Hormone Hunters

— The Discovery of Adrenaline —

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Hormone Hunters: The Discovery of Adrenaline

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entitled “Hormone Hunters: Adrenaline no Hakken.”

To T. N., my honorable friend

Looking back in time, I smile
To have a friend such as you
Sets my mind at rest
Divided though we may be
By the limitless ocean

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In Brief

Prologue

I often remember the dread I felt as a child sitting in the dentist's chair; it was so different from the enjoyable sensation of sitting in the same type of chair at the barbershop, waiting for my turn while listening to the brisk snip-snip of scissors. Recently, I had to visit the dentist to have a wisdom tooth treated. This time, I felt the briefest flutter in my breathing and my heart when the dentist gave me a local anesthetic, but the procedure finished with no pain and no bleeding. The wonders of modern medicine made me realize how much science has advanced. I asked the dentist the name of the drug he had given me, and when I got home I looked up its active constituents. The drug contained the local anesthetic xylocaine, also known as lidocaine, and the vasoconstrictor epinephrine, otherwise known as adrenaline.

My first thought was that most people who have an injection when they undergo dental treatment probably do not realize that they are being given epinephrine (adrenaline). After further investigation, I was surprised to find that there is actually a huge number of drugs containing epinephrine on the market, so there must be a great many people who, just like me, have benefited from epinephrine without even being aware of it.

In the United States, the EpiPen® auto injector is used to deliver a minute dose of epinephrine to those going into anaphylactic shock due to peanut allergy or bee sting as an emergency procedure before the person is rushed to the hospital. Since 2012, schoolteachers in an increasing number of states are being advised to use the EpiPen in these situations.

Adrenaline was the first hormone to be isolated. Hormones are chemical substances produced within the bodies of animals that act in minute quantities on specific organs, enabling them to carry out functions necessary to maintain life. Adrenaline is secreted by the adrenal glands, which are tiny glands that sit one on each kidney. The adrenal glands have two layers of structures, and adrenaline is secreted by the inner layer, called the adrenal medulla. Once secreted, adrenaline is carried by the blood to the heart and other organs, where it exerts its physiological activity.

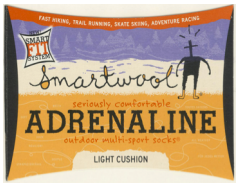
I imagine a golfer making his approach shot to the green for the last hole of the

championship. He is aware that victory hangs on this shot, but he sends it too far, overshooting the green. At an interview after the match, you might hear him say, “I guess I was pumping too much adrenaline out there.” This is the action of adrenaline.

The three figures below illustrate popular images of adrenaline. An old Japanese friend of mine found the canned soft drink on the left at a gas station while we were traveling in New Mexico in 2007 (of course, it contained no adrenaline). The others are advertisement and goods that he had collected for me by that time. In these examples, “adrenaline” refers to the energy and vitality associated with the hormone; these products do not actually claim to contain biologically active adrenaline.

Adrenaline, a drink to keep you awake. The main active ingredient is caffeine.

Fillup with adrenaline, and then just go!



Adrenaline socks that let you walk on and on.

It is by no means uncommon, at least in the U.S., to come across advertising or products like those shown in the above figures. Most people have an accurate understanding that adrenaline is a substance produced within the body that somehow works to make us more lively and energetic. However, there is no doubt that with the exception of professionals specializing in medicine and pharmaceuticals, few people realize that adrenaline is known as epinephrine when it is used in pharmaceutical drugs. Why does one substance have two different names, adrenaline and epinephrine? This is one of three things about adrenaline that has puzzled me for some time. Incidentally, I will use the name adrenaline throughout this book, except in special cases such as in the references or for trade names.

I worked for many years at Sankyo Co., Ltd., now Daiichi Sankyo Co., Ltd. This was Japan’s first pharmaceutical company, and the first company president was Dr. Jokichi Takamine (1854−1922).

Readers may have heard of Takamine, as even today he is greatly respected in the U.S. as the father of modern biotechnology (p-1). At an early age he set out for the new world of America to make a name for himself, and there he made two major discoveries.

His first success was the discovery of highly potent saccharifying enzyme isolated from culture of sake-brewing Kojikabi mold. This became the first enzyme to be extracted and purified on an industrial scale, and it first went on the market in 1895 as Taka-Diastase digestive medicine. This was produced in the U.S. by Parke-Davis and Company, now a subsidiary of Pfizer, Inc., and rapidly became a highly popular product. Not long after, Taka-Diastase appeared in the seminal novel *I Am a Cat* (pub. 1905−06) by the much-loved Japanese author Natsume Soseki. Soseki's alter ego in the book, Mr. Sneeze, was a keen user of Taka-Diastase.

Takamine's second remarkable achievement in the scientific field was the crystallization of adrenaline. Takamine achieved the feat at his laboratory in New York City in July 1900, five years before the word "hormone" was proposed. Crystallizing a physiologically active principle secreted within the body was a historic piece of scientific research, and a fitting prologue to the twentieth century.

Adrenaline was first discovered as a clump of innocuous-looking crystals at the bottom of a test tube. Chapter 1 tells of the excitement of the moment when the discovery was made, and how the substance was subsequently named and an application was filed for a patent on its manufacture. Chapter 2 takes a great leap back in time with a scientific history of how anatomists and physiologists from the time of Galen onward racked their brains to understand the adrenal glands, which were shrouded in mystery. The famous 18th-century French thinker and social commentator Montesquieu also makes an appearance in this tale.

We then witness a ground-breaking discovery in 1856 that instigated the race among scientists to extract pure adrenaline. This was the discovery by the French scientist Alfred Vulpian (1826−1887) that adrenaline has a particular color reaction; if a minute quantity of a chemical substance is added to adrenaline, a reaction takes place that produces a characteristic color. I wondered what had led Vulpian to be interested in a substance originating from the adrenal glands in the first place, and why he was searching for it. This was the second thing about adrenaline that had puzzled me for so long. To get my answer, I carried out a detailed study of research papers primarily from France, Germany, and the UK, which were the most advanced countries in the natural sciences at the time. I discuss the results in Chapter 3, and in Chapter 4, I turn once again to the race to isolate adrenaline.

In Chapter 5, I try to get to the bottom of the third question that was puzzling me. Curiously, it turns out that Takamine carried out no other research into hormones either before or after his isolation of adrenaline. He does not seem to have shown any interest in these active substances produced and secreted in minute quantities within our bodies. In the

44 years from the discovery of the characteristic color reactions of adrenaline until Takamine's success, over 20 researchers took part in the race to extract pure adrenaline, including some of the greatest scientists of that time. So why was it that for 44 years not one of them was able to make it to the finish line, while Takamine, who entered the race all of a sudden, far behind anyone else, succeeded in almost no time at all? This was the third big question puzzling me. The background that helps unravel this mystery is given in detail, and it is here that Parke-Davis and Company had a major role to play.

Chapter 6 examines the clear ruling passed by Judge Learned Hand in a dispute over infringement of the patent on the manufacture of adrenaline. Judge Hand dared to challenge the idea that natural products should not be subject to patents, which was the accepted wisdom among lawyers up until the end of the 19th century. The controversy surrounding this decision continues to this day, and in 2013 the U.S. Supreme Court ruled that a patent could not be granted on naturally occurring DNA.

In Chapter 7, a valuable historical document—a letter to Takamine from one O.W. Smith, president of Parke-Davis and Company—helps unravel the mystery of how the name epinephrine came to be used in the *US Pharmacopeia*. I will also look at the confusion that has been caused by the double naming. This chapter gives a glimpse of Takamine at the end of the nineteenth century, already focused on his mission as he carried out contract research for companies.

With the crystallization of adrenaline in 1900, there was no longer any need for the laborious and time-consuming task of extracting the hormone from the adrenal glands of domestic animals. Scientists were now able to obtain commercially available adrenaline crystals for use in experiments with just a single telephone call. Their subsequent research is described in detail in Chapter 8, and the rapid succession of new advances represented a paradigm shift. While these three chapters may appear to be a lecture transcript on the history of modern biochemistry, they have a great many implications with respect to the integrity that is expected of scientists of the future.

Although the subject matter is inevitably somewhat technical, I have tried to present it in a way that will be readable even for those with no background in chemistry. Where I have used slightly technical terms, I have included simple explanations in separate columns.

Finally, I would like to touch on the reasons why I chose to publish this book. I have spent many years carefully reviewing the literature relating to adrenaline, and other than a brief but meticulous review in Japanese by Dr. Yutaka Sano (p-2), there does not appear to be anything in the vein of a science history book that tells the story of adrenaline from recognition of the

adrenal glands to isolation of the hormone. There are numerous books and articles, particularly in Japanese, relating to Takamine's achievements, but most of these were written to honor Takamine and his collaborator, Keizo Wooyenaka (also spelled Uenaka). While these publications give detailed accounts of the characters of Takamine and Wooyenaka, many are essentially the biographies of important figures; there is almost no treatment of the scientific articles and materials on their historical achievements in the fields of physiology, medicine, and chemistry.

Adrenaline was the first substance to be established as a hormone, and is therefore of monumental importance to human scientific endeavor. Nonetheless, although people from the country where adrenaline was crystallized have made fragmentary records of the history of this research, I have not found any books in English, German, or French that cover all aspects of the story. It therefore became my mission to bring out an English version of this book after it was published by the Kyoto University Press in 2012 in Japanese.

I found out recently that in June 2013, six months after this book was published in Japanese, the science history book *Adrenaline* by Dr. Brian B. Hoffman was published. Dr. Hoffman is a researcher who has studied the cellular action of adrenaline for many years, and is a professor of medicine at Harvard Medical School. Dr. Hoffman's book naturally gives a very full account of adrenaline from the perspectives of physiology and medicine, and he also devotes many pages to the glorious history of advances after adrenaline was first crystallized. I am delighted that Dr. Hoffman's book and mine complement each other perfectly, and his book in no way lessens the significance of making an English version of my own book.

History of the quest for the role the adrenal glands play is of course, linked to the history of scientific development in the particular country where research took place. At the same time, it is an enormously interesting scientific drama that played out across a wide area in an age of rudimentary information technology. It is a drama of courageous challenges and human conflict, as well as the woes of a small country with a minor language.

I hope this book will tell that story, and at the same time illustrate the struggle of scientists working in an age in which technology for separation and analysis of organic compounds was unbelievably primitive by today's standards.

Literature Cited

(p-1) Map of the Woodlawn Cemetery (1990).

(p-2) Sano, Y., "Adrenaline Hakken eno Michi (Road to the discovery of adrenalin)." *Microscopia,* **6**: 194–200 (1989).

Chapter 1

Making History with Hormones

In Paris in 1856, Alfred Vulpian discovered that minute quantities of a substance were secreted from the adrenal glands into the blood vessels, and that this compound reacted with commonly found reagents to produce a characteristic color. Naturally, it seemed only a matter of time before this substance could be extracted. For the next 44 years, numerous eminent scientists from continental Europe, the United Kingdom, and the U.S. sought this elusive compound, but no matter what expertise they brought to bear, the prize remained beyond their grasp.

The scientists who finally succeeded were Jokichi Takamine and Keizo Wooyenaka (Uenaka), who had emigrated to the U.S. from Japan, which was then just a minor country in the Far East.

1. Dawn in a foreign country

Summer in New York City is hot. On July 21, 1900, the last year of the 19th century, the temperature in the city was 88°F (31°C) and the sky was overcast. In the basement of a brick apartment building halfway between Central Park and the Hudson River, there was a small laboratory. Outside, a signboard proclaimed this to be Takamine Laboratory.

The cramped, hot laboratory measured just around 13 square meters. The detailed records that Wooyenaka kept in his laboratory notebook [Note 1-1] lets us see what the laboratory was like inside [Figure 1-1]. Wooyenaka came originally from a mountain village in Hyogo Prefecture, Japan. As he picked up a few test tubes left standing from the previous night's experiments, he was probably thinking that six months had already passed since he came to the U.S. He checked the test tubes by holding them up to the morning light streaming through the window. *Strange, he thought—there seems to be a lump of something stuck to the bottom of one of them*. He had worked hard the day before, but the extraction fluid had given off an unfamiliar smell and the color reaction he had tried had not been promising. *This is not going to be at all easy*, he had thought as he crawled into bed.

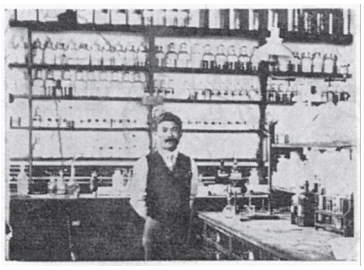


Figure 1-1. Keizo Wooyenaka in the "semi"-basement laboratory in New York City. On July 21, 1900, Wooyenaka became the first person to isolate the hormone adrenaline. (Courtesy of Sankyo Co., Ltd.)

Note 1-1.
The existence of Wooyenaka's laboratory notebook was not widely known for many years following his death, but it had been held at the famous Kyogyoji Temple in his hometown of Najio in Hyogo Prefecture, Japan.

In 1965, Wooyenaka's son Mioji provided science historian Ms. Aiko Yamashita with a copy of the notebook. Through her efforts to decipher the technical terms and the difficult handwriting, Yamashita made the story of adrenaline available to the world and ensured that it would be passed on accurately to future generations (1-1, 1-2).

The notebook has been in the safekeeping of the Kyogyoji Temple, since the time when Dr. Sosogu Nakayama, professor emeritus at Okayama University, had concurrently been the chief priest of the temple. He happened to know that Wooyenaka had been a native of the area of his temple and got acquainted with Wooyenaka's son Mioji, who donated the original notebook inherited from his father to the temple.

In March 2010, Wooyenaka's notebook was designated as Certified Chemical Heritage No. 2 by the Chemical Society of Japan. (A reproduction of the original notebook (1-3) may be viewed at the National Museum of Nature and Science, Tokyo.) [Figure 1-5, 1-7]

"No, it can't be this easy," he mumbled to himself as he removed the lump from the tube. First, he tested its solubility in water and alcohol, and he compared its properties to those of naturally occurring substances that had already been described in scientific works. Finally, he washed the lump in a minute amount of water and dissolved it in a small amount of dilute hydrochloric acid. Using a handmade pipette with a fine tip, he transferred some of the solution into a shallow glass plate (watch glass) about 10 centimeters across. Again using his handmade pipette, he added a drop of dilute ferric chloride solution that he had prepared separately to the solution in the glass plate. The solution instantly turned a deep sea-green color.

This was the characteristic color that Alfred Vulpian [Figure 1-2] had described in French as *glauque* (sea-green) when he discovered the color reaction nearly half a century earlier (1-4).

Wooyenaka was on the verge of euphoria, but he kept himself in check. He repeated the experiment, this time dripping an aqueous solution of iodine onto the solution in the glass plate. Now the solution turned the color that Vulpian had described as rose-carmine [Figure 1-3].

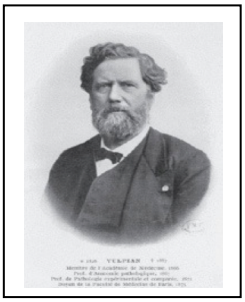


Figure 1-2. Alfred Vulpian, the discoverer of adrenal glands hormone. (Courtesy of the National Library of Medicine)

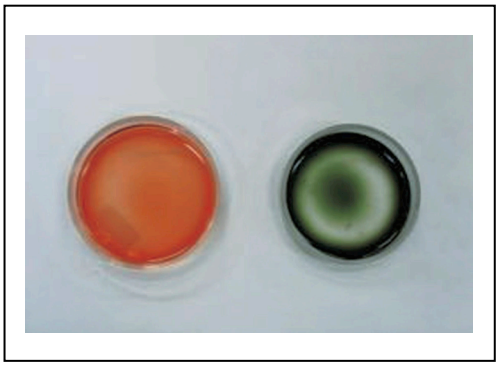


Fig. 1-3. Color reactions of adrenaline discovered by Dr. Alfred Vulpian in 1856. Left: rose-carmine with iodine. Right: glaucous (sea-green) with ferric chloride (results of the reproduction experiments by the author)

The crystals in the test tube Wooyenaka was holding were a hormone, isolated from a living organism for the first time in human history—yet for Wooyenaka it was almost an anticlimax.

A young scientist of just 24, Wooyenaka had given up on Japan, which placed more emphasis on school background than actual ability, and sailed to the U.S. at the end of the previous year. Just five months or so earlier, he had been hired by Jokichi Takamine [Figure 1-4] who had a doctorate in engineering.

Figure 1-4. Jokichi Takamine, who made a name for himself worldwide as a scientist and businessman (1-5).

Wooyenaka was not fully aware that this was an instant that would be indelibly inscribed in the annals of hormone research. Although the sign outside said Takamine Laboratory, the staff consisted of only the director, Takamine, and the newly employed laboratory worker, Wooyenaka.

Much later, at the age of 82, Wooyenaka was asked in an interview about his memories of the laboratory. What was it like at Dr. Takamine's laboratory on New York City's 109th Street? "You couldn't really imagine it in Japan," Wooyenaka replied. "We were renting a basement that was part of the janitor's residence." (1-6).

It would not be until 1903 that a technician by the name of Henry Ford, who had worked in Detroit at a company founded by inventor Thomas Edison, succeeded in starting up an automobile company after twice failing, and commenced mass production of the Model A. Therefore, the motorized age was yet to come, and the street in front of Takamine Laboratory reverberated to the gentle clip-clop of horses' hooves. The laboratory was, of course, without

air conditioning.

When Takamine, who was living at 475 Central Park West at the time (1-7), arrived at the laboratory building, Wooyenaka sensed his presence and went immediately to Takamine's office to give him the results of the experiments. Takamine had only recently come to New York City from Illinois, after finally managing to make a livelihood from the success of his Taka-Diastase digestive medicine. He was 46, and after having overcome various difficulties while living in a foreign country, he was now in his prime as a scientist and businessman.

Takamine looked at the lump of crystals at the bottom of the test tube that Wooyenaka held out to him, and after carefully examining them he turned to Wooyenaka with a smile. Wooyenaka explained the details of the experiments he had carried out and the Vulpian color reaction. Takamine confirmed that an active substance from the adrenal glands had been isolated as crystals, and Wooyenaka immediately asked Takamine to order more organ samples from Parke-Davis and Company.

Returning to the laboratory, Wooyenaka quickly entered the results of the day's experiments into the laboratory notebook, which resembled a large-sized daily planner, which he had started the day before (1-3). On the first page of the notebook he had written "Investigations for Active Principle of Suprarenal Glands" in bold letters with a thick pen [Figure 1-5, right]. The cover of this notebook proclaims "On Adrenalin" in red letters [Figure 1-5, left], but as the word "Adrenalin" had not yet been come into existence at that time, it must have been written at a later stage of the laboratory notebook. His surname is written as Uenaka—although this is the normal spelling, he realized that no one would pronounce this correctly, so shortly after this he started to sign his name as "Wooyenaka."

The first entry on the second page in the laboratory notebook, dated July 20, the previous day, reads, "Dr. Takamine returned from the New York branch office of Parke, Davis & Co. carrying an aqueous extract of adrenal gland, and I was requested to set about the preliminary experiments for the separation of principles"

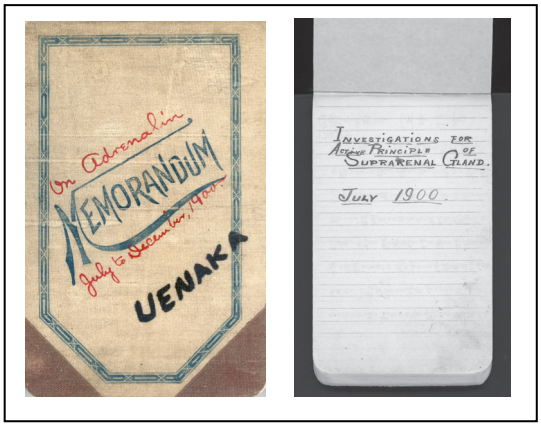


Figure 1-5. Wooyenaka's laboratory notebook.
Left: cover
Right: the first page
(owned by Kyogyoji Temple, Hyogo Prefecture, Japan)

You could perhaps say that in just two days, Takamine and Wooyenaka had earned the laurels that would assure them an indelible place in history. However, what Takamine brought back was aqueous extract of adrenal glands (it is unknown whether this was extract of just the medulla or not), rather than adrenal glands themselves. Rather than supposing that this day marked the start of an agreement for collaborative research between Parke, Davis & Co. and the Takamine Laboratory, it probably makes more sense to assume that Wooyenaka had already been instructed to carry out the preparatory research. Takamine would naturally have received the latest information on the active principles of adrenal glands when he previously visited the headquarters of Parke, Davis & Co. in Detroit, and he would have communicated this to Wooyenaka.

2. Steady progress

In a research laboratory, the work to clinch a discovery like this continues for a surprisingly long time and requires a great deal of patience, as Wooyenaka was to find out. The next entries in his laboratory notebook start from July 30. He records on that day that he weighed a delivery of cattle adrenal glands sent by Parke, Davis & Co., and it came to a total of about 8 kg; approximately 900 g (about 2 lb.) had been lost during transport through evaporation or seepage. At that time, such a delivery would doubtless have been packed in ice for transport. The laboratory had been receiving supplies of aqueous extract of adrenal glands at first, but on that day Wooyenaka started to make their own extract from glands sent to the laboratory. Half of the glands, or 4 kg, were submerged in three times their weight of water, and the remaining 4 kg were submerged in twice their weight of 95% ethanol, and both were heated.

The experiments to obtain the crystallization of the hormone were repeated, and the subsequent entry for August 4 records the following. The laboratory happened to be infested with mice, and Takamine and Wooyenaka managed to catch three of them, which they kept in a bell-shaped glass container. They tried applying a drop of a solution of the crystals dissolved in acid to the eye of a mouse; the eye mucous membrane immediately lost its color and turned pallid. The researchers tried this because they recalled a research report (1-8) that found that the main constituent of adrenal glands caused peripheral blood vessels to contract.

From the next day, Takamine and Wooyenaka energetically continued their work, performing experiments on the chemical reactivity of the crystals and investigating different purification methods with a newly purchased compression filter.

There was something that bothered Takamine, and it bothered Wooyenaka as well. Over the previous three years, Prof. Dr. J. J. Abel of the Johns Hopkins University ［Figure 1-6］ had produced six research reports in close succession, and he was miles ahead of Takamine and Wooyenaka in his research for the means of the isolating active principle. Unable to take their minds off this, they could not suppress the desire to check his research. Abel had persistently worked on a strategy to simplify extraction and purification of the active principles by reacting adrenal gland extracts with benzoyl chloride to convert the active principles and the other constituents in the adrenal glands into benzoyl derivatives. With this method he had obtained a compound from adrenal glands that he believed was the blood pressure-raising principle. This he called "epinephrin," without the final "e" (1-9).

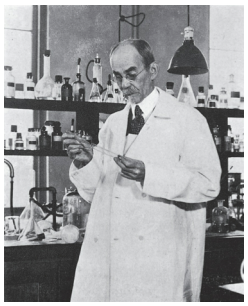


Figure 1-6. John Jacob Abel, who led the way in research into the isolation of the blood pressure-raising principle of the adrenal glands (1-10).

So Takamine and Wooyenaka tried reacting the new crystalline body ［Note 1-2］ of the active principle that they had produced with benzoyl chloride, to see whether they would be able to collect the same substance as Abel. Any scientist commencing this research after Abel would naturally need to perform this experiment. Wooyenaka first performed the experiment on August 10, and showed crystals that appeared to be a benzoyl derivative to Takamine. However, Takamine was doubtful; he thought that the new crystalline body was simply adhering to a crystal of benzoic acid (an acid produced by the reaction of benzoyl chloride and water).

Note 1-2.
Apparently, Wooyenaka was not at all certain whether the crystalline lump he had obtained was a single crystal or not, so at this stage he decided to call the lump a "new crystalline body" for convenience.

Thinking this likely, they labeled this crystal "No. 1" and the new crystalline body "No. 2," and asked Parke, Davis & Co. to perform activity tests on them. The results of the activity tests are not recorded anywhere in the laboratory notebook. Just to make absolutely sure, Wooyenaka performed the same test again on August 21, 1900, but was unable to obtain a compound that was the same as Abel's.

Abel stressed that the active principle did not precipitate (crystallize) in an alkaline medium, but Wooyenaka contradicted this by achieving crystallization in an alkaline

medium. The compound that Abel produced must have been completely different from Wooyenaka's active sample. Yet even though Wooyenaka came to this conclusion, there must have been something still bothering him, because he tried again for a third time on October 30. Nonetheless, he still did not produce "Abel's benzoyl derivative."

3. Aiming for commercialization

Summer was turning to fall, and on the morning of September 10, Wooyenaka heard from the janitor that a resident on the second floor had complained about the noise from the laboratory. The weather was cooler, and Wooyenaka had turned up the power for the warm water extraction that he carried out late into the previous night. Reflecting that this had not been a good idea, he had no choice but to put a stop to his nighttime operations and instead keep the extract until the following morning at the consistency of a watery gruel at about 60ºC. He refused to be downhearted about this, though, and he realized that the fatty substances that came out of the organs and floated to the surface of the water formed a layer that prevented air oxidation. So it turned out that this method was not at all bad, and Wooyenaka soon regained his spirits.

Unsurprisingly, he enthusiastically continued the experiments to find a better way to collect crystals, and frequently sent crystals he obtained to Parke, Davis & Co. The activity of these samples is not recorded in Wooyenaka's laboratory notebook, but the samples were rapidly quantified at the newly established Biological Research Laboratory of Parke, Davis & Co. in Detroit using dogs as experimental animals.

Wooyenaka's research did not always progress as smoothly as described so far. Sometimes he made mistakes and was unable to collect any crystals at all. He writes that when this happened, Takamine consoled and encouraged him. Takamine was his employer, but there must have been a very close relationship of mutual trust between the two men.

The next important experimental result to emerge can be found on the page for September 19. This page has drawings of six crystalline forms of the new crystalline body [Figure 1-7]; the notes record that four of these crystals take on a clear shape as the purification stage progresses, while the other two crystals appear at the crude stage. This shows Wooyenaka's painstaking approach to research, and the laboratory notebook is now an important part of the heritage of scientific history.

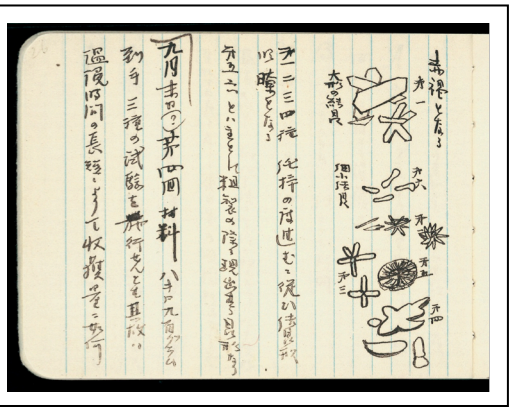


Figure 1-7. A page of the Wooyenaka's Laboratory notebook clearly recording that there are many different crystal forms of adrenaline (owned by Kyogyoji Temple, Hyogo Prefecture, Japan)

One thing that should certainly be mentioned here is the verification of the level of K. Wooyenaka's experimental technique, which comes from an extremely interesting research report that was recently released.

Prof. Dr. Shigeru Saito of the Graduate School of Medicine / School of Medicine, Faculty of Medicine, Gunma University, Japan, extracted and processed 179g of pig adrenal glands according to Wooyenaka's procedure. While he was able to collect a tiny amount of crude crystals of adrenaline, Dr. Saito notes that this crystallization was a very difficult experiment to perform. Describing his impressions, he says, "Carrying out these procedures under the laboratory environment of the time must have been far from easy. This was a tremendous achievement, which only a skilled technician with the temperament of an artisan could have managed. These were no rough-and-ready experiments— success could only have come through the methodical approach, painstaking forethought, and constant diligence of the researchers of the day."(1-11).

Wooyenaka then investigated the conditions for extraction and purification, on a far grander scale than before. Processing some 10 kg of cattle adrenal glands, the equivalent of 700 to 1,000 glands, he obtained of more than 10 g of crystals. The tone of his entries in the laboratory notebook suggests that he felt the task was completely on track with the quantity of crystals now sufficient to meet any demand of research.

Wooyenaka conducted a bold experiment on a living human subject—himself. On October 13, he applied drops of a solution of the crystals he had prepared, dissolving in 1,000 times their weight of water, to one of his own eyes; to the other eye, he applied drops of aqueous extract of adrenal glands prepared by Parke, Davis & Co. He observed the loss of color in the eye mucous membranes using a mirror, finding that his own crystals had a stronger effect. "Dissolution in 1,000 times the weight of water is right for practical use," he recorded in the laboratory notebook. This dissolution rate of 1:1,000 is the same as the "0.1% content of adrenaline solution for external application" that is on the market today, a whole century

later.

Perhaps his chest swelled with pride as he imagined the day in which a new product was presented to the world in a glass bottle labeled "SOLUTION Adrenalin Chloride 1:1,000."

A momentous entry appears on November 7: "At the suggestion of Dr. Wilson, an acquaintance of Dr. Takamine, the new crystal has been named 'adrenalin.'" The name had no final 'e'.

Apparently Takamine had the confidence to talk to a trusted acquaintance about the outcome of the research. The name coined at Dr. Wilson's suggestion combined the Latin *ad*, meaning "by" or "near," with *renal*, meaning "kidneys," and added the suffix *in*, meaning "chemical substance" (penicillin and insulin are examples of other substances using this suffix). The resulting name was both apt and easy to read. Nowadays, a Google search of "adrenaline" generates about 48 million hits. It shows just how widely this word is used.

As fall advanced, Wooyenaka moved to refine the procedure to achieve higher purity. On November 11, he obtained 25 g of white crystals from 40.9 g crude crystals; two days later, he obtained 2 g white crystals from 4 g crude. However, he was still not satisfied. Wooyenaka was beginning to feel that they were approaching the limits of the amount of work that could be done in the tiny laboratory in the semi-basement of an apartment. It was just at this time that Takamine announced that as they could not process greater quantities, he was planning to transfer control of the work to a company factory after the completion of the next experiment, which was their eighth. On November 15, after processing 23 kg of samples of adrenal glands in New York, yielding a total of 2,116 samples, they turned their attention to industrial level production at the premises of Parke, Davis & Co.

In the beginning of December, Wooyenaka accompanied Takamine to the headquarters of Parke, Davis & Co. in Detroit. In the margin of the laboratory notebook, it says, "Refer to the laboratory diary for other reports." Unfortunately, this diary is yet to be found, despite the best efforts of people involved in Wooyenaka's story. In his last years, at age 82, Wooyenaka commented during a conversation, "It would be interesting if the documents still remained from the time when we started."(1-12) Perhaps the results of the experiments performed before July 20, the date of the first entry in Wooyenaka's existing laboratory notebook, have been preserved in the laboratory diary he referenced.

Wooyenaka only records once in the laboratory notebook which animal species the adrenal glands provided by Parke, Davis & Co. used to obtain adrenal glands; on or after July 30 he specifies that the first batch of raw materials were "cattle adrenal glands," but after this there is no other record. In that entry, he records 29 cattle adrenal glands weighing

a total of 8 kg, a mean weight of 275 g each. On October 10, the mean of the glands in the fifth batch of raw materials was only 122 g, or about half the weight of the mean from the first batch, so they were probably collected from calves. Subsequently, on November 15 the laboratory was supplied with adrenal glands that were exceptionally small in comparison. The mean weight of those glands is recorded as 10.6 g, so these were presumably sheep adrenal glands. In the patent that Takamine applied for on the basis of Wooyenaka's experimental results (1-13), the animals from which adrenal glands are removed are described as "cattle, sheep etc."

◇ ◇ ◇

The results achieved in that cramped semi-basement laboratory over just a few short months were to become the link between the labors of many prior researchers from the preceding half century and the illustrious accomplishments in the fields of physiology, chemistry, and medicine that unfolded from that point onward.

Column 1-1.
The extraction and crystallization method developed by J. Takamine and K. Wooyenaka

The main points of the methods for extracting and purifying adrenaline that J. Takamine and K. Wooyenaka put together and then described by Takamine in a patent (1-13) are as follows:

In carrying out my invention I make a fluid extract of the clean suprarenal capsules from animals-such as cattle, sheep, &c.- by disintegrating the said capsules by suitable means, then mixing with about the same weight of water and steeping at a temperature of about 60 to 70°centigrade for the period of about five to ten hours in a suitable vessel, preferably avoiding contact with atmospheric air. This can be to a great extent accomplished by a film of fat floating on top of the liquid or may be done by passing a slow current of hydrogen or carbon dioxide into the top part of the vessel. The object of this is to prevent the oxidation of the extract. The film of fat referred to may conveniently be the fat naturally associated with the glands. At the latter part of the steeping the temperature of the mixture may be raised from 85°to 100°centigrade. The mass is now strained, and the residue is pressed to squeeze out as much as possible. The residue thus pressed out is steeped again with the least amount of water to cover the mass for several hours at the same temperature as above. The two extracts thus obtained are mixed, and the mixture is cooled rapidly and the solidified fat removed. The liquid is now evaporated at a low temperature, preferably in a vacuum pan, admitting, if necessary, a small quantity of hydrogen or carbon dioxide to replace air and to prevent oxidation. The liquid is evaporated until it becomes one-fifth to one-seventh of the original volume. To this concentrated solution two to three times its own volume of alcohol is added, so that the mixture will contain about sixty per cent of alcohol by volume [Details of the following part and the purification method by recrystallization are omitted].

Column 1-2.
The paper by Alfred Vulpian that first reported the adrenaline-specific color reactions

This is an abridged translation of the paper by Vulpian (1-4), which all scientists taking part in the race to isolate adrenaline would probably have consulted (for the color reactions, see also Figure 1-3).

Title: "Note sur quelques reactions propres à la substance des capsules surrénales" (Research carried out at Flourens Laboratory. Reviewers: Dumas, Pelouze, and Bernard)
Summary of the main points: A constituent of the adrenal glands always shows the same specific reaction. The adrenal glands of sheep, like those of other mammals, are comprised of two parts: one part is the cortex, which is fibrous in section and has the same color tone as the kidney; the other is the medulla, which is more homogenous and shows a greyish color tinged with pearl. It is this latter substance that produces these color reactions specifically and almost exclusively. If the surface of the medulla is scraped off with a scalpel after the two parts are separated, a fluid is obtained. Microscopic observation shows that much of this fluid appears to comprise flexible nuclei, and the nuclei appear to be made up of several types of fusiform elements: molecular particles, generally greasy substances, sections of nerve fiber, and a liquid in which these fragments swim. The fluid from the medulla is diluted with distilled water, and the resulting liquid shows the following reactions.

The liquid ranges from neutral to slightly acidic in reaction.

If ferric chloride or ferric oxide is added, the liquid becomes a slightly dark sea-green color with a slight tinge of blue or green.

Ferrous oxide shows the same color reaction, but it is extremely slow. The reaction probably occurs after the iron has been oxidized.

Staining with iodine solution gives a highly unusual color, somewhere between rose and red [In the days before color photographs, a certain amount of ingenuity was needed to express color tones].

[...]

As the various other characteristics are not very clear, I (Vulpian) shall not deal with them here, instead I intend to give an overview of them in the future. The adrenal glands of all mammal species I have investigated from this perspective have caused the same reactions. These animals are human (because human medulla samples are almost always deteriorated, I could not avoid frequent failures in this research), dog, cat, mole, rat, mouse, rabbit, marmot, sheep, calf, cow, and horse. Birds, chickens, and seagulls gave the same reaction. When experimenting with mammal adrenal glands, the cortex can sometimes mask the reaction, making it difficult to distinguish, so from this point of view care should be taken to collect only the medulla.

These reactions are characteristic of the adrenal glands. I created test paper impregnated with ferric chloride and used this to test spleen, thyroid gland, cerebral ventricle, semi-lunar ganglion, nerve, lymph node, liver, pancreas, lung, kidney, all mucous membranes, muscle, colored choroid coat, and blood; none of these produced a reaction.

There is therefore a singular substance endowed with notable chemical properties that to this day is unknown and present only in the adrenal medulla. Consequently there exists a substance that gives this organ its characteristics.

Is this substance present in the medulla preordained to be broken down on the surface of the medulla, or does it permeate into the blood so that it can be carried throughout the circulatory system? I strongly support the latter hypothesis. In sheep there is a main vein that runs along the longitudinal axis of the medulla, and there is a type of venous sinus with its opening at the tip of the adrenal gland. I have consistently observed drops of blood that come from the venous opening, and this blood shows the characteristic reaction with ferric chloride. Similarly, the small clots that solidify in the hollow of the vein near the opening of the adrenal gland vein show the same reactions to the reagents noted above, although the reactions are fairly weak. The substance has thus made a pathway across the membrane lining the vein.

This phenomenon occurs constantly after death, so I reason that the same thing must occur when the organism is alive. This will likely be determined by future experiments. Similarly, the hypothesis that the adrenal glands should be regarded in the same way as the gland tissues usually called bloody glands, or, to put it differently, the hypothesis that this gland sheds its secretions directly into the blood, should be proven definitively in the future. How important is this secretion? I confess that I have no ideas to put forward with regard to the possible purpose of this substance. Consequently, I shall not take the risk of formulating a hypothesis [Underlines added].

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Chapter 2

The Quest to Unravel the Mystery of the Adrenal Glands

The quest to unravel the mystery of the adrenal glands has a history that stretches back a very long time before Jokichi Takamine and Keizo Wooyenaka crystallized adrenaline from the aqueous extract of adrenal glands. Anatomical drawings from 16th century Italy accurately show the adrenal glands, so if we consider that period to be the starting point of the scientific history of the adrenal glands, then the quest has its roots in anatomy and pathology.

1. Searching in the dark

There is a considerable body of references and data relating to adrenaline; most of these begin with descriptions of Addison's disease. The story of Jokichi Takamine, which culminated in the successful crystallization of adrenaline, is no different.

Thomas Addison, who discovered this disease in the mid-19th century, made perceptive observations of anemia and diseases of the adrenal glands as a doctor, but he was not looking at the adrenal glands from the view point of endocrine physiology.

The first description of the adrenal glands appears in Leviticus 3:4 in the Old Testament. One theory holds that this was written around 333 BCE, during the time when Alexander the Great ruled over his empire (2-1); according to another theory, Leviticus was compiled much earlier, around 1,000 BCE (2-2).

The text describes the sacrifice of peace offerings, and the same description appears three times in Leviticus 3, once each for cattle, sheep, and goats, and once for calves in Leviticus 4: "Peace-Offering. The fat that cover the inwards, and all fat that is upon the inwards, and the two kidneys, and the fat that is on them, which is by the loins, and the *appendix* (see on iii. 4) upon the liver shall he take away. And the priest shall *burn* it upon the alter." (2-3). In this passage, "the fat that is on them, which is by the loins" is believed to refer to the adrenal glands (2-1).

Some scholars maintain that the next remaining record of the adrenal glands comes from

around 200 years after the start of the Common Era. Claudius Galenus (L: Galenos in Greek, often called Galen) was the giant of medicine and pharmacy of the Greco-Roman era. He dictated his findings in Greek as he observed animal dissections, and these were recorded on papyrus. Seven of his treatises on dissection have been translated into German.

Galen gives these details of his observations of the veins in the vicinity of the kidney: "A twig from this not inconsiderable vein joins and connects with the spongy flesh (suprarenal gland) lying there. The second vein (renal) goes to this kidney itself." Following this, he states that his findings from observation of the vicinity of the right kidney were clearly different from those of the left kidney (2-4, 2-5). While many of his ideas differ from present-day medical knowledge, Galen is recognized as the first person to describe the adrenal glands of mammals (2-1).

The first accurate illustration of the human adrenal gland that has been preserved was by the enigmatic anatomist Bartolomeo Eustachi [Figure 2-1], Professor of the Collegio della Sapienza in Rome (2-6, 2-7).

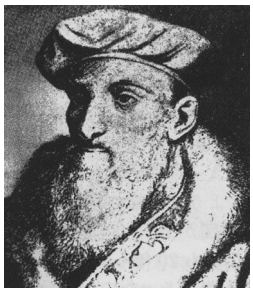

Figure 2-1. Bartolomeo Eustachi of Rome,
the first person to accurately draw the adrenal glands.
(Courtesy of the National Library of Medicine)

His human anatomy drawings, which he completed in 1552, include pictures of the kidney; blood vessels can be clearly seen connecting to a small gland tissue sitting on top of the broad bean-shaped kidney [Figure 2-2]. In his description, which was subsequently added in 1563, Eustachi appropriately named these the "*glandulae renibus incumbentes*" (glands lying on the kidneys), thus leaving for posterity his understanding that the adrenal glands are organs attached to the kidneys (2-1).

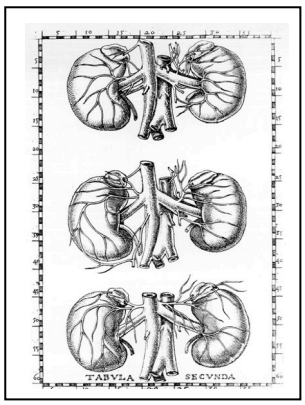

Figure 2-2. Bartolomeo Eustachi's Figure 2.
The blood vessels connecting to the kidney and
the adrenal glands are clearly depicted (2-8).
(Courtesy of HathiTrust).

Eustachi dedicated much of his life to producing copperplate engravings of the human anatomy, and in 1552 he completed 47 plates with the help of a relative, the artist Pier Matteo Pini. Of these, eight were published during Eustachi's lifetime. The remaining 39 plates were kept in the Vatican Library, and were published nearly 160 years later by Giovani Maria Lancisi as *Tabulae Anatomicae* in 1714 (2-8).

The beginning of the 17th century saw great advances in science, and these were accompanied by increased interest in discovering the functions of the kidney and the adrenal gland, which are organs shared by many higher animals.

First, in 1611 the Danish anatomist Casper Bartholin announced his findings that the adrenal glands were hollow organs filled with black bile, and he named them "*capsulae atrabiliarae*." The Bartholin family name is assured a place in medical history—Casper's son, Thomas, left a large body of work on the circulatory system, and Thomas' eldest son, Casper (the same name as his grandfather), discovered Bartholin's gland, a female genital organ.

The first anatomy book in Japan was the well-known *Kaitai Shinsho* (New Book of Anatomy), written in *kanji* characters and published in 1774. The author was Genpaku Sugita, who based his work on the *Tafel Anatomia*, an anatomy book that had been brought to Japan from the Netherlands, and which was itself a Dutch translation of the *Anatomische Tabellen* by the German Johann Adam Kulmus. Sugita had also referred to anatomy books by Casper and Thomas Bartholin, namely *Anatomicae Institutiones Corporis Humani* and *Historarium anatomicarum rariorum*.

Japan at the time was still in a state of national isolation, and trade was only permitted with a very limited number of countries. Because of this, the only clues to Western science, culture, and technology came from the cultural artifacts that were brought to Japan on Dutch ships. Few Japanese people understood European cross-wise writing, and Sugita even translated the names of foreign authors into *kanji* characters or the katakana syllabary (2-9).

Takamine and Wooyenaka, who were both born around a century later, must surely have read this book, deriving from it basic knowledge about the organ that they were to research and the scholars who had been pioneers in this field.

Incidentally, the way that "adrenal gland" is written in Japanese has changed with the times. The *kanji* characters that were used in the first edition of the *Kaitai Shinsho* had the meaning "small kidney," while the *kanji* used in the revised 1826 edition meant "side kidney." The *kanji* normally used today mean "auxiliary kidney."

