Chapter 8

Post Crystallization

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

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

1. Abel's sad decline

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

2. Expanding fields of research

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

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

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

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

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

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

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

3. The name "hormone"

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

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

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

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

4. Synthesis and chemical structure

(1) Structure determination by synthesis

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

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

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

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

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

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

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

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

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

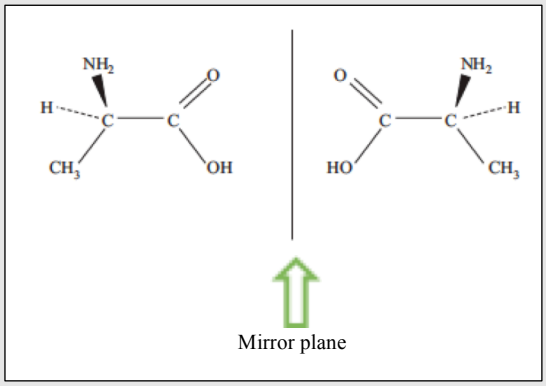
The amino acids that make up proteins and the saccharides that are building blocks for carbohydrates both have spatial structure that cannot be represented by a molecular formula written in planar fashion. The following is a simple explanation of this, illustrated by the example of an amino acid called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

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

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

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

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



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

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

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

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

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

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

(2) Separation of optical isomers

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

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

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

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

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

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

laboratories.

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

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

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

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

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

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

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

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

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

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

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

Figure 8-1. Crystals of adrenaline tartrate.

(3) Decomposition and synthesis in the body

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

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

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

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

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

(4) Looking back over 100 years of research

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

Table 8-2. Literature appeared within ca. 100 years since the discovery of adrenal medulla hormone.

Lit. appeared between	Years	Number of literature	Reference
1856–1899	44	19（4.3%）	1856：Discovery of adrenaline 1893：Discovery of its blood pressure raising activity
1900–1909	10	65（14.8%）	1900：Crystallization of adrenaline 1903：Total synthesis of adrenaline 1908：Optical resolution of adrenaline isomers
1910–1919	10	24（5.5%）	Researches on physiological activity
1920–1929	10	183（41.6%）	Investigation on nerve transmitters
1930–1939	10	133（30.2%）	1936：Nobel prizes to H. Dale and O. Loewi
1940–1953	14	6（1.4%）	1943–46：Proof of the presence of noradrenaline
1856–1953	Total 98	430（100%）	—

Table 8-2 was prepared by the literature cited (8-30).

5. Discovery of noradrenaline, a sympathomimetic compound

The idea that nerve impulse transmission takes place via a body fluid began with Thomas R. Elliott. In 1904–1905, when he was a student at the University of Cambridge and still only in his late 20s, Elliott put forward the outstanding idea that sympathetic nerve cells release a chemical substance from their endings, and that this substance stimulates effector cells to start their action within the body. Elliott thought this substance was adrenaline, and the actual substance was not discovered for a long time (8-49, 8-50). He used the name "adrenalin," without the final "e," for the active principle in both his papers, which had to do with the naming problem described in Chapter 7.

The Nobel Prize winner H. Dale later described in a Sharpey-Schäfer Memorial Lecture titled "Natural Chemical Stimulators" how Elliott worked feverishly on his research after he obtained some of the adrenalin crystals produced by Parke, Davis & Co. (8-51). Dale delivered this lecture at the University of Edinburgh in November 1937, the year after he was awarded the Nobel Prize. However, five years later, there was a surprising announcement; the substance transmitting nerve impulses did not appear to be adrenaline. G. Barger and H. Dale had been unable to confirm that adrenaline was the impulse transmitter of sympathetic nerves, and they stated their view that there was a greater probability that other types of amines showed sympathomimetic ("simulating the effects of the sympathetic nerves") action than adrenaline (8-52).

G. Barger and H. Dale's research, titled "Chemical structure and sympathomimetic action of amines," was reported by the Wellcome Physiological Laboratory in London. In the text, it states clearly that Barger and A. J. Ewins, who appeared to be Barger's helper, were

entirely responsible for the chemical side of the investigation, while Dale was responsible for the physiological experiments. In all, 54 widely differing amines, including adrenaline and noradrenaline, were gathered for thorough testing of their activity. Research of this magnitude was typically carried out only by companies, and it is likely that the aim was not purely academic, but also to search for amines that could be profitable as drugs.

