
1884	4	
1885	2	
1886	5	
1888	9	
1889	4	
1890	5	**1890s:** 58 papers (52.2%) in a 6-year period 9.7 papers/year
1891	10	
1892	17	
1893	7	
1894	7	
1895	12	
Total	111	

As far as possible, I have tried to obtain and read the original texts in order to follow the tracks of all these scientists as accurately, and in as much detail, as possible. After reading these texts, however, I always find it hard not to feel slightly unsatisfied. This is because wherever the researchers of the time wrote *Nebennieren* [suprarenal gland] *extracte* or *la substance de capsules suprarenales*, depending on the language, to describe their samples for research, I wondered if the actual nature of the samples was reliable.

To put it differently, I cannot tell how to evaluate blood pressure data shown by graphs or figures from experiments using research samples taken in an age in which it was not yet possible to isolate and quantify the effects of the adrenal gland.

Furthermore, we now know the details of the mechanism of action of adrenaline and noradrenaline (see Chapter 8, section 5); unfortunately, any discussion of the various studies of medicinal action of adrenal extract in which we have absolutely no idea of the concentration of each of these components is fatally flawed. It is a great shame, but this therefore means that we cannot evaluate these studies.

Nonetheless, as the 19th century drew to a close, research into the principles of the adrenal glands advanced still further [Note 3-13].

Note 3-13.
In an experimental study of the formation of cerebrospinal liquid, the effects of adrenal gland extract are discussed in detail in 1899. This is a paper titled "Erfahrungen über die Nebenniern" by H. Boruttau of

the Physiologischen Institut der Universität in Göttingen in Germany (3-60). It is a broad study, with 27 pages of charts and a discussion that runs to 32 pages. Boruttau reports the action of adrenal gland extract on gut muscle, and it also mentions the paper by C. Jacobj (3-48) published seven years earlier.

The same year, M. Lewandowsky of the Physiologischen Institut der Universität Berlin chose the cat which is an easy experimental animal to use to investigate the effects of intravenous injection of adrenal gland extract on dilation and constriction of the pupils and other smooth muscle action (3-61). He also studied the effects on smooth muscle of the skin of cats and rabbits the following year, reporting that it had no substantial action (3-62).

11. Hemostatic effect and treatment of hay fever and asthma

At around the same time as Oliver made his discovery, three extremely important discoveries were made in the United States. First, on April 20, 1896, by the ophthalmologist William Horatio Bates (3-64) powdered sheep adrenal capsule was dripped into the eye, the conjunctiva of the globe and the lids were whitened in several minutes. He stated that this effect was definite, whereas no medications containing cocaine—which was habitually used at that time—had the same astringent effect.

He also showed various different medical cases, and stated that there was not even one case on which adrenal capsule extract had no effect. Specifically, he showed in great detail the effectiveness of adrenal gland extract in six cases of experimental medical treatment of ocular disease, and in surgery for different symptoms.

This lecture was summarized into a report of around 4,500 words and published in an academic journal; this paper declared that the adrenal capsule extract simplified eye surgery, which until then had been very difficult, and that this preparation surpassed all other drugs in that field. The paper described in detail the method for producing the extract solution, and its chemical properties (3-5).

Bates was born in Newark, New Jersey, in 1860, and opened a practice in New York. He is known for developing the "Bates Method" for improving farsightedness, shortsightedness, and astigmatism, regardless of age, without the use of spectacles (3-65). Having proposed this method, Bates found himself excluded from ophthalmic associations and the spectacles industry of the time, but from his writing it is plain to see that he was a sincere and earnest scientist and physician (3-66) [Note 3-14].

Note 3-14.
Two years after William Bates discovered the hemostatic effect, a paper was published in the *Berlin Klinische Wochenschrift* that did not accept this to be a new discovery. The paper was the record of a lecture M. Radziejewski of the Pharmakologischen Institut der Universität Berlin about the history of research into the physiological activity of the suprarenal gland up to that time. While he does give the grounds for his assertion, it is hard to see why he should add this objection to another ophthalmologist at the end of his lecture (3-67).

The second discovery was in 1898; Solomon Solis-Cohen, physician from Philadelphia, and professor of medicine and therapeutics in the Philadelphia Polyclinic, reported in the field of internal medicine that the adrenal component was effective against hay fever (ragweed coryza).