Eventually in the West, medical scientists appeared who challenged the observation that

the adrenal glands were hollow organs filled with black bile. These were the French anatomists Jean Riolan the Elder, and his son, Jean Riolan the Younger. They asserted that the adrenal glands were not hollow, but support groups of nerves on top of the kidneys (2-1).

An outstanding breakthrough of the time was the discovery of "the circulation of the blood" by the Englishman William Harvey in 1628. He made the discovery of an overall system in which blood courses through the whole body with the heart acting as a pump.

In 1656 the English anatomist Thomas Wharton finally announced a ground-breaking discovery. He was the first to make the connection between the function of the adrenal glands and the nearby nerve plexus. Wharton was greatly impressed by the fact that a small gland was provided with such a large nerve plexus, and he wrote that the adrenal glands receive some substance from the nerves, and pass this to the veins. This predated the present-day concept of neuro-endocrinology by about 250 years.

Numerous scholars, including Wharton's pupil, continued to research and discuss his discovery, but it was not until about 200 years later that Rudolf Albert von Kölliker gave the first complete description of the microscopic anatomy of the adrenal gland, as described later.

In 1785, about 130 years after Wharton put forward his idea, the German Johannes Christophorus Heino Schmidt described his view that endocrine principles were formed in the adrenal glands and were shed into the blood, aiding in the functioning of the heart (2-10). He wrote this penetrating insight in Latin—this was the *lingua franca* of European scholarship until the 18th century—and he passed away just two years later at the early age of 26. While the time in which he lived lacked the kind of rigorous experimentation we require today for a theory to be accepted, Schmidt has been hailed for his tremendous insight. Just four years after Schmidt's announcement, the revolution broke out in France (1785–), marking the start of republicanism.

2. The steady advance of anatomy and embryology

In 1805, a French scholar showed that the adrenal glands were tissues with solid interiors, and he discovered that they had a double-layer structure, with clear morphological differences between the central and outer regions. This was Georges Cuvier, who was a Professor of Animal Anatomy at the Muséum National d'Histoire Naturelle in Paris. Cuvier did not go as far as differentiating the adrenal medulla and the adrenal cortex with separate names, but he stressed that the functioning of the adrenal glands would likely be explained

through comparative anatomical research.

Cuvier was a natural historian, a comparative anatomist, and also the father of paleontology. He served in a number of important positions in the government of the Napoleonic era. One of his pupils, Marie J. P. Flourens, taught a scientist by the name of Alfred Vulpian—thus it was the pupil of one of Cuvier's pupils who would later make the most important discovery in the history of research into adrenaline (2-11). Some 30 years later, Dr. Nagel left a record of how Georges Cuvier was examining the adrenal glands of female snakes (2-12).

Swale Vincent of the Physiological Laboratory of University College, London, took on the ideas of Georges Cuvier, and published four papers on his anatomical research on the adrenal glands of fish, amphibians, and reptiles. He subsequently published reports of his work on the adrenal glands of elasmobranch fish (sharks, rays, etc.), teleosts, and ganoids (2-13).

Sometime after Cuvier's discovery, Friedrich Arnold, the famous Professor of Anatomy of Heidelberg University in Germany, was carrying out embryological research into adrenal glands. In 1831, he showed that the adrenal glands developed from the Wolffian (mesonephric) bodies through the formation of a fissure (2-1). Friedrich Arnold's son, Julius Arnold, continued after his father with a conspicuous career as Professor and Director of the Pathological Laboratory at Heidelberg University, and he also worked on research into the chemistry of adrenaline. Julius Arnold was one of the joint discoverers of the "Arnold-Chiari malformation," one of the causes of hydrocephalus.

Robert Remark and Henry Gray continued the microscopic anatomical research of the adrenal glands, and subsequently in 1836, Dr. Nagel of Germany clearly divided the tissue of the adrenal glands into two types, proposing the terms "cortex" and "medulla" for them. This became the scientific terminology that is still in use to this day [Figure 2-3]. In his 19-page paper on the subject, he wrote the following: "*Die menschlichen Nebennieren bestehen bekanntlich aus seiner Rinden- und einer Marksubstanz; das Verhältniss der ersten zur letzten ist wie 1:2*" (The human adrenal gland is made, as is known, from its cortex and medulla; the ratio of the first to the last is like 1: 2). From the way he expresses this, it looks as though the differentiation into two areas may perhaps have been common knowledge among researchers of the time (2-12).

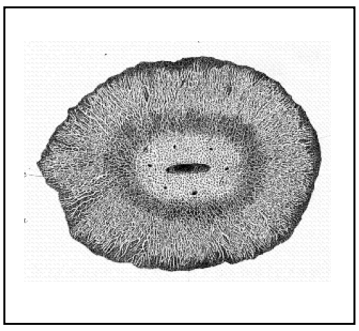

Figure 2-3. Diagram of an enlarged cross section of dissected sheep adrenal gland by Dr. Nagel (2-12).

It was the anatomist Rudolf Albert von Kölliker who announced an extremely important observation concerning the function of these two tissues, confirming the perceptive observations made by the anatomist Wharton some 200 years earlier (Wharton: 1656, Kölliker: 1852). Kölliker published his anatomical diagrams of the adrenal glands, made through microscopic observation, in 1852. In this work, he stated that the functions of the cortex and the medulla were clearly different—he confirmed that because the medulla is connected to a richly endowed nerve plexus, it must be an organ related to the nervous system. Kölliker had made an extremely important point here, but it appears that this was missed by Addison (2-1), a well-known researcher from the same period whom we shall introduce in the next section.

Kölliker, who was born in Zurich, Switzerland and educated in Germany, was later highly commended for his contribution to the field of anatomy, receiving the "Copley Medal" from Britain (see "In Brief 2-1"). The Copley Medal, which predates the Nobel Prize, honors natural scientists that have made a great contribution to their field [Note 2-1].

In Brief 2-1. The Copley Medal

The Copley Medal created by Sir Godfrey Copley is an award given by the Royal Society of London for "outstanding achievements in research in any branch of science, and alternates between the physical sciences and the biological sciences."

Medal-winners relevant to this book, and some others.

1731: Stephen Gray（English, electrical experiment）
1753: Benjamin Franklin（American, lightning rod）
1843: Jean Baptist Dumas（French, chemistry）
1864: Charles Darwin（English, the theory of evolution）
1874: Louis Pasteur（French, microbiology and stereochemistry）
1875: August Wilhelm Hofmann（German, organic chemistry）
1876: Claude Bernard（French, experimental physiology）
1892: Rudolf Virchow（German, medicine）
1897: Rudolf Albert von Kölliker（German, medicine）
1892: Rudolf Virchow（German, medicine）
1897: Rudolf Albert von Kölliker（German, medicine）
1902: Joseph Lister（English, disinfection before operation）
1919: William Bayliss（English, physiology）
1924: Edward Albert Sharpey Schäfer（English, physiology）
1927: Charles Sherrington（English, physiology）
1937: Henry Dale（English, physiology）

Note 2-1.

When Rudolf Albert von Kölliker was Professor of Histology at the University of Würzburg, he attended an academic meeting of anatomy in Berlin in 1889. There he heard a presentation by Santiago Ramón y Cajal (2-14) on anatomical findings of the cerebellum. He was so impressed by Cajal's specimens that he escorted Cajal back to his hotel and is reported to have said, "You discovered neuron synapses, and I discovered you."

Kölliker also had great respect for Camillo Golgi, who is famous for the Golgi staining method. Cajal and Golgi shared the Nobel Prize in 1906, "In recognition of their work on the structure of the nervous system. "

3. Addison's disease

Pathological research into the adrenal glands began in 1849. Thomas Addison was then a doctor at Guy's Hospital, a London hospital established by Sir Thomas Guy in 1712 on par with the celebrated St. George Hospital; at the request of the president of the South London Medical Society, he presented a paper titled "Anæmia: Disease of the Suprarenal Capsules" at a meeting of the society (2-15).

Addison presented three cases in which symptoms of languor had gradually become severe. He made no report of the specific symptoms of deposition of pigment in the skin and hypotension, and in fact did not know how to measure blood pressure. Nonetheless, during autopsies following death due to pulmonary tuberculosis, he noticed that there was disease of the adrenal glands, located immediately above the kidneys. However, he did not present any connection between anemia and abnormality of the adrenal glands. His report was published in the *London Medical Gazette* that year. This was an age in which pulmonary tuberculosis was greatly feared as an incurable disease.

Three hundred years after Eustachi first clearly showed the adrenal glands in his anatomical drawings in 1552, the glands were now shown to be important organs for the maintenance of human life. The date of this academic meeting, March 15, 1849, was seen as a historic moment for the science of endocrinology.

At this time, Britain was at the forefront of modern science. A generation or two before Addison's time, John Hunter, who had a checkered career as an anatomist, and his associates went as far as stealing dead bodies from the cemetery of well-known hospitals in their efforts to create the foundations of anatomy (2-16). Hunter's pupil, Edward Jenner, discovered that smallpox could be prevented with cowpox vaccinations, thus founding the field of immunotherapy (2-17).

Addison became completely convinced that this anemia was a unique syndrome six years after his initial presentation. Encouraged by his peers, he presented a comprehensive scientific report in 1855, titled *On the Constitutional and Local Effects of Disease of the Suprarenal Capsules.* His review described in detail 11 cases, of which six were patients that had contracted tuberculosis (2-18).

This was a momentous report, and while it stirred up considerable debate in England, and Scotland, it was given a very poor reception. John Hughes Bennett of Edinburgh, Scotland was opposed to recognizing this syndrome as a disease.

However, the famous clinician Armand Trousseau of the Hôtel-Dieu hospital in Paris judged the descriptions in Addison's work to be flawless, and named the syndrome Addison's disease (maladie d'Addison, morbus Addisonii) (2-19).

Meanwhile in Japan, in 1855, the year of Addison's scientific report, Jokichi Takamine was one year old. That year, his mother took him from his birthplace in Takaoka, Ettchu Province (present-day Toyama Prefecture), to Kanazawa, Kaga Province (the south of present-day Ishikawa Prefecture), where his father was a doctor working at a public clinic.

That same year, the Universal Exposition was held in the Champs-Élysées in Paris, attracting some 5.16 million visitors. This was a year in which modern civilization and culture were making great leaps.

The discovery of Addison's disease was of enormous importance from the point of view of the history of pathology. However, this disease was caused by abnormality of the exterior "cortex" of the adrenal glands; it had nothing to do with the interior "medulla" of the adrenal glands, which has completely different function from the cortex. It is the medulla that secretes the adrenaline with which we are concerned in this book [Note 2-2].

Note 2-2.
Two famous people are reported to have contracted Addison's disease: one was the English female author Jane Austen, the other was US President John F. Kennedy, who was assassined in 1963 (2-20). Jane Austen, who is famous for works such as *Pride amd Prejudice*, passed away aged 41 in 1817, when Addison had just started as a doctor in London; The presumed diagnosis for Austen is based on her symptoms. There have been differing opinions in recent years, and the cause of death is not definite.

4. What happens if the adrenal glands are removed?

Following on from Addison's discovery, the Frenchman Charles-Édouard Brown-Séquard carried out research aimed directly at the function of the adrenal glands. He presented a total of seven papers—two each in 1856 and 1857, one in 1858, and then later one each in 1892 and 1893—detailing research in which he showed the effects of removing the adrenal glands of animals. He demonstrated plainly that this organ has an important role in supporting the life of the organism (2-21 through 2-27).

The parts directly relating to adrenal glands in his papers can be summarized as follows. In his experiments, Brown-Séquard used dogs, cats, rabbits, mice, and marmots as experimental animals, removing the left, the right, or both adrenal glands. In one experiment, he used 66 rabbits to show that mortality was not an indirect result of the operative procedure. His experiments were on a massive scale, and with a level of detail that had hardly been seen to date. He showed that removal of the two adrenal glands resulted in death more quickly than

removal of the kidneys on both sides.

Brown-Séquard performed this research while working under the physician Pierre Rayer, and he went about it with tremendous vigor. This approach to his work can be seen in his life history and in the following anecdote about him. This was at a time when Brown-Séquard was struggling to find work, traveling between France and the United States. In May1854 he returned to his birthplace, Port Louis in Mauritius, to find a massive outbreak of cholera that had claimed 8,000 victims—he immediately helped organize a response at a hospital. To determine whether or not opium was effective for treating cholera, he ingested material vomited by patients, and apparently nearly died from the dose of laudanum he took (2-28).

Brown-Séquard was born on April 8, 1817 on the island of Mauritius in the Indian Ocean. His father, Charles Brown, was an Irish-American naval officer from Philadelphia, and his mother, Charlotte Séquard, was a cheerful French woman. Unfortunately, his father died fairly soon after marrying when his ship sank. Like the great French physiologist Claude Bernard, Brown-Séquard first aimed to be a playwright, but realizing that he would be unable to gain recognition for his talents, he set his eye on becoming a doctor. He worked under the two greatest clinicians of the time, Armand Trousseau and Henri Louis Roger, until 1842, after which, like Bernard, he chose the path of physiological research rather than continuing his clinical training (2-29).

Even today, Brown-Séquard has a place in the history of medicine for his discovery of Brown-Séquard syndrome, a loss of sensation and motor function (paralysis and anesthesia) caused by the lateral hemisection (cutting) of the spinal cord. This rather strange but earnest character came to take an important part in our story some 33 years later, when he appeared in Paris.

Sometime after Brown-Séquard's research, the Italian Guido Tizzoni, who was Professor of General Pathology at the University of Bologna, published "Ueber die Wirkungen der Exstirpation der Nebennieren auf Kaninchen (On the Effects of Removal of the Adrenal Glands of Rabbits)" in a German journal of pathological anatomy in 1889. This paper was a huge research thesis with a tremendous amount of detail, running to 100 pages with 68 microscopic images of dissected organs in cross section (2-30). This paper included descriptions of the works of other Italian researchers in the same field, including Philippeaux, Foà, Pellicani, and Marino-Zuco.

It was a lengthy journey from Brown-Séquard's conclusion that the adrenal glands were essential to maintaining life until the final proof. Following on from Brown-Séquard's work, some 14 leading physiologists carried out experiments under a great variety of experimental

conditions—different animal species were used, the glands were removed at different ages or different times, and left, right, or both glands were removed—and there was considerable dispute among the researchers over the interpretation of the results. In one experiment, it was found that if the lower part of the left adrenal gland of rabbits was ligated, a compensatory action came into play and the rabbit would become obese, sometimes almost doubling its body weight (2-31).

The final determinant was apparently a scientific report by Strehl and Weiss in 1901, which described experiments using 114 animals. This was 45 years after Brown-Séquard's paper was first published (2-32).

◇ ◇ ◇

The tiny organs on top of the kidneys had puzzled people since ancient times. Medical scientists and physiologists noticed that these organs were likely linked to disease, and they established that removing these organs had a more lethal effect than removing the far larger kidneys. The spirit of enquiry that had led these researchers in their earnest quest to discover the function of the adrenal glands was starting to bloom.

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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).

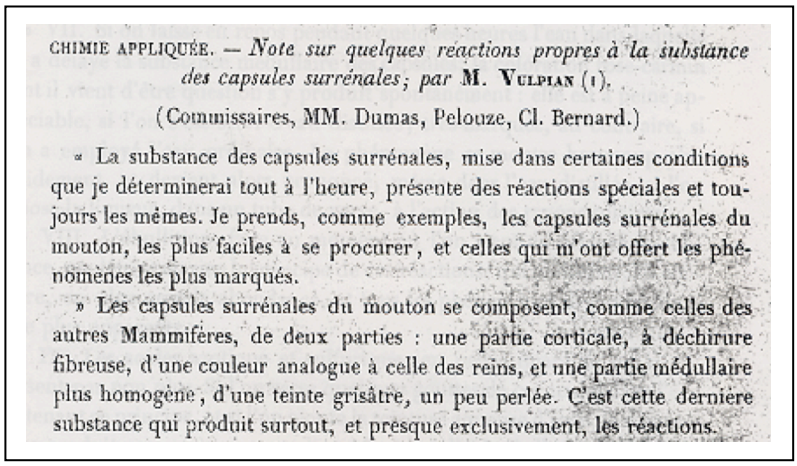
3. Vulpian's keen insight

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).

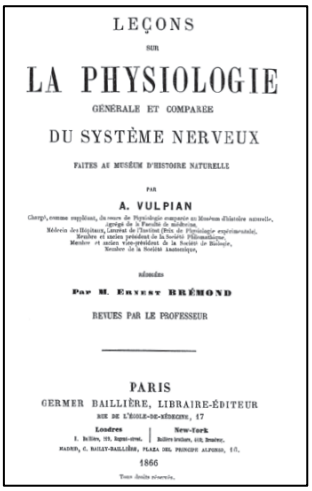

LEÇONS
SUR
LA PHYSIOLOGIE
GÉNÉRALE ET COMPARÉE
DU SYSTÈME NERVEUX
FAITES AU MUSÉUM D'HISTOIRE NATURELLE
PAR
A. VULPIAN
Par M. Ernest BRÉMOND
REVUES PAR LE PROFESSEUR
PARIS
GERMER BAILLIÈRE, LIBRAIRE-ÉDITEUR
Londres
New-York
1866

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

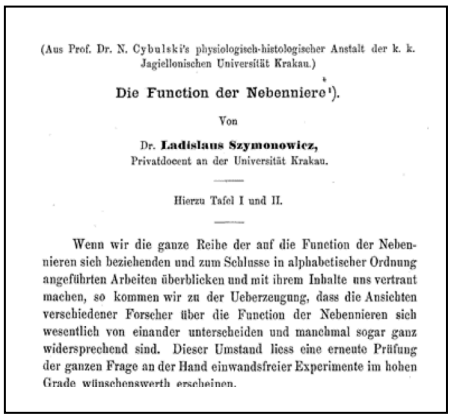
This is something that happens all the time, but the same research as Oliver and Schäfer did was being carried out with the same ideas in a country not so very far away.

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

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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 cat 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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Chapter 4

In Pursuit of the Active Principles

For many scientists working in the fields of medicine and chemistry, isolating the highly active principles of the adrenal medulla was an area of research that was hard to ignore. Over the course of around half a century, more than 20 leading researchers worked on trying to isolate the principles. A fierce competition developed between Britain, which had discovered the blood pressure-raising activity, Germany, then the world leader in organic chemistry, and the United States, then an emerging nation. Eventually, two scientists from Japan made the journey to the United States, where they joined in the race.

1. Physiological curiosity

Alfred Vulpian, who had made the enormous discovery that in many different animal species some extremely interesting specific principles were secreted from the adrenal medulla into the blood, worked with S. Cloez on extracting the principles.

Having made the discovery, it was natural that Vulpian would set to work on extracting the principles in pure form—in other words, isolating—minute quantities of the useful substances from their complex biological system. The two scientists first carefully removed the oily membrane covering the adrenal glands, cut it longitudinally into thin strips, immersed these in 85% aqueous ethanol, and collected the filtrate. A single experiment required at least 1 kg of adrenal glands, which is the equivalent to the glands from 300 to 400 sheep. This was a mammoth task, and it gives us a glimpse of the lengths to which Vulpian and Cloez were prepared to go.

When this filtrate was placed in a glass dish, dried by natural evaporation, and then examined microscopically, crystals of various different shapes could be seen. Vulpian and Cloez then worked through repeated trial and error, but their interests tended toward the chemistry of the components of urine and bile, which was then a major research area, and they were only able to isolate hippuric acid and taurocholic acid.

Nonetheless, they wrote that the damaging effects of air oxidation and light must be

avoided in this process, so their paper shows that their research was heading in the right direction—so much so that had they refined their original methods for extraction and condensation, they might perhaps have obtained adrenaline (4-1).

If they'd had just a little more experience in researching the organic chemistry of natural products, or if a researcher with this experience had been close at hand, the history of the adrenal gland might have been completely different.

The German researcher Rudolf Virchow read Vulpian's first report, and the following year, 1857, he published a short research paper titled "Zur Chemie der Nebennieren" (The Chemistry of Adrenal Glands). This paper mainly dealt with the color reactions of the squeezed liquid of adrenal glands, and Virchow makes absolutely no mention of the extraction and purification of the active principles.

Aged 36 at the time, Virchow was a professor at Berlin University and a leading figure of the medical establishment in Germany. He wrote that the properties of the adrenal glands were related to the nerves and that the presence of sympathetic ganglions could be observed in the glands, from which he deduced that the active principle was probably a completely different substance from the components found in regular cells (4-2). However, Virchow's paper makes no mention at all of research into Addison's disease (see Chapter 2) [Note 4-1].

Note 4-1.
The medical pathologist Rudolf Virchow was born in the Polish town of Świdwin, and studied at a military medical college in Berlin.

He became a professor at Berlin University in 1856. Together with the medical scientist Benno Reinhardt he launched the pathological anatomy and physiology journal *Archiv für pathologische Anatomie und Physiologie, und für klinische Medicin* (Archive of pathological anatomy and physiology, and clinical medicine) in 1847 (the journal changed its name to *Virchow Archiv* in 1902).

Virchow was also important as a politician, stressing the "health of the nation" and "open health education."

The Japanese scientist Katsusaburo Yamagiwa, a professor at the Tokyo Imperial University (now the University of Tokyo) who created the world's first induced cell carcinomas in the summer of 1915, studied in Germany for three years from 1891, and he spent all this time studying under Virchow, who was then a professor at Berlin University (4-3).

Virchow was a man of many talents, and another of his achievements worthy of mention is the huge support he gave to his friend Heinrich Schliemann, who is famous for his excavations of ancient Greek cities and "Trojan antiquities."

In 1860, three years after Virchow published his academic paper, the German Seligsohn published a short, two-page paper titled "Zur Chemie der Nebennieren" (The Chemistry of Adrenal Glands). This comprised extracts of dissertations and professorial theses regarding the coloring of the skin caused by Addison's disease, and the chemistry of the adrenal glands. However, this paper largely follows the results of Vulpian and Cloez, only going as far as the crystallization and collection of hippuric acid and taurine, with nothing that is particularly noteworthy (4-4).

There was subsequently something of a lull in reports of the search for active principles

until 1866, when the German Julius Arnold published a lengthy paper, running to 44 pages, titled, "Ein Beitrag zu der feineren Structur und dem Chemismus der Nebennieren (A contribution to the finer structure and the chemistry of adrenal glands)." This paper was an excellent history of the research to date, but here too, there was nothing with regard to the isolation of active principles that went beyond what Vulpian and Cloez had written. In the year he submitted this paper, Arnold, who was then 31, had just been appointed to the post of Professor of Pathological Anatomy and Director of the Pathological Research Institute at Heidelberg University in Germany, and this paper concentrated on the existing scientific literature (4-5).

The following year, the German F. Holm attempted to isolate the active principles, but was unsuccessful. He used bovine adrenal glands without separating the cortex and the medulla, and after extraction with alcohol he searched for the principles among the substances in the filtrate produced by precipitation with lead acetate and copper acetate. This method is fairly unlikely to be successful (4-6).

After this, the race to isolate the active constituents died down for a while, and for 18 years there were no research results of any note. Then, in 1885, Carl Fr. W. Krukenberg of the renowned Jena University in Germany published a major, 30-page paper. He discovered that the color reaction of pyrocatechol, which is widespread as a structural component of plants, resembled the color reaction of adrenal gland extracts. Using Arnold's method he attempted to extract the active principles, but unfortunately was unable to show the molecular formula (4-7) [Note 4-2]. The similarities in the color reactions were confirmed seven years later, 1892, by Heinrich Brunner of the University of Lausanne (4-8).

Note 4-2.
Krukenberg published *Vergleichende-physiologische Studien* (Studies in Comparative Physiology) at the age of 29 in 1881 when he was at the Physiological Institute of Heidelberg University. Even today, this work is readily available on the Internet.

Several years later, the assumption that there was a pyrocatechol group [Figure 4-1] present in the molecule of the active constituent led to the idea of "benzoylation" as a method for extraction and isolation. This was the idea of the American scientist Prof. Dr. J. J. Abel, who, as we will see later, made a spectacular entrance at the very forefront of research into extraction of active constituents. Ironically, as a result of this idea he entered a blind alley from which he became unable to extricate himself (4-9).

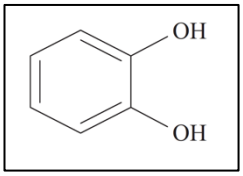


Figure 4-1. Pyrocatechol

Returning to the main topic, the race to isolate the active principles, which had largely died down, once again burst into life in 1894. The spark was provided by the dramatic report by Oliver and Schäfer that blood pressure-raising principles were secreted by the suprarenal glands. Until then, researchers from various different fields had taken the work of Addison and Vulpian as a starting point and for rather vague reasons, had attempted to isolate the active constituents, each with their own expectations that they might perhaps find something. The report provided the definitive goal of finding the principles causing this effect—this was enormously attractive for the scientists of that time, and the situation developed rapidly.

While it is a slight diversion, I would like to think here about presentism, a common risk factor in literary and historical analyses. This refer to evaluating and judging things from long ago with the knowledge available at the present time, and it is a concept that should be borne in mind in particular when describing history.

When evaluating the work of the people that have appeared so far in this story, as well as those scientists that are yet to come, their abilities as researchers are even more impressive if we keep in mind as accurate a picture as possible of the laboratories of the time. Let me give a single example here. I would like to try asking a reader with a background in chemistry a simple question: "Would you be able to extract pure adrenaline from adrenal glands in a laboratory without chromatography?" A present-day researcher thrown into a chemical laboratory like that would most likely be at a complete loss of what to do when faced with a greasy organ in a glass flask.

The separation technique of chromatography was invented by Mikhail Tsvet, a scientist from Imperial Russia, in 1900 (see In Brief 4-1). He separated various pigments in plants by packing a glass column with calcium carbonate powder, pouring a liquid extract of plant material in solvent on top, and pouring solvent on top of this so that it flowed downward. The pigments in the plant material separated out into bands (layers) of different colors due to their different affinities for calcium carbonate.

Tsvet announced this technique at an academic conference in St. Petersburg toward the end of the following year, 1901. Coincidentally, Wooyenaka isolated crystals of adrenaline in 1900 and Takamine announced the results at an academic conference in 1901.

Tsvet's work was published in writing in 1903, and the term "chromatography" was first used in 1906 in a German academic journal. Around half a century after this discovery, the British biochemists Archer J. P. Martin and Richard L. M. Synge, who jointly won the Nobel Prize, discovered paper chromatography (partition chromatography). This led to rapid development of Tsvet's isolation technique, making it readily available to anyone. This

discovery brought about a complete change in fine chemical techniques.

In Brief 4-1. The history of chromatography

1900: Tsvet (Russian) discovered chromatography as a technique for separating the pigments in the leaves of plants. The name Tsvet means "color" in Russian, so perhaps he was fated to make this discovery.

1944: Archer J. P. Martin and Richard. L. M. Synge (both British) invent paper chromatography. This brings about a revolution in separation technology.

1956: Egon Sthal (German) invent thin layer chromatography (TLC), which allows large samples to be readily separated. This led to further advances in separation technology. Since then there have been further developments, and theoretical advances have included partition, adsorption, size-exclusion, and ion exchange chromatography, while technological advances have included gas-liquid chromatography (GLC) and high performance liquid chromatography (HPLC).

2. Painstaking explorations by chemists

Returning again to the main thread of our story, the fierce competition to isolate the blood pressure-raising principles from the adrenal glands developed some years before the separation technique of chromatography came into practical use, and it led to a long period of bitter fighting and confusion that would be unimaginable today.

Benjamin Moore, a biochemistry researcher from Professor Schäfer's laboratory, had seen the discovery of Oliver and Schäfer with his own eyes. With the help of D.N. Nabarro, he set about trying to identify the chemistry of the blood pressure-raising principle (4-10). Moore published a total of six papers stemming from his research from 1894 to 1900 (4-11 through 4-16), but was ultimately unable to extract the active principle. This must have been a serious disappointment for Schäfer's laboratory.

Moore's first paper was four pages long and was titled, "On the Chemical Nature of a Physiologically Active Substance Occurring in the Suprarenal Gland" (4-11). In it, he first notes that he began the research at Prof. Schäfer's request, and then goes on to say that he investigated the chemical properties of the main putatively active principle. He tested activity at various different stages, so his research was something of a frontal attack [Note 4-3].

In the second report (4-12), he reached the important conclusion that the active principle was the same as a reducing substance that presented a green color with ferric chloride. Also in this paper, he insisted that Sigmund Fränkel (whom we will meet later), who believed that the active principle was a pyrocatechol derivative that was soluble in absolute alcohol, was

mistaken. Instead, Moore claimed that the active principle was a pyridine derivative like physiologically active nicotine—however; he later came under criticism because he did not present any evidence (4-17).

The first three of Moore's reports appeared in the *Journal of Physiology (London)*, which was published by the University of London. The next two were published in the *American Journal of Physiology*, and he submitted the last one in German, when he was a professor at the Physiological Laboratory of Yale Medical School in the US, to the German journal *Archiv für die Gesammte Physiologie des Menschen und der Thiere* (Archive for the entire physiology of man and animals). Like the American Abel, he considered German journals to be the best platform for discussion of adrenaline.

Note 4-3.
Although it is a little technical, I will give a summary of Moore's paper "On the Chemical Nature of a Physiologically Active Substance Occurring in the Suprarenal Gland (4-11)," as it is the first report on this topic.

1. The active principle easily dissolves in water. It is soluble in diluted ethanol, but becomes insoluble as the concentration of ethanol rises. It is insoluble in absolute alcohol. It is also insoluble in ether, chloroform, amyl alcohol, carbon disulfide, benzene, and ligroin.
2. It is not broken down by acid or by boiling for a short time, but can be broken down by alkalis, oxidizers, or continuous boiling.
3. The principle does not precipitate with the addition of excess alcohol, saturated ammonium sulfate, mercuric chloride, potassio-mercuric iodide, or tannic acid.
4. Fehling's solution should not be reduced, even after boiling with mineral acids. No crystalline product is produced when heated with phenyl hydrazine.
5. It is not volatile either alone or with water vapor. It dialyses freely through parchment paper and the highly active dialysate so obtained is completely free from proteins.

In 1894, Paul Manasse, an assistant at the Pathological Institute of the renowned Straßburg University, which was then in Germany, was working on animal histological research, when he observed that a certain substance in the veins of the adrenal glands presented a brown color with potassium dichromate. His work was only a report of the internal secretion of the adrenal glands, and did not offer any new findings with regard to the active principle (4-18). In this report, Manasse cites not only the name of Arnold, whom we have already met, but also Eberth and Brunn, as previous researchers in this field. From this, we can suppose that more researchers than we might expect were busy groping their way forward. The pathologist Manasse published a textbook of diseases of the ear in 1917.

After a short while, in 1896, Sigmund Fränkel of the University-Institute for Medical Chemistry in Vienna extracted a syrupy component from the adrenal glands, and believing this to be pure, he named it "sphygmogenin." However, the purity was not constant and he was unable to determine the experimental formula. *Sphygmo* is a Greek connecting word meaning "pulse," and Fränkel probably chose this name because the component he had

extracted had an effect on the pulse. He backed the theory that the active principle was chemically a nitrogen-containing pyrocatechol (4-19). The subtitle of the paper in which he presented his ideas was "Kritik der Arnold-Krukenberg'schen Resultate (Criticism of Arnold-Krukenberg's results)," which gives an idea of the fierce competition to isolate the principle at the time.

The same year, M. Mühlmann of the Chemical Laboratory of Pathological Institute of Berlin University published a comprehensive overview of the literature in a scientific journal, and he emphasized in bold that the active principle was a substance with the properties of a pyrocatechol and was formed in the adrenal medulla. He noted, however, that all the studies had used chemical methods with no activity tests at all, and it had not been possible to purify the active principle (4-20).

Germany at the time was far ahead of other countries in chemistry, and Prof. Schäfer's research group in London, which had discovered the blood pressure-raising effect, saw the threat of Germany joining in the race to isolate the active principle. The scientific literature in this field from Britain and Germany at the time gives a sense of the intense debates among these researchers, who did everything in their power to stake their reputations to achieve victory in the race to obtain the active principle. As an example of how Britain was lagging behind Germany in the field of chemistry, the up-and-coming German chemist August W. von Hofmann was invited to London at the age of 27, where for 20 years until 1865 he taught at the Royal College of chemistry. After returning to Berlin, Hofmann taught the Japanese scientist Nagayoshi Nagai for a long time; Nagai later became professor of the Tokyo Imperial University, and in Chapter 5 we will see in detail how he personally guided Keizo Wooyenaka.

The final thing to spur on the isolation race was the astounding announcement by the American doctor William H. Bates of the hemostatic effect of adrenaline, which was introduced in Chapter 3 (page 55). In the Section in Ophthalmology of the New York Academy of Medicine of April 20, 1896, Bates also showed in a dramatic way that adrenal extracts had the potential for great profits, as they could become leaders of the market for medicines (4-21).

Moreover, the pharmaceutical industry pricked its ears when the medical scientist and physician Solomon Solis-Cohen announced the possibilities of adrenal extract as a therapeutic agent for hay fever and asthma (4-22, 4-23). Adrenal extracts thus seemed to offer great potential as "new medicines"— surgeons and doctors in other fields of medicine had

long desired a hemostatic agent for peripheral veins, while pulmonologists craved an accurate asthma remedy and a miracle drug for hay fever.

3. The climax of the isolation race

In 1897, two very important researchers made their timely entry.

The setting changes to the United States of America, where Professor John J. Abel of Johns Hopkins University (see Chapter 1, Figure 1-6) prepares to take the stage. This university had a well-known medical faculty since it was founded, and in 1893 Abel was appointed to take charge of the newly established Department of Pharmacology. He was a great scholar, who for the next 40 years was at the head of pharmaceutics in America. He studied physiology, medicine, chemistry, and experimental pharmacology for eight years in various countries in Europe that were at the forefront of the exact sciences, and on his return to America he taught at the University of Michigan before being invited to the new Department of Pharmacology of Johns Hopkins University.

Abel announced the first results of his research into the active principle of the adrenal glands through an oral presentation to the Association of American Physicians on May 6, 1897. He submitted the details of the presentation, which he co-authored with Albert C. Crawford, who was in charge of bioassays, to the Johns Hopkins Hospital Bulletin (4-17).

This paper starts with a review of the prior reports in this field, and cites the two breakthroughs, Oliver and Schäfer's discovery of blood pressure-raising activity and Bates' discovery of great efficiency as a hemostatic agent. It is clear that Abel commenced his research with these two works in mind [Note 4-4].

Note 4-4.
Summary of Abel's first report (4-17).

1. The blood-pressure-raising principle of the suprarenal capsule may be completely precipitated from an aqueous extract by treatment with benzoyl chloride and sodium hydrate, according to the Schotten-Baumann method.

2. On decomposing the resulting benzoyl products, a residue is obtained which possesses great physiological activity. It gives the color reactions of Vulpian, reduces silver nitrate and possesses the other specific qualities of suprarenal extracts.

3. With the help of alkalis a carmine-red pigment may also be separated from these decomposition products. The authors take this pigment to be that one of the chromogenic substances of Vulpian which gives the rose-carmine color when suprarenal extracts are treated with oxidizing agents or alkalis.

4. A volatile, basic substance of a coniin-like odor is always found to accompany the crude benzoate. When these substances are removed the active principle is left as a highly active sulfate or hydrochloride, as the case may be. It is therefore a basic substance. Its salts give a color reaction with ferric chloride; they also reduce silver nitrate, but not Fehling's solution.

5. It is not possible to split off pyrocatechin from this isolated active principle.

6. The fact that dry distillation causes the appearance of amines and pyrrole in abundance, taken in connection with its ability to take up acid radicals, its reducing power, its precipitability by cupric

acetate and iodine chloride, and its physiological action, lead the authors to conclude that "our active principle" is to be classed with the pyridine bases or alkaloids.

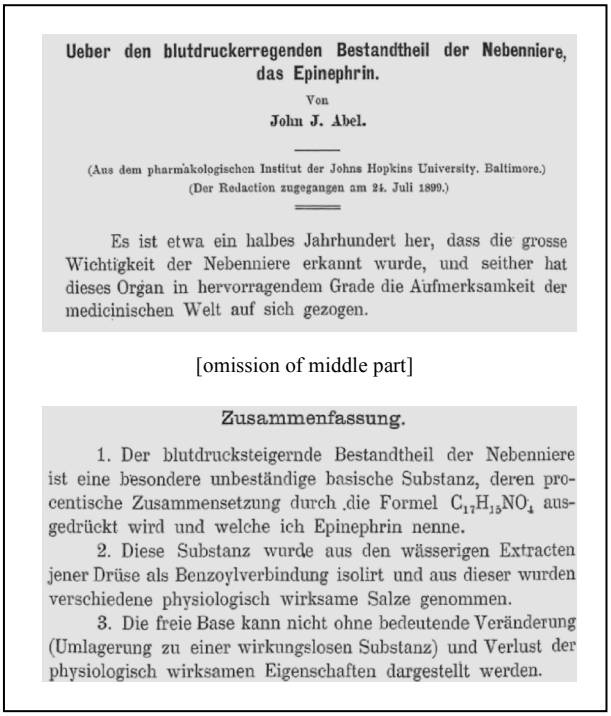
The following year, Abel published his second report, in which he specified the molecular formula $C_{17}H_{15}NO_4$ for the first time (4-24). In this report, he notes that the active principle can be precipitated by treating aqueous extract of the adrenal glands with benzoyl chloride and sodium hydroxide, and this benzoyl derivative is hydrolyzable by adding heated dilute sulfuric acid. He also states that "our active principle" can be obtained in the form of a sticky, tar-like sulfate, which has physiological activity and exhibits the characteristic color reaction and other reactions of the adrenal gland extract. He summarized the overall paper as below: "The active principle of the suprarenal capsule has been isolated in the form of powder of a light gray to brownish color, whose percentage composition is expressed by the formula $C_{17}H_{15}NO_4$. A primary amine and a methylindole are easily split off from the powder by treatment with alkalies. Judging from the elemental composition, this substance is considered to be an unprecedented base that contains only one benzene ring with a substituent in the molecule, as well as a nitrogen-containing heterocyclic compound from which the skatole is derived." In the text that follows, Abel and his co-author put forward a number of concerns that prevent them from saying with certainty that this is the substance they are looking for. The report finishes with a note of gratitude to Dr. Walter Jones, the assistant for chemical analysis (4-24).

As well as these two co-workers, Abel had also relied on another assistant, Thomas Bell Aldrich, since 1893. However, Aldrich was recruited by Parke, Davis & Co., and left Abel's laboratory in 1898. Neither Abel nor his two co-workers would have ever imagined that this assistant, who had been Abel's right-hand man, would later go on to produce the definitive results in the final stage of the story of adrenaline (4-25, 4-26).

A year later, in 1899, Abel published his most important comprehensive research report, which ran to a total of 45 pages, in a German academic journal that boasted the world's widest circulation in the field of physiological chemistry at that time. In this paper, he writes in German that he has a name for the active principle of the suprarenal glands: *"Ich Epinephrin nenne* (I call it "epinephrin")*"* (4-27). However, this statement in Abel's paper subsequently became the source of unexpected confusion in the name that has persisted to this day.

In the body of his paper, on page 320, he states that he names the active principle of the adrenal glands "epinephrin," without a final "e". Likewise, in the summary of the final

section (page 360) he writes that the substance given the molecular formula $C_{17}H_{15}NO_4$ is named "epinephrin" [see Figure 4-2]. However, within a short space of time, "Abel's active principle $C_{17}H_{15}NO_4$" was determined to be completely inactive. The presence of this substance named epinephrin became a source of annoyance to the scientific world—an utterly inactive compound had a name indicating a precise action. [Epi] is Greek for "on," [nephr] means "kidney," and [–in] is used to denote natural active substances. It would not be hard for any expert to guess the meaning of the name, so in that respect it was a good name. Unfortunately, however, it was not accurate [Note 4-5].



Ueber den blutdruckerregenden Bestandtheil der Nebenniere, das Epinephrin.

Von

John J. Abel.

(Aus dem pharmakologischen Institut der Johns Hopkins University. Baltimore.)

(Der Redaction zugegangen am 24. Juli 1899.)

Es ist etwa ein halbes Jahrhundert her, dass die grosse Wichtigkeit der Nebenniere erkannt wurde, und seither hat dieses Organ in hervorragendem Grade die Aufmerksamkeit der medicinischen Welt auf sich gezogen.

[omission of middle part]

Zusammenfassung.

1. Der blutdrucksteigernde Bestandtheil der Nebenniere ist eine besondere unbeständige basische Substanz, deren procentische Zusammensetzung durch die Formel $C_{17}H_{15}NO_4$ ausgedrückt wird und welche ich Epinephrin nenne.

2. Diese Substanz wurde aus den wässerigen Extracten jener Drüse als Benzoylverbindung isolirt und aus dieser wurden verschiedene physiologisch wirksame Salze genommen.

3. Die freie Base kann nicht ohne bedeutende Veränderung (Umlagerung zu einer wirkungslosen Substanz) und Verlust der physiologisch wirksamen Eigenschaften dargestellt werden.

Figure 4-2. Abel's important comprehensive research report (4-27).

Note 4-5.
Reid Hunt, an assistant professor in Abel's laboratory, conducted experiments to show that a component that reduces blood pressure is present in aqueous solution from which epinephrin had been removed and the blood pressure-raising effect had been lost. He argued that this was connected to the components of the living body, but his research did not bear any definite fruit (4-28).

At around the same time, Ludwig Metzger of Würzburg University in Germany was working hard to isolate the active principle of suprarenal glands for his doctoral dissertation under the guidance of A. Gürber. However, he did not manage to show a definite chemical formula. The samples were minute, so it is possible that he was unable to analyze them properly [Note 4-6].

Note 4-6.
Metzger made an extract of rabbit adrenal glands with a dilute aqueous solution of tartaric acid, dried this on pumice stone, and extracted the residue with diethyl ether. The compound dissolved in diethyl ether did not exhibit Vulpian's reaction, but a white mass sticking to the wall of the diethyl ether vessel was soluble in water and in warm ethanol, it demonstrated Vulpian's reaction, and it showed high physiological activity.

Metzger subsequently investigated the chemical properties of this compound (4-29). His mentor, A. Gürber, made an oral presentation of the results of this research at a meeting of the Physical-Medicinal Society in Würzburg in June 1897, and the results were also published in an academic journal. Unfortunately, there was no record of the values used to measure the physiological activity, nor was the chemical formula shown (4-30).

It was at this time that the other major figure, who became Abel's opponent, appeared on

the European stage. He was Otto von Fürth, an assistant of the Physiological-Chemical Institute of Straßburg University. He was born in Strakonitz, Bohemia, in 1867 and studied at Straßburg, later working in Vienna, where he spent the rest of his life [In Brief 4-2]. His first research result was published in 1897 in a noted German physiological journal. The title of his paper was "Zur Kenntnis der brenzcatechinähnlichen Substanz der Nebennieren (Knowledge of the catechol-like substance in adrenal glands)," and in this he forcefully set out his idea, based on his knowledge of chemistry, that Vulpian's color reaction was analogous to a pyrocatechol reaction (4-31).

In Brief 4-2. Strasbourg (France): City of culture at the mercy of Franco-German disputes

In the region known as Alsace-Lorraine on the border between France and Germany lies a cultural city that was founded in the 4th century.

In recent times the city has been tossed around by the fortunes of war between the two countries; its name was destined to keep changing between Strasbourg (French) and Straßburg (German). Gutenberg, the inventor of typographical printing, and the theologian Calvin spent part of their lives in this region; so too did Goethe and Mozart.

This city is home to the University of Strasbourg. Founded in 1631, it has a long history and is one of Europe's leading universities. The city was forced to suffer as a result of ever-changing geopolitics, but both France and Germany— whichever happened to have control of the city—alternately sent their finest scholars and researchers to the university as a matter of national prestige, and these great minds maintained the university at the very highest level.

