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).

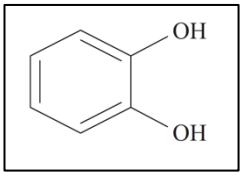


Figure 4-1. Pyrocatechol

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.

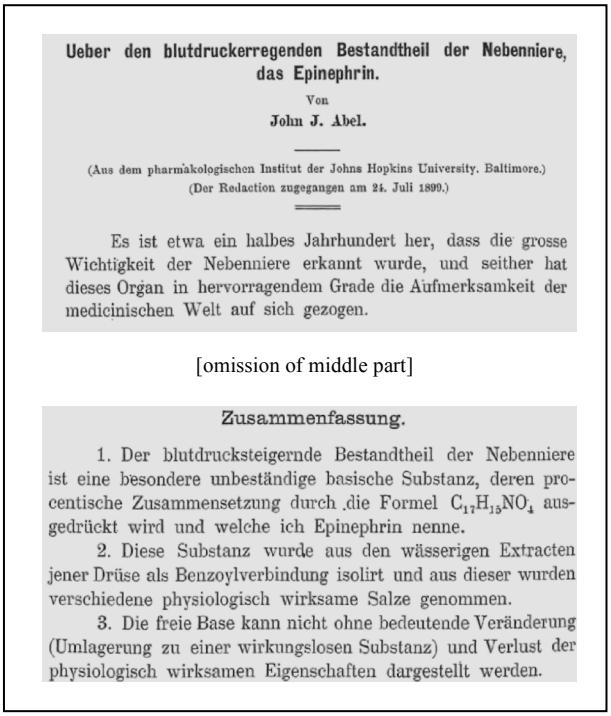
The following year, Abel published his second report, in which he specified the molecular formula $C_{17}H_{15}NO_4$ for the first time (4-24). In this report, he notes that the active principle can be precipitated by treating aqueous extract of the adrenal glands with benzoyl chloride and sodium hydroxide, 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

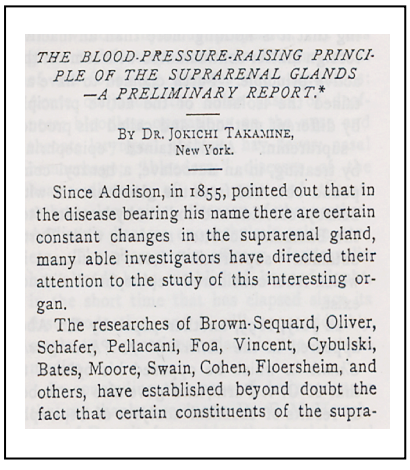
Please think back to the Takamine's hot, semi-basement laboratory in New York City with no air conditioning that we first encountered in Chapter 1 during the heat of the summer of 1900. The young scientist Keizo Wooyenaka, who at the age of 24 had just been taken on by Jokichi Takamine, successfully 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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