Barger co-authored a paper with H. Jowett of the Wellcome Chemical Laboratory in 1905 (8-53), so he may have transferred to the Physiological Laboratory and taken charge of the compound after all the fuss of the heated exchanges between H. Dale and the owner of the company, H. S. Wellcome, over the use of the name adrenaline had died down. In this paper, Barger and Dale deal with the discovery of the structure-activity relationship in detail. Amongst others, the paper states that catechols have no activity (indicating the need for an amine group), and catechols with a methylamino group (adrenaline, etc.) have an inhibitory action on the sympathetic nervous system, whereas catechols with an amino group that has no methyl group (a primary amine) have the opposite effects. Noradrenaline is a typical primary amine, and von Euler praised this discovery in a review published 56 years later (8-54), stating that this paper gave "the first hints as to its relationship with the sympathetic nervous system" [Note 8-3].

Note 8-3.
Ulf von Euler was born into a scientific family. His father, German-born Hans von Euler-Chelpin, won a Nobel Prize in Chemistry in 1929, which he shared with Arthur Harden of the UK, for his work on "Investigations on the fermentation of sugar and fermentative enzymes." His mother was a teacher of chemistry, botany, and geology.

Ulf's parents divorced after 10 years of marriage. His maternal grand-father, Per Teodor Cleve, discovered the two rare earth metal elements Thulium (Tm) and Holmium (Ho).

Dale had carried out a wide range of research of considerable depth, including the job mentioned above, after the physiological and pharmaceutical research into ergot of rye using adrenaline that led to the clash with his boss, the owner of the company. In 1936 Dale was awarded the Nobel Prize jointly with the German researcher Otto Loewi for "their discoveries relating to chemical transmission of nerve impulses." For Loewi, the Nobel Prize was an acknowledgement of his diligent work since confirming that Stolz's synthetic suprarenin was less efficacious than samples extracted from organs when working at Hans Meyer's laboratory at the University of Marburg.

Returning to nerve impulse transmission, the momentous explanation given by Barger and Dale subsequently received the continued support of a great many researchers, but eventually, in 1933, another researcher seemed even closer to unlocking the door of the mystery. This was Heinz Schild, who was working at the National Institute for Medical Research in the London suburb of Hampstead. Dale had been the first director of this institute.

Schild very carefully conducted bioassays using cats, and he observed that the blood pressure-raising effect of suprarenal extract was stronger than would have been expected from the adrenaline content of the extract. However, even with the chance to discover an active substance other than adrenaline (noradrenaline) in front of him, he could not open the door. His paper was an investigation of papers about quantification methods mainly using color reactions that had been developed over about 20 years since 1912. The paper examined the work of some 25 authors, and showed the difficult struggle of researchers before chromatography came into practical use (8-55).

At around this time, a three-sided research race developed. This was a fierce competition between the research group led by Walter B. Cannon of the Laboratory of Physiology of Harvard Medical School, the group led by Peter Holtz, Professor of Pharmacology of the Physiologisch-chemischen Institut and the Pharmakologischen Institut of the Universität Rostock in Germany, and the group led by Ulf von Euler of the Karolinska Institutet in Stockholm, Sweden.

First, Cannon co-authored a paper in 1931 with Z. M. Bacq, who had gone to Harvard from Liége in Belgium to study, titled "A hormone produced by sympathetic action on smooth muscle." In this paper he announced a previously unknown hormone, for which he proposed the name "sympathin" (8-56). Two years later, Cannon co-authored a paper with A. Rosenblueth, in which he reported the existence of two types of sympathin, which he distinguished as sympathin E and sympathin I (8-57).

Bacq returned to Liége, where he continued his work at the Institut L. Fredericq. The following year he announced that sympathin I was adrenaline, which had inhibitory properties, and sympathin E was noradrenaline (8-58).

The following year, Cannon again co-authored a paper with Rosenblueth, this time describing detailed research into the effect of sympathin and adrenaline on the iris. In the experiments they write "adrenine," but they clearly specify that they used commercial adrenalin supplied by Parke, Davis & Co. They write that they used sympathin that they obtained by stimulating cardio-accelerator and liver nerves, but the chemical identity in this substance was not clear. This shows the difficulty of research at that time (8-59).