Famous academics who have taught there include the microbiologist and chemist Louis Pasteur and the organic chemist Adolf von Baeyer. Countless notable figures have studied there, including Klemens von Metternich, the Austrian statesman who long ago presided over the Congress of Vienna, and Paul Ehrlich, the physician who established histological staining and immunology and developed various medicines.

The following year, a second report with the same title was published (4-32). Von Fürth finely chopped fresh adrenal glands from pigs and made an extract with ethanol. He filtered this and added neutral lead acetate solution, and after removing the precipitate he was left with a substance with a high nitrogen content. However, the quantity was tiny—from 2,000 rabbit adrenal glands he obtained just 0.4 g of this substance, and the search for the chemical composition ended in frustration [Note 4-7].

Note 4-7.

At that time, 0.4 g was absolutely not enough to carry out a satisfactory chemical analysis (4-32). Nonetheless, analysis experiments were carried out 10 times, from which elemental analysis figures for the extracted principles were obtained seven times. The samples were not pure, but eventually, from the results of acetylated compound analysis, von Fürth assumed that the compound was tetrahydro dioxypyridine, $C_5H_9O_2N$, or dihydroxy pyridine, $C_5H_7O_2N$ (4-32). He later rejected this assumption.

As an indication of the substantial network among German scientific researchers at that time, in most of his papers von Fürth specified that he received cooperation with the chemistry from the protein chemist Franz Hofmeister, a professor at Straßburg University, and with the physiological activity tests from Rudolf Gottlieb, a professor of Heidelberg University. Such cooperation would normally be hard to come by.

Two years later, the American scientist Abel published his most important paper on epinephrine that we saw earlier (4-27), this time in German, in the same academic journal as von Fürth. This marked the start of a war of words between the two researchers, waged in German in the same journal.

Von Fürth's most important research report was the one he submitted in December 1899 to the same journal as the two previous papers. In this, for convenience he gave the name "suprarenin" to the compound that he assumed to be the active principle. He coined this name by combining the Latin supra, meaning "above," and ren, meaning "kidney." Unlike Abel, he did not apply the name to a chemical compound with a specific, known chemical composition, so when he isolated a compound resembling an iron salt, he could safely describe it as "a suprarenin derivative." In his report, von Fürth discussed in detail the results of a chemical and physiological comparison of the principle he isolated and Abel's epinephrin. Finally, based on this, he asserted that epinephrin had absolutely no blood pressure-raising activity. He then went on to give an extremely detailed account of the results of animal studies examining the chemistry and physiological activity of suprarenin (4-33).

Meanwhile, Abel followed on from his comprehensive research report described earlier by publishing three reports in succession in 1899 in the *American Journal of Physiology (Proceedings of the American Physiological Society)* (4-34, 4-35, 4–36). These were long papers, but I very carefully read them all. Unfortunately, what he referred to as "my epinephrin" was not the active principle; in fact it was adrenaline with a benzoyl group attached that had lost its activity. Not only this, but the attached benzoyl group would not come off, no matter what he did.

As I read the papers, an image of Abel wrestling with this problem for all he was worth came to mind, and it was impossible not to feel for him [Note 4-8]. Sadly, however, the papers cannot be rated as valuable from a scientific perspective.

Note 4-8.
Benzoyl adrenaline is formed when either the hydrogen atom bound to the nitrogen atom or the hydrogen atom nearest to this (which is bound to a carbon atom) within the adrenaline molecule is replaced by a benzoyl group.

Abel believed he could collect crystalline adrenaline by removing the benzoyl group, thus freeing the adrenaline. Unluckily for him, the position of these two hydrogen atoms is such that it causes

intramolecular rearrangement of the benzoyl group. If under certain conditions you try to remove the benzoyl group bonded to the carbon atom, these conditions will be favorable for the vicinity of the nitrogen atom; if, on the other hand, you change the conditions, the vicinity of the carbon atom is then favored. This pattern simply repeats itself, and is a labyrinth that will not allow adrenaline to be isolated. Abel became stuck in this labyrinth, from which he could not extricate himself.

In European terms, it was as if Abel played two parts at the same time: the architect, Daedalus, who built the Labyrinth on the Mediterranean island of Crete, and the Minotaur, who was imprisoned in the labyrinth and could not escape. Some 30 years later, Abel looked back on his research, and realized that the cause of his failure was not concentrating the adrenal gland extract fluid sufficiently in the way Takamine subsequently did. He had then thought that his extract was no good, and reacted it with benzoyl chloride to produce a derivative that was not active. Abel self-deprecatingly described his methods as the "blunders of a pioneer" (4-37).

4. The war of words between American, British, and German researchers

Moore in London, von Fürth in Straßburg, and Abel in Baltimore, USA, were engrossed in the isolation of the active principle, each believing he was in the lead. The three were endlessly concerned about the progress of their competitors' work, studying each other's papers in great detail and criticizing them in the harshest of terms. There is not enough space here to introduce these papers one by one, but the reader will perhaps feel something of the heat surrounding research at that time.

First, Moore criticized the paper published by Fränkel in Germany in 1896 (4-19) because the chemical composition was not shown, and while Fränkel wrote that the compound dissolved in absolute alcohol, Moore found this dubious. It was not as if Moore had any results of his own to put forward, so perhaps this was his attacking thrust.

Moore then came under scathing criticism from Abel, who pointed out that Moore's judgment that his compound was a "pyridine derivative" was based on no more than the odor of pyridine, and had no proper evidence (4-17). The colleagues of Moore and Abel found themselves dragged into the fray, and two opposing camps formed as the dispute widened [Note 4-9].

Note 4-9.
Moore, together with co-author C. Purinton, published a paper titled "On the Effects of Intravenous Injection of Minimal Doses of Suprarenal Extract upon the Arterial Blood Pressure" in an academic journal in 1899 (4-14). In response to this, Reid Hunt, who was part of the Abel camp, published a report with the same title, except the words "Suprarenal Extract" were changed to "Epinephrine Sulphate," in the same American journal two years later in 1901. In this, he asserted that their sulphate showed far

greater activity. While it was not yet an age of quantitative research, this paper can only be seen as an excessively blunt defense of Abel (4-38).

In 1899, Abel published a discussion of the analysis values of an acetylated derivative of his compound, after which he stated his suspicion that the substance that von Fürth had analyzed was epinephrine mixed with other substances. He declared that just by preparing and isolating a great number of salts and derivatives it would be possible to determine the purity and deduce a credible molecular formula (4-39).

When von Fürth found out about this, he was incensed. "He thinks the substance I analyzed is impure epinephrine," he raged, and launched a full-on retaliatory attack against Abel by stating in the same journal, "It may be declared that epinephrin has absolutely no blood pressure-raising effect at all" (4-33).

Abel submitted his paper to the editorial department on July 24, 1899, and von Furth submitted his on December 23 of the same year. It really was a closely fought competition.

By 1903, the contest had already been decided by Takamine and Wooyenaka's successful crystallization of adrenaline. Even after this, however, von Fürth declared that epinephrin was clearly not a natural product and use of this term would invite misunderstandings, so he would avoid using it. He insisted that the whole of the Abel camp, including Abel's assistant, Samuel Amberg, was mistaken (4-40).

It seems that von Fürth's anger was not yet assuaged—in the final paper on the isolation of the active principle, he rammed his point home by stating that epinephrin had absolutely nothing to do with the active principle, and the real blood pressure-raising principle was a completely different substance that had adulterated the epinephrin in small quantities.

Toward the end of his life, Wooyenaka spoke of his impression of the exchanges between these researchers: "Both von Fürth and Abel were fighting without having obtained the principle" (4-41).

5. Success at Last

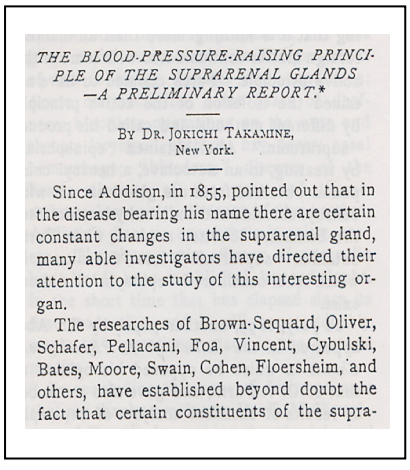
Please think back to the Takamine's hot, semi-basement laboratory in New York City with no air conditioning that we first encountered in Chapter 1 during the heat of the summer of 1900. The young scientist Keizo Wooyenaka, who at the age of 24 had just been taken on by Jokichi Takamine, successfully isolated adrenaline; the bitter struggles between the leading scientists of the day that have been described at length in this chapter seem somehow unbelievable.

More than this, during the 44 years since Vulpian's first attempts in 1857, the number of scientists that left a record of published papers exceeds 20, and Wooyenaka was successful where all of them had failed. He achieved this in an extremely short space of time, and, moreover, he was able to crystallize the active principle beautifully.

The 1880s in Europe had been a golden decade of tremendous development in science, in which new scientific achievements appeared in rapid succession.

1900 marked the start of an exciting new era, to which these results would be passed (In Brief 4-3). Takamine was both a scientific businessman and a patent attorney (a patent professional), and he soon drew up a draught for a manufacturing patent, which he submitted as a patent application to the US Patent Bureau on November 5 (4-42). He made a similar application in his home country, Japan, on April 29 of the following year, 1901 (4-43).

After submitting the patent application in America, Takamine embarked on a vigorous public relations campaign focusing on academic meetings during the following year. First, he gave an oral presentation titled "The Blood Pressure-Raising Principle of the Suprarenal Glands—A Preliminary Report" [Figure 4-3] to the annual meeting of the New York State Medical Society in January 1901 (4-44). This was his first public presentation. Next, he gave a similar lecture to the Section of Laryngology of the same society on March 27. Following Takamine onto the podium, Emil Mayer MD reported 35 clinical studies of adrenaline used in the treatment of cases such as acute vaso-motor rhinitis and for hemostasis during surgery, with favorable results (4-45).



THE BLOOD-PRESSURE-RAISING PRINCIPLE OF THE SUPRARENAL GLANDS —A PRELIMINARY REPORT.*

By Dr. Jokichi Takamine, New York.

Since Addison, in 1855, pointed out that in the disease bearing his name there are certain constant changes in the suprarenal gland, many able investigators have directed their attention to the study of this interesting organ.

The researches of Brown-Sequard, Oliver, Schafer, Pellacani, Foa, Vincent, Cybulski, Bates, Moore, Swain, Cohen, Floersheim, and others, have established beyond doubt the fact that certain constituents of the supra-

Figure 4-3. Jokichi Takamine's first paper on adrenaline, published in 1901 (4-44).

Mayer had received a hydrochloric acid solution of adrenaline from the ophthalmologist Dr. W. H. Bates (see page 55, in Chapter 3) in December 1900 and started testing it, but he lost the sample through an accident. Takamine obligingly provided him with a dilute solution of adrenaline and pure crystals soon after this in 1901, and Mayer continued his clinical

studies. Of the 35 cases, he used tablets of adrenaline tartrate with two of them; he wrote that the tablets "when dissolved in enough water to fill the ordinary atomizer bottle, will be all sufficient for the patient's use (4-45)." It seems likely that there were considerable exchanges of information among Takamine, Bates, Mayer, and Parke, Davis & Co.

In later years, in an obituary to Takamine, Mayer wrote the following: "This paper was read before the Section of Laryngology of the New York Academy of Medicine, the late Dr. W. K. Simpson being President. Dr. Takamine and his associate, Mr. Keizo Wooyenaka, were present, and took part in the discussions, as did Dr. W. H. Bates, who was the first to call attention to the value of the suprarenal extract." From this obituary, we can surmise that it was well known in academic societies that the crystallization of adrenaline was the result of joint research by Takamine and Wooyenaka, and that their work was already highly regarded by a number of clinicians (4-46).

However, Wooyenaka was to recount later that Takamine had no contact with universities or other academic institutions in the United States. Asked if Dr. Takamine had held a high position as a teacher in the United States at that time, he replied, "No, it wasn't like that." When asked if had been connected to universities, Wooyenaka said, "There were no connections at all. He studied at the University of Glasgow as an overseas student, but that was all" (4-47).

When Takamine first presented his research, the scholars and researchers listening must have been unable to hide their inner surprise to hear an Asian with this background launch into an explanation of such momentous results. Takamine went on to publish a detailed paper titled "Adrenalin—the Active Principle of the Suprarenal Glands and its Mode of Preparation" in the *American Journal of Pharmacy* in November of 1901 (4-48).

In this paper he notes that Aldrich of Parke, Davis & Co. collaborated in the research, and he reports the results of Abel's confirmation tests of the benzoyl derivative. In this paper, Takamine gives the estimated experimental formula of adrenaline as $C_{10}H_{15}NO_3$, but this does not agree with Aldrich's correct molecular formula of $C_9H_{13}NO_3$. At the end of the paper, Takamine thanks Dr. Elijah Mark Houghton, the Research Director of Parke, Davis & Co., for his highly accurate activity tests, and records his high appreciation of Wooyenaka's achievements. He gives Wooyenaka's position as "my associate."

Returning to the topic, on the next page of his paper in the *American Journal of Pharmacy*, Takamine mentions a paper by Elijah Mark Houghton, titled "The Pharmacologic Assay of Preparations of the Suprarenal Glands," which gives an extremely detailed method for assaying activity.

In Brief 4-3. Science at the end of the 19th century and the start of the 20th century

1898 ・Marie Curie (France) discovers radium.

1900 ・Max Planck (Germany) announces "Planck's Black-body Radiation Law" in relation to energy radiation. This was the foundation for quantum theory, which was taken over by Einstein (America) and Bohr (Denmark).

・Takamine and Wooyenaka (Japan) isolate the first hormone, adrenaline, as crystals.

・Tsvet (Russia) discovers chromatography, starting a revolution in separation technology.

・Zeppelin (Germany) completes airship No. 1 (LZ-1).

・Mendel's paper on the Chromosome Theory of Inheritance is evaluated 16 years after his death, marking the start of modern genetics.

1901 ・The Nobel prize is established. The first three winners were Röntogen (Germany), who discovered X-rays; van't Hoff (Netherlands), who discovered the osmotic pressure of liquid; and von Behring (Germany), who established diphtheria serum therapy.

1902 ・The three winners of the second Nobel prize were:

[In Physiology or Medicine] Ronald Ross, "for his work on malaria, by which he has shown how it enters the organism and thereby has laid the foundation for successful research on this."

[In Chemistry] Hermann Emil Fischer, "in recognition of the extraordinary services he has rendered by his work on sugar and purine syntheses."

[In Physics] Hendrik Antoon Lorentz and Pieter Zeeman, "in recognition of the extraordinary service they rendered by their researches into the influence of magnetism upon radiation phenomena.

・Hisashi Kimura (Japan): discovery of Z term in variation of latitude.

Houghton's paper had previously been orally presented before the St. Louis meeting of the American Pharmaceutical Association in September of that year. Many physicians and medical scientists read this paper together with Takamine's, and must undoubtedly have seen that a groundbreaking new medication had made its appearance (4-49).

Houghton studied at the University of Michigan in the United States, and he obtained his doctorate of medicine in 1894 and worked there as a pharmaceutical research assistant until 1895. Abel established the country's first Department of Pharmacology at this university in 1891, and as he was a professor there until 1893, it seems likely that Houghton would have taken Abel's classes when he was a student.

Houghton was invited to join Parke, Davis & Co. as supervisor of the research laboratory in 1895. At a lecture to the Detroit College of Medicine, he stated that he himself carried out the activity tests on the sample of adrenaline sent by Takamine (4-50). Houghton, Aldrich, and Abel somehow all seemed to be fated to cross paths.

The following year, 1902, Houghton presented a paper to the Section on Materia Medica, Pharmacy and Therapeutics of the 52nd Annual Meeting of the American Medical Association, which was held in St. Paul, Minnesota, on June 4–7. He covered the history of the principle of the adrenal gland, starting from Addison's disease, and then gave a detailed account of his own physiological research (4-51). Following this, Takamine gave a lecture about the crystallization of adrenaline (4-52), and the record of both lectures can be found together in the *Journal of American Medical Association* of that year.

During his lecture, Houghton touched briefly on the researchers whose achievements had been introduced before those of Takamine, and starting with Addison, this ran to 37 people. The record of these pioneers left a great impression on the audience, and it was after this that Takamine took the podium. The researchers—and particularly those racing to isolate the active principle in Europe—would most likely not have known how to pronounce the name Takamine when this sensational report was presented to them.

This was the age of rough-and-ready adventurers with dreams of getting rich by striking gold like Charlie Chaplin in The Gold Rush; it was also the time of the folk song "Oh My Darling Clementine." Perhaps the "mine" in Takamine would have conjured up images of a goldmine. Seeing the name written, people must have asked, "Who is this Taka-mine fellow? Where is he from? What does he do?" They would have been further confused to find that this name did not appear anywhere in *Chemische Zentralblatt*, the German journal, trusted worldwide, that summarized the current literature in chemistry (the American journal *Chemical Abstracts* had not yet been printed).

At this time, Japan was in the period between the First Sino-Japanese War (1894–95) and the Russo-Japanese War (1904–05), and was mired in its own problems. The country was largely indifferent to the fact that mankind had obtained the first hormone, adrenaline.

6. The gentleman of Parke, Davis & Co.

In the summer of 1900, slightly after Wooyenaka crystallized adrenaline, Aldrich, [Figure 4-4] who was then the head of the adrenaline project at Parke, Davis & Co., isolated an active principle. He was working independently of Takamine and Wooyenaka, and used a slightly

different method, but it was possible to show that this principle was identical to adrenaline.

Figure 4-4. The American chemist Thomas Aldrich of Parke, Davis & Co. conducting experiment (4-53).

Aldrich submitted the details of his work to the Journal of American Physiological Society, the same journal to which Abel, his former teacher and also his rival, had submitted most of his papers in the following year, 1901 (4-25). In summary, Aldrich's paper was as follows:

"I regard Takamine's adrenaline as a huge discovery. A little after Takamine, I also obtained some crystals. Several months later, I was able to prepare a sufficient quantity to conduct combustion elementary analysis. I soon established that Takamine's crystals and my own were similar, and with further research I found that the two crystals corresponded in every aspect, and were identical. The crystals I obtained in my first experiment contained a tiny amount of ash, but I was able to eliminate this in subsequent experiments. The crystals I received from Takamine were purified by my assistant, Mr. Beckwith, and we obtained samples with no ash at all. The purified product had a yellow-white or whitish color that lasted for six months, and there was no loss of physiological activity. Purity was tested by our physiological activity measurement test".

Aldrich next gives the results of an elementary chemical analysis of the crystals, leading to a very important paragraph: "A comparison of these analytical data shows that the two substances obtained are identical, and using them as a basis for calculating an empirical formula the simplest body obtainable is represented by the formula: $C_9H_{13}NO_3$."

The empirical formula put forward by Takamine and Wooyenaka, as we have already seen, was $C_{10}H_{15}NO_3$, which was bigger than Aldrich's empirical formula by just CH_2. Nonetheless, if a molecule differs by CH_2, the conventional wisdom in chemistry says it is a completely different substance. Aldrich analyzed Takamine's sample after purification, and found it to be identical to his own [Figure 4-5].

A comparison of these analytical data shows that the two substances obtained are identical, and using them as a basis for calculating an empirical formula the simplest body obtainable is represented by the formula: $C_9H_{13}NO_3$.

Figure 4-5. The important part in Aldrich's paper, in which he writes that his crystals and Takamine's were the same (4-25).

Takamine's crystals were the result of joint research commissioned by Parke, Davis & Co., while Aldrich had obtained crystals entirely through his own efforts—no one would have objected if he had said that his own crystals were $C_9H_{13}NO_3$ and Takamine's were a different substance, $C_{10}H_{15}NO_3$. However, he wrote that the crystals were the same in his submission to the academic journal.

Aldrich also showed consideration for Prof. Dr. Abel, for whom he had worked as an assistant for five years from 1893, by including the following paragraph: "It is interesting to note in this connection that if we subtract a benzoyl residue from Abel's formula for "epinephrine" —$C_{17}H_{15}NO_4$—we obtain a formula $C_{10}H_{10}NO_3$ which is not very far removed from that of adrenalin—$C_9H_{13}NO_3$—a difference that can be readily explained if we suppose either of the substances to be contaminated with other bodies."

Finally, Aldrich writes that he already had isolated a sufficient quantity of crystals to be able to proceed with the research. At the time, elementary analysis by combustion was the only means available for molecular estimation, and there were none of the micro and non-destructive analytical methods that we have today. Being able to obtain samples of sufficient size was a deciding factor in research. In 1905, Aldrich published an accurate, in-depth review of the history of research into the active principle of the adrenal glands, centered around the results he had obtained himself (4-26) [Note 4-10].

Note 4-10.
In this paper (4-26), Aldrich writes, "In August of 1901 the writer succeeded also by a method differing slightly from that of Takamine's in isolating a body, which was shown to be identical with adrenalin." In his paper published four years earlier, however, he wrote it correctly as "in the summer of 1900" (4-25). It is a trifling point, but it is amusing to imagine how even the normally composed Aldrich could be so busy that he made a mistake with such an important record, the year of his success with crystallization.

7. Reaction to the crystallization

One year after Takamine's dramatic report, the German von Fürth published a report titled "Zur Kenntnis des Suprarenins (Information of Suprarenin)," in which he gave the details of the preparation method, chemical properties, physiological activity, and elemental composition of the compound (4-54). In the second half, von Fürth has the following to say: "Recently Dr. Jokichi Takamine, a chemist from Parke, Davis & Co., used an undisclosed method to obtain a crystalline preparation of blood pressure-raising principles from the adrenal glands, which has the trademark 'adrenalin.' Preparing the principles in this way is worthy of respect as a well-known advance in this field. The sample is a pale yellowish powder, and microscopic observation shows that it is present as a round aggregate made up

of short needle crystals of considerable width. This substance hardly dissolves in cold water but is soluble in dilute acidic water and free alkali, and vividly shows the characteristic color reaction and reducing activity of the active principle of the adrenal glands."

Von Fürth continued by describing how he obtained the results of quantitative activity tests on dogs from Parke, Davis & Co., and carried out experiments to compare these to the effects on dogs of the suprarenin he had prepared himself. He also conducted an elemental analysis using a sample of Takamine's substance that he fully dried himself. He compared the results of suprarenin, and integrating all the results he concluded that there was little margin for doubt that both substances were the same (4-54).

In a footnote to his paper, von Fürth notes that it is a great shame that he is unable to give a final conclusion because he was not able to learn Jokichi Takamine's method for preparing adrenaline, and therefore could not make a sample himself to study. While von Fürth celebrates Takamine's success, he seemed to be frustrated by not being able to reach a final answer.

Takamine's method for preparing adrenaline was first publicised in the *Journal of the Society of Chemical Industry* in July 1901 as "Eng. Pat. 1467, January 22, 1901" (4-55). Takamine's research results also appeared in the Proceedings of The Physiological Society, a well-known British journal with a long history, at around the same time, with an extremely detailed description of the extraction and purification methods using sheep and oxen suprarenal glands (4-56). Von Fürth had either failed to notice these, or had missed the publication of the society's journal.

In a conversation in later life, Wooyenaka recalled Takamine providing von Fürth with a sample of adrenaline. "He sent von Fürth a gram or so of the crystals he had prepared, and von Fürth replied with a very courteous letter in which he thanked Takamine for his kindness in sending the sample, and congratulated him on his success," Wooyenaka said. (4-41)

It is likely that under the terms of the contract with Parke, Davis & Co., Takamine was unable to disclose the details of the preparation methods until the patent had been secured, and it appears that neither Takamine nor Parke, Davis & Co. did anything underhanded —such as holding onto samples.

Von Fürth compiled his work into a large paper of some 30 pages a year later in 1903. The paper comprised seven sections: (1) Representation of crystallized Suprarenin (adrenaline), (2) Analysis of crystallized Suprarenin (adrenaline), (3) Rapid decomposition of crystallized Suprarenin, (4) Decomposition of Suprarenin by mineral acids, (5) Acyl and alkyl

derivatives of epinephrine (adrenaline), (6) Oxidation experiments, and (7) Decomposition of Suprarenin by alkalis.

The research results were summarized into nine items, and von Fürth gave the following conclusion: "If the molecular formula $C_9H_{13}NO_3$ put forward by Aldrich is correct, the following chemical structure may be suggested: $[(CH_3)NC_2H(OH)]$ $C_6H_6(OH)_2$. It is to be hoped that the truth is clarified through further decomposition and synthesis research." Von Fürth makes it clear at the start of this paper that the crystals he used in the experiments were prepared in accordance with the method of Takamine and Aldrich (4-40, 4-57).

Although it relates more to Chapter 8, there is one thing I would very much like to introduce at this point. In von Fürth's final paper, there is an experimental result that shows he really was a top-class researcher. He used the chemical decomposition method to find the chemical structure of his suprarenin. First he found that the molecule did not contain a methoxy group (CH_3O). He then determined that there was a methyl amide group (CH_3N) present, but while the theoretical value for the % content of this group was 8.2%, the results of two experiments gave very low values of 5.19% and 4.79%. This indicated that there were still problems with his suprarenin sample. As we will discuss in more detail in Chapter 8, the crystals of adrenaline prepared by Takamine and Wooyenaka and by Aldrich, as well as von Fürth's suprarenin crystals, were not pure, but included a considerable amount of the analogue noradrenaline. Noradrenaline does not have a methyl amide group, so this result is unsurprising to us today—however, von Fürth had shown experimental results that predicted the existence of this analogue.

Von Fürth finished his research into the adrenal gland active principle at this stage, and found work as an outside lecturer (Privatdozent) at the University of Vienna [Note 4-11].

Note 4-11.
Von Fürth gave an oral presentation of the research outlined here to an academic conference in Vienna on March 5, 1903; the details of his presentation appeared with exactly the same content in two academic journals published in Vienna (4-40, 4-57), and he noted that he received financial support for his research expenses from the Akademie der Wissenschaften in Vienna. It therefore appears that the invitation from Straßburg to the University of Vienna had already been decided.

He wrote a great many specialized reference works, and two of his best-known books are *Lehrbuch der physiologischen und pathologischen Chemie* (Textbook of physiological and pathological chemistry) and *Vergleichende chemische Physiologie der nieder Thieren* (Comparative chemical physiology of lower animals).

Von Fürth's assistant at the Physiological-Chemical Institute of Straßburg University was E. Friedmann, who took over from von Fürth when the latter departed for the University of Vienna. From 1904 onward, Friedmann produced reports of experiments to find the chemical structure of adrenaline, and in 1906 he published the results of in-depth research (4-58, 4-59).

In Britain, where the hormone effects of the adrenal glands was first discovered, John Newport Langley, Deputy Professor of Physiology at the University of Cambridge, was using cats as experimental animals for activity tests and, in most of his experiments, adrenal glands from dogs to investigate the effects of adrenaline. He published his results in 1901(4-60). Although it was later shown that adrenaline has different α- and β-effects (cf., Chapter 8, section 10 (1)), this was not yet known when Langley was carrying out his research. He conducted painstaking research that amounted to a full frontal attack [Note 4-12].

Note 4-12
Langley put the effects of adrenaline in order of intensity:

1. Rise of blood-pressure
2. Inhibition of the sphincter of the stomach and of the intestine (rabbit)
3. Inhibition of the bladder
4. Dilation of the pupil (cat)
5. Withdrawal of the nictitating membrane (cat)
6. Separation of the eyelids (cat)
7. Contraction of the uterus, vas deferens, seminal vesicles, etc. (rabbit)
8. Salivary and lachrymal secretion
9. Inhibition of the stomach
10. Inhibition of the gall-bladder and increased bile secretion
11. Dilation of pupils (rabbit)
12. Inhibition of the internal anal sphincter (rabbit)
13. Contraction of the internal anal sphincter (cat)
14. Contraction of the internal generative organs (cat)
15. Contraction of the muscles of the hairs
16. No contraction of the tunica dartos of the scrotum
17. No secretion of sweat

This research was carried out before Parke, Davis & Co. in America released their crystal adrenaline onto the market, so Langley used tablets of suprarenal glands, called "Supra-renal Tabloids," that were marketed by Burroughs-Wellcome Co. of London.

Although animal organ drugs had inconsistent effectiveness, they were already being supplied by the major pharmaceutical companies. A good example of this is Solis-Cohen from Philadelphia, who, as we saw in Chapter 3 (page 56), treated the asthma of a woman with labored breathing by carefully administering consecutive doses of tablets of adrenal glands; the patient made a dramatic recovery (4-23) [Note 4-13].

Note 4-13.
Today, the word "tabloid" is associated with the newspaper format of half the size of a conventional newspaper, but the word is actually a trademark of the British company Burroughs-Wellcome Co., which devised it as a brand name for tablets that were compressed dosage forms. As the company's business expanded, it used this word in the names of a wide range of products, such as "Tabloid first aid kits and medicine chests," and "Tabloid tea" (4-61).

At around the same time, the Research Laboratories of the Royal College of Physicians and Surgeons, London, published an in-depth paper in 1904 on the effect of adrenal gland

extracts as part of their research into the physiology of the lung. The first footnote states: "In our first experiments the solution of suprarenal extract employed was one prepared from the tabloids of Burroughs and Wellcome. In the later experiments the 1 in 1000 solution of adrenalin of Parke Davis and Co., was used. The results were of a much more uniform character when this latter preparation was taken." (4-62).

Wooyenaka had taken part in the research by Parke, Davis & Co. to develop a method for manufacturing adrenaline on an industrial scale, and it can be seen that even academic societies had confidence in the reliability of this method. In a paper titled "The Action of Adrenalin," Thomas R. Elliott, a highly talented student who had taken over the traditions of the Langley Laboratory at the University of Cambridge, succinctly and accurately acknowledged the value of the achievements of Takamine and Wooyenaka: "Takamine's isolation of the definite chemical substance adrenalin, as the active principle of the suprarenal glands, has made the further study of the question easier, for it permits an exact quantitative determination of the extent as well as of the nature of the reactions which are produced by it in the body (4-63)"

Germany was at the forefront of chemistry research, and after von Fürth finished his work the country did not remain on the sidelines of research into the "chemistry of adrenaline." In 1903, Pauly of the University of Bonn first acknowledged Takamine's achievements, and then showed that the elemental composition of the active principles he had collected agreed with that of Aldrich's sample, but the nitrogen content of Abel's epinephrin was lower. He went on to measure the optical rotation of the samples; this is the rotation of the plane of linearly polarized light about the direction in which it travels (see Chapter 8, Column 8-1). He found that this principle of living animals was optically active, and this activity was levorotatory, meaning that light passing through the substance is rotated counterclockwise as it approaches the observer, with the angle of rotation $[\alpha]_D = -43°$ (4-64).

In 1904, Emil Abderhalden, a student at the Chemical Institute of Berlin University, which was hallowed ground for natural product chemistry research, examined which was correct: Abel's formula of $C_{10}H_{13}NO_3 \cdot 1/2H_2O$, or Aldrich's (or Pauly's) formula of $C_9H_{13}NO_3$. Abderhalden, who was 27 at the time, was studying under the famous chemist Emil Fischer, who won the second Nobel Prize in Chemistry in 1902. He presented data showing that Aldrich had the correct formula, and put forward five possible molecular formulae for adrenaline as a substituted pyrocatechol. It looked as though Abderhalden already had a mental picture of the correct chemical structure (4-65).

That same year, Gabriel Bertrand in France collected 125 g of purified adrenaline crystals

from the suprarenal glands of nearly 4,000 horses, and conducted repeated elemental analysis. He combined this with the results of molecular weight measurement using a cryoscopic method, and in his paper he stated that for future research, adrenaline should be defined as $C_9H_{13}NO_3$ (4-66). (This paper determined the molecular formula of adrenaline. Strictly speaking, the previous formulae showing the elemental composition of adrenaline were empirical formulae, but in this book the more familiar expression "molecular formula" has been used where appropriate.)

Hooper A. D. Jowett, who was in charge of the adrenaline project at the Chemical Institute of Burroughs-Wellcome Co., the company that sold the Supra-renal Tabloid, published two papers in 1904 on the chemical structure of adrenaline (4-67, 4-68). He first maintained the elemental composition put forward by Aldrich was correct, and then put forward three possible chemical structures. He matched these against the results of chemical reaction research, and finally narrowed them down to two planar structures. He correctly guessed that one of these was highly likely to be the structure of adrenaline, and he also reported that the specific rotation of a purified sample was $[\alpha]_D = -32.6^{\circ}$ (4-68) [Note 4-14].

Note 4-14.
Burroughs-Wellcome Co. had marketed their "Supra-renal Tabloid" as an animal organ medicine, and they probably put Jowett in charge of determining the chemical structure with the aim of manufacturing synthetic adrenaline.

8. Abel refuses to give up

Even after reading the research report by Takamine and Wooyenaka, Abel was not prepared to give up without a fight. He submitted seven papers in rapid succession to academic journals, in which he developed his own opinions (4-69 through 4-75). There would not be much point in introducing them in detail at this stage, but I will just mention the controversy that Abel started.

In 1902, Abel published a paper (4-71) titled "On a Simple Method of Preparing Epinephrin and its Compounds" in the journal of his university. In this, he described von Fürth's suprarenin, with an average molecular formula of $C_{8.5}H_{12.2}NO_x$, as no more than a "rough approximation to the truth." He had just six words for adrenaline: "Crystalline it is, but not pure" (4-72). In 1903 he purchased some 32 g of commercially available adrenaline, and after purifying it and conducting elementary analysis, he concluded, "It is very evident then that adrenalin cannot yet be spoken of as having a 'constant composition' (Takamine), and as being a pure chemical individual (4-73). Takamine did not once respond to Abel's provocative

verbiage in academic journals, but Aldrich put forward careful but scathing rebuttals (4-26, 4-76).

It should be pointed out that when judged from the present-day level of science, the points Abel made were, in fact, legitimate, although the knowledge we have now was not available to Abel. As we will see later in Chapter 8, the crystals that Wooyenaka collected from bovine adrenal glands contained noradrenaline, which is a "sister compound" in terms of its chemical and physiological effects, in quantities that were not negligible. However, given the technical level of that time, it is easy to see how Aldrich felt—it must have been hard to accept objections to the purity of highly active adrenaline from someone who had not actually isolated the active principle.

9. Lack of a group to determine activity

Looking back over the struggle lasting 44 years in which more than 20 isolation researchers, including some of the leading researchers of the day, took part, it is strange that they all seemed to lack the most important, obvious method: there were almost no researchers (or research institutions or laboratories) that measured the physiological activity of the extracted substances and carried out experiments with these as indices.

As the researchers were searching for endocrine substances that caused raising of the blood pressure, it would be natural to prepare a method for measuring the activity of the extracted substances at each step of the purification. Moreover, as this was an age in which techniques for chemical analysis were entirely undeveloped, there was an even greater need for this, so it seems very curious that a method for measuring activity was not established.

Of course, using laboratory animals such as live dogs or cats to quantify blood pressure increases would require a considerable number of animals if each sample was measured even just three times and the mean value calculated at each purification step—this was probably too much for laboratories with limited staff and budgets.

In an era with no chromatography, fine chemistry research was truly a difficult science. Nonetheless, Aldrich of Parke, Davis & Co. was fully prepared to quantify activity test results, and his paper titled "Is Adrenalin the Active Principle of the Suprarenal Gland?" puts forward a wealth of activity test values (from Aldrich's colleague, Dr. Mogk) to match the chemical data (4-76).

One more thing that could be pointed out is that the handling of the experimental samples does not appear to have been very careful, and this is common to the many researchers that

were unsuccessful in their bid to isolate the active principle. They seem to have ignored the nuanced words of Vulpian, the discoverer of the color reaction, who wrote in his first and most important report: "*Lorsqu'on essaye les capsules des animaux mammifères, il faut, avec beaucoup de précaution, ne prendre que la substance médullaire, car la substance corticale masque quelquefois la réaction, au point de la rendre méconnaissable* (When trying to extract suprarenal capsules of mammals, we must be very careful and only take their medulla, the corticolous substance which sometimes masks the reaction beyond recognition)".

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More than 20 of the Western world's top-level scientists, who recognized that Vulpian's color reaction was the key, pursued the identity of the hormone over the space of 44 years. Eventually, it was to be two researchers from the East that revealed the hormone. The moment of isolation in crystalline form was the starting point from which a plethora of fascinating research developed spectacularly across the world.

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Chapter 5

Good Fortune

Following the isolation of adrenaline, there was a wealth of fascinating research. This will be examined in Chapter 8, but for the moment we will go back in time again. As I noted at the beginning of this story, Jokichi Takamine was not an expert in the physiology or chemistry of physiologically active substances in the body. So what gave him the opportunity to start working on the extremely challenging research area of the "isolation of adrenaline," and why did he take this chance? To find out, we need to trace the difficult path he took and learn of the fateful encounters along the way.

1. Takamine's first turning point

Jokichi Takamine—hereafter referred to as Takamine—was born on November 3, 1854, in his mother's family home in present-day Takaoka, Toyama Prefecture. His father, Seiichi, and mother, Yukiko, had five sons and seven daughters, of which Jokichi was the eldest.

The year before Jokichi was born, Commodore Matthew C. Perry of the United States Navy led a fleet of ships, known in Japan as the "Black Ships," to Japan to try to force the country to open up to trade. Perry had a letter from US President Millard Fillmore, which was delivered to Shogun Tokugawa Ieyoshi.

Perry returned in 1854, the year Takamine was born, and after landing at Uraga Bay, signed the Convention of Kanagawa with the government of Japan at Yokohama Harbor, thus marking the start of substantial trade between Japan and the United States. It was indeed a historic year.

Takamine's father Seiichi had studied Dutch medicine, and was a doctor with a high social position as an employee of the lord of Kaga Domain. He lived near Kanazawa Castle, the home of the domain lord, and when Jokichi was one year old he went with his mother to live with his father.

The fourth feudal lord of Kaga Domain, Tsunanori Maeda (1643–1724) had been greatly interested in scholarship, and he had invited eminent scholars from all around the country to

the domain, where he compiled an encyclopedia, introduced Noh drama, and was also committed to collecting, organizing and safeguarding ancient documents.

Since this period of benevolent rule, when it was said that "there were no beggars in Kaga," the domain had remained as one of Japan's leading cultural spheres, with an extremely high educational level. Jokichi grew up in this environment, and he was strictly disciplined in the academic atmosphere of a doctor's house.

In 1865, three years before the Meiji Restoration (when imperial rule was restored under Emperor Meiji), Jokichi left Kaga to study medicine in Nagasaki City at the age of 11. Once there, he realized that the times were changing—Dutch had previously been the main foreign language, but it was being replaced by English. Jokichi studied the basics of English at a private school under Reishi Ga (Noriyuki, 1840–1923), and subsequently studied English for about two years under Guido Verbeck (1830–1898) at a Saga domain school "*Chienkan*" in Nagasaki (5-1).

During this time, Japan underwent a period of great upheaval: the *Boshin* Civil War (1868–1869) broke out, leading to the demise of the *Shogunate*. Takamine bided his time reading Western books at a military school in Kyoto as he waited for the disturbances to die down. After the Tokugawa Shogunate was overthrown, which ushered in a new period in Japanese history, Takamine's ardent love of learning was to set him on a course that would determine the rest of his life.

In 1869, *Osaka Igakko* (Osaka Medical School, attached to Osaka Temporary Hospital) was established to the southeast of Osaka Castle by Koreyoshi Ogata (1843-1909) on the wishes of Emperor Meiji (5-2). The Dutch doctor Anthonius Franciscus Bauduin (1820-1885), a specialist in ophthalmology was put in charge of education at the school. In a precious photograph of the opening of *Osaka Igakko*; Takamine, who at the age of 15 had yet to lose his boyish looks, cuts a rather charming figure (5-3). Takamine had first started to study medicine with the aim of carrying on in his father's footsteps, and he had no hesitation in enrolling in the new medical school. Here, he was finally able to settle down and apply himself to scholarship.

The basic conception for education in chemistry in Japan was drawn up by Bauduin. He had traveled from the Netherlands to Nagasaki in 1862 to be the successor to the Dutch doctor Johannes Lijdius Catharinus Pompe van Meerdervoort (1829–1908), who had left behind a record of considerable achievement in Nagasaki City. Bauduin established chemistry and physics, which until then had been part of medical education, as a separate subject; and he sent for his pupil, Koenraad Wolter Gratama (1831–1888) from the

Netherlands, to take charge of the practical business of teaching this new field. The foundations for education in chemistry and physics in Japan during the turbulent period from the demise of the Shogunate through establishment of the new Meiji Government were built by the enthusiastic foreign teacher, Gratama.

Leaving out the subsequent rather complicated history that followed, let us just note that the college for chemistry and physics that was established was *Osaka Seimi-Kyoku*, which was built on the western side of Osaka Castle and opened its doors on May 1, 1869. The aforementioned *Osaka Igakko* was then opened to the southeast. *Osaka Seimi-Kyoku* was renamed *Rigakko* (Institute of Western Science) that year, and the number of students increased rapidly. In the year 1871–72, some 59 auditing students came to this college from the nearby *Osaka Igakko*. Bauduin's basic plan to establish chemistry and physics independently of medical education turned out to be remarkably successful due to the fact that the institute for chemistry and physics was established so close to the *Igakko* that students of the latter were able to walk there (5-4).

Rigakko was subsequently re-organized and became *Osaka-kaiseijo Rigakusho*, and Gratama, the first head of the institute, completed his term of office at the end of 1870. His position was taken over by Herman R. Ritter, a German chemist. Ritter had been invited to the forward-thinking Kaga Domain as a foreign teacher in 1869, but, like many other domains, Kaga Domain found itself in financial difficulty during the period of political turmoil, and was unable to employ Ritter. He had no choice but to seek employment with the new government. Ritter had gained considerable business experience since leaving his native Germany after completing his PhD at the University of Göttingen under Friedrich Wöhler, who in 1828 became the first person to synthesize an organic compound, urea, from inorganic compounds.

Seiichi Takamine, Jokichi's father, was a doctor, but he also had a vast knowledge of chemistry. He was responsible for extracting nitrogenous constituents from disused silkworm pupae, used to manufacture nitric acid salts in order to bolster Kaga Domain's store of gunpowder. Conjecturing from this, it is possible that inviting Ritter to Kaga may have originally been Seiichi's idea. And later, as his beloved son was studying in Osaka, Seiichi may well have let him know that a German chemist by the name of Ritter would soon be on his way to the same city.

When Ritter took up his new post at *Rigakusho*, he enthusiastically set about teaching German modern chemistry in English. In 1872, he performed three types of chemical experiment in front of Emperor Meiji, who was making an imperial visit to Osaka (5-4).

Jokichi Takamine had acquired a good level of English at Verbeck's school in Nagasaki City, so Ritter, coming to Japan from Germany, the world leader in chemistry, and lecturing in English, was better fortune than he could ever have wished for. As well as grounding in theoretical knowledge, Ritter also had experience in the chemical industry, and his lectures must have been fascinating for Takamine. This was to be the decisive turning point that changed Takamine's path away from medicine and toward chemistry instead.

Ten years after this, Nagayoshi Nagai, who was Takamine's senior by nine years, was studying in Germany as an assistant under the organic chemist A. W. Hofmann. He was so captivated by Hofmann's lectures that he changed from medicine to chemistry; interestingly, this is the same as the change that Takamine made during his time in Osaka, when he switched his goal from becoming a doctor to becoming a chemist. Moreover, when he returned to Japan, Nagai worked at the Tokyo Imperial University, where he taught Wooyenaka. It seems nothing short of destiny that Wooyenaka should then take his talents overseas to the United States, where he became Takamine's assistant and achieved the crystallization of adrenaline.

