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.

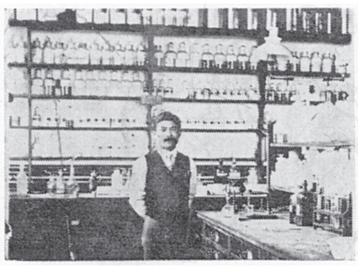

Figure 1-1. Keizo Wooyenaka in the "semi"-basement laboratory in New York City. On July 21, 1900, Wooyenaka became the first person to isolate the hormone adrenaline. (Courtesy of Sankyo Co., Ltd.)

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.

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

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].

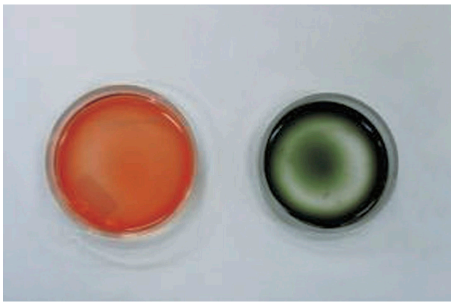

Fig. 1-3. Color reactions of adrenaline discovered by Dr. Alfred Vulpian in 1856.
Left: rose-carmine with iodine.
Right: glaucous (sea-green) with ferric chloride
(results of the reproduction experiments by the author)

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"

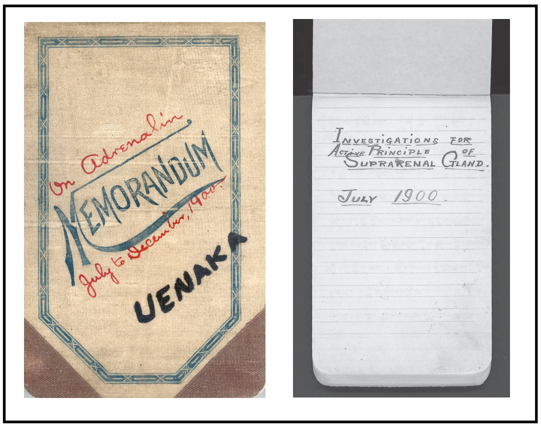

Figure 1-5. Wooyenaka's laboratory notebook.
Left: cover
Right: the first page
(owned by Kyogyoji Temple, Hyogo Prefecture, Japan)

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).

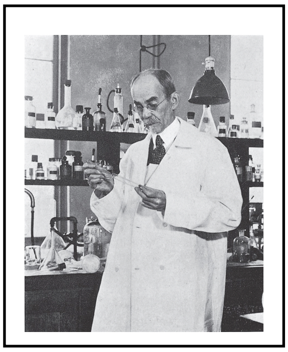

Figure 1-6. John Jacob Abel, who led the way in research into the isolation of the blood pressure-raising principle of the adrenal glands (1-10).

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.

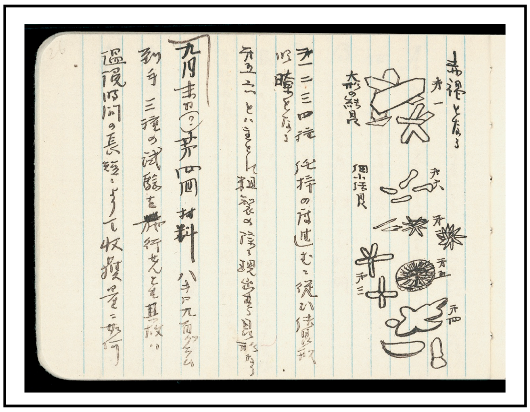

Figure 1-7. A page of the Wooyenaka's Laboratory notebook clearly recording that there are many different crystal forms of adrenaline (owned by Kyogyoji Temple, Hyogo Prefecture, Japan)

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].

Literature Cited

(1-1) Yamashita, A.,"Laboratory Note on adrenalin (in Japanese)." *Kagakushi kenkyu* (Research on Science History), No. 79, 143–148 (1966)
(1-2) Yamashita, A., "Research note on adrenaline by Keizo Uenaka in 1900." *Biomedical Research* **23**(1): 1–10 (2002).
(1-3) Facsimile edition of the Wooyenaka's Laboratory Notebook (in Japanese), Collection in National Museum of Nature and Science, Tokyo. The original notebook is in the possession of Kyogyoji Temple, Nishinomiya City, Japan.
(1-4) Vulpian, E. F. A., "Note sur quelques reactions propres à la substance des capsules surrénales." *Comptes rendus hebdomadaires des séances de l'Académie des sciences*, **43:** 663–665 (1856).
(1-5) Agnes de Mille, *Where the Wings Grow*, the page next to p. 192. N.Y.: Doubleday (1978).
(1-6) "Autobiographical interview, 2-2 (in Japanese)." *Yakkyoku no Ryoiki* (Field of Pharmacy), **7**(10): 52–54, 57–58 (1958).
(1-7) *New York City Directory*, **114**, p.1311 (1900).
(1-8) Bates, W. H., "The use of extract of suprarenal capsule in the eye." *New York Medical Journal*, pp. 647–650, May 16 (1896).
(1-9) Abel, J. J., "Ueber den blutdruckerregenden Bestandtheil der Nebenniere, das Epinephrin." *Hoppe-Seyler's Zeitschrift für Physiologische Chemie*, **28**: 318–362 (1899).
(1-10) Voegtlin, C., "John Jacob Abel." *The Journal of Pharmacology and Experimental Therapeutics*, **67**: 373–406 (1939).
(1-11) Shigeru Saito, "Keizo Uenaka: The main researcher in adrenaline purification." *The Journal of Japan Society for Clinical Anesthesia*, **32**(4): 573–581 (2012).
(1-12) "Autobiographical interview, No. 2 (in Japanese)," *Yakkyoku no Ryouiki* (Field of Pharmacy), **7**(9): 46–52 (1958).
(1-13) Jokichi Takamine, US Patent No. 730,175, "Process of obtaining products from suprarenal glands" (patented on June 2, 1903).