His paper was written with a bit of humor: “The success attending, in my hand, the treatment of exophthalmic goiter and other forms of vasomotor ataxia with suprarenal substance, led a further trial of the power of this agent in controlling neurovascular disorder. The experiment was made *in corpore vile*—myself.” For more than 20 years (he was 41 at the time), even when living in the city, during most of June and July his desperately itchy eyes and constantly running nose had been unbearable, and he would retreat to the coast or high ground. He later took a more medical approach, and considering that his condition started only after he had moved to eastern America, he self-diagnosed his complaint as hay fever. After this he consulted with doctors and tried administering various different medications to himself to decide their effectiveness, finally finding that adrenal substance was very effective. The actual drug he used was Supra-renal Tabloid, produced by Burroughs and Wellcome of London, and he records his pleasure at finding that while there had been some deterioration of the drug, there were no side effects on his heart (3-68).

Thirdly, Solis-Cohen further reported that adrenal active substance had therapeutic effects against asthma. In June 1897 he carefully examined a 22-year-old woman admitted to hospital with labored breathing, and although he racked his brains and tried giving her various different drugs, nothing had a satisfactory effect. He therefore tried the Supra-renal Tabloid that had been effective against hay fever, gradually increasing the dose, and succeeded in bringing about a dramatic recovery. The patient was discharged in October. The same therapeutic effects were later found in other patients, and Solis-Cohen gave a detailed account of his findings in an academic paper in 1900 (3-69).

◇ ◇ ◇

From Britain and Poland came the groundbreaking discoveries of physiological activity, and then from America came the three research reports from clinical practice. The British and Polish reports attracted tremendous interest in the fields of medicine and physiology, while the work from America garnered interest in the fields of medical treatment and pharmaceutics. It was the start of fierce competition in research to find the active principle.

Column 3-1.
What is the truth of Oliver's experiment on his son?

An investigative paper by Barcroft and Talbot can be summarized as follows.

"In the autumn of 1893, Oliver gave his son an injection of suprarenal gland extract, while his son wore a device attached to the wrist to detect minute changes in the internal diameter of the radial artery that Oliver had devised.

Finding that there were changes in the diameter of the artery, Oliver went to London to visit Schäfer. Oliver persuaded Schäfer to inject the extract into the vein of a dog, and as they watched, the mercury of a blood pressure gauge went off the scale. This episode is recounted by Henry H. Dale on the basis of a strong tradition that survived at the Department of Physiology of the University College, London, when he was a professor there from 1902 to 1904.

According to T. R. Elliot of the Physiology Laboratory of Cambridge University, Oliver had developed an apparatus to measure the diameter of human peripheral arteries. He gave his son a glycerol extract of adrenal glands orally, which made his son sick and caused the radial artery to constrict. Most people would have simply assumed that the extract was toxic; not Oliver.

Believing that half an explanation is worse than none, he went to see Schäfer at University College, London, to test the extract experimentally.

This was in 1893, when hormones and glands were unknown to physiology. Schäfer agreed to Oliver's request, and he injected some of the extract that Oliver brought into a <u>cat</u> on which he had been performing a separate blood pressure experiment. The blood pressure gauge shot off the scale before Schäfer's eyes, opening up the new physiology field of research into internal secretion glands."

This was what Barcroft and Talbot found in their investigation (3-42). From a scientific point of view, the conclusion would probably be the same regardless of whether it was a dog or a cat. From the point of view of the "tale of a major breakthrough," the author would like to know which one was actually used.

The investigation continues. Schäfer's recollection of Oliver's visit to his laboratory is as follows: "In the autumn of 1893 there called upon me in my laboratory at University College a gentleman who was personally unknown to me, but with whom I had a common bond of interest—seeing that we had both been pupils of Sharpey, whose chair at that time I had the honour to occupy.

I found that my visitor was Dr. George Oliver, already distinguished not only as a specialist on his particular branch of medical practice, but also for his clinical appreciation of physiological methods.

Dr. Oliver was desirous of discussing with me the results which he had been obtaining from the exhibition by the mouth of extracts of certain animal tissues, and the effects which these had in his hands produced, upon the blood vessels of man, as investigated by two instruments which he had devised one of them the haemodynamometer, intended to read variations in blood pressure, and the other, the arteriometer, for measuring with exactness the lumen of the radial or any other superficial artery.

Dr. Oliver ascertained, or believed he had ascertained, by the use of these instruments, that glycerine extracts of some organs produce decrease in caliber of the arteries and the increase of pulse tension, of others the reverse effect."

Schäfer spoke of his recollection at an invited lecture in London some 15 years after the event, but he states that Oliver himself reported giving his son, Charles, the extract by oral administration (3-3).

There are two more things from Barcroft and Talbot's investigation that should be added; these are points that the two authors write that they are not satisfied with. One is that none of Charles' children or his cousins recalls having heard of Charles being the subject of his father's experiment. The other is that adrenaline has almost no effect when administered orally, so the question is how the experiment as described by Charles could have produced this major breakthrough.

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