Ritter's lectures were published as an outstanding textbook of chemistry and physics, and this is still carefully preserved in libraries such as Waseda University. Ritter became a teacher of mining studies at *Tokyo Kaisei School* (now the University of Tokyo) in 1873, but he sadly later contracted smallpox, and despite the efforts of the German doctor Theodor Hoffmann (a German naval and army doctor and professor at the *Daigaku Tohkou [University Eastern Campus]*, the forerunner of the Faculty of Medicine of the University of Tokyo) to treat him, he passed away on December 25, 1874 at the age of 47. The Japanese government paid out condolence money with a value equivalent to 7 million yen today. His remains were interred at Yokohama Foreign General Cemetery, and his students had a splendid monument in his honor erected at Ueno-Yanaka Cemetery in Tokyo [Figure 5-1].

In the class registers of *Osaka-kaiseijo Rigakusho* (5-5), there is one name that cannot be overlooked. This is Mitsuzo Hida, who was in a lower grade than Takamine, but took the same classes in physics, chemistry, and mathematics. Hida remained on good terms with Takamine as Takamine's junior, and later, when he was working in the section for analyses of the Ministry of Agriculture and Commerce, he was to recommend an important character in this story to Takamine.

Figure 5-1. The monument to Herman Ritter at Ueno-Yanaka Cemetery in Tokyo. The monument was proposed by his students, and erected the year after his death. Even today, there are German visitors to the monument. (Photo taken by the author)

In 1872, Takamine left for Tokyo as a technical trainee sponsored by the newly established Ministry of Engineering. In 1879 he graduated at the top of the first graduating class of chemistry from the Imperial College of Engineering in Tokyo (the forerunner of Faculty of Engineering, the University of Tokyo), and was dispatched by the Japanese government to study for three years in the United Kingdom, at Anderson College in Glasgow, Scotland.

He studied chemical engineering across a wide range of fields. After returning to Japan, he started to aim at using scientific methods to turn around Japan's traditional industry as a senior member of the Ministry of Agriculture and Commerce (5-6).

2. Takamine's second turning point

In December 1884, the World Industrial and Cotton Centennial Exposition was held in New Orleans, in the southern United States. At this time, a century had passed since slaves had been brought from Africa and sold to work in the cotton fields. The government of Emperor Meiji in Japan decided to send the up-and-coming Jokichi Takamine, who had only returned from his three years in Scotland the previous year, to the exposition. Accompanied by Ichizo Hattori and Kizo Tamari (Agronomist), the 30-year-old Takamine, who was then working at the Ministry of Agriculture and Commerce, set sail from Yokohama in September, heading for New Orleans.

A detailed article praising the high quality of the Japanese display at the Industrial Exposition has been preserved. In particular, Takamine and Hattori, who were present at the display throughout the exposition and gave unfailingly courteous answers to even the most trivial of questions, gained a tremendous reputation for their manners (5-7).

The long overseas trip had a profound effect on the later course of Takamine's life, both professionally and personally [Figure 5-2] (5-8). During the time of the exposition he was invited to dinner by a local dignitary, and was immediately smitten by his daughter, whose name was Caroline Hitch [Figure 5-3] (5-9). They eventually became engaged and married three years later, when Takamine returned to the United States.

(Left) **Figure 5-2.** Jokichi Takamine at 30-year-old, when he returned to Japan after studying in Scotland and was working for the Ministry of Agriculture and Commerce.

(Right) **Figure 5-3.** Caroline Hitch (later Caroline Hitch Takamine), daughter of a noted local family.

At the exposition venue, Takamine noticed a display of phosphate rocks. After the exposition had finished, he visited Charleston, South Carolina, in order to get some of these phosphate rocks to take back to Japan with him. With these rocks, he founded a company called Tokyo Artificial Fertilizer Company, which is now Nissan Chemical Co. (5-10) (5-11). He probably gained the basic knowledge for manufacturing fertilizers when he visited a superphosphate factory in Newcastle, England, during his time studying in Scotland.

Among the people Takamine called on for investment in his new company were two leaders of the financial world during the Meiji Period: one was Eiichi Shibusawa, who founded Daiichi National Bank, and the other was Takashi Masuda, who was a head clerk in the Mitsui *zaibatsu* (family-owned business). Takamine remained friends with these two throughout his life. I will devote a little space here to what they had to say about Takamine.

Shibusawa: "Dr. Takamine is an extremely gentle person, and while originally a scholar, he is also a capable businessman. He is an elegant character, rather different from what is generally seen as the scholarly type, and he is a gentleman who would never go to extremes to compete with anyone else. However, no matter how capable he is of running a business, he is never so hurried that he tries to simply cut through difficult problems. This is because he is, after all, a scholar more than anything else." (5-12).

Masuda: "I first met Takamine in 1886, and I thought he was a splendid person. I felt like I had known him for 10 years, and we soon became such good friends it was almost as if we were related. I knew a little bit about chemistry, and the first time Takamine came and I heard him talk about various things, I thought that he was a credible scholar and I had to help him in a big way to realize his dream. That's why I agreed immediately to the artificial fertilizers. I dare say it was I, Masuda, who brought him out into the world." (5-13).

There were two more important encounters as a result of the exposition. One of these was the meeting between Patrick Lafcadio Hearn, a journalist covering the exposition, and Ichizo Hattori (5-11). The two became close friends. When Hearn later visited Japan, Hattori, then a bureaucrat at the Ministry of Education, intervened to help him find work as an English teacher at Matsue Middle School. Hearn married Setsu Koizumi while he was in Matsue, and became a naturalized Japanese. He adopted the name Yakumo Koizumi, and made a name for himself as a fiction writer. He was buried in Zoushigaya Cemetery in Tokyo, side-by-side with his wife Setsuko.

The other meeting was when Takamine provided the head of the display judging

committee, Ferdinand Lascar, with a sample of diastase that he himself had prepared and brought with him. Lascar reported in an academic journal that he was surprised by the extraordinarily high activity of this substance (5-14, 5-15). This shows that at this time Takamine already had the idea of applying diastase to alcohol fermentation and digestive enzymes. Lascar wrote that the sample he received from Takamine was an "extract of malt," but judging by the strength of its activity it was very likely to have been diastase prepared from *Nihon kojikabi*, a strain of fungus which will be discussed below.

After various twists and turns, Takamine was able to establish the technique for applying the microorganism used in brewing Japanese sake, *Nihon kojikabi* (*Aspergillus flavus* var. *oryzae*), to the manufacture of whiskey. This eventually developed into Takamine's first discovery, TAKA=DIASTASE, which he was able to build into an international business with the business acumen he had inherited from his mother's home. Takamine's mother, Yukiko, was the daughter of the Tsuda family, who were sake brewers from Takaoka (present-day Toyama Prefecture along the Japan Sea coast).

The site where *Nihon kojikabi* was grown on the rice grains used to brew sake had been an excellent place for the young Jokichi to play [Figure 5-4]. From the close proximity of such activities, he was more than familiar with the color and flavor of the microbes, the aroma of sake during fermentation, and the complicated steps of the brewing process.

Takamine eventually devised a way to prepare a powder of dried spores of *Nihon kojikabi* microbes so that they could be stored. He packed this powder into a travelling bag along with the results of experiments in the whiskey manufacturing process, and, accompanied by Kosuke Fujiki, a specialist in brewing techniques, and his wife and two sons, left for the new land of the United States in November 1890.

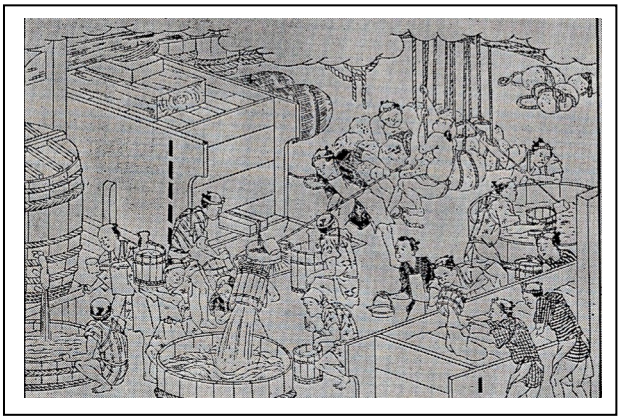


Figure 5-4. A late 18th century sake brewery in Japan. Robert William Atkinson: *The Chemistry of Saké-brewing*, 1881, Tôkiô: University of Tôkiô.

Fujiki was given the job of assistant to Takamine on the recommendation of Mitsuzo Hida, whom we met earlier as Takamine's junior at *Osaka-kaiseijo Rigakusho* and at the

government's Ministry of Agriculture and Commerce.

Simply explained, whiskey is made by using the power of enzymes to change starch into soluble sugars inside a tank, and these saccharides are then converted to ethanol by the action of yeast. In the traditional method, the enzyme used for saccharification is a diastase extracted from malt; the preparation of malt required considerable time and effort and yet the diastase thus prepared was not very powerful, so this method was inefficient. Takamine developed a groundbreaking new brewing method using a diastase that contained amylase [Note 5-1], an extremely potent enzyme that could be collected from *Nihon kojikabi* in a very short time (5-16).

Note 5-1.
A diastase is a group of enzymes which catalyses the breakdown of starch into soluble sugars. Amylase is a group of enzymes that catalyze the hydrolysis of **α**-1→4 glucosidic linkages of polysaccharides such as glycogen, starch, or their degradation products.

Takamine first took up residence in Chicago, and the following year, 1891, he moved to Peoria, halfway between Chicago and St. Louis. There he established the Takamine Ferment Co., with the aim of perfecting his brewing technique with diastase. In 1892, his business was progressing well enough for him to build a pilot plant.

However, Takamine's good fortune was not to last. Faced with the impending huge success of his plan, the local malt manufacturers saw their jobs threatened and they mounted a furious opposition that was little short of intimidation. In the spring of 1893, Takamine's pilot plant was destroyed by a suspicious fire. Seeing the wreckage of the burned-out factory before his eyes, Takamine stood rooted to the spot, unable to hold back his tears. To make matters worse, he was visited by further ill fortune when he was hospitalized for a chronic liver ailment. His life plan had hit a major setback.

3. Fighting back with a new idea

However, Takamine was not one to give up easily. During the period when his prospects in the whiskey industry were bleak, the way forward seemed to have been blocked off, yet he still devoted himself to research. Even on his sickbed, he thrashed out a new idea: the powerful enzymes of *Nihon kojikabi* were highly effective in the saccharification tank, so they should also work in the stomach, the body's internal tank. He reasoned that the enzymes would surely digest the starch in barley, wheat, or corn that had been eaten.

Having polished his idea, he made a fresh start by perfecting a method for the industrial production of the digestive medicine TAKA=DIASTASE (5-16, 5-17). In all likelihood, he

recalled how he had given Ferdinand Lascar a sample of powdered diastase at the exposition in New Orleans nine years earlier, and how Lascar had been enthusiastic in his praise for the extraordinary activity of the powder (5-7).

Takamine later recalled that the concept for industrial production of the digestive medicine first came about in early 1892, and we can assume that it took shape around the time that he called Tetsukichi Shimizu [Figure 5-5], his junior from the Imperial College of Engineering, to come to Peoria from Tokyo. Shimizu was working in the Japanese government's Ministry of Agriculture and Commerce when he received the invitation from Takamine in 1892. Aged 28, he resigned from his job and traveled to the United States, where he joined the research at the laboratory in Peoria that was aiming to develop an industrial process for making whiskey. However, it was shortly after this that the project was thrown into disarray by the fire that destroyed the pilot plant. For a short while, Shimizu found himself deprived of his goal.

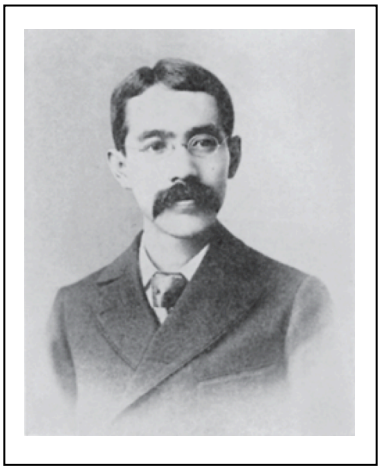


Figure 5-5. Tetsukichi Shimizu, Takamine's junior at the Imperial College of Engineering, who traveled to the United States to act as his assistant (5-18).

In the project to develop the technique for producing whiskey with *Nihon kojikabi*, Takamine had been entirely reliant on the professional sake brewer Kosuke Fujiki. They had resolved to develop the technique and had traveled to the United States together. However, after that dream was shattered and Takamine abruptly changed course and set his sights on the development of the digestive medicine TAKA=DIASTASE, the most important person was his trusted friend Shimizu, who had studied the same applied chemistry at the same university.

In 1902, after Takamine had achieved success in the United States and returned in triumph to his home country, he was invited to give a lecture to the Japan Federation of Engineering Societies, of which Shimizu had also been a member. In his lecture, he courteously and publicly expressed his gratitude to Shimizu, saying that much of the credit for the success of TAKA=DIASTASE went to Shimizu's hard work (5-17).

Sadly, however, Shimizu never returned to his home country. He contracted

tuberculosis—whether this was because he was so engrossed in his work he was unable to relax, or because he took on too much work when Takamine was hospitalized, we will never know—and he died in Chicago in 1896 at the age of only 34. His remains were buried there.

Not far from where Takamine was working, there was a man taking a particular interest in the TAKA=DIASTASE digestive medicine. This was George Davis, one of the proprietors of Parke, Davis & Co., a pharmaceutical company. In the letter shown below [Figure 5-6], which was sent to Takamine just before Christmas of 1893 by J. B. Russel, a department manager at Parke, Davis & Co., we can see that the company had confirmed the uncommonly high enzymatic activity of the powder and was very keen to take charge of its commercialization.

Parke, Davis & Co.

Detroit, Mich., December 22'1893
Jokichi Takamine, Esq. 2111 N. Jefferson Ave. Peoria, Ill.

Dear Sir;

We have been delayed in conveying our final report with regard to the Takamine Diastase, owing to the writer's absence from his desk.

Tests made subsequent to these already reported fully convince us that the product is possessed of unusual strength, and if it can be produced in the dry form at a reasonable cost, it is our impression that we could create for it a large scale. Certain it is that our reputation and facilities for reaching the medical profession are such that we could introduce it more quickly and perhaps more successfully, than any other house in this country, and we therefore hope that you will decide to make us terms which will warrant our accepting them.

We shall be very glad to have you call upon us here in Detroit, or if necessary, the writer will meet you in Chicago.

Very truly yours.

(signed) Parke, Davis & Co.
J. B. Russel

Fig. 5-6. A letter from Parke, Davis & Co. (Courtesy of Yutaka Yamamoto)

Takamine had most likely sent samples to a number of possible companies and was considering the results of their evaluations. Receiving a reply like this from a trustworthy company such as Parke, Davis & Co. must have given Takamine confidence in his product. On February 23 of the following year (1894) he applied for a US patent for TAKA=DIASTASE and obtained the patent rights on September 11 (5-19).

In Brief 5-1. Parke, Davis & Co., the leader of the pharmaceutical industry (5-20).

1874: Catalog listed 254 types of fluid extracts, 300 types of sugar coated pills, 74 solid extracts, 53 concentrations, 46 medicinal elixirs, 23 medicinal syrups, 15 medicinal wines, 8 alkaloids, and chloroform.
1879: A process for standardization by chemical assay was developed.
1886: Initiation of the practice of using lot numbers on the labels of all products. This was made obligatory by the United States government 76 years later, in 1962.
1890: Branch was established in London (extending sales into Europe).
1893: Introduction of desiccated thyroid gland as a treatment for glandular disorder.
1895: TAKA=DIASTASE goes on sale. On March 19, the first injections in the United States of diphtheria therapeutic serum made by Parke, Davis & Co. are given. Two years later, Parke, Davis & Co. market a therapeutic serum for streptococcus and tetanus.
1897: Product quality control through bioassays begins (20 years later, 1,100th product tested).
(**1899:** Sankyo Shouten established in Yokohama, Japan.)
1913: Daughter company Sankyo Co., Ltd., Japan's first pharmaceutical manufacturing company founded in Tokyo. Jokichi Takamine appointed as the first company president.

The Detroit, MI research institute of Parke, Davis & Co. (1900). Inside are laboratories for performing bioassays and elementary analyses, and Takamine and Wooyenaka were probably frequent visitors for their collaborative research.

Davis was a very shrewd businessman, and he was in charge of all areas of the company except for the finances, which were the responsibility of the joint proprietor, Hervey Parke. Various letters sent to Takamine around 1894 have been preserved, and they show that Takamine sent out samples of TAKA=DIASTASE to different companies and also engaged a technical lawyer when he was looking for a company to buy the product. There are letters showing that the lawyer repeatedly urged Takamine to meet with Davis as soon as possible (5-21, 5-22).

Davis was a highly original individual, and he had been at the forefront of the American pharmaceutical industry since the early 1870s by building and developing novel system models in areas such as quality assurance and stock management (see In Brief 5-1, above).

Davis and Takamine met during a period of tremendous growth in the pharmaceutical industry, and Davis was greatly impressed. He saw Takamine's talent, and immediately signed a contract with him. The following year (1895) a digestive medicine in fine powder form went on sale under the trademark "TAKA=DIASTASE" [Figure 5-7]. This new product quickly gained popularity, and sales increased rapidly (5-20, 5-23).

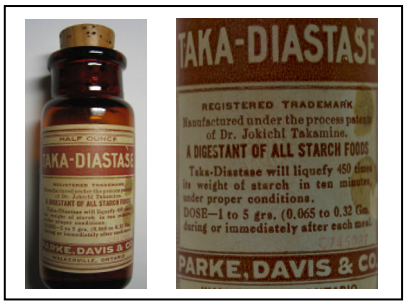
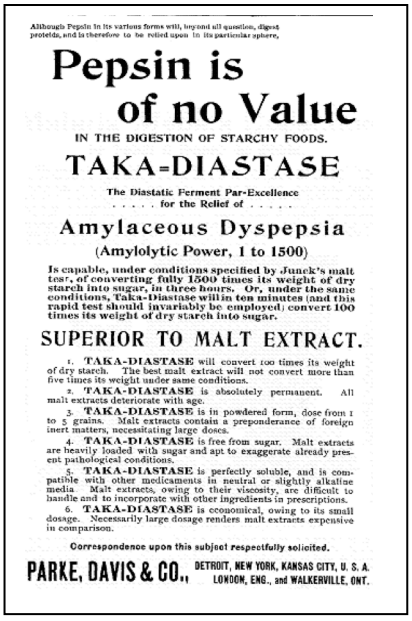


Figure 5-7. A half-ounce bottle of TAKA=DIASTASE, which went on sale in 1895. The label states, "Manufactured under the process patents of Dr. Jokichi Takamine," and the lot number, "C745087" is written to the upper right of the company name (Photo by Kouichi Inoguchi)

The digestive medicine TAKA=DIASTASE was indeed fortuitous for Takamine, but why did it sell so well? The answer can be seen in an advertisement. Figure 5-8 shows an advertisement for TAKA=DIASTASE that Parke, Davis & Co. posted in the *American Journal of Pharmacy*, an academic journal, in 1895 (5-23).



Although Pepsin in its various forms will, beyond all question, digest proteids, and is therefore to be relied upon in its particular sphere,

Pepsin is of no Value

IN THE DIGESTION OF STARCHY FOODS.

TAKA-DIASTASE

The Diastatic Ferment Par-Excellence
. . . . . for the Relief of . . . . .

Amylaceous Dyspepsia

(Amylolytic Power, 1 to 1500)

Is capable, under conditions specified by Junck's malt test, of converting fully 1500 times its weight of dry starch into sugar, in three hours. Or, under the same conditions, Taka-Diastase will in ten minutes (and this rapid test should invariably be employed) convert 100 times its weight of dry starch into sugar.

SUPERIOR TO MALT EXTRACT.

1. **TAKA-DIASTASE** will convert 100 times its weight of dry starch. The best malt extract will not convert more than five times its weight under same conditions.
2. **TAKA-DIASTASE** is absolutely permanent. All malt extracts deteriorate with age.
3. **TAKA-DIASTASE** is in powdered form, dose from 1 to 5 grains. Malt extracts contain a preponderance of foreign inert matters, necessitating large doses.
4. **TAKA-DIASTASE** is free from sugar. Malt extracts are heavily loaded with sugar and apt to exaggerate already present pathological conditions.
5. **TAKA-DIASTASE** is perfectly soluble, and is compatible with other medicaments in neutral or slightly alkaline media. Malt extracts, owing to their viscosity, are difficult to handle and to incorporate with other ingredients in prescriptions.
6. **TAKA-DIASTASE** is economical, owing to its small dosage. Necessarily large dosage renders malt extracts expensive in comparison.

Correspondence upon this subject respectfully solicited.

PARKE, DAVIS & CO., DETROIT, NEW YORK, KANSAS CITY, U. S. A. LONDON, ENG., and WALKERVILLE, ONT.

Figure 5-8. An advertisement for TAKA=DIATASE that appeared in the advertisement supplement of the *American Journal of Pharmacy* (1895). (5-23)

Reading the benefits and merits of this medicine, we can imagine that doctors confidently prescribed it for patients with stomach problems. The "starch-digesting diastase" that had been marketed up until then was manufactured through extraction from malt with water and concentrating it. However, the constituents extracted from malt with water are

overwhelmingly sugars and other carbohydrates, which form a starch syrup when concentrated. Even if this product is separated into smaller quantities at the factory, measuring it out and packaging it is difficult and time-consuming for doctors when they prescribe it to patients. Also, as it cannot readily be mixed with other medicines, administration of this product is very inconvenient for doctors.

Compared to this, the powder medicine TAKA=DIASTASE was not only stable, but it was ground-breaking in the way it could be separated into smaller quantities and mixed with other medicines. It is easy to see why not only manufacturing plants but also doctors and patients welcomed this new product, and TAKA=DIASTASE was even more successful than expected.

Back in Japan, Jokichi Takamine's homeland, this medicine caught the eye of a young businessman. He was Matasaku Shiobara (1877-1955), who ran a silk trading company. Shiobara had heard about the good reputation TAKA=DIASTASE enjoyed in the United States from a friend, Shotaro Nishimura (1864–1945), and after gaining the trust of Takamine through the intermediation of the Japanese consul in Chicago, Tatsugoro Nose (1857–1911), Shiobara successfully acquired a license to import and sell TAKA=DIASTASE in Japan.

As a result of meeting Takamine, Shiobara was able to expand his business by establishing Sankyo Co., Ltd. in 1913; he asked Takamine to take the post of president and carry out research and development of new medicines while still in the United States, and he himself concentrated on running the business in Japan. This was Japan's first pharmaceutical manufacturing company.

For a long time, the catalogue of Parke, Davis & Co. had eighteen different TAKA=DIASTASE compound drugs in a wide variety of formulations, which included combinations of TAKA=DIASTASE with pepsin and strychnine (5-24). Without the benefit of a stable, fine powder that could be mixed with other medications, this would have been unimaginable.

Takamine subsequently worked to improve the fungus strains and the culture conditions in order to obtain even greater activity. Wooyenaka, who was employed by Takamine in 1900, started work straight away with research into TAKA=DIASTASE. He later recalled that although he interrupted this research to work on the crystallization of adrenaline, once that was finished he once again devoted himself to screening for higher TAKA=DIASTASE production strains over a long period, and in 1907 he established an excellent patent.

The key that led to the technical success of TAKA= DIASTASE was the use of the branny

parts of grain for the cultivation of *Nihon kojikabi*—this was the same as when Takamine was producing whisky.

In the patent description, he explains the advantage of using these parts (5-19) as follows: "They not only are materials practically and economically suited for the purpose of my invention, but also they have the following merits, viz: First. Being of a loose and coarse nature, they afford a large surface for the growth of the fungus, and a ready access of air, one of the necessary conditions to its growth. Second. Being rich in albuminoids and phosphates, they supply the most necessary ingredients for the production of the enzyme. Third. They contain a large percentage of woody fiber, which renders their use of special advantage in the process of extraction as described below. Fourth. They are cheap and abundant, and in constant supply at all seasons of the year."

This shows Takamine's superb powers of observation, which bordered on divine revelation.

After cultivation, the grains were air-dried and the diastase was then extracted with water or water containing alcohol. The diastase was concentrated and settled with alcohol, and after drying was made into a powder. This principle of the extraction is the same method that was used by the French scientist Anselme Payen (1795–1878) and his coworkers when they discovered diastase in 1833 (5-25).

The idea of producing TAKA=DIASTASE in this way represents the beginning of biotechnology, in which microbes are used industrially to produce useful chemicals. This concept subsequently developed through its application to various different fields. In recent years especially, it has been used in genetic engineering, and has shown rapid growth as an indispensable technique in a wide range of fields, such as the economical production of insulin and other medicines, and amino acids such as *l*-glutamic acid. For this reason, Jokichi Takamine is now seen as "the Father of Modern Biotechnology" in the United States.

4. The meeting with Davis

As Takamine approached the milestone of his fortieth birthday, fortune seemed to be on his side. He was lucky to be given the chance to work on the challenging problem of the "isolation of adrenaline," which had continued for more than 40 years, and we can guess that this chance came from the corporate culture of Parke, Davis & Co., which had been built up by Davis, a man brimming with curiosity. Takamine's meeting with Davis was a fateful

encounter, and is worth looking at in a little detail.

On October 26, 1866, Dr. Samuel Duffield, a pharmacy manager, jointly established a pharmaceutical company on the northeast shore (*le Côté du Nord-Est*) of Detroit with Hervey Parke, the 38-year-old manager of a mining and steel company.

The following year, 1867, an ambitious young salesman, George Davis, joined the company at the age of only 22, and business started to take off. Duffield later retired due to poor health, and Parke took charge of the company's finances while Davis was responsible for all the other areas, which included research, development, manufacturing, and sales.

This marked the start of Parke, Davis & Co, which linked the two men's names. In both his business and his private life, Davis was somewhat unconventional. He was born into a noted Detroit family, and after graduating from high school he elected to go into business rather than continuing his education to university. By the time he joined Parke, Davis & Co., he had already established himself as an outstanding salesman. In their dispositions, Parke and Davis were completely different— Parke was taciturn and had an air of authority behind his luxuriant white beard, while the young Davis was more showy and overflowing with ideas for expanding the business.

The company showed unprecedented growth under the leadership of these two men, but they always stuck to the motto that Duffield had originally adopted: *Medicamenta vera* (Pure medicine) [Note 5-2]. The first project Davis worked on was the collection and commercialization of *Cephaelis ipecacuanha*, a plant native to South America that is effective as a therapeutic remedy for amoebic dysentery, for preventing vomiting, and as an expectorant. He had to overcome a great many problems, but he was successful and he boosted the company's reputation through the development of a new product.

Note 5-2.
Albert B. Lyons (1841–1926) was a technician who put in place a system for Parke, Davis & Co. to conduct quality control through chemical analysis. He subsequently established the Scientific Division of the United States Pharmaceutical Manufacturers Association (5-26). Parke, Davis & Co. started to market the world's first standard solutions that were guaranteed by chemical analysis in 1883.

His subsequent business development was extremely creative, and it is no exaggeration to say that he largely put in place the structure of the modern pharmaceutical industry. For example, he published a bidirectional research journal as a means of ensuring collaboration with doctors and pharmacists and dissemination of accurate information on drug efficacy and side effects. The company's policy of putting a lot number on all its products to ensure quality even after sales was ground-breaking at that time (5-20). I own an old 1/2-ounce brown TAKA= DIASTASE bottle, on the label of which is printed the lot number "C745087" [Figure

5-7].

At the time when the company was steadily developing under Davis' outstanding leadership, he had an interview with Takamine. Davis was greatly impressed and he could see Takamine's abilities, so he immediately signed a contract with Takamine as a consultant. Judging by a letter that has been preserved from the contract agent George Whitney to Takamine dated November 5, 1894, this must have been around late fall of 1894. About the same time, the United States became caught up in the panic of 1893, an economic depression that had its epicenter in the United Kingdom. The collapse of an American railroad company was the trigger for the panic in the United States, and by the following year the unemployment rate had surged to between 12.3% and 18.4% and society was in turmoil. It was a similar situation to the panic brought on by the Lehman shock due to subprime loans at the start of the 21st century, which is still a recent memory.

Davis had invested heavily in land in California, and the losses were so huge that his income of Parke, Davis & Co. was insufficient to cover them. He became bankrupt in November 1896, and was forced to resign from the company [Note 5-3].

Unfortunately, because of Davis' unexpected resignation as a result of his bankruptcy, Takamine and Davis only had close dealings for about three years or so.

Note 5-3.
Davis was a lifelong bachelor, but there were incessant rumors linking him to glamorous women. At the height of his success, he lived in a fabulous mansion and owned 500 acres of farmland, where he kept racehorses. He had a magnificent yacht on Lake Saint Clair and lived an extravagant lifestyle, and there was no one in Detroit who did not know his name.

He was a great admirer of Napoleon, and had an extensive collection of items that had belonged to him. He also collected first editions of books, and by 1886 he had a library of over 5,000 volumes. So great was his fall that when he died in 1930 at the age of 85, it is said that only a handful of people attended his funeral. It was indeed a life of extremes (5-20).

However, before Takamine's assistant Tetsukichi Shimizu died in Chicago at an early age, he was able to savor the excitement surrounding the launch of TAKA=DIASTASE in 1895 thanks to Davis' decisive action. This must have been a great comfort for Shimizu, who had put his heart and soul into the development of a technique for industrial production of the digestive medicine TAKA=DIASTASE. For Takamine as well, the commercialization of the product within just one year of the patent application was enormously good fortune that gave him sufficient time and income from royalties to set up his base in New York.

5. The participation of Keizo Wooyenaka

Takamine would never have been able to achieve his success in the United States without the collaboration of Parke, Davis & Co. I have devoted considerable space to TAKA= DIASTASE, which was the world's first biotechnology product, because although it was a digestive enzyme with no direct relation to hormones, it was the first strong link between Takamine and the company. Another key to Takamine's success was the participation of Wooyenaka. This could be seen as a predestined encounter between two people brought together by history, but a closer look reveals that it was a link between scientists that you might call the destiny of wisdom.

Table 5-1. Board members of the Tokyo Chemical Society

Year	Chairman	Permanent board members	Secretary, clerk	Notes
1887	Nagai	—	Shimizu and nine others	—
1888	Nagai	Takamine and four others	Shimizu and three others	Society constitution decided
1889	Nagai	Takamine and four others	Shimizu and three others	—
1890	Nagai	Takamine and four others	Shimizu and three others	Takamine leaves for the United States
1891	Nagai	Five people	Shimizu and three others	—
1892	Matsui	Tamemasa Haga and four others	Four people	Shimizu leaves for the United States
1893	Takamatsu	Tamemasa Haga and four others	Four people	—

[This table is prepared from Kozo Hirota, *Meiji no Kagakusha* (in Japanese), Tokyo Kagaku Dojin, Tokyo (1988)].

Please look at Table 5-1, showing the Board members of the Tokyo Chemical Society during the early days of its establishment. This shows the relationship between Nagayoshi Nagai, Jokichi Takamine, Tamemasa Haga, and Tetsukichi Shimizu. First, let us start by looking at Wooyenaka's teachers, Nagayoshi Nagai and Jokichi Takamine. Table 5-1 shows that these two scientists were companions that helped develop the Tokyo Chemical Society, Japan's first academic society in the field of chemistry, which came into being in 1878. Takamine was a permanent member of the board of the society until he left for the United States.

We have already looked at Tetsukichi Shimizu during the period of development of the TAKA=DIASTASE digestive medicine, and this table shows that as secretary of the society

he would have been in contact with Nagai, who was chairman of the society, for five consecutive years.

Shimizu undertook the important task of drawing up the Tokyo Chemical Society constitution in 1888 in collaboration with Jintaro Takayama and Iwata Nakazawa, which tells us that he was an indispensable figure for the society.

Wooyenaka and Shimizu did not cross paths, as Wooyenaka joined a non-regular course at the Pharmaceutical Department of Tokyo Imperial University in 1893, the year after Shimizu went to the United States. However, Wooyenaka had a big problem in that had he been a student on a regular course, he would have had free access to all the documents in the library, but the discriminatory treatment of non-regular course students meant that they were not allowed to take books from the bookshelves at will.

After graduating from the non-regular course, Wooyenaka worked on the isolation of natural active substances as an assistant to Professor Dr. Nagai, and during that period he would undoubtedly have heard about Shimizu from his mentor.

It was probably also a good opportunity for Wooyenaka to become familiar with Takamine's outstanding activities in the United States. Wooyenaka would have heard from Nagai about Takamine's success in launching "TAKA=DIASTASE" in 1895, shortly before Shimizu fell ill. He must have been excited to learn that Shimizu's work had been instrumental in this success.

The third person to appear in the table above is Professor Tamemasa Haga of the Faculty of Science of the Tokyo Imperial University. Haga was Takamine's junior by two years at the Department of Chemistry of Imperial College of Engineering, and thus two years senior to Shimizu.

Both Takamine and Haga were students of Edward Divers, a foreign chemistry teacher at the Imperial College of Engineering (later incorporated into the Tokyo Imperial University) brought in to assist Japan on its path to modernization at that time. Haga later had studied at the University of Kiel in Germany and returned to Japan in 1898, and he took over the inorganic chemistry course taught by Professor Divers. Haga's lectures were somewhat difficult to understand, but he took great care of his students (5-27). He would most likely have been close to Nagai through the Tokyo Chemical Society, and he wrote a letter to introduce Wooyenaka to Takamine.

In the spring of 1899, at the age of 23, Wooyenaka left his job as an assistant at Prof. Nagai's laboratory. After studying English he left for the United States at the end of 1899, carrying the letter of introduction from Haga. He arrived at Takamine's laboratory in New

York in the beginning of February 1900 [Figure 5-9].

Figure 5-9. The site of the Takamine Laboratory in a basement in New York. The windows near street level were probably the windows of the laboratory (5-28).

Wooyenaka was taken on by Takamine in February, and the first job he was given was to cultivate *Nihon kojikabi*. This had been the job of the previous assistant, a student of Columbia University called Yoneda who had helped with the research into diastase before leaving for France. Wooyenaka next helped with the job of a researcher called Matsuo, who was in charge of developing a fire retardant using ammonium phosphate. For his third job, he was directed toward research into the active principle of the suprarenal glands. Wooyenaka did not complain about being given jobs that were not his area of specialty as it was an age in which you did whatever was needed in order to earn a living (5-29).

6. The diligent preparations of Parke, Davis & Co.

Takamine and Wooyenaka would probably never have achieved their success were it not for the scrupulous research system that Parke, Davis & Co. had built. For 44 years, researchers at numerous institutions and laboratories in Europe and the United States had failed in their attempts to isolate the active principles of the adrenal glands; the one thing they all lacked was a systematic approach.

One thing about the successful team that is worth noting is that in 1894, Davis, one of the proprietors of Parke, Davis & Co., scouted two medical scientists from the University of Michigan, Elijah M. Houghton (See page 78) and Charles McClintock, to establish the first biological laboratory in the United States for the company's launch of a diphtheria therapeutic serum. It is likely that without this, the success of adrenaline isolation would never have come about. In an age without chromatography or spectroscopic analysis, research into endocrine substances required highly accurate, rapid activity tests to be carried out in large numbers. We have already seen the importance of this in Chapter 4, and the rivals in the race to isolate the adrenal principle did not have this capability. The isolation of natural active substances was a task that was beyond chemists working on their own.

The two medical scientists from Michigan were able to finish the job of developing the diphtheria therapeutic serum within just a few months. After this, only Houghton remained with the company, and he became head of the research department while at the same time taking teaching jobs at Detroit Medical College and the University of Michigan.

Parke, Davis & Co. turned its attention to putting together an adrenaline project team. The team was headed by Houghton, and Thomas B. Aldrich, the assistant to Prof. Abel, who was the researcher at the forefront of the world of chemical research into the active principle of the adrenal gland, was scouted to be an advisor. Two laboratories, one for activity tests and one for elemental analysis, were set up, and the company signed an agreement with Takamine.

Aldrich had completed his doctor's degree in Germany and then returned straight away to the United States, and the following year (1893), as soon as Abel had been welcomed by the newly established Johns Hopkins University Pharmaceutical Department as a professor, Aldrich became Abel's assistant. He was a solid organic chemist, working diligently on research for five years. Consequently, when Wooyenaka arrived from Japan, he was able to commence research with everything more or less in place. As we saw in Chapter 1, as Wooyenaka carried out his experiments, he sent the crystals that he obtained to Parke, Davis & Co. and he received the results of the activity tests without delay so that he could use them for planning the next experiments.

In his later years, Wooyenaka remembered the elementary analysis device in the chemical laboratory of Parke, Davis & Co. that he used: "The laboratory combustion furnace that I was using on loan for elementary analysis was faulty, and the amount of hydrogen after combustion was too high. I got the result $C_{10}H_{15}NO_3$, whereas the sample analyzed in the same laboratory by the German chemist Aldrich gave the correct result of $C_9H_{13}NO_3$" (5-30) [Note 5-4].

> **Note 5-4.**
> According to the *American Webster's Biographical Dictionary* (5-31), Aldrich was American, born in Port Jefferson, NY. Apparently, Wooyenaka mistakenly thought he was German because he had obtained his PhD at Jena University, Germany, in 1892.

At the time, the only available method for analyzing the constituent elements of a molecule was a destructive analytical method, and this method required large quantities of samples. At the end of one paper, Aldrich wrote, "Samples of sufficient quantity have already been obtained." This was because at that time, this analytical method was the only way to determine the composition of a substance (5-32).

7. Takamine's continuous advertising

In Chapter 4, we saw in detail how Takamine embarked on a vigorous program of publicity at academic conferences from the year following the successful crystallization of adrenaline, but following this his publicity activities were more attuned to the business side of science. That was an age without the abundant means of rapid information dissemination we enjoy today, and the most effective way for researchers to communicate the results of their research was for them to go out into the world personally and move from place to place, announcing the results. As this research was carried out under a contract with Parke, Davis & Co., Takamine put Wooyenaka in charge of developing a manufacturing method for pure adrenaline that would not run counter to the company policy of "*Medicamenta vera*," while he was in charge of communicating their achievements to the world.

During these publicity activities, Takamine must certainly have felt very happy to give an invited lecture at the Meeting of Medical Men, which was held at the School of Medicine in Edinburgh, Scotland, on December 3, 1901 (5-33).

Twenty-one years earlier, he started his three-year period as a student abroad in Glasgow, one of the centers of the Industrial Revolution, which was not far from Edinburgh. Having achieved research results that excited the scientific world, he was now making a triumphant return to his adopted home.

On March 20, earlier that same year, Thomas Maben had given a lecture at a pharmaceutical society meeting in Edinburgh (5-34) with a very suitable explanation and presentation of Takamine's announcement from New York the previous year (5-35), so we can imagine that there was an enthusiastic reception when Takamine appeared on the stage.

The following year, Takamine returned in glory to Japan, his native country, where he gave lectures in different parts of the country. The first was a public lecture at the Mitsui Assembly Hall in Hibiya, Tokyo, on February 27, 1902. This was very likely at the request of Takashi Masuda of Mitsui. In his lecture, Takamine looked back over the many hardships along the way to his success with TAKA=DIASTASE and adrenaline, recounting his memories with passion. He ended by expressing his thanks to his assistants, Tetsukichi Shimizu and Keizo Wooyenaka, for their achievements (5-36).

Takamine next spoke at the annual meeting of the Osaka Medical Society in April (5-37), and the annual meeting of the Engineering Society in Tokyo in September (5-17). At these lectures as well, he announced in detail the research processes of TAKA=DIASTASE and adrenaline. That same year, he published a report with more or less the same content in the

journal of the Tokyo Chemical Society, which he himself had nurtured (5-38).

In his lecture in Osaka, Takamine presented the mistaken formula for the adrenaline molecule. Later, in his doctoral thesis for his Dr. of Pharmacy, in September 1906, seven years after he was awarded his Doctorate of Engineering, he presented his mistaken C_{10} molecular formula along with Aldrich's correct formula (C_9) and the formulae put forward by von Fürth and Abel. He stated that in the future, it would be shown which of these four formulae was correct. As far as he was concerned, as long as the molecule showed high activity, the exact molecular formula was secondary and was not a particularly big problem. This seems typical of Takamine, the "practical scientist."

While on the topic of practical science, the following passage from the end of Takamine's paper in the *American Journal of Pharmacy* of November 1901 mentioned earlier (5-39) clearly shows that "chemical industry" always occupied his mind: "There are several useful applications of adrenalin in arts and industry; for instance, a developer of photographic plates, as a reducing agent in chemical analysis, art of dyeing, etc."

Medical scientists and physiologists were probably dumfounded at the thinking of this Japanese scientist that an endocrine substance secreted in minute quantities from a small internal organ could be provided to the chemical industry. However, Takamine was not one to be constrained by the narrow preconceptions to which specialists are prone, and the following research, which emerged a short while later, is evidence that this was his way of thinking.

In 1907, five years after his lectures in Japan, two British scientists published a report in the *Pharmaceutical Journal* of joint research into a method to detect the iron content of commercial products containing oleic acid, an unsaturated fatty acid. Oleic acid was produced in huge quantities at that time, usually as an ingredient of soap, but it was also listed in the Pharmacopoeia as a solvent for use in medicines. An analysis method was needed because of the restrictions on adulteration with iron, which mainly came from the iron vessels used in production.

Readers will remember Vulpian's color reaction, introduced in Chapter 3, in which adrenaline reacted with ferric chloride presents a characteristic sea-green color. At that time there were no satisfactory analysis methods, so it hardly needs saying how useful a reaction giving a specific color with just a small quantity was. These scientists were proposing a method that could be carried out straight away, anywhere and by anyone, using only the naked eye as the analyzer—they had come up with the idea to use adrenaline to control the product quality of medicinal oleic acid to meet the specifications of the Pharmacopoeia (5-40).

Perhaps Vulpian would have smiled at Takamine's abundant creativity and the unexpected use of his own discovery.

8. Ensuring stable product quality

There is one important thing that is not mentioned in detail in the many papers and essays about Jokichi Takamine and adrenaline. This is the history relating to making adrenaline commercially available as a medicinal product that could be used with confidence by doctors after it had been extracted from animal organs and purified, which was extremely important from the point of view of society in general.

As we saw earlier, before Takamine and Wooyenaka succeeded in crystallizing adrenaline, the American doctor Solis-Cohen used himself as an experimental animal to examine the effects on hay fever of the tablet "Supra-renal Tabloid," which was manufactured by the London company Burroughs, Wellcome & Co. and sold worldwide. However, he recorded that one lot of the tabloids had deteriorated in quality (5-41). There were also probably times when this passed unnoticed, and we can assume that cases like this may have been common.

Apparently, a problem with liquid medicines containing adrenaline that were available on the general market was that discoloration became visible over time. In 1908, the detailed results of a study of discoloration of liquid medicines were published in the United Kingdom. This study used the term "makers" in the plural, so it is likely that products from more than one company were investigated [Note 5-5].

In his later life, Wooyenaka said that for four or five years after the success of crystallization, he was busy with the task of completing the product. He was in charge of the field of technical development for mass production, and he seems to have devoted himself wholeheartedly to this job. TAKA=DIASTASE has a very wide range of permissible doses, and even today it is available over the counter. Adrenaline, on the other hand, is a very active substance that requires extremely strict control, and only doctors are allowed to prescribe it.

Note 5-5.
If we look at just the section headings of the 1908 report, we can see that the study covered considerable ground: "Action of Alkali," "Atmospheric Oxidation," "Influence of Light," "Influence of Iron," "Possible Cause of the Colouration," and "Physiological Activity of Coloured Solution." The study concluded that discolored products showed marked reduction in their effectiveness (5-42).

The authors of this study, Alex Gunn and E.F. Harrison, reported the development of simple qualitative confirmation tests for adrenaline the previous year, in which they state that they used a product manufactured by Hoechst A. G. as well as a number of other products, with names such as Suprarenaline and Solutio Haemostasin Hydrochlor (5-43).

There were two extremely difficult problems that needed to be resolved in order to perfect an adrenaline product that doctors could use with confidence. The first of these was that the results of the activity tests of adrenaline were not always constant, but depended on the type and age of the animal from which the suprarenal glands had been taken, so that the adrenaline preparation needed to be adjusted accordingly. Wooyenaka must have eagerly continued to make great efforts to manufacture a formulation that would be reliable enough for use in medical settings even after the launch of the product.

The other problem was preventing decomposition of the formulation and breakdown of the active ingredient. The adrenaline solution that went on sale in 1901 was mixed with chloretone (chlorobutanol) as a preservative (5-44).

A stable product was achieved, and Parke, Davis & Co., which always aimed to live up to its motto "*Medicamenta vera*," did not change the compositional formulation of its extracted adrenaline even after the advent of synthetic adrenaline until 1975; this is testament to the confidence the company had in the techniques developed at the start by Wooyenaka and Aldrich (5-24).

Once these problems were overcome, the "Solution Adrenalin® Chloride" [Figure 5-10] with its stabilized product quality was launched, and soon every doctor always had a bottle in his bag. It was also an extremely attractive medicine for athletes; boxer James Joseph "Gene" Tunney, "the Fighting Marine" who defeated the great Jack Dempsey to become heavyweight champion in a legendary bout in 1926, apparently always had adrenaline in his hand when he stepped into the ring (5-45).

Figure 5-10. An advertisement for new products from Parke, Davis & Co. with a photo of "Solution Adrenalin® Chloride" (appeared at the end of *Therapeutic Notes*, **9**(4), 1902). Hay fever is shown as the complaint for which adrenaline may be used.

The wide use of adrenaline can be seen from the 1907 *Journal of the American Medical Association*, which gave a thorough explanation of the appropriate methods of use of adrenaline against a wide range of diseases, and the side effects that might occur (5-46).

We have already seen how being able to perform activity tests using experimental animals gave Takamine and Wooyenaka, and consequently Parke, Davis & Co., a huge advantage in

research into the isolation of adrenal active principles.

At least according to the literature and data available from that time, none of the research institutions in Europe or the United States that were involved in the quest for the adrenal active principles appear to have been equipped with laboratories that could quantitatively check the activity of a large number of extracts.

It has already been pointed out that there was no spectroscopic analysis or chromatography at this time, and the techniques for isolating organic compounds were largely undeveloped—it is easy to see how effective activity tests would have been a powerful weapon in the hunt for physiologically active substances.

However, investigating whether there was physiological activity was not especially difficult; the problem was that demonstrating this with statistically significant numerical values was a very high-level technique for that time. It called for workers with high technical capability and a great many homogenous test animals, such that the costs were very high. Parke, Davis & Co. stated this publicly.

This valuable research system was managed and directed by Dr. Houghton, with the understanding of the owner of the company, Davis. Houghton's reports in academic journals were written in great detail, even including the methods used for dealing with—in other words, the welfare of—dogs used in adrenaline activity tests after the tests were finished. As he reported the methods for preparing the standards for activity tests up until adrenaline was crystallized, it seems likely that he started the preparations for a special laboratory straight after Oliver and Schäfer's blood pressure-raising effect and Bates' hemostatic effect were announced, probably in 1897 (5-47).

In Japan, Sankyo Shouten (the forerunner of Sankyo Co., Ltd.) signed an exclusive distribution agreement with Takamine. The company imported "Solution Adrenalin® Chloride," "Adrenalin Ointment," and "Adrenalin Inhalant," and these first went on sale on May 10, 1902 (5-48) [Note 5-6].

Note 5-6.
Adrenalin Ointment was a preparation of 0.1% adrenaline chloride packaged in a container that allowed easy application to the nose, the urethra, and the external ear. Adrenalin Inhalant was a preparation of 0.1% adrenaline chloride dissolved in perfumed natural oil with 3% chloretone added, which was administered using a nebulizer inhaler (5-49).

9. An English translation of Vulpian's paper

Judging by their past histories, Takamine and Wooyenaka were both undoubtedly

proficient in English and German, but I imagine they might have struggled with French. When Parke, Davis & Co. signed the adrenaline research contract with Takamine, it is not unreasonable to suppose that the company would have delivered all the relevant scientific literature to him.

This is purely my own conjecture, but if that were the case, I would assume that the documents would have included an English translation of Vulpian's report, which was originally in French. Consequently, Takamine and Wooyenaka may perhaps have been fully aware not only of the experimental methods used by the previous researchers who had wrestled with adrenaline, but also of Vulpian's elaborate techniques.

Parke, Davis & Co. was established in Detroit, MI which was originally a town opened up by the Frenchman Antoine de la Mothe Cadillac for French people doing business with the indigenous Chippewa people. Detroit became well known as the center of the automotive industry, and the name Cadillac became synonymous around the world with luxury cars. The name Detroit is from the French le détroit, meaning "strait," and the reason for this is apparent if you look at a map.

Because of the history of the city, there must have been many employees at the research center who spoke French at home, so it would undoubtedly have been easy to find a technician to translate Vulpian's papers.

In an interview later in his life, Wooyenaka recounted, "Fifty years earlier, which would now be about 100 years ago [1856], Vulpian showed that there was something in the adrenal medulla that gave a green color with ferric chloride (5-30)." "As I said before, Vulpian had written everything down properly, so it was already clear. So really, if someone had followed Vulpian's experimental method, they would have been able to isolate adrenaline before me" (5-50).

Even if Wooyenaka was unable to read French, it is surely reasonable to suppose that he would have read an English translation, and would therefore have started his work fully aware that Vulpian's report was pivotal. If he had heard that Vulpian was seen in France as the "discoverer of adrenaline," he would have probably nodded in agreement.

10. Wooyenaka stays faithful to Nagai's teaching

Wooyenaka reflected on that time on July 21, 1900, when he observed crude crystals of adrenaline at the bottom of the test tube. "The very first thing I found was at the bottom of the test tube," he said. "Even so, I remembered what Prof. Nagai taught. Whatever he was

working with—alkaloids or anything else—instead of test tubes, he always used watch glasses for observing reactions. For example, he would line up watch glasses of about 10 cm in diameter on a piece of white paper, put a sample in each one, and then add the test reagent made up to different concentrations with his own self-made narrow tipped pipette, first one drop and then a tiny drop at a time. He could then judge by the color as the reagent sank into the sample what the most suitable amount of reagent to use was. This was an extremely effective method." (5-30).

I remember that when I first read this, I could not help feeling the difference between the young Japanese today and that of in Meiji era. Compared to today's youth, the young people in Meiji era were very patient. Wooyenaka, like his teacher Nagayoshi Nagai, was a typical example of that kind.

Reading Nagai's paper on ephedrine (5-51, 5-52, 5-53), it is clear that the task of purification requires a degree of repetition and perseverance that would be intolerable for most people. Wooyenaka saw this dedicated figure while studying the non-regular course at the Pharmaceutical Department of the Tokyo Imperial University, and was doubtlessly reminded of Nagai when he worked in the semi-basement laboratory in New York.

Figure 5-11. Former teacher of Wooyenaka: Nagayoshi Nagai, Head of Pharmaceutical Department, Tokyo Imperial University and his wife Therese（Courtesy of The Pharmaceutical Society of Japan）

However, Wooyenaka did not simply do everything just as Nagai [Figure 5-11] had taught him. In the interview in his later life, he said, "Unfortunately I don't have it now as I lent it to someone, but I bought a book in the United States around that time that shows an experimental method where you select crystals that have formed in the precipitate you get as I explained, and from the state of the crystals you can distinguish different types of alkaloid. Not many people used this method, but it seemed to work very well."

11. Should Wooyenaka be a co-author?

Many Japanese people have been unhappy that in all the academic papers on the crystallization of adrenaline, including the historic first report (5-35), the author is given only

as Jokichi Takamine, with Keizo Wooyenaka [Figure 5-12] not credited as a co-author. This is felt to be rather unfair.

Figure 5-12. Keizo Wooyenaka and his wife Yaeno, in 1905, shortly after they were married (5-54).

However, the people making this argument do not appear to have made any attempt to verify the accepted practice of scientific circles at that time. Let us look at how researchers in leading positions at that time decided whether to make coworkers or assistants co-authors.

Wooyenaka was perhaps closest to his former teacher, Dr. Nagayoshi Nagai, a professor of the Tokyo Imperial University. What did Nagai do with respect to co-authorship? Let us look at his research paper on the alkaloid "ephedrine," from the plant mahuang (*Ephedra sinica*), which brought him sudden, worldwide fame. His paper on ephedrine is a masterly work on the organic chemistry of natural products, comprising five reports totaling a considerable number of pages, but the authorship is credited to Nagai alone. In the introduction to the first report, Nagai writes, "Mototada Yamashina worked on the analysis of *mahuang* at Osaka Inspection Station and collected a type of alkaloid, but he died suddenly, leaving the extracted liquid with a minute quantity of needle crystals. I carried on the research with Yuzo Hori as assistant (5-51)."

Not even Yamashina, who had extracted the alkaloid as far as the crystallization stage, was credited as an author. Dr. Kinnosuke Miura of the Medical Department of Tokyo Imperial University received a sample of ephedrine from Dr. Nagai, and he used this to carry out pharmacological research. Miura reported the mydriasis effect in the German journal *Berliner Klinische Wochenschrift* (5-55), and he is credited as the sole author.

Next, let us look A.W. von Hofmann, Nagai's mentor at Berlin University. Hofmann had been enchanted by Italy, where his father took him on a trip at a young age, and he aspired to literature. However, he ended up becoming captivated by the lectures and experiments of Justus Freiherr von Liebig, the father of German organic chemistry, who was teaching the latest advances in chemistry at Hofmann's hometown of Gießen, and he went on to make a name for himself in organic chemistry. Over the course of 25 years, Hofmann published some 150 papers, of which only nine were credited with a co-author, while all the rest were

credited to Hofmann alone. In Germany at that time, students would sometimes be credited as co-authors, but they could not be authors once they had graduated and become salaried assistants, even if they played a substantial role in the research of a professor. So if someone was receiving a salary for carrying out your research, the common practice at that time was to regard that person simply as a worker (5-56). Looking next at Abel, his first paper on epinephrine credited Crawford as a co-author, but while some of his other papers give names of people who collaborated, none of these people are made co-authors.

My understanding is as follows. The extraction and purification of adrenaline was research carried out under a contract between Takamine and Parke, Davis & Co., and Takamine appointed Wooyenaka to the research after making arrangements for all the reference documents, the materials for the experiments, and the expenses. This was accepted academic practice for the time, and Keizo Wooyenaka, who had not published an academic report in this field, was an assistant.

At least Takamine gave Wooyenaka the title of "associate" in the first academic report (5-39), and this can be interpreted as Takamine showing his appreciation for Wooyenaka's exceptional achievements. Given the normal thinking of the time, it is not reasonable to criticize Takamine. It might perhaps have been extremely forward-thinking to make Wooyenaka a co-author, but from the point of view of the general etiquette among scientists of the time, it would probably have seemed inappropriate. In an interview when he had reached an advanced age, Wooyenaka himself looked back over the research: "This adrenal medulla hormone, adrenaline, was just a question of running into it by chance," he said. "I just happened to be the one that did the experiment at that time, and adrenaline was easy to obtain. Parke, Davis & Co. wanted to use the story for their publicity, so they exaggerated it a bit. It was rather as though we simply collected them!" he laughed (5-30). "I was very disappointed that my husband didn't receive a PhD in Japan," recalled Wooyenaka's wife, Yaeno, at the same interview. It was only natural she should feel that way after the merit of crystallizing adrenaline became ever more highly commended.

Wooyenaka himself would have probably felt the same way if he had finally found success after struggling with research into adrenaline extraction as an assistant for many years. But he had been a young man of 24 who had only arrived in the United States six months earlier, and to come across the crystals like that was actually something of an anticlimax. It was as though in a high hurdle race he had skipped over the hurdles with no run-up at all—only when looking back from the finishing line did he appreciate how high he had soared. In the same way, perhaps he only gained a true understanding of his achievement

as he grew older.

Takamine later shared with Wooyenaka the benefits he obtained from adrenaline and TAKA=DIASTASE. He arranged work at Sankyo Co., Ltd., the company to which he had granted exclusive sales rights in Japan for the ground-breaking new medicines, and gave these jobs to Wooyenaka, ensuring that the latter was well looked after until late in his life [Note 5-7].

Note 5-7.
An example of the jobs that Takamine arranged for Wooyenaka is "Bakelite." This was the discovery of Leo Henricus Arthur Baekeland, a close friend of Takamine's from the Chemical Society of New York. Baekeland was born in Gent, Belgium, and went to the United States where he successfully developed "Velox," the first photographic printing paper. He then created the world's first plastic by combining formalin and phenol, aptly named "Bakelite." Takamine was enormously interested in this, and Baekeland provided his friend with the technology, free of royalties, and authorized production in Japan by Sankyo Co., Ltd in 1911. Baekeland and Takamine had both worked to the utmost to realize their dreams in the unforgiving society of a new continent, a long way from their home countries, and thus must have felt a strong desire to support each other.

Wooyenaka returned to Japan in 1916, and devoted himself to the pharmaceuticals business at Sankyo Co., Ltd., respecting the wishes of Takamine, even after he had passed away, by continuing to play an active role there, which included a long spell in the United States in 1926 to work on problems with the technology for manufacturing Bakelite. Japan's first domestic plastic industry was taken over by Sumitomo Bakelite Co., Ltd., and has developed into a worldwide business.

With respect to the consideration that Takamine showed him, Wooyenaka felt throughout his life that he was fully rewarded for the contributions he had made [Figure 5-13].

Figure 5-13. Mr. and Mrs. Wooyenaka at the tomb of Jokichi Takamine in Aoyama Cemetery, Tokyo (5-57).

12. Applied and pure science

Science is like a two-wheeled cart, needing both logic and practical application to advance. The success of Takamine and Wooyenaka is clearly the latter.

Their main interest, and also that of Parke, Davis & Co., the company to which they were contracted, was purely in marketing the active principle they extracted as a therapeutic medicine. Their interest in the scientific principles of hormones was secondary—it was for this reason that they were so careless with the molecular formula of adrenaline.

Logic and practical application go hand-in-hand to make science evolve, but the people that aim for this all have different characters and different circumstances. This is shown by the example of the crystallization of adrenaline [Note 5-8].

Takamine took classes in pure scholarship at the Imperial College of Engineering, but for the rest of his life following his period of study in the United Kingdom, his work was based on practical applications. In an interview, Wooyenaka had this to say about Takamine: "Dr. Takamine had no contact at all with universities in the United States. When he was studying in the UK, he studied at the University of Glasgow, but then after that as a scientist he was involved in big business, pioneering a very wide range of work, such as the exposition and the chemical fertilizer company (5-30)."

Note 5-8.
There is a very interesting history in the relationship between applied science and pure science. The Japanese scientist Katsusaburo Yamagiwa (1863–1930) was interested in the irritation theory of Rudolf Virchow (1821–1902), whom Yamagiwa had studied under as a student at Humboldt University in Berlin. Yamagiwa successfully created the world's first induced cell carcinomas by continuous application of coal tar, and should have been awarded the Nobel Prize. After this historic discovery, Yamagiwa chose to work on treatments for cancer rather than clarifying the substances that caused it. The cancer specific substances in coal tar were found by the Briton E. L. Kennaway (1881–1959) and his co-workers. Kennaway's interest was practical: he wanted to control the onset of skin cancer in chimney sweeps, which was seen as an occupational disease. He first determined that the strength of the carcinogenicity of the substances stuck to the inside of the chimney depended upon the site in the chimney, and from samples with high concentrations of the carcinogen he determined that the causative compound was 3, 4-benzpyrene. He then tried to reduce skin cancer in chimney sweeps by guiding them to work in such a way that they would not absorb this substance (5-58).

Anderson College in Glasgow, at the time when Takamine, then a young man with a promising future, mainly studied chemical engineering. Takamine's family on his mother's side, the Tsuda family, ran a sake brewing business in Takaoka City, and from an early age he had grown up watching sake brewing. At Anderson College, he had lectures in basic chemistry from Dr. E. J. Mills, and he also studied the latest fermentation science (5-6). It was this combination that would lead to the success of TAKA=DIASTASE.

In those days, it was not uncommon for Japanese students who studied in a developed country to act arrogantly based on their adherence to the teachings of their former teachers or the country of study on their return to Japan. Takamine shows no traces at all of having been influenced in that way; he was a self-reliant scientist who stressed knowledge and experience, and fully applied these to his chosen subjects.

The adrenaline crystals were born of practical science, and they subsequently led to the magnificent and dramatic flowering of the logic of hormones, nurturing the development of many researchers in both theoretical and applied science, and even yielding a number of Nobel Prize winners.

◇ ◇ ◇

These are two of the many quotes that the great French scientist and patriot Louis Pasteur left behind, expressing his convictions (5-59).

"In the field of observation, chance favours only the prepared mind."

"If science has no country, the scientist should have one, and ascribe to it the influence which his works may have in this world."

Parke, Davis & Co. established a research organization, and the company was motivated to call on Takamine to collaborate in the planning by his record of achievements as a pioneer of biotechnology. He was joined by Wooyenaka, who had shown perseverance in refining his methods for handling natural products as an assistant to Nagayoshi Nagai. Chance undoubtedly favored these two minds, and the two men both took a great many benefits from their work back to their home country.

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Chapter 6

The Historic Ruling in the Patent Dispute

Should natural constituents formed in our own bodies be patentable? This was a particularly taxing problem for legal experts, and the first answer is believed to be that given by Learned Hand, the American judge who presided over the lawsuit involving infringement of the patent for the manufacture of adrenaline.

Jokichi Takamine, who wrote the patent for the manufacturing process himself, was instructed by the authorities to divide the patent claims into separate patent applications, and his work as a patent attorney to divide the original patent was well regarded. Judge Hand inspected the applications and passed the judgment that Takamine's manufacturing method was patentable, and he commended Takamine's research results in the courtroom as something that no one else had achieved until that time.

1. Unreliable nineteenth century medicines

In the latter half of the 19th century, the jobs of pharmacists and physicians were made harder by the fact that the activity of commercially available medicines, and particularly medicinal extracts of animal organs or herbal medicines, was very unpredictable.

Product quality varied enormously—some of these products had so little activity that they were virtually useless, while others were so strong that they put the lives of patients in danger. Needless to say, no pharmaceutical company wanted one of its products to be responsible for the death of a patient, so there was inevitably a tendency for the activity of medicines to become weaker.

As a result, physicians either made up their own prescriptions or dealt only with pharmacists they knew and trusted. Techniques for analysis and testing had yet to be developed, and so it was extremely difficult for sellers, buyers, or users to have any confidence in medicines.

George S. Davis, the charismatic proprietor of Parke, Davis & Co., had a shrewd grasp of the situation. He gathered together chemists and set about finding a way to solve this

problem. In 1879, he developed standardization processes using chemical assays, and the world's first "standardized medicine" went on the market. By 1883, the company's product list included 20 different types of "normal liquid."

At the start of the 1890s, pharmacological researchers began to notice that animal gland tissues were potential raw materials for new medicines. Parke, Davis & Co. was quick to respond to this new development, and in 1893 released dried thyroid for therapeutic use in treating hypothyroidism.

The techniques available at the time did not allow for even the most basic chemical analysis to be performed on medicines made from animal products, so in 1897 the company began introducing standardized assays for physiological activities that used experimental animals. We saw in the previous chapter how this quality control system was started on the understanding of Davis, the owner, with Dr. Houghton in charge.

Twenty years after the first system was introduced, the 1,100th product was standardized using this method (6-1). Doctors were particularly stringent in their demands for reliable quality control with useful products such as adrenaline, in which a mistaken dose could be life threatening, and this was of utmost importance for ensuring confidence in the pharmaceutical industry [Note 6-1].

Note 6-1.
Obtaining crystals of a substance does not necessarily guarantee its purity. However, if after repeated purifications the results of elemental analysis do not change and the numerical values for physiological activity do not rise any further—in other words, both sets of values reach a plateau—you can be more or less certain that you have the active principle in its pure form.

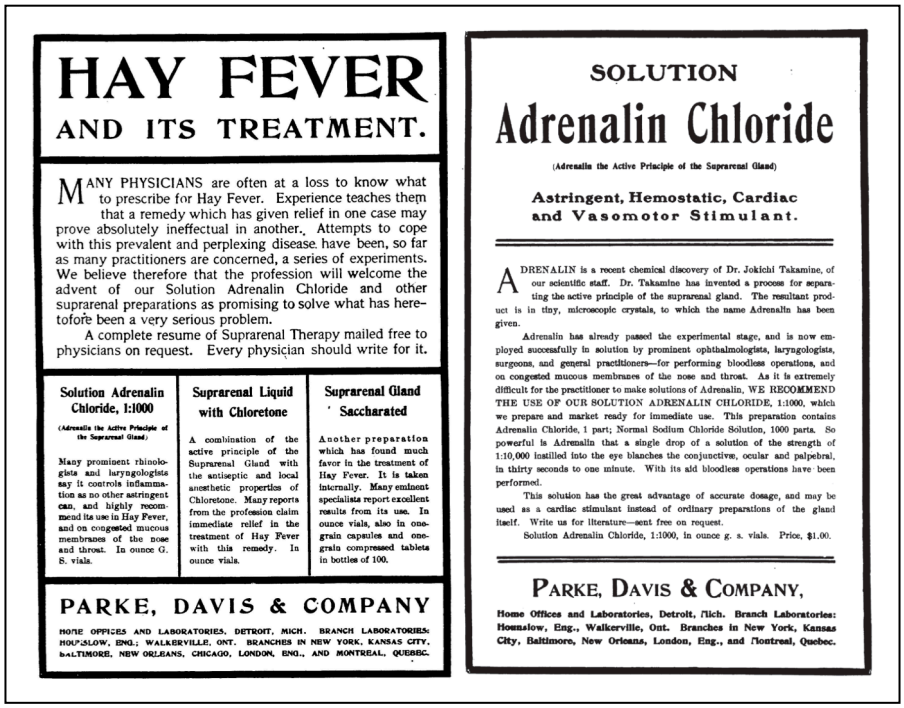
Abel gave the impression he was right at the fore front of the race to isolate the active principles of the adrenal glands. Inferring from his reports, it appears that he was receiving economic support for things like experimental materials from companies (6-2), but none of his research reports give an accurate description of activity, expressed as numerical values, corresponding to the purification stage.

2. Smooth commercialization

Parke, Davis & Co. steadily put its system of biological activity tests in place from around 1897 onward, and to make absolutely sure, the company also put together a chemical group for adrenal extracts under the leadership of Aldrich. The company launched its product "Solution Adrenalin Chloride" in 1901, the year after Wooyenaka and Aldrich successfully crystallized adrenaline. Aldrich wrote in a report that same year that he and Takamine had already collected samples of sufficient quantity for thorough research in the future (6-3), and it appeared that he was confident in the manufacturing method that had already enabled the company to put the product on the market.

The parent patent and the manufacturing expertise accumulated during that time were extremely valuable to Takamine and to Parke, Davis & Co. from the point of view of both social responsibility and business development. At the same time, the company was aggressively marketing its adrenaline products with publicity campaigns aimed at hospitals and doctors.

An advertisement from that time shows not only the ordinary preparations of the adrenal gland (saccharated) that had already been on sale, but also two other products: 0.1% adrenaline solution and this solution combined with chloretone (chlorobutanol) for its preservative and local anesthetic effects (6-4) [Figure 6-1].



HAY FEVER
AND ITS TREATMENT.

MANY PHYSICIANS are often at a loss to know what to prescribe for Hay Fever. Experience teaches them that a remedy which has given relief in one case may prove absolutely ineffectual in another. Attempts to cope with this prevalent and perplexing disease have been, so far as many practitioners are concerned, a series of experiments. We believe therefore that the profession will welcome the advent of our Solution Adrenalin Chloride and other suprarenal preparations as promising to solve what has heretofore been a very serious problem.

A complete resume of Suprarenal Therapy mailed free to physicians on request. Every physician should write for it.

Solution Adrenalin Chloride, 1:1000
(Adrenalin the Active Principle of the Suprarenal Gland)

Many prominent rhinologists and laryngologists say it controls inflammation as no other astringent can, and highly recommend its use in Hay Fever, and on congested mucous membranes of the nose and throat. In ounce G. S. vials.

Suprarenal Liquid with Chloretone

A combination of the active principle of the Suprarenal Gland with the antiseptic and local anesthetic properties of Chloretone. Many reports from the profession claim immediate relief in the treatment of Hay Fever with this remedy. In ounce vials.

Suprarenal Gland Saccharated

Another preparation which has found much favor in the treatment of Hay Fever. It is taken internally. Many eminent specialists report excellent results from its use. In ounce vials, also in one-grain capsules and one-grain compressed tablets in bottles of 100.

PARKE, DAVIS & COMPANY

HOME OFFICES AND LABORATORIES, DETROIT, MICH. BRANCH LABORATORIES: HOUNSLOW, ENG.; WALKERVILLE, ONT. BRANCHES IN NEW YORK, KANSAS CITY, BALTIMORE, NEW ORLEANS, CHICAGO, LONDON, ENG., AND MONTREAL, QUEBEC.

SOLUTION
Adrenalin Chloride
(Adrenalin the Active Principle of the Suprarenal Gland)

Astringent, Hemostatic, Cardiac and Vasomotor Stimulant.

ADRENALIN is a recent chemical discovery of Dr. Jokichi Takamine, of our scientific staff. Dr. Takamine has invented a process for separating the active principle of the suprarenal gland. The resultant product is in tiny, microscopic crystals, to which the name Adrenalin has been given.

Adrenalin has already passed the experimental stage, and is now employed successfully in solution by prominent ophthalmologists, laryngologists, surgeons, and general practitioners—for performing bloodless operations, and on congested mucous membranes of the nose and throat. As it is extremely difficult for the practitioner to make solutions of Adrenalin, WE RECOMMEND THE USE OF OUR SOLUTION ADRENALIN CHLORIDE, 1:1000, which we prepare and market ready for immediate use. This preparation contains Adrenalin Chloride, 1 part; Normal Sodium Chloride Solution, 1000 parts. So powerful is Adrenalin that a single drop of a solution of the strength of 1:10,000 instilled into the eye blanches the conjunctive, ocular and palpebral, in thirty seconds to one minute. With its aid bloodless operations have been performed.

This solution has the great advantage of accurate dosage, and may be used as a cardiac stimulant instead of ordinary preparations of the gland itself. Write us for literature—sent free on request.

Solution Adrenalin Chloride, 1:1000, in ounce g. s. vials. Price, $1.00.

PARKE, DAVIS & COMPANY,

Home Offices and Laboratories, Detroit, Mich. Branch Laboratories: Hounslow, Eng., Walkerville, Ont. Branches in New York, Kansas City, Baltimore, New Orleans, London, Eng., and Montreal, Quebec.

Figure 6-1. On the left is an advertisement for Solution Adrenalin Chloride from the year following the successful crystallization (*Homœopathic News*, May 1901). The advertisement states that the product is the invention of Dr. Jokichi Takamine, a member of the technical staff of Parke, Davis & Co., and it gives the conditions for which the product is indicated, the directions for use, and the content of the active ingredients. The price of a one-ounce bottle is one dollar. The advertisement on the right (*Homœopathic News*, August 1901) introduces two new products from adrenaline crystals alongside the suprarenal extract medicine that was already on sale. As we saw in Chapter 3, America was a country of vast grassy plains with many sufferers of hay fever—this was a market that no drug company could overlook. (Courtesy of HathiTrust)

A subsequent Parke, Davis & Co. medicine information magazine disclosed the composition of the basic Solution Adrenalin Chloride 1:1,000 solution, which was hydrochloric acid salt of adrenalin dissolved in physiological saline solution with 0.5% chloretone added (6-5).

Parke, Davis & Co. provided doctors with a detailed technical document, the introduction of which stated, "A member of our scientific staff, Dr. Jokichi Takamine, had finally perfected a process for separating the active principle of the suprarenal gland, to which he gave the name 'Adrenalin.'" The conditions for which the medicine was indicated were divided into 24 separate items. There was an introduction to clinical use of the medicine for each item that included the very latest information released the same year. These items were coryza, tonsillitis, eye disease, nasal surgery, epistaxis, hay fever, heart disease, surgery, otitis media, rhinitis, chronic hypertrophic, uterine inertia, metrorrhagia, hematuria, hemoptysis, post-partum hemorrhage, chloroform syncope, opium and morphine poisoning, rachitis, exophthalmic goiter hematemesis, pain, Addison's disease, laryngeal papilloma, and miscellaneous (6-6).

The information on the launch of a new product with guaranteed quality had a tremendous impact on doctors and researchers, who either prepared adrenal gland extract themselves or used medicinal extracts of animal organs such as the "Supra-renal Tabloid" of the British company Burroughs, Wellcome & Co.

Very soon, these doctors and researchers began to switch their allegiance to the new product from Parke, Davis & Co. A good example of this transition phase is the treatment report of the Late House Surgeon of New York City Hospital. This report includes three clinical cases, with a freshly prepared suprarenal emulsion used to treat two of these, and a 1: 5,000 adrenalin solution used to treat the other. The report states that the positive effects that were expected were found in all three cases (6-7).

3. The appearance of a rival product

The label of Solution Adrenalin Chloride lists the efficacy of the medicine, noting that it sustains the heart and prevents depression, and is effective for bloodless operations, congested mucous membranes, and hay fever among others. The list also included asthma from the initial launch onward.

We have seen in previous chapters that adrenal extract was known to be effective for all these conditions before adrenaline was crystallized, but when Parke, Davis & Co. made high-quality adrenaline available to doctors, cases of clinical treatment using the drug were reported in rapid succession.

An example of these is a report of the treatment of asthma by two doctors at Montefiore Home for Chronic Invalids, a hospital in New York.

The paper gives an explanation of the mode of action of adrenaline and then details the clinical treatment of five cases, three women and two men ages 17 to 63. In one case in particular, adrenaline brought great relief to an asthmatic patient (6-8, 6-9): "Male, aged sixty years; peddler; Wheezing and sonorous râles all over the chest. Five minutes after the injection of 6 minimus of adrenalin chloride, the respiration dropped to 30, and the pulse to 100; all râles disappeared and the patient slept quietly. A number of other cases were treated with adrenalin chloride hypodermatically with precisely the same results." The paper concludes, "In conformity with the angioparetic theory of an attack, the dose must be such as will cause prompt general vasoconstriction."

A new medicine with so many benefits was, of course, an irresistible target for pharmaceutical companies. It was only a matter of time before companies such as the German pharmaceutical manufacturers Hoechst A. G. and Bayer A. G. attempted to respond with their own synthetic compounds, while other companies rapidly developed similar products using manufacturing techniques that did not infringe on the patent for the production of adrenaline.

However, this was no easy matter—as we will discuss in more detail in Chapter 8, manufacturing a synthetic product for marketing as a medicine requires a high level of technological development.

In 1906, an academic report on a method of extraction and purification of adrenal gland extract was published by the chemical laboratory of the American company H. K. Mulford Co. (6-10). This was a complex process, in which the proteins were removed using trichloroacetic acid and then lead compounds, in order to extract the active principles. The references cited in this report included papers by Abel and von Fürth, but even though the authors were evidently aware of Parke, Davis & Co., no mention at all was made of the research results of Takamine or Aldrich. While no documents have been found to show exactly when this was, H. K. Mulford Co. began to market a dry powder preparation of the active principle of adrenal glands under the brand name "Adrin." One can easily suppose that the company did not make it a liquid preparation in order to avoid similarity to the products of Parke, Davis & Co.

4. The Patent dispute and a landmark ruling

Takamine had already transferred the patent license to Parke, Davis & Co., and the company naturally filed a lawsuit to prevent infringement of their patent. Presiding over the

patent infringement lawsuit was Judge Learned Hand of the Circuit Court of the Southern District of New York, who passed his ruling on April 28, 1911 (6-11): the lawsuit was won by Parke, Davis & Co. Takamine's method was ruled to be patentable, and the method of H. K. Mulford Co.was found to infringe upon the patent rights of Parke, Davis & Co.

This lawsuit called for an extremely difficult legal judgment, as it addressed the common understanding of the legal field of the day—that a product of nature could not be patented. At the same time, a challenging question such as this could not be tackled without a sound understanding of organic chemistry.

Judge Hand had no background in chemistry; nonetheless, he presided over the court and the judgment paper he read out was extremely detailed and showed a deep understanding of the science, clearly indicating the extent to which he had studied chemistry and other related fields to prepare himself for this lawsuit.

The paper ran to about 12,000 words, citing the work of Moore, von Fürth, and Abel, and was of a very high scientific level. The highlight is Judge Hand's declaration, "For he has been author of a valuable invention and has succeeded where the most expert have failed." The "he" in this case naturally referred to Takamine.

The judgment paper notes that Takamine was instructed by the patent office to divide the original patent (U.S. Patent No. 730,175), and even includes the details of the difficult negotiations he conducted during the application process. The original U.S. patent was divided into four patents (marked * in Table 6-1), and Hand acknowledged Takamine's work as a patent attorney [Note 6-2].

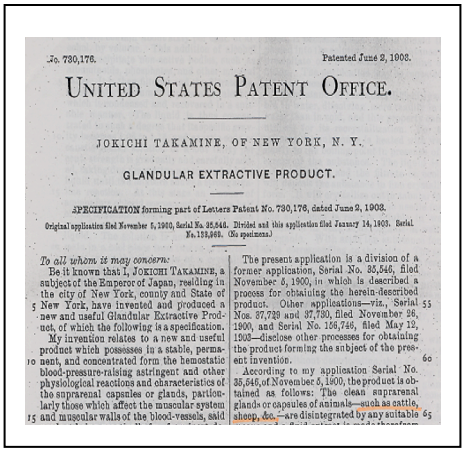
Note 6-2 Judge Hand found that H. K. Mulford Co. had infringed upon nine of the patent claims of U.S. Patent No. 730,176 (6-12) and four of the patent claims of U.S. Patent No. 753,177. The other four patents were not covered by the lawsuit (6-13), because "Adrin" was a dry powder preparation, and therefore only patents that corresponded to this were considered for possible infringement.

This was an historic ruling in the field of natural sciences that gave patent rights to a substance containing natural products, and has been the subject of numerous treatises. Since the discovery of the structure of DNA, the ruling is still studied by legal experts even today in relation to natural products that control life. Most recently, in November, 2010 the U.S. Patent and Trademark Office wrote in a brief that it would not recognize patents for DNA, and on June 13, 2013 the U.S. Federal Supreme Court ruled that patents for human genes would not be recognized.

Table 6-1. U.S. Patents applied by Jokichi Takamine

Patent No.	Date of application	Patented date	Divided patent		Summary of patent contents and sentence treatments
			Date of Acception	Patented date	
730,175 (Serial No. 35,546)	Nov. 5, 1900	June 2, 1903			This is the original patent which was asked to divide. This was patented with 4 other divided patents on the same date. In this patent, methods to extract crystals by combining such as extraction of impurities by using solvent, by changing pH of solution and so on. Number of claims: 9.
730,176* [Fig 6-2]	Nov. 5, 1900		Jan. 14, 1903	June 2, 1903	This is the patent divided from the patent No. 730,175. This was designated as the mother patent in the patent dispute. This has the suprarenal activity and show the characteristic color reactions and free from the inactive glandular tissues. Off white powder or crystal. Melting point: ca 207 °C. This shows alkalinity. This has blood pressure raising and hemostasis activities. 9 out of 16 patent claims were awarded as patent infringement.
753,177 Serial No. 156,747	May12, 1903	Feb.23, 1904			The patent applied on 1903 separately from the original patent and adopted as the patent in the patent suit. A stable method for the preparation of adrenaline aqueous solution. 4 claims out of 8 were recognized as patent infringement.
730,196*	Nov. 5, 1900		Nov. 26, 1900	June 2, 1903	Minerals and proteins were removed with alcohol. After removing pigments with ether, adrenaline was separated by using alkali, especially ammomia. Number of claims: 9.
730,197*	Nov. 5, 1900		Nov. 26, 1900	June 2, 1903	Minerals and proteins were removed with alcohol. After removing pigments with ether, adrenaline was separated from its alkaline solution by using neutralizing agents favorably with carbon dioxide. Number of claims: 9.
730,198*	Nov. 5, 1900		Jan. 8, 1901	June 2, 1903	Methods of preparation of aqueous crude extracts of suprarenal gland by using precipitation agents such as alcohol. Number of claims: 5.

The mark * indicates divided patents. Two patents under gray zone are the patents discussed in the patent suit together with the mother patent.



No. 730,176. Patented June 2, 1903.

UNITED STATES PATENT OFFICE.

JOKICHI TAKAMINE, OF NEW YORK, N. Y.

GLANDULAR EXTRACTIVE PRODUCT.

SPECIFICATION forming part of Letters Patent No. 730,176, dated June 2, 1903.

Original application filed November 5, 1900, Serial No. 35,546. Divided and this application filed January 14, 1903. Serial No. 138,969. (No specimens.)

To all whom it may concern:

Be it known that I, JOKICHI TAKAMINE, a subject of the Emperor of Japan, residing in the city of New York, county and State of New York, have invented and produced a new and useful Glandular Extractive Product, of which the following is a specification.

My invention relates to a new and useful product which possesses in a stable, permanent, and concentrated form the hemostatic blood-pressure-raising astringent and other physiological reactions and characteristics of the suprarenal capsules or glands, particularly those which affect the muscular system and muscular walls of the blood-vessels, said

The present application is a division of a former application, Serial No. 35,546, filed November 5, 1900, in which is described a process for obtaining the herein-described product. Other applications—viz., Serial Nos. 37,739 and 37,730, filed November 26, 1900, and Serial No. 156,746, filed May 12, 1903—disclose other processes for obtaining the product forming the subject of the present invention.

According to my application Serial No. 35,546, of November 5, 1900, the product is obtained as follows: The clean suprarenal glands or capsules of animals—such as cattle, sheep, &c.—are disintegrated by any suitable

Figure 6-2. The patent for adrenaline in the U.S. (U.S. Patent No. 730,176, shown in Table 6-1).

Learned Hand was an ideal judge, an outstanding man with a broad understanding of legal theory coupled with profound common sense. After he passed judgment in the adrenaline lawsuit, he went on to state his own personal philosophy in the courtroom.

"There is no surer way to misread any document than to read it literally," he once aptly said, and as a true patriot, he was worried about the confusing administration of justice in America and always encouraged efforts to improve it (6-14) (see Column 6-1 at the end of this chapter).

Hand was never appointed to be one of the nine Justices of the Supreme Court, but he was often seen as the "tenth justice" and has a place in American legal history as one of the country's most influential judges. After a lifetime in which his influence came to be felt across America, he passed away on August 18, 1961.

5. Maintaining the manufacturing method and product quality that were praised

When Judge Hand gave his opinion in the courtroom after ruling in favor of Parke, Davis & Co., it must have been an unexpected compliment for the two researchers, Takamine and Wooyenaka. As well as encouraging the two men, it must have given momentum to use the judgment in favor of the plaintiff, Parke Davis & Co., to the maximum to expand the company's business.

The quality and stability of "Adrenalin chloride (Takamine)" were highly regarded not just in the medical field but also among researchers. For example, a well-known research report by the American physiologist W. B. Cannon and his associates clearly describes this (6-15).

As a natural consequence of this, Parke, Davis & Co., which had a monopoly on the market with their product bearing the name of the active principle as "Adrenalin chloride (Takamine)," constantly paid large sums in patent and technical fees to Takamine.

Combined with the income from Taka=Diastase, this made him a very wealthy man. In his later life, his activities contributing to non-governmental diplomacy between Japan and the United States earned him the title of "Unofficial Ambassador."

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Jokichi Takamine studied the basics of patent law in the United Kingdom, and researched the latest information on the American patent system after the close of the exposition in New Orleans (1885). He continued his efforts even after that, and Judge Hand showed his

appreciation with the comment, "The applicant, after some struggles with the Patent Office, decided voluntarily to divide out the product claims."

However, as we shall see in the next chapter, the patent rights for which Takamine fought and won had a big impact on a ruling involving trademark rights.

Column 6-1.

Hand's philosophy

Judge Learned Hand's philosophy, which he put forward in the courtroom, was as follows:

"Whatever confusion the intricacy of the subject-matter causes, one fact stands out, which no one ought fairly to forget. Before Takamine's discovery the best experts were trying to get a practicable form of the active principle. The uses of the gland were so great that it became part of the usual therapy in the best form which was accessible. As soon as Takamine put out his discovery, other uses practically disappeared; by that I do not mean absolutely, but that the enormous proportion of use now is of Takamine's product. There has been no successful dispute as to that; hardly indeed any dispute at all. What use remains is, so far as the evidence shows, of the old dried glands, which everyone concedes to have been dangerous, at least for intravenous use. All this ought to count greatly for the validity of the patent, and Takamine has a great start, so to speak, from such facts. It is true that he overstates the degree of stability of his acid solution without any preservative. Strictly it is not in that form fit for sale about in drug stores where it may be kept for long even in a stoppered bottle; but commercial or practical stability is a somewhat elastic term, and this is a case where he should be entitled to a lenient construction, for he has been author of a valuable invention and has succeeded where the most expert have failed.

I cannot stop without calling attention to the extraordinary condition of the law which makes it possible for a man without any knowledge of even the rudiments of chemistry to pass upon such questions as these. The inordinate expense of time is the least of the resulting evils, for only a trained chemist is really capable of passing upon such facts, e.g., in this case the chemical character of Von Furth's so-called 'zinc compound,' or the presence of inactive organic substances. In Germany, where the national spirit eagerly seeks for all the assistance it can get from the whole range of human knowledge, they do quite differently. The court summons technical judges to whom technical questions are submitted and who can intelligently pass upon the issues without blindly groping among testimony upon matters wholly out of their ken. How long we shall continue to blunder along without the aid of unpartisan and authoritative scientific assistance in the administration of justice, no one knows; but all fair persons not conventionalized by provincial legal habits of mind ought, I should think, unite to effect some such advance." (6-11).

Judge Hand showed tremendous ability, and Hand's formula, which was the classic source of defect standards for planning defects, was used as a method for calculating liability for defects. It became the keystone of the Product Liability Law.

He was a strong protector of freedom of speech, and left behind many written works that encapsulated the spirit of the United States of America. *The Spirit of Liberty* (6-16), a collection of his essays and lectures, and *The Bill of Rights*, edited from a series of lectures, are his major legacy to the United States.

A short but moving oratory Hand gave in New York's Central Park on May 21, 1944 left his audience of thousands entranced. He spoke on faith, explaining that liberty lies in our minds and that when it dies, the constitution, the laws, and the courts can do nothing to save it.

"The spirit of liberty is the spirit which is not too sure that it is right; the spirit of liberty is the spirit which seeks to understand the minds of other men and women," he said.

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Chapter 7

Confusion over the Name

At one time or another, adrenaline has had four different names. Even until very recently there was a great deal of confusion, caused around the world because the substance that the American John J. Abel extracted from the suprarenal glands and named "epinephrin," believing it to be the active principle, turned out to be inactive.

The authorities wanted to use "adrenaline" as the official name in the *United States Pharmacopeia*, and a request was made to Parke, Davis & Co., which until then had dominated the market for the drug with this name. However, this came at a time when the company was desperately defending its trademark, "ADRENALIN," because the manufacturing patent had expired. The company was unable to accept the request, which further added to the confusion over the name.

1. Four people, four names

The adrenal medulla hormone was the first hormone to be isolated. Because this was in an age when separation and analysis technology had yet to be developed, the road to extracting the hormone in pure form and verifying it as a separate chemical substance was long and tortuous.

This in turn meant that the process of naming the hormone had various twists and turns, and it was given a different name by each of the four researchers that extracted it. In chronological order, it was variously named sphygmogenin, epinephrin, suprarenin, and adrenalin.

In 1894 to 95, Oliver and Schäfer verified that there were blood pressure-raising principles in the adrenal gland. Their student and a member of physiology research group at University College London, Benjamin Moore, attempted to isolate the specific compounds, but he did not give a name to the principles.

Neither did William H. Bates, who discovered the clear hemostatic effect. There was little point in naming the principles when no one knew for sure if there were one, two, or even

more components.

The first name, Sphygmogenin, was given by Sigmund Fränkel of the University-Institute for Medical Chemistry in Vienna to the syrupy constituent he extracted from the adrenal glands. He was convinced the constituent was pure, but the purity was inconsistent and he was unable to clarify the chemical formula, and there the matter rested (7-1).

Chronologically, the next name was epinephrin, which was given by John J. Abel of Johns Hopkins University. The most important part of the text of his research report, from the point of view of the present story, is the part in which he explains how he named the active component, so I will include the essential parts of the text in their original form. In his most important paper (7-2), he gives the name in two places. The first section of the main body of the paper is titled Epinephrin, and he writes, "*Ich nenne daher die blutdruck-steigerunde Substanz in Uebereinstimmung mit Hyrtl's Nomenclatur Epinephrin* (Therefore, I call this vasopressor substance "epinephrin" according to Hyrtl's nomenclature)". This means that he named the active component by defining it as the blood pressure-raising principle. Abel followed the nomenclature of Hyrtl, an Austrian anatomist. Hyrtl was keen on Greek, so for "kidney" in the stem of the word he used the Greek *nephr-* rather than the Latin *ren.* Incidentally, Hyrtl and the German anatomist Henle, whose research into the tissue of the adrenal glands using the chromaffin reaction we saw in Chapter 3, both published well-known books on dissection that have become textbooks used globally.

Now, however, we come to a dead end. In the final summary (*Zusammenfassung*) of the same paper, Abel writes, "*die Formel $C_{17}H_{15}NO_4$ ausgedrückt wird und welche ich Epinephrin nenne* (The formula of the substance which I call epinephrin is $C_{17}H_{15}NO_4$)". He named the active principle in the body of the paper, so after defining the chemical structure there would seem to be no problem with using the name for this as well. Thus in the final summary he writes the molecular formula $C_{17}H_{15}NO_4$ and specifically gives the name "epinephrin" to this compound. This means that he applied the name to this molecule rather than to the active principle, which is an abstract concept.

Unfortunately, the molecule in question has absolutely no activity at all. Abel was convinced that this molecule was the active principle, so he cannot really be blamed, but the dual nature of this name subsequently caused a degree of confusion that he would never have been able to imagine.

In 1903, Abel's assistant Samuel Amberg published a short report titled "The Toxicity of Epinephrin (Adrenalin)" (7-3). It is interesting to note that he used the name "epinephrin" given by his teacher, Abel, together with the name "adrenalin." However, von Fürth read this

article and haughtily declared that as epinephrin was not the true active principle, he would not use this term (7-4). The German von Fürth subsequently put forward the third name, suprarenin. From 1897 onward he published a series of reports of his quest to find the physiologically active adrenal principles in a German physiological chemistry journal (*Hoppe-Seyler's Zeitschrift für Physiologische Chemie*). In the third of these, published in 1900, he wrote "*Der Kürze wegen will ich für die von mir als wirksam angesprochene Substanz die Bezeichnung Suprarenin benutzen* (To put it simply, I would prefer to call the substance that I regard active "suprarenin")" (7-5).

The fourth name is adrenalin(e), which was announced by Takamine five years after the first name, sphygmogenin. In a paper published in an academic journal in 1901, he wrote, "I have therefore, termed my substance, as I isolated, 'Adrenalin.'" Takamine had isolated the substance himself and verified its activity, and he gave it this name regardless of its molecular formula—strictly speaking, this was therefore not a compound identified by its chemical formula (7-6).

As we saw earlier, the molecular formula Takamine put forward was soon found to be mistaken. However, as he had not applied the name adrenalin to a specific molecular formula, this did not present a problem.

2. Trademark rights

We saw in Chapter 1 how Takamine decided the name "adrenalin" on the advice of Norton Wilson, a friend of his. Takamine applied to register the trademark ADRENALIN in the US, and it was registered on April 16, 1901. The rights to the trademark were transferred to Parke, Davis & Co. five years later, on May 14, 1906 (7-7). Twenty years later, when adrenal medulla hormone was listed in the *US Pharmacopeia* for the first time, these trademark rights were to become the reason for a very important decision, as we will see. Takamine also applied to register the trademark in Japan on March 28, 1902, and it was registered on May 3 of the same year. The name "epinephrin" was coined by Abel, the university professor—unlike Takamine with "adrenalin," he had no intention to acquire trademark rights.

3. The War of Words

The first person to find a problem with "epinephrin," the name given by Abel, was von Fürth from Germany. In one of the footnotes to his own scientific report (1903) (7-4), he

stated that the term epinephrin was not appropriate, and that he would therefore avoid using it. The reason for this, he declared, was that the substance isolated by Abel was not a natural active substance.

In 1904, the year after von Fürth's initial objection, another German, Pauly, argued strongly against the name epinephrin in an academic paper titled "Zur Kenntnis des Adrenalins." (7-8) In a long footnote (26 lines), he put forward his view that Abel's names epinephrin and epinephrin hydrate were not appropriate terms because the concept of the words was vague, they did not include the active principle, and there were cases of researchers who had been under the misunderstanding that Abel was the first person to collect the adrenal active principle.

In the war of words over adrenaline and epinephrine, the argument that took place in London in 1906 seems to overwhelm the others in its ferocity and intensity. I will give some of the details here. It was an extremely interesting incident, and it occurred during the transitionary period in medical history from an age of trickery, fraud, and chicanery by many peddlers of patent medicines and quack cures to ethical drug manufacture and sales. The dispute was sparked off by a physiological and pharmacological research report by Henry H. Dale of the Physiological Research Laboratories of the pharmaceutical company Burroughs, Wellcome & Co. The laboratories were established in 1894 for research into biological medicines and therapeutic serums. Dale was conducting research into ergot, a group of alkaloid-producing *fungi* that grow on *rye* and related plants, and his report included the results of an experiment using adrenaline.

In the six weeks following publication, a fierce dispute broke out, with over 40 letters on the topic exchanged. E.M. Tansey of the Wellcome Institute for the History of Medicine gives an account of the affair in a paper titled, "What's in a Name? Henry Dale and Adrenaline, 1906." This paper is 18 pages long and is an extremely detailed report, but it reads like a short novel, unexpectedly drawing the reader in (7-9).

When Dale wanted to publish his research report, he first submitted it to the Research Director, Walter Dowson, in order to get approval for publication. The proposed publication came to the attention of Henry Wellcome, the owner of the company, who said that use of the word adrenaline was to be avoided because this was a registered trade name of Parke, Davis & Co., and "epinephrine" should be used in its place. Dale, however, refused. The Wellcome laboratories were managed independently of the everyday commercial activities of the company, and he believed that the researchers should be able to carry out their activities freely. Dale may have been an employee of the company, but he was also a

world-class scientist; he made no attempt to follow the orders of the owner of the company.

Only Dale, who exactly 30 years later would go on to win a Nobel Prize, could have shown such conviction and fighting spirit. The reason was perfectly clear: the Physiological Society (UK) had decided that the term "adrenaline" should be used in writing to refer to the physiologically active principles of the adrenal medulla, and it was common knowledge that this did not refer to any specific commercial product. Dale considered the term "epinephrine" to be inappropriate and inaccurate. Dale's immediate boss, Dowson, gave him all the support he could, but Wellcome would not change his instructions to use "epinephrine."

The deputy director of the Chemical Research Laboratories (established a year after the Physiological Research Laboratories), Hooper A. D. Jowett, joined the dispute—he had already used the term "epinephrine" in his own research reports, and he urged the director Dowson to abide by the wishes of the company owner. Dale, however, was totally unprepared to accept the order. He stood firm in his view, supported by a paper by Dr. Thomas R. Elliott of the University of Cambridge that used the term "adrenalin" and an official statement by Prof. John N. Langley in support of this as the scientific name. Dale's view was still not accepted, so in the end he declared that if the paper was not published he would be unable to remain in the laboratory and would thus be forced to tender his resignation. The result for this was a complete about-face, and Wellcome approved Dale's manuscript.

It looked as though the matter had been settled, but less than 24 hours later the research director, Dowson, was surprised to receive a telegram from Wellcome saying that approval for publication had been withdrawn. Jowett had suggested the company could face litigation over the use of a trademark. A protracted dispute ensued, into which a number of prominent intellectuals were dragged. There is insufficient space here to go into the details, but eventually Wellcome agreed to approve Dale's manuscript on the condition that it included a cautious footnote explicitly stating that the research was unrelated to any trademark.

For several months after this, Jowett continued his campaign from the Chemical Research Laboratories to use the name epinephrine, but the Physiological Research Laboratories paid him no attention whatsoever (7-9). At the top of a paper published in 1904 titled "The Constitution of Epinephrine," Jowett noted that various names had been given to the active component, but he maintained that even with an impure extract the name first given by Abel—epinephrin— should be used. Interestingly, at the end of this paper Jowett added a section, in which he noted that Abel still insisted on the existence of a "hydrate" that included $1/2 \cdot H_2O$, but that he and his colleagues had shown experimentally that there was no

water formed with the crystallization.

Even though Jowett did not consider Abel's chemistry to be correct, he still maintained that epinephrine was the legitimate term—he appeared to be indifferent to the question of the physiological activity of the substance. Jowett was a chemist who simply could not engage with Dale, who emphasized physiological activity (7-10). Strangely enough, however, at around the same time Jowett co-authored a paper with G. Barger of the Physiological Research Laboratories, titled "The Synthesis of Substances Allied to Epinephrine," at the end of which the following note was added: "The necessary physiological experiments in connection with this inquiry were performed by Dr. H. H. Dale, to whom we wish to tender our best thanks." (7-11).

In 1907, Thomas Maben (his position was given only as "F.C.S.," which appears to mean that he was a fellow of the Chemical Society) published a concise and accurate history of the active principle of the adrenal glands in the *Pharmaceutical Journal*, a British publication. He noted that the name "adrenalin" was not accorded the same status as "epinephrin," but he put forward his explanation that adrenalin was the only name for the substance in question and said that Jowett's argument for the legitimacy of "epinephrin" could not be justified (7-12). At the end of his paper arguing for the appropriateness of "adrenalin," Maben expressed his hope that this could be used as a generic name without trademark rights being asserted. However, this could not be accepted by the business world, for which trademark rights are assets. We will examine the reasons in detail later on in this chapter.

Maben prided himself on his speed. Six years earlier, Takamine gave an oral presentation in New York in January 1901—by March of that year, Maben had given his own concise lecture on adrenalin at a Pharmaeutical Society meeting in Edinburgh, and just three days later a summary of his lecture was published in the society's journal. He probably felt a certain responsibility as well (7-13).

To add a brief word about the owner of Burroughs, Wellcome & Co., Henry Wellcome was invited by his friend, Silas Burroughs, to establish a company together, and the two men established Burroughs, Wellcome & Co. in London in 1880. After Burroughs died, the company became the Wellcome Foundation Ltd., which grew to become one of the world's leading pharmaceutical companies; both men were American. Wellcome's life is an incredible story of success: he came from a poor farming community in northern Wisconsin, and his parents, who traveled around the area as Adventist preachers, brought him up strictly. He broke free by studying at the Philadelphia College of Pharmacy, where he was able to demonstrate his innate talent. He eventually took British nationality and became a member of

the British aristocracy.

Before Wellcome went to London, he worked as a traveling salesman for two of America's top pharmaceutical companies, and he later had to labor hard to retain the rights to the "Tabloid" trade name of the "Tabloid products" that brought rapid success to Burroughs, Wellcome & Co. This, together with the fact that he was extremely well attuned to industrial property rights and was an enormously careful manager with respect to American products, may perhaps explain why he wavered over the decision concerning Dale's paper. We have already seen in Chapter 4 that Burroughs, Wellcome & Co. was marketing tablets of adrenal glands under the name "Supra-renal Tabloids" (see page 84).

In the conclusion of his paper on the war of words that took place within Burroughs, Wellcome & Co., Tansey notes that there were legal, scientific, and human lessons to be learned from the dispute at various different levels. However, I have been unable to find any documents that shed light on whether the circumstances of this altercation ever reached the ears of J. J. Abel in the US, who first named the substance "epinephrin."

4. The struggles of a researcher: the fifth name

Correctly using the various different names that appeared throughout this history was a major headache for academics and researchers. A fifth generic name, "adrenine," appeared in the United Kingdom. An article was published in the *Pharmaceutical Journal* of August 3, 1907 that would probably have been of great interest to Takamine. This was a report of research into a method for detecting the iron content of oleic acid, a type of unsaturated fatty acid, in medicinal products. With this method, adrenaline was added to the product and the Vulpian color reaction indicated the presence of iron. This has already been briefly mentioned in Chapter 5. The footnote on the first page of the report reads as follows: "The substances used in the experiment and included under the name "adrenine" were adrenalin, suprarenin, suprarenalin and various commercial solutions of the blood-pressure-raising principle of suprarenal gland". Even before this, the term "adrenine" is used in the title of the paper (7-14). From this time onward, "adrenine" came to be widely used in papers published in the United Kingdom (7-15), and even Prof. Schäfer, one of the researchers who discovered the blood pressure-raising effect, stated that the name was appropriate during an invited lecture he was giving (7-16).

Another example is a brief paper dated May 23, 1908, which was written by A. R. Cushny of the Pharmacological Laboratory of University College, London, who demonstrated that the synthetic product "suprarenin" produced by the German company Höchst A.G. had only

half the activity of adrenaline extracted from adrenal glands; the title was "Synthetic Suprarenin or Adrenine" (7-17). In another full paper he uses the term "adrenalin" in the title, and in a footnote he states, "The bodies used were really "suprarenin," but as natural suprarenin is identical with adrenalin and the latter is the more familiar term I have adopted it throughout" (7-18). It was three years after this (1911) that the historic ruling was passed in the US patent lawsuit recognizing the inventiveness of the method of producing adrenaline, a natural product, as we saw in Chapter 6.

Moving forward to 1913, the American physiologist Walter B. Cannon, who was well known for proposing the concept of homeostasis (the steady state of the organism), attempted to verify in his final work that adrenaline was the chemical transmitter in adrenergic nerve impulses. He was unsuccessful, but in a paper titled "The Depressor Effect of Adrenalin on Arterial Pressure," he used the term "adrenalin" (small "a," no final "e"), and he specified that he used the fresh product of Parke, Davis & Co. in the experiments (7-19).

However, in his celebrated work on physiology published about 20 years after this, *The Wisdom of the Body*, the explanation of adrenalin (small "a," no final "e") that starts on page 44 has the following brief definition in the margin [Figure 7-1]: "'Adrenin' is the natural secretion of the adrenal medulla; 'adrenalin' is a commercial extract of the adrenal glands" [underlines added] (7-20). Perhaps Walter B. Cannon recognized that the name epinephrine should not be used in research reports.

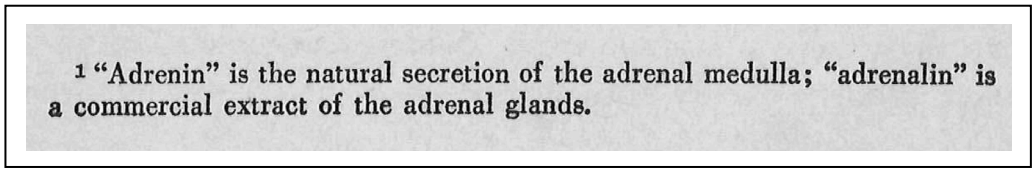

[1] "Adrenin" is the natural secretion of the adrenal medulla; "adrenalin" is a commercial extract of the adrenal glands.

Figure 7-1. The footnote on page 44 of the classic *The Wisdom of the Body* (7-20), which was the lifework of the American physiologist W. B. Cannon.

5. The letter that decided the name in the *US Pharmacopeia*

In 1926, the adrenal medulla hormone was listed for the first time in the *US Pharmacopeia* with the name "EPINEPHRINA Epinephrine." Why was the name "epinephrine" used? The answer can be found in a letter from that time that has been preserved. This was a business letter, dated November 19, 1921, from O. W. Smith, the president of Parke, Davis & Co., to Dr. Jokichi Takamine. The existence of this letter was referred to in an essay on Takamine's will that appeared in a magazine (7-21).

The letter has two paragraphs, the second of which reads,

"I might say, incidentally, that the revision committee of the U. S. P. expects to include Adrenalin Solution in the next edition. We have been asked to supply specifications. We have done so, but we have suggested to the sub-committee, of which Mr. Rosengarten is chairman, that the word "Adrenalin" is registered, is a valid trademark, and that under the circumstances the committee would probably use the word "Epinephrin" in the Pharmacopeia. We are afraid that if the word "Adrenalin" is used it may encourage manufacturers to use it also, whereas so far all of them have kept off the grass" [underlines added].

By this time, the trademark rights had already been transferred from Takamine to Parke, Davis & Co., and Adrenalin Solution had a monopoly of the market. This letter clearly shows that Parke, Davis & Co. still had the trademark rights to the name "Adrenalin," and the company refused to authorize use of the name in the *Pharmacopoeia* because it wanted to protect its monopoly. However, the company was not opposed to the name "Epinephrin" being used instead; as a result, this is the name that was adopted for use in the *US Pharmacopeia*.

In fact, even before this stage was reached there had been various discussions, which included exchanges of opposing views, aiming to find a generic name for the adrenal medulla principle that could be widely used regardless of any interests or rights. Let us look at these in chronological order.

First, there was an editorial in the *Journal of the American Medical Association* of March 25, 1911, titled "The Name 'EPINEPHRIN' versus the Name 'ADRENALIN,'" which was followed by the minutes of a heated discussion on "Proprietary versus Unprotected Names" (7-22). The latter article filled six A4 sized pages, and included downright antagonism between medical scientists and doctors on the one hand and Parke, Davis & Co. on the other. It is an engrossing read, but the contents are quite astonishing—what clearly emerges is that the culture, rules, and ethics of the academic and the business worlds were very different from what they are today.

On April 28, 1911, just 34 days after the publication of this journal, Judge Learned Hand ruled in favor of Parke, Davis & Co. in the lawsuit relating to the infringement of the patent for the manufacture of adrenalin that we saw in detail in Chapter 6. As a result, the company's monopoly of the sale of adrenalin became even more profitable [Note 7-1].

Note 7-1.
The names of the non-prescription medications containing adrenal medulla principle that were given in the article in the journal were: Adnephrin (Frederick Stearns & Co.), Adrenalin (Parke, Davis & Co.), Adrin (H. K. Mulford & Co.), Supracapsulin (Cudahy Co.), and Suprarenalin (Armour & Co.) in the US; and Atrabilin, Chelafrinum, Epirenan, Hemostasin, Ischemin, Paranephrin (Merck), Renoform, Supranephran, Suprarenin (Hoechst), Suprarenin synthetic, Tonogen, and Vasoconstrictin in Europe and elsewhere. In addition, the article mentions that the same year in London the name "adrenine" was proposed for the *Yearbook of Pharmacy* in place of "adrenaline."

It is worth noting that the *Journal of the American Pharmaceutical Association* (7-23) published in 1915, four years later, discussed a similar ongoing problem with naming in relation to Aspirin (acetyl salicylic acid, an analgesic and antipyretic). This drug, which was produced by the German company Bayer A.G., was at the time a huge product on a scale comparable to Adrenalin.

The disputes over the name continued one after another, until 1920—this was the year in which the patent for the manufacture of Adrenalin expired. Adrenalin had grown since its launch to become a massive product, and there were 30 to 40 rival products of different quality containing adrenaline. The US government must have decided that it could no longer ignore the complexity of this competition or the confusion that it was causing in medical settings, and it took the opportunity to commence the preparations for listing the official name in the *US Pharmacopeia*.

The *US Pharmacopeia* is revised every 10 years, but the preparations for the revision begin several years earlier. The committee for drawing up the draft version of the 10th revision, which was to be made in 1926, released a detailed report on May 11, 1920, six years before the revision and, very aptly, the year in which the patent for the manufacture of adrenalin expired (7-24). The report proposed that adrenaline should be named "EPINEPHRIA (Latin name), Epinephrine (English name)." The members of the committee included Dr. Houghton, the project leader from Parke, Davis & Co. who had played an important part in the crystallization of adrenaline, and Frank O. Taylor from the same company. The final proposal was probably decided after they had put forward their company's position and come to a mutual agreement with the other members.

Let us return to the business letter to Takamine from O. W. Smith, the president of Parke, Davis & Co. This letter is dated November 19, 1921, one year after the draft for the 10th revision was submitted proposing "Epinephrine" as the official name. We will probably never know why Dr. Rosengarten, the Chairman of the Organic Chemicals Sub-committee, was trying once more to confirm the view of Parke, Davis & Co., because the Revision Committee members most likely had a duty of confidentiality. However, it seems to me that

with the expiry of the manufacturing patent, he probably wanted to check for the last time whether Parke, Davis & Co. would negotiate the use of adrenaline as a worldwide generic name without asserting trademark rights. Anyone in his position would have wanted to use the catchy name that was already firmly rooted in the medical profession as the name in the pharmacopoeia, so I sympathize with him.

Following the legal expiry of the manufacturing patent, the only way Parke, Davis & Co. could prevent products from other companies entering the market that it had monopolized was to protect its widely known trademark "Adrenalin" to the last.

There was no particular problem with this line of thinking, and the company certainly cannot be criticized for it. However, much of the ensuing confusion might have been avoided if Parke, Davis & Co. had suggested a name such as "adrenin(e)," which had been advocated by several other people (7-14 through 7-17, 7-20), instead of "Epinephrin," which was the name used for an inactive substance that was not the active principle.

6. The review that caused an unfortunate misunderstanding

In 1927, Abel published a long review titled, "Chemistry in relation to biology and medicine with especial reference to Insulin and other hormones" in the well-known scientific magazine *Science* (7-25). This review included two important descriptions.

The first of these is the following passage, in which he recollects how Takamine visited him at the time when he was struggling with his adrenal gland research: "After I had completed the above described investigation and while I was still endeavoring to improve my processes I was visited one day in the fall of 1900 (as I recall it) by the Japanese chemist, J. Takamine, who examined with great interest the various compounds and salts of epinephrine that were placed before him. He inquired particularly whether I did not think it possible that my salts of epinephrine could be prepared by a simpler process than mine, more especially without the troublesome and in this case wasteful process of benzoylating extracts of an animal tissue."

The other important description is almost unnoticeable, but it should not be overlooked. On page 341 of the review, toward the bottom of the left-hand column, it says, "[…] their secretory product, adrenalin or epinephrine (U.S.P.)." Thus, Abel wrote "adrenalin" first, followed by "or epinephrine." He must have confirmed that it had been listed for the first time in the *US Pharmacopeia* under the name "epinephrine" the year before the review was published, and then written "adrenalin" before "epinephrine." Abel clearly harbored no ill

will toward Takamine when he wrote this review.

Unfortunately, Abel's review was to lead to a major misunderstanding, which he could surely never have imagined. "Epinephrin(e)," which was written by Horace W. Davenport, a professor at the University of Michigan who had made the study of the sympathetic nervous system his life's work. This was published in 1982, over half a century since both Abel and Takamine had passed away. Davenport had great respect for Abel, who had been the first professor of the Department of Pharmacology at the University of Michigan. "When I was young," he begins, going on to reminisce about the past, "I was taught to say adrenalin only when I specified the Parke, Davis product. Otherwise, I should say epinephrine. In addition, a faint air of scandal seemed to hang over adrenalin, something about a stolen secret. No one seemed to know the facts. Tracing the origin of the scandal, if there were one, added piquancy to my investigation." (7-26)

This passage served to spread the baseless rumor widely among researchers in the field that Takamine had stolen the experimental procedure from Abel, who was ahead of him in the isolation race.

While it is something of a postscript, entry no. 3650, Epinephrine, in the well-known *Merck Index* (13th Edition, 2001) lists the following names: adrenaline, levorenin, Bronkaid Mist, Epiglaufrin, Eppy, Glauposine, Primatene Mist, Simplene, Sus-phrine, and Suprarenaline, besides Epinephrine. Even today adrenaline remains as just one of the 10 alternate names. In spite of this, the references cited for "isolation from animal adrenal glands" are one work each from Takamine and Aldrich, which are the very works cited in Chapter 4 of this book (4-55 and 4-25). Despite the fact the entry is a listing of Epinephrine, Abel, who named it, is not mentioned and his work is not cited.

Davenport's review (7-26) contains a significant factual error. One paragraph starts, "Takamine never gave the source of his starting material." This is simply untrue. In US patent 730,175 of November 5, 1900, Takamine gives examples of the animals used: "The clean suprarenal glands or capsules of animals such as cattle, sheep, &c." He then describes the extraction process. (This patent was later divided, and the same is recorded in patent 730,176, which was the mother patent).

It may have been difficult for Davenport as an academic to get hold of the patent specifications, but I include this detail for the sake of Takamine's good name. In addition, Vulpian's 1856 paper on the color reaction lists the names of many animal species in which he discovered secretions showing the color reaction, and it had been clear for over half a century that if the animals were readily available domestic animals, there was no need to

specify the species.

Davenport attempts to verify his theory of Takamine's plagiarism by comparing Abel as an academic and researcher, largely on the basis of Abel's recollections, with the conduct of Takamine as a businessman. However, he ends without obtaining any positive evidence. Considering that Abel did not have a method that could successfully crystallize adrenaline, and consequently there was nothing to steal, the whole idea of a "motive to steal" is thus utterly illogical and indeed incomprehensible.

Among the Japanese researchers who read Davenport's review, some felt very unhappy for the suspicion against Takamine and the exclusion of adrenaline from the Pharmacopoeia, and raised blames towards Abel. After everything became clear, however, this really was a great shame (7-27).

Davenport's review caused the misunderstanding by running contrary to what were probably Abel's true intentions, and contained expressions that Abel would perhaps not have wanted. Nine years after this review was published, Davenport once again touched on the subject of adrenaline, this time in a comprehensive scientific history titled, "Early History of the Concept of Chemical Transmission of the Nerve Impulse" (7-28). Curiously, Abel's name does not appear in the text, and not one of the 84 works cited was by Abel. Takamine is mentioned in five places; and one of these, a short sentence of just seven words, is quite clear: "The man who succeeded was Jokichi Takamine." It looks as though Davenport had come to realize with the passage of time that in the history of adrenal gland chemistry, it was not Abel whose achievements should be trumpeted [Note 7-2].

Note 7-2.
At the start of this latter review, Davenport writes that one reason for his interest in epinephrine was the fact that the leading American physiologist Walter B. Cannon, whose lifework was the sympathetic nervous system, had tried to prove in his final work that the adrenergic nerve transmitter was epinephrine but had been unsuccessful; Davenport wanted to discover the reason for this (7-28).

It was the Swedish physiologist Ulf von Euler who later succeeded, and he was honored with a Nobel Prize for his work. In a commemorative lecture titled "Twenty Years of Noradrenaline," von Euler praised Cannon for his huge achievements in this field (7-29).

7. The name of the adrenal medulla hormone in different Pharmacopoeias

So far we have looked at the reasons for, and the background of, the five names given to the active principle of the adrenal glands. We have already seen that only two of these names —adrenaline and epinephrine—have survived, and Table 7-1 shows which of these names is currently used in the respective pharmacopoeia of various major countries.

England is proud of its ground-breaking discovery that the blood pressure-raising

principles are secreted from the adrenal glands. Jeffrey K. Aronson of the Department of Clinical Pharmacology, University of Oxford, who gives a thorough guide to the naming of the adrenal medulla hormones to date in a paper published in 2000 (7-30), states, "There is […] clear historical and etymological evidence that epinephrine is an inappropriate name to use."

He goes on to conclude, "Assuming that you don't want to call it dihydoroxy-phenylmethylaminoethanol, which name should you use—adrenaline or epinephrine? All the arguments and evidence suggest that you should prefer adrenaline." This reaffirms the "validity of adrenaline" that Thomas Maben had emphasized nearly 100 years earlier (7-12).

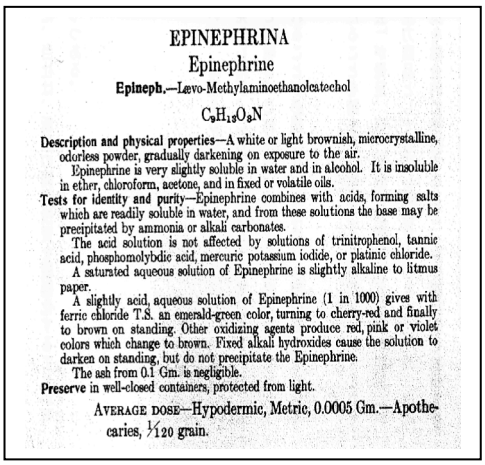
In the United States, however, which was the land where adrenaline was crystallized, there has been no change in the listing in the pharmacopoeia from when it first appeared in 1926 as "epinephrine" up to the present day [Figure 7-2]. The name "adrenaline" has never appeared together with "epinephrine.

Table 7-1. Names of suprarenal medulla hormone in several pharmacopoeias

Country	**Adrenal medulla principle**	**Other names**
USA	Epinephrine	No description
Japan	アドレナリン Adrenaline	Epinephrine
Britain	Adrenaline/ Epinephrine	No description
European*	Adrenaline （Adrenalinum）	No description
P.R. of China**	腎上腺素	Epinephrine
WHO international	Epinephrine	Adrenaline
Cf.Merck Index13th ed.	Epinephrine	Adrenalin[Parke-Davis] Adrenaline

* The *European Pharmacopoeia* is published by the European Directorate for the Quality of Medicines & Health Care (EDQM) of the Council of Europe/Strasbourg.

** P.R. of China adopted 'Adrenalinum Adrenaline (Epinephrine)'in English, in 1992.



EPINEPHRINA

Epinephrine

Epineph.—Lævo-Methylaminoethanolcatechol

$C_9H_{13}O_3N$

Description and physical properties—A white or light brownish, microcrystalline, odorless powder, gradually darkening on exposure to the air.

Epinephrine is very slightly soluble in water and in alcohol. It is insoluble in ether, chloroform, acetone, and in fixed or volatile oils.

Tests for identity and purity—Epinephrine combines with acids, forming salts which are readily soluble in water, and from these solutions the base may be precipitated by ammonia or alkali carbonates.

The acid solution is not affected by solutions of trinitrophenol, tannic acid, phosphomolybdic acid, mercuric potassium iodide, or platinic chloride.

A saturated aqueous solution of Epinephrine is slightly alkaline to litmus paper.

A slightly acid, aqueous solution of Epinephrine (1 in 1000) gives with ferric chloride T.S. an emerald-green color, turning to cherry-red and finally to brown on standing. Other oxidizing agents produce red, pink or violet colors which change to brown. Fixed alkali hydroxides cause the solution to darken on standing, but do not precipitate the Epinephrine.

The ash from 0.1 Gm. is negligible.

Preserve in well-closed containers, protected from light.

AVERAGE DOSE—Hypodermic, Metric, 0.0005 Gm.—Apothecaries, 1/120 grain.

Figure 7-2. The page of the *US Pharmacopeia* released on January 1, 1926, showing the entry for "Epinephrine." The name has not changed since then.

Table 7-2 shows the names used in successive pharmacopoeias in Japan, the homeland of Takamine and Wooyenaka, the scientists who successfully crystallized the active principle of adrenal glands and called it adrenalin.

Table 7-2. Names in the Japanese Pharmacopoeia

Revision	Year	Name	Other names
5th - 7th	1932 - 1961	Epirenamine	—
8th	1971	Epinephrine	Epirenamine
9th - 12th	1976 - 1991	Epinephrine	Epirenamine, Adrenaline
13th - 14th	1996 - 2001	Epinephrine	—
15th	2006	Adrenaline*	Epinephrine
16th	2011	Adrenaline	Epinephrine

* On December 14, 2001, a request for the adoption of Adrenaline as the Pharmacopoeia name, was submitted to the Ministry of Health, Labour and Welfare of Japan.

We can see that the name was changed twice following the first listing in 1932, and by 2006 had finally settled with "adrenaline."

I will close this chapter by mentioning one work that shows how the isolation of adrenaline is seen in the specialized field. This is the *Handbook of Physiology*, a major work published by the American Physiological Society. The only researchers that are recorded as having extracted adrenaline from the adrenal glands are Takamine and Aldrich (7-31).

◇ ◇ ◇

The history of naming the adrenal medulla hormone must surely be more convoluted than that of any other principle of the living body. No less than five names have been proposed over the course of the century and a half since the isolation race began and two of these—adrenaline and epinephrine—have lasted.

In the Prologue, I showed three photographs illustrating how widely the word "adrenaline" has come to be used in fields other than medicine in the US. Returning to use of the official name of the hormone would inevitably cause a considerable degree of chaos. No one is going to buy a bottled soft drink advertising blood pressure-raising activity from a vending machine, and no one is likely to imagine that buying a drink that says "Epinephrine" on the label will make them feel livelier.

The name "adrenaline," given to a hormone—an organic compound—was born in the United States of America. For me, it is a great shame that this name will probably never be

used again in the fields of either government administration (the pharmacopoeia) or science in the land of its birth.

Nonetheless, I am pleased to be able to show the disputes over the trademark and the generic name, and the events through which the *US Pharmacopeia* came to use the name "epinephrine," as clear historical facts through the letter in this chapter. I am also happy to be able to show through some of the writings left by Abel and Takamine that there was no ill feeling between these two men, with regard to either their work or the naming of adrenaline.

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Chapter 8

Post Crystallization

At the start of the 20th century, when the technologies for separation and analysis were still in their infancy, obtaining crystals of a highly active natural principle that is only present in the body in minute quantities was a more revolutionary development than we can possibly imagine today.

With crystals of adrenaline readily available, capable researchers now had free reign to exercise their talents, and there were huge, significant advances in pure and applied physiology, medical science, and pharmacology. These achievements included many that led directly to Nobel Prizes. At the same time, adrenaline made its mark in the world outside academia. Like the diastases of Taka-diastase type, also discovered by Takamine, it has been a hugely important medicine for over 100 years.

1. Abel's sad decline

This book does not aim to give a detailed history of the research that continued after the successful crystallization up until the present day, and I shall limit myself to presenting a few topics that will perhaps be of interest to readers.

Abel continued his efforts to shake off his "blunder," but most of these ended up being fruitless. As an example of this, in 1903–04 he reported that epinephrine was a hydrate with $1/2 \cdot H_2O$ attached to it (8-1, 8-2), but Jowett of the Wellcome Chemical Laboratories in the UK went out of his way to append an extra section to his own paper in which he rather harshly pointed out that there was no such thing (8-3).

Over the space of 19 years from 1901, Abel published a total of 10 papers (8-1, 8-2, 8-4 through 8-11). None of these were of any great value when judged by today's level of technology, but bearing in mind the huge efforts to which he went, I will introduce just two of them.

The first is a paper that appeared in the *American Journal of Pharmacy* in 1903 in which Abel describes his own ineptitude over the experimental method. He states that he regrets his lack of thoroughness: "Although I had demonstrated that epinephrin is a basic substance, and

although I had repeatedly shown that it can be precipitated as a flocculent substance by ammonia, the fact that it could be precipitated in a physiologically active and crystalline condition directly from gland extracts had escaped me because I used either an insufficient quantity of ammonia or too dilute a solution of the active principle" (8-2).

The other is a paper that was published the same year in a German academic journal. In this, Abel clearly states, "The momentous observation that the crystalline principle can be precipitated out of a concentrated extract of adrenal glands by ammonia and other alkalis is thanks to (*verdanken wir*) Takamine" (8-9). He cites a paper by Takamine (8-12), stating, "Later still, Aldrich also collected the same substance."

John Jacob Abel was born on May 19, 1857 in Cleveland, Ohio, the son of farmers George Abel and Mary Becker. John lost his mother at the age of 15 when she contracted puerperal fever after the birth of her eighth child. He graduated from high school in Cleveland at the top of his class, and in 1876 he entered the University of Michigan.

He had to leave after completing his third year because of a lack of money, and over the following three years he worked as a schoolteacher and then principal. He became the superintendent of education in La Porte, Indiana, where he met a high school teacher named Mary Hinman, who was later to be his wife.

During the three years he stayed in La Porte, he set his heart on moving into medicine. From the very beginning, he intended to go into research rather than practice. He returned to the University of Michigan in 1882 to complete his undergraduate studies, spending most of this time studying under the physiological chemist Victor Vaughan and the physiologist Henry Sewall in the Department of Medicine.

After graduating in 1883 he married Mary Hinman (they later had 3 children), and they moved to Baltimore. There he worked in the laboratory of the physiologist Henry Newall Martin of Johns Hopkins University for a year.

Abel was fascinated by Germany, which at the time was highly regarded as the world center of pharmacological research, and in 1884 he managed to get a job at the laboratory of the famous physiologist Carl Ludwig in Leipzig. However, he soon realized that he lacked the basic foundations for carrying out medical research at a high level, and he enrolled in a medical college in Leipzig in order to reinforce his knowledge of basic physiological and medical research. He spent a total of six and a half years studying at universities in Germany, Austria, and Switzerland, after which he acquired his Doctorate of Medicine from the University of Straßburg, which was a part of Germany, in 1888.

After long years of diligent study while working to support himself, Abel returned to his

native America. There he enjoyed considerable success, making a name for himself as an outstanding educator in the history of American pharmacology. His most noteworthy research result was the crystallization of insulin in 1926, which many other researchers had attempted without success. Insulin is a hormone produced by the pancreas, and is important as a medicine for diabetes.

Abel reported that insulin was a type of peptide, which is a chain of amino acids forming the constituent unit of proteins. At the time, it was generally held in academic circles that peptides, which are not small molecules like adrenaline, did not exhibit any physiological activity. Abel's discovery was therefore viewed with skepticism, but he was eventually proved right (8-13).

Despite successfully crystallizing insulin, Abel's desperate struggle to crystallize adrenaline came to nothing. It is possible that the fortunes smiled on Abel with insulin; crystallization begins around a nucleus of zinc, so it may have been the case that he was blessed with a solvent that happened to contain traces of zinc.

In November 1900, just four months after Wooyenaka obtained adrenalin crystals, Abel lost the sight of one eye due to an explosion in his laboratory during an experiment. However, he was not a man who gave up easily, and he still continued with his experiments. The last of Abel's 10 papers (8-10), which may not appear to be particularly significant today, was published in 1905 on the first page of the *Journal of Biological Chemistry* Volume 1, Number 1. This journal was launched in that year by Abel himself, and the paper was a research report titled, "On the decomposition products of epinephrin hydrate."

The following year, the *Biochemical Journal* was launched in the UK by Benjamin Moore and Edward Whitley. Moore's achievements were examined in detail in Chapter 4. He had previously crossed swords with Abel during the race to purify the active principle of the adrenal glands, but their journals, started at around the same time in the UK and the US respectively, went on to become important journals in which all researchers in this field aspired to have their work published. Even now, over 100 years after they were launched, both journals enjoy this reputation. The highly talented Moore passed away in March 1922 at the age of 55, and two weeks later a memorial article appeared in the well-known British scientific journal *Nature* (8-14). Coincidentally, Jokichi Takamine passed away in the summer of the same year.

Five years later, the Nobel Prize winner F. G. Hopkins, the founder of biochemistry and Moore's senior, submitted an article together with a portrait of Moore to an academic journal, in which he lamented Moore's death and praised his achievements (8-15).

In Abel's report, the one before the last, about the adrenal principle (8-1), he compared the results of elemental analysis for C, H, and N put forward by Takamine, Aldrich, von Fürth, and Pauly with those of his benzoyl derivative, and discussed the differences in the methods of sample preparation and elemental analysis. One cannot help feeling sorry for him, going to such lengths when the vast majority of scientists had already concluded that everything was settled and there was really no need to say anything further on the subject. He really was an earnest scholar and researcher.

2. Expanding fields of research

The discovery that adrenaline was an organic compound built around a skeleton of nine carbon atoms was enormously interesting to physiologists around the world, and it spurred on advances in research in various different specialized fields. Table 8-1 shows the title and year of research reports concerning adrenaline, giving an idea of the development of the research.

In the table, there are 11 reports that used Adrenalin products manufactured by Parke, Davis & Co.; these are marked with an asterisk. In the remaining studies, seven different types of commercially available adrenaline products were used. This shows that a research environment was in place in which it was no longer necessary for researchers to prepare their own extracts in order to carry out their work, and suggests that the speed and accuracy of the research was improving significantly.

Table 8-1. Rapid progress of researches on the action of adrenaline in several countries.

Country	Year	Title of research report
France	**1902**	Poehl, A. de, "Influence des agents de catalyse sur le fonctionnement de l'organisme: spermine, cérébrine et chloradrénal." *Comptes rendus hebdomadaires desséances de l'Académie des sciences*, **135**: 1141–1143 (1902)*****
France	**1902**	Bouchard, Ch. et H. Claude, "Recherches expérimentales sur l'adrénaline." *Comptes rendus hebdomadaires des séances de l'Académie des sciences*, **135**: 928–931 (1902)
Germany	**1902**	Bulm, F., "Weitere Mittheilungen zur Lehre von dem Nebennierendiabetes." *Archiv für die gesammte Physiologie des Menschen und der Thiere*, **90**: 617–629 (1902)
Great Britain	**1903**	Paton, D. N., "On the nature of adrenalin glycosuria." *Journal of Physiology (London)*, **2**(93): 286–301 (1903)*****
Germany	**1903**	Scheidemandel, E., "Über die durch Adrenalininjektionen zu erzeugende Aortenverkalkung der Kaninchen." *Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*, **181**: 363–382 (1903)*****
Austria	**1903**	Exner, A., "Über die durch intraperitoneale Adrenalininjektion verursachte Verzögerung der Resorption von in den Magen eingeführten Giften." *Archiv für Experimentelle Pathologie und Pharmakologie*, **50**: 313–318 (1903)*****

USA	**1903**	Vosburgh, C. H. and A. N. Richards, "An experimental study of the sugar content and extravascular coagulation of the blood after administration of adrenalin." *American Journal of Physiology*, **9**: 35–51 (1903)*****
Germany	**1903**	Aronsohn, Ed., "Die Zuckerausscheidung nach Adrenalin - Injektionen und ihre Beeinflussung druch künstlich erzeugtes Fieber." *Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*, **174**: 383–392 (1903)
Germany	**1904**	Friedmann, E., "Zur Kenntnis des Adrenalins (Suprarenins)." *Beiträge zur chemischen Physiologie und Pathologie*, *Zeitschrift für die gesamte Biochemie*, **6**: 92–93 (1904)
Great Britain	**1904**	Drummond, W. B. and D. N. Paton, "Observations on the influence of adrenalin poisoning on the liver, with special reference to the glycogen." *Journal of Physiology (London)*, **31**: 92–97 (1904)*****
USA	**1905**	Wiggers, C. J., "On the action of adrenalin on the cerebral vessels." *American Journal of Physiology*, **14**: 452–465 (1905)*****
Germany	**1905**	Wolownik-Charkow, B., "Experimentelle Untersuchungen über das Adrenalin." *Archiv für pathologische Anatomie und Physiologie und für klinische Medizin*, **180**: 225–238 (1905)*****
Great Britain	**1905**	Paton, D. N., "The effect of adrenalin on sugar and nitrogen excretion in the urine of birds." *Journal of Physiology (London)*, **32**: 59–64 (1905)*****
Great Britain	**1905**	Dakin, H. D., "On the Physiological Activity of Substances Indirectly Related to Adrenalin." *Proceeding of the Royal Society of London, Ser.B*, **76**: 498–503 (1905)
Great Britain	**1905**	Dakin, H. D., "The synthesis of substances allied to adrenaline." *Proceeding of the Chemical Society, London*, **21**: 154–155 (1905)
Great Britain	**1906**	Dakin, H. D., "The physiological action of synthetical substances allied to adrenalin." *Journal of Physiology (London)*, **32**: xxxiv–xxxvi (1905)
Germany	**1906**	Meyer, O. B., "Über einige Eigenschaften der Gefäßmuskulatur mit besonderer Berücksichtigung der Adrenalinwirkung." Zeitschrift für Biologie, 48: 352–397 (1906)*****
Germany	**1906**	Ehrmann, R., "Zur Physiologie und experimentellen Pathologie der Adrenalinsecretion." *Archiv für Experimentelle Pathologie und Pharmakologie*, 55: 39–46 (1906)
Germany	**1906**	Biberfeld, J., "Pharmakologische Eigenschaften eines synthetisch dargestellen Suprarenins und einiger seiner Derivate." *Medizinische Klinik*, Nr. 45, 1177–1179 (1906)
Germany	**1906**	Rupp, E., "Konstitution und Synthese des Adrenalins." *Apotheker-Zeitung*, **21** (75): 793–794 (1906)
Germany	**1906**	Friedmann, E., "Die Konstitution des Adrenalins." *Beiträge zur chemischen Physiologie und Pathologie, Zeitschrift für die gesamte Biochemie*, **8**: 95–120 (1906)
Great Britain	**1906**	Elliot, T. R. and H. E. Durham, "On subcutaneous injection of adrenalin." *Journal of Physiology (London)*, **34**: 490–498 (1906)*****
Germany	**1907**	Kahn, R. H., "Über die Beeinflussung des Augendruckes durch Extrackte chromaffinen Gewebes (Adrenalin)." *Centralblatt für Pysiologie*, **20**: 33–40 (1907)
Germany	**1907**	Biberfeld, J., "Beiträge zur Lehre von der Diurese. XIII Über die Wirkung des Suprarenins auf die Harnsekretion." *Archiv für die gesammte Physiologie des Menschen und der Thiere*, **119**: 341–358 (1907)
Great Britain	**1907**	Sohn, C. E., "Adrenaline and its synthesis." The *Pharmaceutical Journal*, May18, 1907, pp. 623–624

The mark * indicates the use of Adrenalin, commercial product of Parke, Davis & Co.

The lengthy research report of Oskar B. Meyer of the Physiological Institute of Würzburg University in Germany (8-16) gives the results of experiments using liquid extracted from the Supra-renal Tabloids of Burroughs, Wellcome & Co., summarized into 14 concise items. One of these, *"Eine adrenalinähnliche Substanz findet sich im Blute,"* (an adrenaline-like substance found in the blood) seems to predict the existence of noradrenaline; another one states that atropine, cocaine, and curare all exhibit vessel widening activity of different intensities, and that this action is antagonistic to that of adrenaline.

3. The name "hormone"

In 1902, one year after Takamine first announced the isolation of adrenaline, two British physiologists, William Madock Bayliss (1860–1924) and Ernst Henry Starling (1866–1927), discovered a second hormone through experiments using dogs. The two co-researchers had a family link, as Starling was married to Bayliss' younger sister.

They discovered that if dilute hydrochloric acid is injected into the small intestine of dogs, alkaline pancreatic juice is rapidly secreted to neutralize the acid. This reaction was not, as was thought at the time, a reflex action of the nerves; Bayliss and Starling found that some unknown substance was produced in the mucous membrane of the intestine, which was carried in the blood and stimulated the pancreas. This was the discovery of *secretine*.

It took three years after this until Bayliss and Starling proposed the name "hormone" for physiologically active endocrine substances such as secretine and adrenaline, the latter having already been the subject of a great deal of interest. This new name came from a suggestion by the physiologist William B. Hardy of the University of Cambridge in the UK.

How did Hardy come up with this name? The answer to this can be found in a valuable description left by Joseph Needham, a Cambridge biochemist and distinguished scholar of China (8-17). Needham explains that one day Starling was invited by Hardy to dine at the dining hall of Caius College, Cambridge. The two scientists agreed that a suitable name was needed for the active principles that were secreted into the blood and stimulated other parts of the body. They consulted the classical scholar W. T. Vesey, who came up with the Greek word *ormao*, meaning to "excite" or "arouse." Using this as a base, Starling and Bayliss coined the term "hormone."

4. Synthesis and chemical structure

(1) Structure determination by synthesis

The major German dye and fine chemical manufacturer Hoechst A. G. began marketing the active principle of adrenal glands extracted by von Fürth as a product named "Suprarenin" in 1900. It is not known whether this product guaranteed the same effects, side effects, and stability as the substance isolated by Takamine and Wooyenaka, but it was recommended for use as a hemostatic agent in various different medical settings. Naturally, rival products soon appeared in the market, and one company advertised their product as a cure for rickets and epilepsy (8-18).

Hoechst A. G. followed by starting a project to synthesize adrenaline, led by Friedrich Stolz, Director of the Chemical Division of the company's laboratories. The synthesis research was successful, with three patent applications made between August and December of 1903 (8-19). The first two applications were for methods of synthesizing intermediates, and the third was a process for deriving adrenaline as a final product. The project was managed in a manner befitting a company, with an academic conference held between the second and third patent submissions (8-20).

For the terminology, the company used the chemical name in the text of the patent, and in papers submitted to the academic conference the name "adrenalin" (without the final "e") was used rather than the name of the company's product, suprarenin. A sample of Stolz's synthesized compound was tested for activity by Hans Horst Meyer (1853–1939) and Otto Loewi (1873–1961) of Marburg University, Germany. A sample of the product was displayed and a provisional oral presentation made at the International Congress of Physiology in Brussels, Belgium, on August 30, 1904 (8-21).

A report in the *Pharmazeutische Zeitung* of October 12, 1904 notes that it was announced at the international conference that Roser had successfully synthesized adrenaline at Meyer's suggestion. However, no clues are given regarding any subsequent developments (8-22). Meyer and Loewi published the results of their completed research in an academic journal in 1905, the year after they transferred to the Pharmacology Institute of the University of Vienna (8-23). In this report, they confirm that the synthesized product had the same blood pressure-raising activity as a sample extracted from organs, but found that the efficacy of the synthesized substance was inferior to that of the natural sample.

This was the initial research result for Loewi, who took his first stride as a pharmacologist by becoming Meyer's assistant in 1898, after earning his PhD at Straßburg University.

Loewi subsequently won a joint Nobel Prize in 1936 with Henry Dale, whose achievements were described in Chapter 7, for "their discoveries relating to chemical transmission of nerve impulses" in relation to their work on acetylcholine.

Prior to Stolz's work, Hermann Pauly of the University of Bonn had found in 1903 that samples taken from adrenal glands were optically active. He measured the optical rotation, and found that the substance in the body was optically active, with a specific rotation of −43° (laevorotatory) (See Column 8-1; (8-24)).

Column 8-1.
Optical isomers: The same elemental composition but completely different biological activity.

Rather than being planar, almost all of the organic chemical substances that make up the living body have what is known as stereostructure.

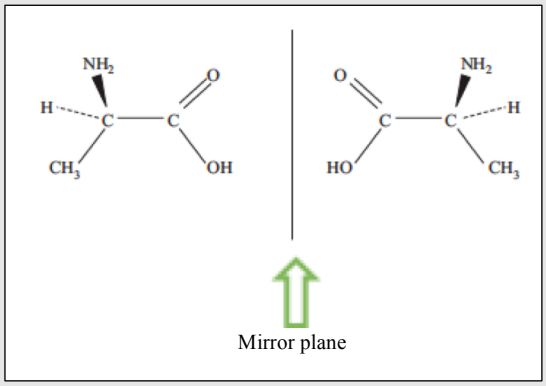
The amino acids that make up proteins and the saccharides that are building blocks for carbohydrates both have spatial structure that cannot be represented by a molecular formula written in planar fashion. The following is a simple explanation of this, illustrated by the example of an amino acid called alanine.

In the diagram, the broken line is a binding bond pointing downward with respect to the plane of the page, and the triangular line is a binding bond angled upward relative to the page. The vertical line in the middle represents a mirror. The molecule on the left is alanine, and the molecule on the right is its mirror image. The elemental composition is exactly the same, but they cannot be superimposed on each other because the binding bonds are in opposite directions. These two molecules are optically different from each other, and are known as "optical isomers." The distinguishing feature of these two molecules is that the four groups (radicals) on the carbon atom to which the -NH_2 is bonded are not arranged in the same way, and carbon atoms such as this are called "asymmetric carbon."

To illustrate this with something more familiar, place the palms of your hands together—the left and right hands seem to have the same shape. However, one hand cannot be superimposed on the other. The palms have the same composition, but cannot be made to match one another. If you put a mirror to your face, you cannot match your own left ear to the left ear of the mirror image. In these two examples, the pairs of bodies are called "enantiomers," or "mirror image isomers."

Molecules with this stereostructure exhibit particular characteristics with regard to light. If light that oscillates in a fixed plane, known as "polarized light", passes through this molecule, the plane of the polarized light is rotated. The angle of this rotation is called the "rotary power" of the molecule. Molecules that rotate this plane to the left are called "laevorotatory" and those rotating light to the right are "dextrorotatory," and are written as laevum (*l*-) and dextrim (*d*-).

In chemical formulae, the angle of this rotation is expressed as "$[\alpha]_D^{23.5} = -43°$", where α is the polarized light angle, 23.5 is the temperature in Celsius at the time of measurement, and D is the wavelength of the polarized light, which is typically referred to as the "sodium D line" and is about 589 nm.



A year later, Hooper Jowett of Wellcome Chemical Laboratories in the UK (where similar studies were being conducted) announced several values for the optical activity (8-25). Given these results, Stolz must surely have considered that the difference in efficacy of the synthesized product and the natural substance might be due to the presence of inactive optical isomers mixed into the synthesized product [Note 8-1].

Note 8-1.
Before joining Hoechst A. G., Friedrich Stolz worked as an assistant to Professor Adolf von Baeyer of München University (and also a teacher at Straßburg University), who was famous for the synthesis of indigo, a deep blue pigment. As well as his historic achievements in the synthesis of adrenaline, Stolz also made a name for himself through the development of aminopyrine (antipyretic and painkiller) and the structure determination of complex compounds (8-26).

One year later than Stolz, H. D. Dakin of the Laboratory of Pathological Chemistry at the Lister Institute of Preventive Medicine in the UK independently achieved success with the synthesis of adrenaline, and confirmed its physiological activity (8-27).

When the German pharmaceutical company Bayer A.G. learned of this activity, it realized it could not afford to delay and immediately set about synthesis research. From 1905 to 1913, Bayer applied for four patents for methods of synthesis (8-28).

In addition, Professor Nagayoshi Nagai of the Department of Pharmacy at Tokyo Imperial University, who had always had an enormous interest in the chemistry of natural products, brought his great stock of knowledge to bear, and in 1917 he applied for a patent for the total synthesis of adrenaline (8-29).

In 1954, the German researcher Hans Loewe marked the 50th anniversary of Stolz's synthesis of adrenaline by compiling a history of the synthesis of adrenaline and its derivatives, over which chemists had been in fierce competition. This was published as a marvelous review that cited over 500 papers (8-30).

(2) Separation of optical isomers

The problem of the different hormonal activity of the two optical isomers had still not been settled with any degree of clarity. J. Bieberfeld of the Pharmacological Institute of Breslau University, which was then part of Germany, compared data on the blood pressure-raising effect resulting from intravenous injection in rabbits and found no difference in efficacy between the synthesized and natural products (8-31).

However, in 1908, A. R. Cushny of the Pharmacological Laboratory of University College,

London, compared both products using dogs, and reported that the synthesized compound showed weaker efficacy. He concluded that the dextrorotatory isomer must be inactive to be consistent with the evidence. He explained that the relationship between the activity of these two optical isomers was the same as the relationship between the natural anticholinergic substances atropine, which is a mixture of equal amounts of dextrorotatory and laevorotatory isomers (a racemic mixture), and hyoscyamine, which is the laevorotatory active principle (8-32, 8-33). Cushny's interpretation of the discrepancy was that the rabbit, which Bieberfeld had used as his experimental animal, might not be suitable as it straightaway showed resistance to adrenaline.

At almost exactly the same time, Stolz and Franz Flächer from Hoechst A. G. co-authored a paper titled "On synthetic suprarenin," describing the difficulty of understanding the scientific and technical reports of activity at that time. They pointed out that when synthetic and natural compounds were being compared, very often the purity of the samples was not made clear, so that ultimately comparisons could not be made (8-34). At that time, optical isomers could only be distinguished by animal experiments, while separation technology had not yet been developed; researchers really were groping around in the darkness. This was a particularly taxing problem, which highly talented people were struggling to overcome. With the experiments available today, a postgraduate student could come up with the perfect solution in just a week or so.

Stolz had successfully carried out the total synthesis of adrenaline, but it pained him that this success did not extend as far as separating and obtaining the active optical isomer. In September 1906, he delivered a lecture to the Assembly of German Scientists and General Practitioners in Stuttgart in which he carefully explained the history of chemistry since Takamine crystallized adrenaline, and he went on to say that the research into synthesis of suprarenin (adrenaline) had not been fully successful as it had not been possible to separate the optical isomers. He noted that overcoming this difficulty would be no easy task because suprarenin did not readily form salts that could be crystallized (8-35).

The reputation of the Hoechst A. G. project team rested on breaking down this obstacle. It was Stolz's subordinate, Franz Flächer, who was able to clear this formidable wall and gave a clear verification of the reason for the low efficacy of synthesized adrenaline in comparison to the natural product. Flächer had studied under Professor Abderhalden at the Chemical Laboratory of the University of Berlin, and he had learned how to separate dextrorotatory and laevorotatory synthetic suprarenin. He joined Hoechst A. G. after graduating, and was part of the suprarenin project carried out by the chemical division of the company's

laboratories.

Flächer first tried the methods pioneered by the French chemist and microbiologist Louis Pasteur. Pasteur carried out detailed research into tartaric acid, the main component of the tartar found at the bottom of wine casks. He developed his experimental methods, which are of great historical importance, when he discovered the existence of optical isomers, which are responsible for the stereostructure of some organic chemicals, in 1848. Pasteur's first method was to use microbes to decompose one isomer by digestion while leaving the other isomer. However, Flächer was unable to achieve this goal because the fungus he used was weak, and simply died out. He had a stroke of luck, though—during the course of the experiments, he discovered that the active principle extracted from the adrenal glands readily combined with tartaric acid to form a salt. Having made this discovery, Flächer could use Pasteur's second method. This was to form a salt by reacting the compound with optically active tartaric acid, and then optically resolving the isomers by making use of differences in the crystallizing properties (solubility) of their respective salts.

Flächer discovered that the *d*-form of tartaric acid readily formed a salt with active adrenaline that was less soluble in methanol and crystallized more readily than the salt formed with inactive adrenaline. This was an elegant crystallizing resolution of the *d*-form and the *l*-form. He was able to report that the rotation value of the *l*-form of adrenaline, which had the same physiological activity as natural adrenaline, was $[\alpha]_D^{20} = -50.40^{\circ}$, while the rotation value of the inactive *d*-form was $[\alpha]_D^{19.8} = +51.88°$. His report appeared in an academic journal in 1908 and is a glittering page in the history of the pharmaceutical industry (8-36), alongside the achievements of Stolz, who succeeded in the total synthesis of adrenaline. Hoechst A. G. was now able to market a synthetic product with the same activity as naturally-occurring adrenaline.

While the development of a process for the artificial manufacture of a material identical to the active principle by the two men from Hoechst A. G. is engraved in history, it is interesting to note that Flächer's teacher, Abderhalden, was vigorously working on this subject at about the same time.

On August 26, 1908, three months earlier than Flächer's report, Abderhalden and Markus Guggenheim jointly presented a paper at an academic conference in which they stated that the rotation value $[\alpha]_D^{20}$ of the *l*-form of adrenaline was –50.72° and of the *d*-form was +50.49° (8-37).

Just 72 days after this, a report co-authored by Abderhalden, this time working with Franz Müller, arrived at the office of the academic society (8-38). This report gave the results of

research into the blood pressure-raising effects of intravenous injection of the *l*-form, the *d*-form, and a mixture of equal quantities of both, *dl*-form. It was just 20 days after this that the journal received Flächer's historic paper (8-36).

Thus in one year, 1908, these three papers appeared one after another in the same highly reputed German journal of physiological chemistry. Normally, the credit for the "optical resolution" of adrenaline should have gone to Abderhalden, the teacher who described the rotation values of the *d*-form and the *l*-form, as a matter of course. However, in the paper he co-authored with Guggenheim he noted in the margin that the details of the method for resolution of the *l*-form and *d*-form would soon be announced, and he made absolutely no mention of the experimental method or the result. If Abderhalden and Flächer had been unconnected rivals this would probably have led to a dispute, but Abderhalden showed himself to be a true teacher in the second paper he co-authored, in which he recognized his pupil's achievements by saying that Flächer of Hoechst A. G. had recently succeeded in separating the *l*-form and the *d*-form, with good yields of both. He also wrote that he had obtained the experimental sample of synthetic suprarenin through the kindness of Dr. Ammelburg of Hoechst A. G.

Abderhalden next threw himself wholeheartedly into comparative research of the physiological action of the *d*-form and the *l*-form, which are the optical isomers of adrenaline, and he published four papers in a row in the same academic journal in 1909. Abderhalden mainly used frogs, rabbits, dogs and mice as experimental animals, and measured physiological reactions including pupil widening, volume of urine, nitrogen content of urine, sugar concentration in urine, body weight change, and minute-by-minute body temperature. In particular, he made an exhaustive investigation into whether the inactive *d*-form changed to show activity in the body, and whether the activity of the *l*-form grew weaker. From his results, he concluded that the *d*-form was completely inactive (8-39 through 8-42).

As the efficacy of the synthetic product was weaker than that of the natural product, Cushny, working in London, had concluded that the only explanation was that the *d*-form was inactive. He was subsequently provided with pure samples of the *l*-form and the *d*-form by Hoechst A. G., and carried out research into the physiological action at the same time as Abderhalden's group, later reporting his results. Cushny explained clearly that (1) the blood pressure-raising effect of the *l*-form is 12–15 times higher than that of the *d*-form, (2) the activity of the *l*-form to cause glycosuria is 12–18 times higher, (3) the toxicity of the *l*-form is stronger in about the same proportion, and (4) there is no evidence for a point of action other than the receptor of the sympathetic myoneural junction (8-43).

In Chapter 4, we saw that Wooyenaka recalled receiving a letter of thanks from someone he called "Furehitah," who was von Fürth's assistant or co-researcher. This person had been provided with a sample of adrenalin crystals, and intended to carry out an elemental analysis and then attempt to synthesize the substance (8-44). At that time, Hoechst A. G. was working on the commercialization of suprarenin, so it is likely that von Fürth was working closely with Flächer—this suggests that Wooyenaka's "Furehitah" may perhaps have been Flächer. In a different interview, Wooyenaka is recorded as referring to Abel as "'Eberusu' in America," and to Vulpian as "Warupian," so it is quite possible that he also mispronounced Flächer, or that the interviewer misheard him (8-45).

As the active *l*-adrenaline is unstable in air, it is currently sold as the stable tartaric acid salt of the *l*-form [Figure 8-1]. When the pure *l*-form is needed, it is liberated from the salt and prepared, and then used straightaway in experiments.

Figure 8-1. Crystals of adrenaline tartrate.

(3) Decomposition and synthesis in the body

Von Fürth, who had played a major part in the isolation of the adrenal medulla active principle, published a paper titled "Über die Zerstörung des Suprarenins (Adrenalins) im Organismus (About the decomposition of suprarenin (adrenaline) in the organism)" in 1904 (8-46). Since Oliver and Schäfer made their discovery, many physiologists had speculated on the reason why blood pressure rose immediately after injection of adrenaline, but this effect was short-lived.

In response to von Fürth's report, a report of research into the same theme was published by the Physiological Institute of Königsberg University in what was then Preußen (present-day Kaliningrad in Russia) (8-47). The authors stated in a footnote that von Fürth had used suprarenin, and they had used adrenalin chloride solution from Parke, Davis & Co. While it seems a little unfair to compare the content of these papers with research that a present-day postgraduate student could finish off in a week using the radioisotope labeling method, the research was of a level that would scarcely seem worth introducing. However, I mention them because the authors were ardently investigating the action and behavior of adrenaline one after another.

In 1906, two years after the chemical structure of adrenaline was confirmed by Stolz's total synthesis, a researcher rose to prominence with his investigation of how adrenaline was manufactured within the body. This was Walter L. Halle of the Laboratory of L. Spiegler-Stiftung in Vienna—he had been taught by Fränkel of the University-Institute for Medical Chemistry, whom we met in Chapter 4. Halle hypothesized that the synthesis pathway was a process like this: (1) phenylalanine → (2) tyrosine → (3) DOPA → (4) dopamine → (5) NH_2-methylated dopamine → hydroxylated adrenaline (8-48) [Note 8-2]. Halle's estimated pathway of biosynthesis was correct as far as step (4). We now know that in this pathway, step (4) is initially hydroxylated to become noradrenaline, and finally an amino group is methylated to form adrenaline.

Note 8-2.
To investigate this hypothesis, Halle added tyrosine [(2) in the above passway] to ground pig or cattle adrenal glands, and left it to stand for seven days at 37ºC. He then compared the adrenaline content of the liquid with a similar preparation to which tyrosine had not been added, and his results showed a 14% to 33% increase in adrenaline in the samples with added tyrosine.

Noradrenaline, an extremely important sympathomimetic compound, finally makes its entrance. Halle's research results were published just two years after the structure of adrenaline was confirmed, and two years before the separation of optical isomers was announced. The paper is testament to the level of chemistry and biochemistry in the German language sphere at that time. However, it was to take over 30 more years of continuous hard work by researchers until the biosynthetic pathway was established.

(4) Looking back over 100 years of research

We have followed the unbroken tracks of adrenaline researchers up until the present stage in this story, and finally, in order to give an overview of the research, the scientific literature cited in a review published to mark the 50th anniversary of the synthesis of adrenaline (8-30) is presented in a table that groups the references together chronologically [Table 8-2]. It can be seen that research forged ahead once crystallized adrenaline became readily available, but then there was a period of calm during the 1910s. Seventy percent of all the research results that were published over the full 98 years appeared during the 20-year period from 1920, after which there was a transition to a period in which the fire appeared to be quenched.

Table 8-2. Literature appeared within ca. 100 years since the discovery of adrenal medulla hormone.

Lit. appeared between	Years	Number of literature	Reference
1856–1899	44	19（4.3%）	1856：Discovery of adrenaline 1893：Discovery of its blood pressure raising activity
1900–1909	10	65（14.8%）	1900：Crystallization of adrenaline 1903：Total synthesis of adrenaline 1908：Optical resolution of adrenaline isomers
1910–1919	10	24（5.5%）	Researches on physiological activity
1920–1929	10	183（41.6%）	Investigation on nerve transmitters
1930–1939	10	133（30.2%）	1936：Nobel prizes to H. Dale and O. Loewi
1940–1953	14	6（1.4%）	1943–46：Proof of the presence of noradrenaline
1856–1953	Total 98	430（100%）	—

Table 8-2 was prepared by the literature cited (8-30).

5. Discovery of noradrenaline, a sympathomimetic compound

The idea that nerve impulse transmission takes place via a body fluid began with Thomas R. Elliott. In 1904–1905, when he was a student at the University of Cambridge and still only in his late 20s, Elliott put forward the outstanding idea that sympathetic nerve cells release a chemical substance from their endings, and that this substance stimulates effector cells to start their action within the body. Elliott thought this substance was adrenaline, and the actual substance was not discovered for a long time (8-49, 8-50). He used the name "adrenalin," without the final "e," for the active principle in both his papers, which had to do with the naming problem described in Chapter 7.

The Nobel Prize winner H. Dale later described in a Sharpey-Schäfer Memorial Lecture titled "Natural Chemical Stimulators" how Elliott worked feverishly on his research after he obtained some of the adrenalin crystals produced by Parke, Davis & Co. (8-51). Dale delivered this lecture at the University of Edinburgh in November 1937, the year after he was awarded the Nobel Prize. However, five years later, there was a surprising announcement; the substance transmitting nerve impulses did not appear to be adrenaline. G. Barger and H. Dale had been unable to confirm that adrenaline was the impulse transmitter of sympathetic nerves, and they stated their view that there was a greater probability that other types of amines showed sympathomimetic ("simulating the effects of the sympathetic nerves") action than adrenaline (8-52).

G. Barger and H. Dale's research, titled "Chemical structure and sympathomimetic action of amines," was reported by the Wellcome Physiological Laboratory in London. In the text, it states clearly that Barger and A. J. Ewins, who appeared to be Barger's helper, were

entirely responsible for the chemical side of the investigation, while Dale was responsible for the physiological experiments. In all, 54 widely differing amines, including adrenaline and noradrenaline, were gathered for thorough testing of their activity. Research of this magnitude was typically carried out only by companies, and it is likely that the aim was not purely academic, but also to search for amines that could be profitable as drugs.

Barger co-authored a paper with H. Jowett of the Wellcome Chemical Laboratory in 1905 (8-53), so he may have transferred to the Physiological Laboratory and taken charge of the compound after all the fuss of the heated exchanges between H. Dale and the owner of the company, H. S. Wellcome, over the use of the name adrenaline had died down. In this paper, Barger and Dale deal with the discovery of the structure-activity relationship in detail. Amongst others, the paper states that catechols have no activity (indicating the need for an amine group), and catechols with a methylamino group (adrenaline, etc.) have an inhibitory action on the sympathetic nervous system, whereas catechols with an amino group that has no methyl group (a primary amine) have the opposite effects. Noradrenaline is a typical primary amine, and von Euler praised this discovery in a review published 56 years later (8-54), stating that this paper gave "the first hints as to its relationship with the sympathetic nervous system" [Note 8-3].

Note 8-3.
Ulf von Euler was born into a scientific family. His father, German-born Hans von Euler-Chelpin, won a Nobel Prize in Chemistry in 1929, which he shared with Arthur Harden of the UK, for his work on "Investigations on the fermentation of sugar and fermentative enzymes." His mother was a teacher of chemistry, botany, and geology.

Ulf's parents divorced after 10 years of marriage. His maternal grand-father, Per Teodor Cleve, discovered the two rare earth metal elements Thulium (Tm) and Holmium (Ho).

Dale had carried out a wide range of research of considerable depth, including the job mentioned above, after the physiological and pharmaceutical research into ergot of rye using adrenaline that led to the clash with his boss, the owner of the company. In 1936 Dale was awarded the Nobel Prize jointly with the German researcher Otto Loewi for "their discoveries relating to chemical transmission of nerve impulses." For Loewi, the Nobel Prize was an acknowledgement of his diligent work since confirming that Stolz's synthetic suprarenin was less efficacious than samples extracted from organs when working at Hans Meyer's laboratory at the University of Marburg.

Returning to nerve impulse transmission, the momentous explanation given by Barger and Dale subsequently received the continued support of a great many researchers, but eventually, in 1933, another researcher seemed even closer to unlocking the door of the mystery. This was Heinz Schild, who was working at the National Institute for Medical Research in the London suburb of Hampstead. Dale had been the first director of this institute.

Schild very carefully conducted bioassays using cats, and he observed that the blood pressure-raising effect of suprarenal extract was stronger than would have been expected from the adrenaline content of the extract. However, even with the chance to discover an active substance other than adrenaline (noradrenaline) in front of him, he could not open the door. His paper was an investigation of papers about quantification methods mainly using color reactions that had been developed over about 20 years since 1912. The paper examined the work of some 25 authors, and showed the difficult struggle of researchers before chromatography came into practical use (8-55).

At around this time, a three-sided research race developed. This was a fierce competition between the research group led by Walter B. Cannon of the Laboratory of Physiology of Harvard Medical School, the group led by Peter Holtz, Professor of Pharmacology of the Physiologisch-chemischen Institut and the Pharmakologischen Institut of the Universität Rostock in Germany, and the group led by Ulf von Euler of the Karolinska Institutet in Stockholm, Sweden.

First, Cannon co-authored a paper in 1931 with Z. M. Bacq, who had gone to Harvard from Liége in Belgium to study, titled "A hormone produced by sympathetic action on smooth muscle." In this paper he announced a previously unknown hormone, for which he proposed the name "sympathin" (8-56). Two years later, Cannon co-authored a paper with A. Rosenblueth, in which he reported the existence of two types of sympathin, which he distinguished as sympathin E and sympathin I (8-57).

Bacq returned to Liége, where he continued his work at the Institut L. Fredericq. The following year he announced that sympathin I was adrenaline, which had inhibitory properties, and sympathin E was noradrenaline (8-58).

The following year, Cannon again co-authored a paper with Rosenblueth, this time describing detailed research into the effect of sympathin and adrenaline on the iris. In the experiments they write "adrenine," but they clearly specify that they used commercial adrenalin supplied by Parke, Davis & Co. They write that they used sympathin that they obtained by stimulating cardio-accelerator and liver nerves, but the chemical identity in this substance was not clear. This shows the difficulty of research at that time (8-59).

Bacq's hypothesis that sympathin E was noradrenaline was carefully re-examined by a four-person research group headed by C. M. Greer at the prestigious Vanderbilt University School of Medicine in Nashville, Tennessee. They presented hard evidence to show that consistency emerged if the transmitter for at least one type of sympathomimetic effect was assumed to be noradrenaline (8-60).

The following year, Cannon presented a paper titled "Evidence for Adrenaline in Adrenergic Neurons" at an academic conference. Although he showed that adrenaline and sympathin were not the same, he struggled unsuccessfully to specify the nature of sympathin (8-61).

At exactly the same time, Ulf von Euler, who later garnered a Nobel Prize for his flawless work in this field, was investigating sympathin. However, his group failed to notice noradrenaline—this was because for their experiments they were using rabbits, the adrenal glands of which contain practically no noradrenaline (8-54). Similarly, O. Loewi (whom we met on page 161) discovered the presence of adrenaline in the heart of frogs, and asserted that it was a sympathetic nerve impulse transmitter in 1937. However, the reason for this discovery was that frogs are exceptional in that they only have adrenaline in the adrenergic nerve system (8-62).

Von Euler later wrote, "It is of interest to note that in the thirties both Stehle and Ellsworth in Canada and Tiffeneau in France commented upon the quirk of nature which made use of a compound like E when the non-methylated compound seemed to serve the purpose better and was chemically simpler" (8-54). This comment serves to highlight the troubles of the researchers of this age before the development of isolation technology.

Then a rapid succession of reports that marked a turning point in the history of adrenaline appeared. The first came from W. Raab, of the Departments of Biochemistry and Medicine, University of Vermont. He published a paper titled "Adrenaline and Related Substances in Blood and Tissues", in which he announced that the sympathetic nerve impulse transmitter was not adrenaline but a substance containing a catechol group that was found in the extract of the spleen (8-63).

The next important research results to appear were in a paper by Holtz and his associates titled "Über das sympathicomimetische pressorische Prinzip des Harns ("Urosympathin") (About the sympathomimetic pressor principle of urine ("Urosympathin"))" in which they demonstrated the existence of noradrenaline in the adrenal glands and the normal urine of cats (8-64) [Note 8-4].

> **Note 8-4.**
> Strangely, although the academic journal in which this paper appears records that it received the manuscript on October 8, 1944, the paper was not actually published until three years later, in 1947. From then onward, Holtz and his team worked feverishly, publishing 17 papers over the course of five years (8-30).

Two years later, in 1946, the decisive blow was finally delivered. Using biological methods, von Euler proved the existence of a substance extremely similar to noradrenaline in fresh extracts of bull and cow spleen and bull, horse, and cat heart, as well as in the

sympathetic nerve chain (8-65). This discovery marked a turning point in the history of research into natural catechol amines, and as a result the value of the observations of Barger and Dale mentioned earlier (8-52) came to be acknowledged.

Von Euler followed this up in 1948 with a definitive paper with the long title, "Identification of the Sympathomimetic Ergone in Adrenergic Nerves of Cattle (Sympathin N) with Laevo-Noradrenaline" (8-66).

That same year, W. O. James of the Department of Botany of Oxford University proved that the only catechol amines present in the normal adrenal gland were adrenaline and noradrenaline (8-67). He separated these two catechol amines for the first time in 1948, using paper chromatography.

Von Euler worked for many years on the quantitative separation of adrenaline and noradrenaline using biological tests, which are difficult methods. In 1949, he co-authored a paper with Ulla Hamberg in which they announced the separation of the adrenal principles of cattle by paper chromatography. The separation can be vividly seen in a figure of their paper (8-68).

We have already seen in Chapter 4 how Mikhail Tsvet of Imperial Russia invented the technique of chromatography as a method for separating plant pigments in1900, the same year that Wooyenaka obtained adrenaline crystals. Half a century later, a researcher of botany, the same field as Tsvet worked in, used chromatography to clearly and unquestionably separate adrenaline and noradrenaline, proving beyond doubt that the crystals obtained by Wooyenaka had been mixed with noradrenaline. History goes around in strange ways.

Von Euler went on to develop an extremely elaborate biological test method, and using this he conducted a quantitative analysis of the adrenaline and noradrenaline within the spleen and its nerves. He reported that of the total catechol amines, adrenaline accounted for 7.2% in spleen extracts and just 2.2% in spleen nerves; noradrenaline accounted for most of the catechol amine content, and a part of it was methylated adrenaline (8-69) [Figure 8-2]. The same year, the results of measurement of noradrenaline in organs and pharmacopoeia products were reported by both universities and companies (8-70, 8-71).

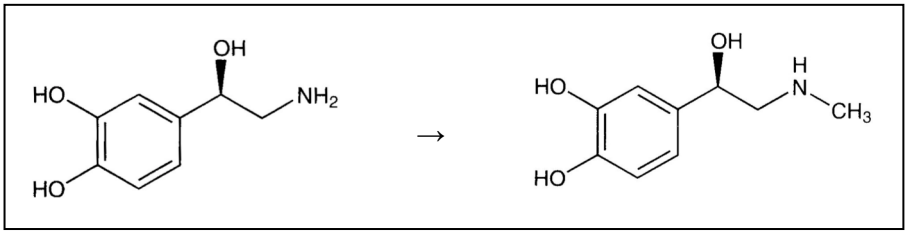


Figure 8-2. noradrenaline → adrenaline
(primary amine) → (secondary amine)
(NH_2 methylated *in vivo*)

6. More Nobel Prizes

Most of the research by von Euler described above was built around extremely detailed tests using animals, and was carried out at a research laboratory with absolutely none of the chemical isolation and analysis techniques such as chromatography and spectroscopy. In 1951, he wrote a detailed review paper in which he cited 243 research works (8-72). Von Euler was honored for these achievements with a Nobel Prize in 1970, which he received jointly with Julius Axelrod of the US and Bernard Katz of the UK.

In a paper he compiled in 1966, titled "Twenty Years of Noradrenaline," von Euler gives an extremely interesting and easy to understand overview (8-54). He starts by commenting humbly that the work of his research group was accepted only because of the outstanding work of the other two research groups mentioned earlier, i.e. W.B. Cannon's Harvard group and P. Holtz's Rostock group, and of many other researchers as well; von Euler was a very modest man.

Julius Axelrod, the joint prizewinner, was the child of Polish Jews that emigrated from Poland to the US. His Nobel Lecture entitled, "Noradrenaline: Fate and Control of its Biosynthesis," which he delivered on December 21, 1970, was a very well explained account. It was striking that he, an American researcher, did not use the name "norepinephrine," which appeared in the *US Pharmacopeia*, but instead announced the name "noradrenaline" to the world in his acceptance speech.

The third prizewinner, Bernard Katz, was born to a Jewish family in Leipzig, Germany, and at the age of 24 he fled from the Nazis, going into exile in the UK. He won the prize for his huge achievements in research into the mode of action of transmitters in synapses, and his Nobel Lecture was titled "On the Quantal Mechanism of Neural Transmitter Release." Katz was knighted in his later life.

The development of analytic methods gradually revealed some very interesting facts. I will give two examples of those here. The first of these is the data obtained by four researchers at the Department of Medicine and Biochemistry, College of Physicians and Surgeons, Columbia University and Presbyterian Hospital, New York, who were using paper chromatography (Table 8-3) (8-73). It is astonishing first of all to see that the composition of the *US Pharmacopeia* reference standard samples and commercially available *U.S.P.* epinephrine were not consistent. It is also surprising to realize that when this study was conducted in 1949, after World War II, there was still only this level of pharmaceutical technology and regulation. The research also showed that pheochromocytoma (a type of cancer) cells contain abundant noradrenaline.

Table 8-3. Abundance ratio of adrenal medulla principles in pharmaceutical products and cancer cells (8-73).

Exp. No.	Sample	Epinephrine %	Norepinephrine %
1	*U.S.P.* reference standard sample 1	81.5	18.5
2	*U.S.P.* reference standard sample 2	64	36
3	*U.S.P.* reference standard sample 3	84	16
4	Commercially available *U.S.P.* epinephrine sample 1	88	12
5	Commercially available *U.S.P.* epinephrine sample 2	100	0
6	Pheochromocytoma sample 1	47	53
7	Pheochromocytoma sample 2	12	88
8	Pheochromocytoma sample 3	10	90

*Each value represents at least 5 parallel determinations (concordant within 10%)

The second fact to emerge is that the ratio of the two active principles to each other varies greatly depending on the species of animal. This was shown by data from a literature review carried out by G. B. West of the Department of Pharmacology and Therapeutics, Queen's College, Dundee, Scotland in 1955, which are shown in Table 8-4 (8-74). In 1856, Vulpian found that the adrenaline color reaction occurred in the adrenal medulla of 14 animal species, and I simply assumed that the amount would be more or less the same in all mammals. West's findings were a revelation.

Table 8-4. The amount of adrenaline and noradrenaline in the suprarenal glands of various adult animals (8-74)

Species	Adrenaline (mg/g)	Noradrenaline (mg/g)	Noradrenaline %
Whale	0.15	1.50	91
Fowl	2.02	8.08	80
Dogfish	0.90	2.40	73
Lion	0.20	0.30	60
Cat	0.60	0.37	38
Sheep	0.50	0.25	33
Dog	1.16	0.40	26
Ox	1.20	0.42	26
Mouse	0.75	0.25	25
Man	0.52	0.10	16
Rat	0.91	0.10	9
Rabbit	0.48	trace	2
Guinea-pig	0.21	trace	2
Baboon	0.83	0	0

I am unaware of any record of how much noradrenaline was contained in the Solution Adrenalin Chloride that Parke, Davis & Co. produced by extraction from bovine adrenal glands, but going on the data in the above table for the ox, one can conjecture that there must have been sufficient quantity to have a significant effect on the activity of the product.

If adrenal glands from different animal species were used, the risk of different compositions would have made it impossible to guarantee efficacy or side effects, and even with glands from the same species, differences due to sex and age would have had to be taken into account.

This is the difficulty of animal-based medicines. Even after it became possible to prepare adrenaline cheaply through synthesis, Parke, Davis & Co. hesitated for a long time to make the change from a preparation of animal extracts to a synthetic product; this was presumably because the company would be unable to avoid deviation in the efficacy that it had guaranteed for many years through activity tests using preparations that contained noradrenaline (8-75).

While it is a digression, von Euler referred to a very interesting fact in relation to this. Dr. Goodall, who studied under von Euler at the Karolinska Institutet in Stockholm in around 1950, had a private plane and flew to Africa to collect and analyze the adrenal glands of various different animal species. The purpose of this was apparently to test Goodall's hypothesis that predators have relatively higher levels of noradrenaline and prey species have relatively higher levels of adrenaline, which strongly affects their decision to flee. Some researchers are born into great wealth (8-54).

The natural world is full of mysteries, and one such mystery is the existence of noradrenaline in plants. This discovery was made through joint research between L. J. Haynes and K. E. Magnus of the Department of Chemistry at the University College of The West Indies, Jamaica, and P. C. Feng from the Department of Pharmacology at the same university. They discovered that purslane (*Portulaca oleracea* L.; Figure 8-3), a species of plant that is believed in Jamaican folklore to have therapeutic effects for cardiovascular disease, contains abundant noradrenaline. They reported that the noradrenaline content may be 2.5 mg per 1 g fresh weight of plant (0.25%), which is more than the content of animal adrenal glands. They also reported the presence of noradrenaline in different banana and potato varieties (8-76).

Figure 8-3. *Portulaca oleracea* L contains noradrenaline in high concentrations. (photo taken by the author in Yokohama, Japan)

The first person to synthesize noradrenaline was Friedrich Stolz, the same German researcher of Hoechst A.G. who first synthesized adrenaline. However, he did not synthesize it out of any awareness that it was a compound with important physiological properties. During the course of his research, he was experimenting with a compound that could be reduced to form adrenaline—in other words, a precursor. He treated this with liquid ammonia, and wrote down that the compound that was created was a "free base." He then performed an elemental analysis twice and wrote the values he found against the theoretical values, and these results were published in a paper in 1904 (8-20). The molecular formula shown in the paper has one carbon atom in excess, and even my calculations show that there is clearly a mistake. The numerical values have the low level of accuracy of the time, but looking at the chemical reaction process, later researchers have recognized that the compound was the first synthesized noradrenaline (8-30, 8-54). Hoechst A. G. gave this the trademark "Arterenol," and there have been a few papers here and there that have used this name (e.g., 8-70, 8-71).

Noradrenaline is optically active, as is to be expected in the natural world, and laevorotatory *l*-noradrenaline is the physiologically active isomer. Stolz synthesized a dextro and laevorotatory mixture (called a "racemate"), and this was separated into dextro and laevorotatory forms by B. F. Tullar and his colleagues at the Sterling-Winthrop Research Institute, an American pharmaceutical company, 40 years after Flächer's optical resolution of adrenaline (8-77, 8-78). Some 10 years later, the three-dimensional structure of active noradrenaline was clarified by P. Pratesi and associates at the Istituto di Chimica Farmaceutica, Università di Pavia, Italy (8-79).

In the discussion above, we have followed the struggles of many physiologists and chemists after the crystallization of adrenaline. After von Euler showed the importance of the action of noradrenaline, the field of research expanded exponentially, and it saw astounding development during the 20 years between 1940 and 1960.

J. Malmejac of the Faculty of Medicine of the University of Paris gives an overview of this development in a long review paper titled "Activity of the Adrenal medulla and its Regulation," which was published in English in an American journal in 1964 and which cites 365 different works, including 76 of the author's own (8-80).

7. New medicines developed by chemical structure modification

Finally, we come to the invention of the fourth man to win a Nobel Prize in 1988 after Dale, Loewi, and von Euler. This was the Scottish chemist and physiologist Sir James Whyte

Black (1924-2010), who was honored for work that is monumental in the history of the quest for β-blockers (beta-adrenoreceptor antagonists) (8-81).

Until Black's research, all work in this field had been groping in the dark. Simply put, his achievement was the invention of a miracle drug for *angina pectoris*, which is a difficult problem in the field of cardiology. Black developed a new drug by modifying the structure of adrenaline, and won a Nobel Prize in Physiology or Medicine in 1988. While it gets a little technical, I have shown a group of chemical structures side-by-side, as it may be of interest to readers to have a visual image of the concepts that medical and pharmaceutical researchers pursue and the process of trial and error they use.

As the chemical structures in Figure 8-4 show, by just a small structural modification (technically, by inserting CH_2O), Black arrived at propranolol (V) from pronethanol (IV), which he had previously invented. Pronethanol had a fatal flaw as a drug candidate—it was carcinogenic. Black's new invention, propranolol, successfully eliminated the carcinogenic properties to yield a safe, new drug.

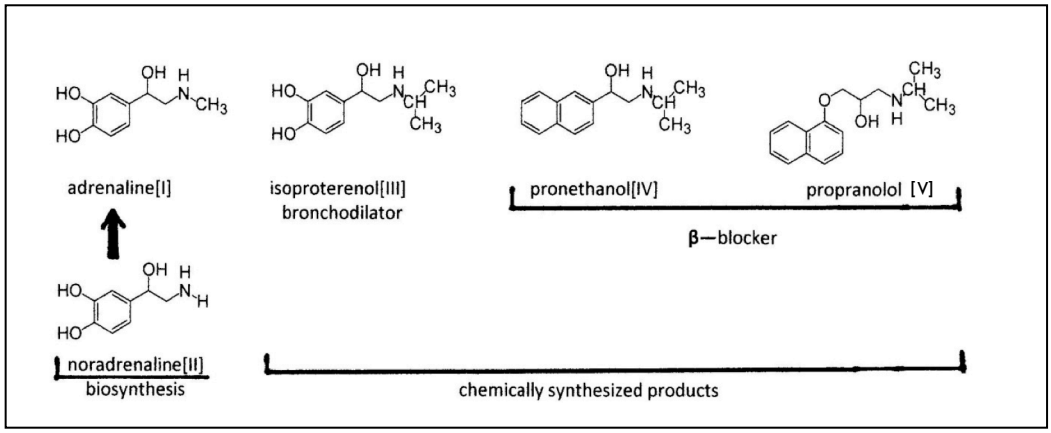


Figure 8-4. Invention of new medicines by chemical structure modification.

Propranolol is a β-blocker, and as well as the curative effect on *angina pectoris*, it acts to lessen the impact of bad memories if taken immediately after recollection of a trauma. It is also taken by performers to lessen the effects of stage fright. Black was awarded the Nobel Prize in Physiology or Medicine in 1988 together with Gertrude B. Elion and George H. Hitchings for their discoveries of important principles for drug treatment [Note 8-5].

Note 8-5.
Black studied medicine at the physiology department of the University of St. Andrews in Scotland before beginning his research. He worked for a time as a teacher at the University of Malaya in Kuala Lumpur, and then returned to Scotland, where he was instrumental in establishing the Physiology Department at the University of Glasgow. Black was a highly talented man, who worked for ICI (ICI Pharmaceuticals),

SKF (Smith, Kline and French), and Wellcome (Wellcome Research Laboratories), three major pharmaceutical companies, before accepting a post as professor of pharmacology at the University of London and King's College.

8. Later information on the two American scientists

(1) Aldrich

Aldrich (see Figure 4-4 in Chapter 4) made a glorious name for himself as a chemist by isolating crystals of adrenaline, somewhat later than Takamine but independently, and by deducing the correct molecular formula. But what did he work on after that? I searched the scientific literature to find out.

Some of his subsequent research was in fields other than constituents of the living body. In 1911, he carried out research into methods of manufacturing a drug named brometone [$(CH3)_2C(OH)CBr_3$] (8-82); in 1912, he carried out an analysis of the iodine content of cattle thyroid (8-83); and in 1915, he worked on the trial manufacture of an experimental apparatus (8-84). However, his main work was with hormones. First, in 1912 Aldrich published a report on the development of the anterior pituitary (adenohypophysis) in puppies (8-85), and then from 1915 he published preliminary research reports relating to the posterior pituitary (8-86).

Later, in 1928, he published his findings as one of the five members of the pituitary hormone project of Parke, Davis & Co. This was a groundbreaking work that separated and identified the two physiologically active substances secreted from the posterior pituitary: vasopressin (the blood pressure-raising constituent) and oxytocin (the mammalian neurohypophysical hormone). These two substances are both peptides comprising nine amino acids (Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2) and have a single ring structure. They also have the same terminal structure which determines their reactivity. Given the separation technology that was available at the time, it was no easy task for the group to arrive at their results (8-87).

(2) Houghton

Dr. Houghton, who directed the research group of Parke, Davis & Co. that tested the activity of the adrenal principles, was born in New York in 1867, and entered the University of Michigan at the age of 22. He acquired his PhD in medicinal chemistry and medical science in 1893, and was welcomed into Parke, Davis & Co. two years later.

On March 24, 1904, the German medical scientist Paul Ehrlich, who was visiting the US,

was invited to give a lecture at Parke, Davis & Co. Ehrlich was a towering presence who had established the chemistry of immunization; he later received the Nobel Prize in Physiology or Medicine in 1908 and had been respected as "a researcher for life" for perfecting the technology to produce diphtheria therapeutic serum. Parke, Davis & Co. was the first company in the US to start production and sales of diphtheria therapeutic serum, and Houghton, who was involved in this project, was probably on good terms with Ehrlich.

Ehrlich had been a colleague of the Japanese bacteriologist Shibasaburo Kitasato during the decade that was later known as the "golden 1880s"; the two had established the theory of the antigen (toxin)-antibody reaction at the same time in the Koch laboratory in Berlin. Houghton may perhaps have met Kitasato when the latter visited the US in 1904 at Takamine's invitation. Takamine acted as Kitasato's guide in the US, and there is a photograph still in existence that shows them sailing on Lake Michigan in a yacht owned by a director of Parke, Davis & Co. (8-88).

Houghton took up residence in 1905 in a mansion at 680 Longfellow in the Boston-Edison district of Detroit, where the great automobile pioneer Henry Ford lived. Boston-Edison, which is in the heart of the city, was home to a great many celebrities. The mansion is renovated following colonial time architectural style. Today it is designated as an historical landmark. Houghton passed away in 1937 (8-89).

9. A ring connecting Nagai, Takamine, and Wooyenaka

We have already seen how Wooyenaka, who was the first person to obtain adrenaline crystals, thus energizing hormone science, joined a non-regular course at the Pharmaceutical Department of Tokyo Imperial University. There, he diligently studied the chemistry of natural substances under Professor Nagai. Nagai became well known around the world for his research into the alkaloid ephedrine, which was the active principle of *mahuang* (*Ephedra sinica*), a Chinese medicine. The research had been completed and a report published in an academic journal by the time Wooyenaka joined Nagai's course, so Wooyenaka did not have any direct involvement in this job. However, it is likely that he learned about the extraction and purification of ephedrine and carried it out himself, as part of the course. Even if that were not the case, he would have listened carefully to the details at every opportunity, as an example of handling natural products. Later in his life, Wooyenaka stated that he remembered the teaching of Professor Nagai as being the key to his own success (see Chapter 5, section 10).

The results obtained by Wooyenaka led to rapid development of the study and application

of hormones. One outcome was a report in 1903 by two New York doctors, Jesse Bullowa and David Kaplan, that subcutaneous injection of adrenaline chloride was very effective against asthma. This led to the spread of adrenaline injections as a treatment for asthma (8-90, 8-91). In fact, the statement of efficacy of adrenaline chloride solution from 1905, in the initial stage of sales in the US, states that its primary indication is "for asthma."

We have already seen in Chapter 3 how in 1897, the American Solis-Cohen brought about a dramatic recovery in a 22-year-old woman who was suffering respiratory difficulty by administering Burroughs & Wellcome's Supra-renal Tablets, and he published a paper on the efficacy of adrenal substance against asthma. The news that the product of Parke, Davis & Co., which was of consistent quality, was effective must have been welcomed by respiratory doctors.

Some 10 years after adrenaline was reported to be highly effective for asthma, two researchers began a full study of the medicinal action of ephedrine, which has a similar chemical structure [Figure 8-5]. These were Hajime Amatsu and Seiko Kubota, assistants at the Medicinal Science Laboratories of Kyoto Medical College, Kyoto, Japan (8-92, 8-93). They published two research reports demonstrating that this substance was effective against asthma in experimental animals, but unfortunately their findings were never applied to clinical trials on humans in Japan, and because their reports were written in Japanese, the content was never disseminated beyond the shores of Japan.

Ten years later, in 1924, K. K. Chen and Carl F. Schmidt at the Laboratory of Pharmacology of the Union Medical College, Peking, China, published a report of the great efficacy of ephedrine against asthma in English. They were credited with this discovery (8-94), even though Chen cited the work of Amatsu and Kubota in the report. It was unfortunate for Amatsu and Kubota that scientific research in Japan was still undeveloped and Japanese was a minor language.

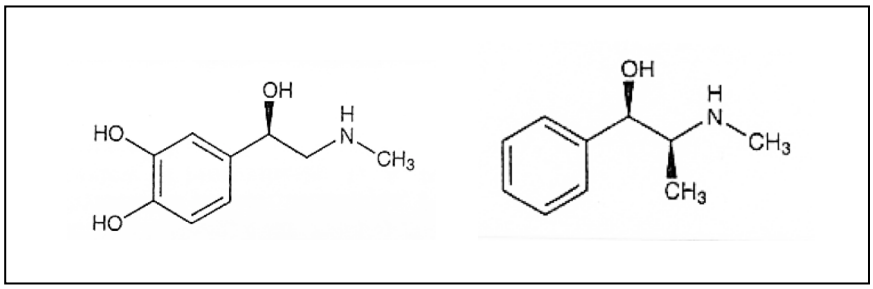


Figure 8-5. **Left:** Takamine and Wooyenaka crystallized an adrenal hormone "adrenaline"
Right: Nagayoshi Nagai extracted a plant alkaloid "ephedrine"

Leaving that aside, Nagai's methods for natural product research, in particular ephedrine, led to the success of Wooyenaka, who had crossed the ocean to New York and taken on the task of the crystallization of adrenaline in Takamine's laboratory.

The news that adrenaline showed great efficacy against asthma made its way back to Japan, where two young medical researchers in Kyoto discovered the efficacy of ephedrine on bronchial smooth muscle—thus Nagai's methods traveled full circle. The researchers were members of the Tokyo Chemical Society, which Nagai and Takamine had developed from 1888 onward as president and permanent member of the board, respectively.

While this is something of an aside, the biography of Friedrich Stolz, the head of the Chemical Department at Hoechst A. G. who succeeded in the total synthesis of adrenaline, states that he devoted his later life to chemical research into ephedrine (8-27). Stolz outlived Takamine and Nagai, but right up until the end they were bound by a curious thread of fate.

10. Adrenaline still in use today

I have condensed the results of the diverse research into adrenaline during the period of over a century since Wooyenaka first obtained the crystals, and I would like to give a brief account of how the physiological action of adrenaline is used in medical treatment today.

Our bodies do not stop producing adrenaline even for an instant, and it continually demonstrates its action as an essential hormone. At the same time, adrenaline that has been extracted (or today, adrenaline that has been chemically synthesized) acts differently within the body. Its action is effective for hemostasis and against asthma and food or pollen allergies, so adrenaline has a double role.

In the US there have recently been many cases in which young children suddenly have lost their lives as a result of severe allergy (anaphylaxis) to some foods, particularly peanuts. Because of this, the EpiPen (a pen-like device that delivers an injection of epinephrine) is kept on hand at schools and teachers are trained to be able to give prompt administration (injection) of adrenaline without any mistakes. Japan has learned from the system in the US, and similar systems are starting to go into place there as well.

(1) Physiological activities

Adrenaline and noradrenaline both act directly on the heart muscle to increase contraction and raise the pulse rate. However, while they are similar in this respect, they are not entirely

the same. Adrenaline acts strongly to increase glucose levels in the blood by glycogenolysis, whereas noradrenaline has marked blood vessel contraction action.

Adrenaline and noradrenaline are both classified into the group known as catechol amines. Body tissues have special parts that act when they receive hormones—these are called "receptors."

There are two types of receptor for catechol amines: α- and β-receptors. The α-receptors receive both adrenaline and noradrenaline, while the β-receptors receive adrenaline.

When α-receptors act, there is blood vessel contraction. When Oliver and Schäfer were astounded to see the column of mercury in the sphygmomanometer rise in 1893, they were witnessing the α-receptors acting to make vascular smooth muscle contract so that the blood pressure rose sharply.

When β-receptors act, there are effects such as vasodilation, accelerated cardiac output, and bronchial tree dilation. The α- and β-receptors have been studied in detail, and it has been shown that α-receptors can be further classified into α_1 and α_2, and β-receptors into β_1, β_2, and β_3. The different actions of each of these have been clarified.

(2) Adrenaline products listed on Pharmacopoeia (combination drugs are excluded)

(a) US Pharmacopeia

(i) Epinephrine Inhalation Aerosol
(ii) Epinephrine Injection
(iii) Epinephrine Inhalation Solution
(iv) Epinephrine Nasal Solution
(v) Epinephrine Ophthalmic Solution
(vi) Epinephrine Bitartrate
(vii) Epinephrine Bitartrate Inhalation Aerosol
(viii) Epinephrine Bitartrate Ophthalmic Solution
(ix) Epinephrine Bitartrate for Ophthalmic Solution
(x) Epinephrine Borate Ophthalmic Solution

(b) Japanese Pharmacopoeia

(i) Adrenaline Solution
(ii) Adrenaline Injection

11. Other contributions by Jokichi Takamine

Takamine granted Matasaku Shiobara, who at that stage was a manager of a pharmaceutical company, a monopoly to market Taka-Diastase in Japan.

In 1913, Takamine established Sankyo Co., Ltd. together with Shiobara in order to develop the business. While still living in the US Takamine took the position of company president, and he gave his undivided attention to new product development.

However, his extensive knowledge and rich experience did not stop there.

(1) Toyota Motor Corporation

In his later years, Takamine was said to be the best-known Japanese person in America, and many of the Japanese people who crossed the Pacific Ocean to the new continent called to visit him at his mansion in Manhattan.

One of these was Sakichi Toyoda, the founder of Toyoda Automatic Loom Works, who was known as the "King of Inventors." At a time when combined businesses in Japan were stagnating and there seemed to be no hope for the future, he had left Japan in despair. He landed in Seattle and crossed America by train, eventually arriving in New York. It was 1910, and he was 43 years old.

Takamine, then 56, welcomed him kindly. Toyoda was feeling lost, and Takamine patiently spoke of the path he himself had followed. In his autobiography, Toyoda records what Takamine told him: "Many profitable inventions often end in failure and are consigned to oblivion. Society is at fault, but the inventor himself is also responsible. I believe that for an invention to be successful, the inventor must never be separated from it. It is the inventor's responsibility to apply the invention. This means that the inventor cannot part from the invention until there is the prospect that the invention will be fine even if he lets society use it.

Before an egg becomes a bird, it may die or be incapacitated if someone else gets hold of it. The inventor has the responsibility to carefully watch over it until it grows fine, strong wings, so that wherever it is set free it has the prospect of being able to fly safely. That is the way to perfect an invention." Listening closely to Takamine, Toyoda felt his confidence returned.

After returning to Japan, he pushed forward with his business. Having seen the huge growth of motorization in the US, he believed the future lay with the automobile industry. Although he did not live long enough to achieve his desire, he continually insisted to his

family that the automobile would be the next big thing. It was from Sakichi's dream that today's Toyota Motor Corporation developed (8-95, 8-96).

(2) RIKEN

Another seed that Takamine sowed was the Rikagaku Kenkyusho (the Institute of Physical and Chemical Research, usually known as RIKEN) in Japan, which looks set to bring great benefits to humanity in the near future.

Takamine developed the concept and put forward the proposal for a new research institute, and with the support of his acquaintances in the business world such as Eiichi Shibusawa and Takashi Masuda, RIKEN was established over a century ago, in 1917 (8-97).

Dr. Masayo Takahashi of RIKEN has been carrying out joint research with Prof. Dr. Shinya Yamanaka, who won the 2012 Nobel Prize in Physiology or Medicine, into tissue therapy for age-related macular degeneration (AMD) using induced pluripotent stem (iPS) cells. She recently announced the start of clinical tests. The Japanese government gave its approval in July 2013, and around the world, patients of this as yet incurable disease are hoping for their success as soon as possible.

12. Takamine as an unofficial ambassador

The year 2012 marked the 100th anniversary of the planting of the famous Japanese cherry trees that flower every year along the banks of the Potomac River in Washington and the Hudson River in New York. The gift of the cherry trees came about as a result of the love of travel writer Eliza R. Scidmore and First Lady Helen Taft for Japan's cherry blossom, the efforts of the Japanese Consul General Kokichi Mizuno, and an offer for donation by Jokichi Takamine. On the Japanese side, Foreign Minister Jutaro Komura took a leading role in facilitating the donation (8-98).

Figure 8-6 shows a photograph of a meeting of the Japanese delegation for the peace negotiations of the Russo-Japanese War, taken at Metropolitan Club, New York, in 1905. The delegation was about to leave for Portsmouth, New Hampshire, carrying the responsibility for Japan's future. Jokichi Takamine joined in the meeting as the only non-government person to give encouragement to the delegation.

Figure 8-6. Japanese delegation for peace negotiation with Russia at the end of the Russo-Japanese War in 1905. Sitting from right to left in the front row: Jokichi Takamine, Koichiro Tachibana, Kogoro Takahira, Kentaro Kaneko, Jutaro Komura (Courtesy of Masayoshi Matsumura).

One member of the delegation, Kentaro Kaneko, had attended Harvard University at the same time as the then U.S. President Theodore Roosevelt, and he was instructed to tour America to explain the Japanese position in the negotiations. As he was not accompanied by his wife, Takamine's wife, Caroline, accompanied Kaneko in her place to his lectures in different parts of the country. Kaneko later recalled that he could not possibly have fulfilled his responsibility without the help and cooperation of Dr. and Mrs. Takamine.

Takamine, then living in America, was fully aware that the task of the chief delegate, Jutaro Komura, was to fight for the destiny of Japan, and he spared no effort in giving support to his home country.

Takamine and Komura had both learned English from the same teacher in their younger days—this was Guido Verbeck, the teacher of English at the *Chienkan* School in Nagasaki (8-99). Takamine had already returned to Kaga Domain by the time Komura enrolled at the school, so the two were never classmates.

Strangely enough, however, they were brought together 36 years later at the Russo-Japanese peace conference, where the fate of Japan was to be decided. They must surely have shared memories of their youth in Nagasaki, and Takamine would have given heartfelt encouragement to his junior from the *Chienkan* School by wishing the delegation well as it left for the negotiations.

13. Here lie the Japanese hormone hunters

In the early morning of July 22, 1922, Jokichi Takamine passed away at the age of 67 after

a full and eventful life. He had fought against illness for two years, but the medical treatment proved ineffective. At the end of his life, Takamine was cared for by his wife and two sons; his younger sister, Junko Takehashi; and Keizo Wooyenaka.

Takamine always said that he wished to spend his final years in his home country of Japan. However, Eiichi Shibusawa and Takashi Masuda, the two leaders of the financial world to whom Takamine felt himself obliged, begged him to stay in America to work for Japan-America friendship and diplomacy. Takamine's last years were tinged with sadness, as he finally had no choice but to give up all hope of returning to his homeland.

Three days later, on July 25, a majestic Catholic funeral service was held for Takamine at Saint Patrick's Cathedral in New York. Some 600 mourners, including many Japanese and American dignitaries, packed the cathedral.

His widow, Caroline, spoke at the funeral. She thanked the mourners for attending, and then went on to talk of her deceased husband. The records say that the widow's tear-choked words resonated strongly in the hearts of those who heard her; "Jokichi loved Japan until the very end, he missed Japan, he yearned for Japan. I ask you all to speed Jokichi on his way to heaven by singing together the Japanese national anthem."

A memorial ceremony was later held at the Nippon Club, and several hundred people made flower offerings.

Apparently, an anonymous American woman laid a bunch of flowers accompanied by a note; the note simply gave thanks for the joy of life that had been returned by the drug Adrenalin, and hoped that Dr. Takamine's soul would rest in eternal peace.

The name Adrenalin had become well known among the American people, and its effectiveness was very highly regarded (8-100, 8-101).

The day after Takamine's death, *the New York Times* published an obituary. As the headings accurately reflect the esteem in which Takamine was held, I will include them here:

> "JOKICHI TAKAMINE, NOTED CHEMIST, DIES"
> "Japanese Who Discovered Adrenaline and Takadiastase Had Been Ill Two Years"
> "FOUNDED THE NIPPON CLUB"
> "He Was Widely Known for His Work for Friendly Relations between Japan and United States"

Jokichi Takamine lies with his beloved wife Caroline in Woodlawn Cemetery in a suburb of New York, the city where he was so successful [Figure 8-7]. Some of his hair was buried in

Aoyama Cemetery in Tokyo. His friend Jutaro Komura also lies in the same cemetery.

Figure 8-7. Sepulcher of the Takamine Family in Woodlawn Cemetery, suburb of New York City (photo taken by the author).

Keizo Wooyenaka lived for many more years. In his later life he was an auditor for Sankyo Co., Ltd. for seven years from 1928, and after his retirement he continued his pursuit of knowledge through reading, acquiring the German language, and other activities, until he breathed his last at his home in Nakano Hikawacho, Tokyo, on January 11, 1960 at the age of 84.

He lies in Kodaira Cemetery on the outskirts of Tokyo with his wife, Yaeno, who shared his hardships, and their beloved children [Figure 8-8].

Figure 8-8. Grave of the Wooyenaka Family in Kodaira Cemetery, Tokyo (photo taken by the author)

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Through the sheer hard work of the "Hormone Hunters," it became possible to obtain a specific adrenal active principle in crystalline form. Until then, researchers around the world had only been able to conduct experiments that gave no more than frustrating glimpses of their target—now, they could make vigorous advances toward verifying the hypotheses they had pictured in their minds.

Animal physiology and pharmacology entered a completely new era, and for pharmaceutical manufacturers the way was open to a future in which they could dream of a vast range of new products.

Those who contributed to this, many of whom went on to explore new paths, left behind magnificent achievements for posterity.

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Epilogue

My major was organic chemistry, and I knew absolutely nothing about animal hormones. It just so happened that Sankyo Co., Ltd., a pharmaceutical company I was once working for, wanted a detailed study of the work of the first company president, Jokichi Takamine, and as I sorted through all the documents and information I acquired during the course of the study, I found myself becoming more and more interested in adrenaline, an animal hormone.

And then something struck me. While there were papers looking at different parts of the long and convoluted history of research into this fascinating active substance, I could not find a single work that gave a complete overview.

I had to keep in mind that when talking about adrenaline, there is no one single viewpoint. At the very least, there are the three perspectives of physiology, medicine, and pharmaceutical products.

As representatives of these, Oliver, Schäfer, and Abel investigated adrenaline from the perspective of physiology and pharmacology, Bates and Solis-Cohen studied it from the perspective of clinical medicine, while Parke, Davis & Co., Takamine, and Wooyenaka were interested in this active substance as a medicinal product.

Following the science history of the suprarenal glands from the anatomical lectures of Galenos, the medical giant of ancient Greece, to the accurate anatomical drawings of the Roman Eustachio, I noticed that this history gave a perfect illustration of how at any given time the development of the natural sciences is related to the influence of countries.

We saw that in the review published in 1896 by the Polish researcher Szymonowicz, who discovered the blood-pressure raising effect of the principles of suprarenal medulla (Table 3-1, pages 53-54), over 50% of the 111 papers he cited were in French, followed by German and Italian.

English, however, accounted for only a small percentage of his publications. It was remarkable how French and Italian all but vanished from that time onward, while the vast majority of work in this field came to be in English or German.

Incidentally, the *lingua franca* of academia in Europe up to the 18th century was Latin.

When Schmidt made his astute observation in 1785 that the adrenal gland secreted something into the blood that acted on the heart, he wrote it in Latin. I well remember feeling greatly moved when I learned that he passed away just two years after this, at age 26.

One thing I feel when I study the history of natural science is that there are those who have found success by attacking a problem head-on, and there are others who have made great breakthroughs in a more unobtrusive manner.

In the history of the race to extract adrenaline, over 20 researchers pounded away at the problem for 44 years without success. Eventually, a researcher appeared who was not quite so ambitious. His approach was to wait, quietly and intently, and he was able to readily isolate the crystals that the other researchers had found so elusive.

As Wooyenaka himself said, it was as if the prize had been snatched from under their noses. I hope the researchers of today, who carry the responsibility for the future, will carefully heed the different paths taken by these pioneers, and learn to take the right path at the right time.

After I finished writing the long story that led to Takamine isolating adrenaline, I spent many days mulling over the question of what exactly had been the key to his success. Finally, it came to me—the key was his use of wheat bran, which was industrial waste, for the cultivation of *Nihon Kojikabi* (*Aspergillus flavus* var. *oryzae*) (see Chapter 5, section 3).

Takamine had shown abundant wit and intelligence through his difficult journey until then, but I concluded that this was the point at which fate really smiled on him. His association with Parke, Davis & Co., lead to the production of the world's first biotechnology product, Taka-diastase, and then to the first ever crystallization of a hormone, adrenaline. The wealth Takamine acquired as a result allowed him to act as an "unofficial ambassador," and gave him a luxurious lifestyle.

Vulpian's paper describing the discovery of the secretion of adrenaline from the suprarenal glands was written in French. I read it while keeping a mental picture of the level of science and technology of the time, and I remember how impressed I was by the high quality of the content, and later how my heart beat at the instant when I dripped ferric chloride and iodine solution into an aqueous solution of adrenaline in a glass dish and saw the color reaction for myself (see Chapter 1, Figure 1-3). As I pressed the shutter button for what I imagined was probably the first ever color photograph of the Vulpian reaction, I shared the emotion Wooyenaka must have felt around 100 years earlier in that cramped New York laboratory. It was an unforgettable moment. I would like to thank my late parents, who gave me the gift of a French-Japanese dictionary and let me study French at university.

Sometimes in life, the completely unexpected occurs. The year after this book was published in Japanese, a medical scientist called Dr. Brian B. Hoffman, who had been tracing the story of adrenaline far away in Boston, US, published a magnificent book, titled *Adrenaline*. I hope readers will be able to see for themselves that the two books were written from different viewpoints, and have many parts that complement each other. I was delighted to receive an e-mail from Dr. Hoffman, in which he said, "I am sure the two of us represent the most interested in adrenaline people in the world."

This has been a long path—I learned that people in the times of the Old Testament used the suprarenal glands, which they regarded as "lumps of fat," as offerings to God. I recreated the Vulpian's color reaction and delved deeply into the fierce research race between France, Germany, and the US.

With the crystallization of adrenaline by Takamine and Wooyenaka as a second starting point, I saw how the seeds of their work bore fruit in the fields of physiology, medicine, and chemistry.

I am deeply grateful for the good fortune to have been able to follow the tracks of the Hormone Hunters across the vast expanse of their 500-year history, which has been a source of limitless fascination.

(Please note that honorific titles have been omitted in historical descriptions.)

Acknowledgements

I would like to thank Dr. Yutaka Sano, who allowed me to read valuable documents when I first decided to write this book; Mr. Yutaka Yamamoto, who provided a copy of the letter that was to prove so important to the problem of naming the hormone; my German friend Mr. W. Kreutner, who went to great lengths to obtain a portrait of von Fürth; Dr. Kazuo Sato, who obtained judicial records of the patent dispute; and the former Sankyo Agrochemical Research Laboratories, which allowed me to use laboratory space and reagents to recreate Vulpian's color reaction.

I am also greatly indebted to Dr. Tsutomu Nakatsugawa, Professor Emeritus at State University of New York, for his highly objective judgment of a number of key reference works and his extremely helpful advice about the manuscript of this book.

I am very grateful to the people who kindly helped me to obtain documents and data for this book, in particular the staff of the investigation department of Yokohama Central Library and the staff of the Kashiwa Library at the University of Tokyo, who always gave exactly the right help when I needed to search for an old reference or copy a document.

I am enormously grateful to Dr. Tsuneo Fujita, Professor Emeritus at Niigata University, who kindly read my manuscript and gave many helpful suggestions when I was trying to get my book published in Japanese and even agreed to write a letter of recommendation. I was deeply saddened by Dr. Fujita's sudden death in February 2012, and he remains in my prayers. I would also like to thank Dr. Kozo Kuchitsu, Professor Emeritus at the University of Tokyo, who gave valuable advice from a specialist viewpoint, and science journalist Mr. Shinpei Miyata, who gave me tremendous encouragement.

When this book was published in Japanese in 2012, I was honored to receive letters of recommendation from Dr. Eiichi Negishi, Distinguished Professor at the Department of Chemistry of Purdue University, the winner of the 2010 Nobel Prize in Chemistry, and Mr. Takashi Shoda, the former chairman of Sankyo Co., Ltd, the company which has passed on "*Medicamenta vera*" as its corporate creed since the time of Jokichi Takamine. I would like to publicly thank these two gentlemen.

I would like to express my deepest gratitude to my honorable friend Dr. Kazutake Kyuma, Professor Emeritus at Kyoto University, Mr. Tetsuya Suzuki, managing director and editor-in-chief of Kyoto University Press, and Ms. Yuko Fukushima, editor of Kyoto University Press, who brought both the Japanese and the English versions of this book to publication.

I would like to thank the translator Mark Phillips and Forte Science Communications, Tokyo for their great cooperation in translating into English the Japanese edition of this book.

Finally, I would like to thank my wife, Yukiko, for looking after my health and encouraging me throughout the process of researching and writing this book, right up to the preparation of the English version.

March, 2017 Mitsuo Ishida

Profile of Jokichi Takamine

Year	Event
1854	Born in Takaoka in Ettchu Province, present-day Toyama Prefecture. His father, Seiichi, was a doctor practicing Western medicine; his mother, Yukiko, came from a family of sake distillers. Jokichi was their first son. Moves to Kanazawa the following year due to his father's work.
1865	Goes to Nagasaki to study medicine at the age of 11. Studies English under Reishi Ga and Verbeck.
1868	Bides his time in Kyoto and Osaka at the time of the Battle of Toba–Fushimi.
1869	Studies at *Osaka Igakko* and *Rigakusho*, where he meets the German teacher Ritter and sets his sights on applied chemistry.
1872	Goes to Tokyo to study chemistry as a technical trainee sponsored by the Ministry of Industry.
1879	Graduates at the top of his class in applied chemistry from Imperial College of Engineering (aged 25).
1880	Sent by the Ministry of Industry to study in the UK for three years. Studies at the University of Glasgow and elsewhere.
1883	Returns to Japan via the US. Becomes an official at the department of industry promotion in the Engineering Bureau of the Ministry of Agriculture and Commerce.
1884	Dispatched to the New Orleans Exposition (World Industrial and Cotton Centennial Exposition). Journalist Lafcadio Hearn covers this exposition. Takamine becomes close to Caroline Hitch.
1885	Engaged to Caroline Hitch. Becomes interested in phosphate rocks on display at the exposition and buys some at his own expense to take back to Japan.
1886	Concomitantly becomes vice-director of the Patent Bureau and head of the analysis department of the General Affairs Bureau. Becomes friends with Eiichi Shibusawa and Takashi Masuda.
1887	Begins preparations for establishing a company making chemical fertilizers, travels to the US and Europe to carry out surveys and purchase equipment. Marries Caroline Hitch in New Orleans. Establishes "Tokyo Artificial Fertilizer Company" in Fukagawa, Tokyo, with the help of Shibusawa and Masuda.
1888	Begins production of fertilizer. Resigns post as a government official. Eldest son born. Builds a private medicine manufacture laboratory and begins research into fermentation.
1889	Second son born.
1890	Takamine and his family move to the US at the invitation of the Hitch family, accompanied by Kosuke Fujiki. Contracts liver disease during the voyage and is hospitalized in Chicago for treatment (aged 36).
1891	Establishes a method for distilling whiskey using *koji* mold. Moves to Peoria to begin full-fledged research into brewing.
1892	Establishes a method of fermentation using *koji* mold at the pilot plant. Tetsukichi Shimizu, Takamine's junior from the Imperial College of Engineering, comes to America to join him. The business develops well.
1893	Meets with opposition from malt manufacturers and his factory burns down. Relapse of liver disease. Abandons whiskey business, changes track and **begins research and development of the digestive medicine Taka-diastase.** Parke, Davis & Co. show great interest in this medicine, and commence negotiations.
1894	Applies for US patent for Taka-diastase and concentrates on research into increasing the power to saccharify starch.
1895	**Parke, Davis & Co. begins sales of "TAKA=DIASTASE,"** which becomes a hugely popular product (aged 41).
1896	Tetsukichi Shimizu dies suddenly. Kosuke Fujiki carries his ashes back to Japan.

1897	Takamine moves to New York with his family. Opens the Takamine Laboratory for the adrenal gland blood pressure-raising principle; The American researcher Abel announces the name "Epinephrin," Fürth names it "Suprarenin."
1898	Matasaku Shiobara, the founder of Sankyo Co., hears about the digestive medicine Taka-diastase from his friend, Shotaro Nishimura.
1899	Sankyo Shouten in Tokyo begins import and sales of Taka-diastase. Takamine is conferred a PhD in engineering in Tokyo.
1900	Keizo Wooyenaka is employed by Takamine Laboratory. Successful crystallization of the active principle of the adrenal medulla in July through collaboration with Wooyenaka. **Takamine names it "Adrenalin" and applies for a patent** (aged 46).
1901	Trade name "Adrenalin" registered in the US. Takamine begins a vigorous program of announcing the crystallization of adrenaline at academic conferences in the US and the UK. Parke, Davis & Co. begins sales of "SOLUTION Adrenalin Chloride."
1902	Japanese trade name "Adrenalin" registered. Sankyo Pharmaceutical Company acquires exclusive rights to market Taka-diastase in Japan and also imports and sells Adrenalin solution.
1904	Takamine and his wife give great assistance to Kentaro Kaneko, a special envoy sent to the US to garner support for Japan in the Russo-Japanese War. Takamine appears in a newspaper article publicizing the modernized Japan.
1905	Takamine re-erects the *Shoufuuden* ("Pine Maple Pavilion") at Merriewold, outside New York, and works for Japan-America relations. Establishes a Nippon Club in New York for Japanese people living in the US, and becomes the first chairman. In New York he gives encouragement to the Russo-Japan War peace delegation, which is headed by Jutaro Komura, a fellow alumnus of the *Chienkan* school in Nagasaki (aged 51).
1906	Conferred a PhD in pharmaceuticals in Tokyo.
1907	An American influential person from the financial world establishes the "Japan Society" in New York, Takamine becomes honorary vice president.
1909	Takamine and the Japanophile Eliza Scidmore discuss planting rows of Japanese cherry trees in the US, and enlist the support of First Lady Helen Taft. The cherry trees are donated by the city of Tokyo, but are not planted because they are infested with pests and have to be incinerated.
1911	Takamine wins a patent infringement lawsuit against a similar product that appeared after his own, and his patent receives praise.
1912	The Japanese cherry trees he long hoped for are planted around the tidal basin of the Potomac in Washington and along the Hudson River in New York.
1913	Establishes Sankyo Co., Ltd. and is appointed the first company president while still residing in the US. Proposes establishment of the National Chemistry Laboratories (now the Institute of Physical and Chemical Research) (aged 59).
1914	Sankyo Co. begins production of Taka-diastase in Japan. Takamine's friend Dr. Leo Baekeland gives him the license for Bakelite for free, and Sankyo commences production in Japan.
1917	*Rikagaku Kenkyusho* (the Institute of Physical and Chemical Research, RIKEN) was established according to Takamine's suggestion. Establishes the Takamine Laboratory in Clifton, New Jersey.
1918	Takamine proposes and works for construction of a hydroelectric plant for producing aluminum on the Kurobe River in his native Toyama Prefecture.
1920	Sankyo Co. commences production of Adrenalin solution in Japan.
1921	Parke, Davis & Co decline a request from the Revision Committee of the *US Pharmacopeia* for the use of the name adrenaline, so the name in the *Pharmacopeia* has been "epinephrine" ever since in the US. Takamine was undergoing medical treatment, but he continued to work hard at his Japan-America friendship activities and eventually collapses at the end of the year.
1922	**Takamine dies at age 67 in New York on 22 July.** **He is buried in Woodlawn Cemetery.**

Profile of Keizo Wooyenaka

Year	Event
1876	Born in Najio, Shiose Village, in the Arima district of Hyogo Prefecture (present-day Najio, Nishinomiya City), the fifth child (third son) of Jihei Uenaka (or Wooyenaka), a paper manufacturer, and his wife, Yano.
1886	After graduating from elementary school, he leaves for Osaka. At age 10, he commutes to the *Taisei Gakkan* school from the house of his eldest brother, Osamu.
1888	Graduates from *Taisei Gakkan*. To pursue his studies, he works as a live-in employee of Ishizu Pharmacy.
1891	Leaves Ishizu Pharmacy and enters *Osaka Yakugakko*, a school of pharmaceutical sciences in Osaka.
1893	Graduates from *Osaka Yakugakko*, passes the pharmacist exam, and enrolls in a non-regular course (an elective pharmaceutics course) at the Pharmaceutical Department of Tokyo Imperial University (aged 17).
1895	Graduates from the elective pharmaceutics course. Continues working as an assistant at the same university in Nagayoshi Nagai's laboratory. During this time, at the request of Dr. Kenjiro Yamakawa of the College of Science, he successfully prepares and supplies barium chloroplatinate for use in fluorescent screens for X-rays.
1896	Works at Matsuzaka Pharmacy in Shibecha, Kushiro in Hokkaido for a period. Returns to Tokyo and is appointed chief pharmacist of Eiraku Hospital, also carries out research as an assistant to Yoshizumi Tawara of Tokyo Health Laboratory. Analyzes samples of pollution from Ashio Copper Mine, prepares and tests nicotine sulfate for use as a pesticide.
1899	Decides to resign and move to the US in the spring, studies English in preparation. Leaves for the US at the end of the year with a letter of introduction from Tamemasa Haga, professor of the College of Science at the Tokyo Imperial University.
1900	Gets a job at Takamine Laboratory in New York in February. Begins research into *koji* mold, in charge of research into the adrenal principle. **Successfully crystallizes adrenaline in July** (aged 24).
1905	Marries Yaeno Yasui in Seattle. Moves into a new home in New York. Continues to work to increase the productivity of Adrenalin and Taka-diastase (aged 29).
1906	Eldest son born.
1907	Eldest daughter born.
1909	Second son born.
1910	Second daughter born.
1913	Third daughter born.
1914	Returns to Japan to help plan increased production of Taka-diastase by Sankyo Co.
1916	Retires from Takamine laboratory in New York, returns to Japan and gets a job at Sankyo Co. (aged 40).
1921	Travels to North and South America for a long period on business. Nurses Takamine, who is undergoing treatment.
1922	Assists the Takamine Laboratory in the US. Surveys and procures bovine adrenal glands in Brazil and Argentina. Present at the deathbed of Jokichi Takamine (aged 46).
1926	Travels to the US to import technology for Bakelite, a phenol resin (aged 50).
1930	Travels to the US to support the management of the Takamine Laboratory.
1936	Resigns as auditor of Sankyo Co.
1960	Dies in Tokyo on January 11, aged 84. Buried in Kodaira Cemetery, Tokyo, with his wife, Yaeno.

Personal History of Mitsuo Ishida (June 25, 1931–April 2, 2018)

EDUCATION AND EXPERIENCE

1954: Graduated from Kyoto University, Japan, majoring in Biological Organic Chemistry.

1954: Employed by Sankyo Co., Ltd. (now Daiichi Sankyo Co., Ltd.), a pharmaceutical manufacturing firm in Tokyo, Japan.

1962: Got a study leave from the company to study at Iowa State University (ISU), Ames Iowa, USA with research assistantship.

1964: Master of Science from ISU.

1968: Granted Doctor of Agriculture Degree from Kyoto University.

1990–1996: A member of the board of Sankyo Co., Ltd., General Manager of Agricultural Chemicals Division.

1999: Retiring from Sankyo Co., Ltd.

2006: Set up a nonprofit organization "Research Conference on Modern Creative Japanese Scientists," acting as the general manager.

2015: Appointed the president of "Dr. Jokichi Takamine Research Foundation," a specified nonprofit corporation.

PUBLICATIONS

in Japanese

Hormone Hunters: the discovery of Adrenaline. Kyoto: Kyoto University Press, 2012; "Paul Ehrlich and Sahachiro Hata, their contribution to medicine," *Microscopia* **25**(3)-**26**(2), 2008-2009; *Cherry trees on Potomac Basin*. Yokohama: Research Conference on Modern Creative Japanese Scientists, 2011; and many other short essays.

in English (co-edited with Dr. Tsutomu Nakatsugawa, emeritus Professor of State University of New York)

Jokichi Takamine: The Man Who Gave "Adrenaline" to the World: English translation of the Panels Exhibited at the National Science Museum, Tokyo, Japan, December 10, 2004–January 10, 2005 in Commemoration of the 150th Anniversary of Takamine's Birth. Yokohama: Research Conference on Modern Creative Japanese Scientists, 2007.

Postscript to English version of *Hormone Hunters*

Mitsuo Ishida, the author of *Hormone Hunters*, passed away on April 2nd, 2018, from pneumonia at the age of 86. He was working hard in his last days to complete the English version of his book. He had finished more than 98% of the work, but left the last touch to his son, Jun-ichi. He fulfilled father's wish during the scarce time to spare in his busy regular work at Honda Motor Co., Ltd.

The author graduated in 1954 from Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, majoring in Organic Chemistry. Then, he worked for Sankyo Co., Ltd., present-day Daiichi Sankyo Co., Ltd., mainly in the agro-chemicals division. While there, he had an opportunity to complete a Master of Science degree under Dr. Paul A. Dahm at Iowa State University, Ames, Iowa, in early 1960s. Later in 1968 he was granted Doctor of Agriculture degree from Kyoto University for the in-depth investigation of the research he initiated during his MS work on the metabolic processes of a then widely used agrochemical, benzene hexachloride (BHC).

The Japanese Edition of *Hormone Hunters*, published in 2012 by Kyoto University Press, was welcomed by a wide range of readers. As is written in the Prologue of the English version, Prof. Brian B. Hoffman of Harvard Medical School published *Adrenaline* in 2013 in the U.S. After reading this book, Dr. Ishida thought that contents of the two books would complement each other and so it would be worth making an English version of his own book. Dr. Ishida put his heart and soul into this work. He deleted some parts of his Japanese version that would have facilitated understanding of Japanese readers, while adding new paragraphs to supplement novel information for the readers in English. This change might have made some wording of his acknowledgement irrelevant to the English version, but it was kept as was written by the author at an earlier date to convey his profound gratitude to those whom he felt he owed in various ways.

Dr. Kazutake Kyuma, Professor Emeritus at Kyoto University and a classmate of the author's Kyoto University days, and Ms. Yuko Fukushima, Kyoto University Press, editor of *Hormone Hunters* in its Japanese edition, assisted Mr. Jun-ichi Ishida in completing the English version.