Bacq's hypothesis that sympathin E was noradrenaline was carefully re-examined by a four-person research group headed by C. M. Greer at the prestigious Vanderbilt University School of Medicine in Nashville, Tennessee. They presented hard evidence to show that consistency emerged if the transmitter for at least one type of sympathomimetic effect was assumed to be noradrenaline (8-60).

The following year, Cannon presented a paper titled "Evidence for Adrenaline in Adrenergic Neurons" at an academic conference. Although he showed that adrenaline and sympathin were not the same, he struggled unsuccessfully to specify the nature of sympathin (8-61).

At exactly the same time, Ulf von Euler, who later garnered a Nobel Prize for his flawless work in this field, was investigating sympathin. However, his group failed to notice noradrenaline—this was because for their experiments they were using rabbits, the adrenal glands of which contain practically no noradrenaline (8-54). Similarly, O. Loewi (whom we met on page 161) discovered the presence of adrenaline in the heart of frogs, and asserted that it was a sympathetic nerve impulse transmitter in 1937. However, the reason for this discovery was that frogs are exceptional in that they only have adrenaline in the adrenergic nerve system (8-62).

Von Euler later wrote, "It is of interest to note that in the thirties both Stehle and Ellsworth in Canada and Tiffeneau in France commented upon the quirk of nature which made use of a compound like E when the non-methylated compound seemed to serve the purpose better and was chemically simpler" (8-54). This comment serves to highlight the troubles of the researchers of this age before the development of isolation technology.

Then a rapid succession of reports that marked a turning point in the history of adrenaline appeared. The first came from W. Raab, of the Departments of Biochemistry and Medicine, University of Vermont. He published a paper titled "Adrenaline and Related Substances in Blood and Tissues", in which he announced that the sympathetic nerve impulse transmitter was not adrenaline but a substance containing a catechol group that was found in the extract of the spleen (8-63).

The next important research results to appear were in a paper by Holtz and his associates titled "Über das sympathicomimetische pressorische Prinzip des Harns ("Urosympathin") (About the sympathomimetic pressor principle of urine ("Urosympathin"))" in which they demonstrated the existence of noradrenaline in the adrenal glands and the normal urine of cats (8-64) [Note 8-4].

> **Note 8-4.**
> Strangely, although the academic journal in which this paper appears records that it received the manuscript on October 8, 1944, the paper was not actually published until three years later, in 1947. From then onward, Holtz and his team worked feverishly, publishing 17 papers over the course of five years (8-30).

Two years later, in 1946, the decisive blow was finally delivered. Using biological methods, von Euler proved the existence of a substance extremely similar to noradrenaline in fresh extracts of bull and cow spleen and bull, horse, and cat heart, as well as in the

sympathetic nerve chain (8-65). This discovery marked a turning point in the history of research into natural catechol amines, and as a result the value of the observations of Barger and Dale mentioned earlier (8-52) came to be acknowledged.

Von Euler followed this up in 1948 with a definitive paper with the long title, "Identification of the Sympathomimetic Ergone in Adrenergic Nerves of Cattle (Sympathin N) with Laevo-Noradrenaline" (8-66).

That same year, W. O. James of the Department of Botany of Oxford University proved that the only catechol amines present in the normal adrenal gland were adrenaline and noradrenaline (8-67). He separated these two catechol amines for the first time in 1948, using paper chromatography.

Von Euler worked for many years on the quantitative separation of adrenaline and noradrenaline using biological tests, which are difficult methods. In 1949, he co-authored a paper with Ulla Hamberg in which they announced the separation of the adrenal principles of cattle by paper chromatography. The separation can be vividly seen in a figure of their paper (8-68).

We have already seen in Chapter 4 how Mikhail Tsvet of Imperial Russia invented the technique of chromatography as a method for separating plant pigments in1900, the same year that Wooyenaka obtained adrenaline crystals. Half a century later, a researcher of botany, the same field as Tsvet worked in, used chromatography to clearly and unquestionably separate adrenaline and noradrenaline, proving beyond doubt that the crystals obtained by Wooyenaka had been mixed with noradrenaline. History goes around in strange ways.

Von Euler went on to develop an extremely elaborate biological test method, and using this he conducted a quantitative analysis of the adrenaline and noradrenaline within the spleen and its nerves. He reported that of the total catechol amines, adrenaline accounted for 7.2% in spleen extracts and just 2.2% in spleen nerves; noradrenaline accounted for most of the catechol amine content, and a part of it was methylated adrenaline (8-69) [Figure 8-2]. The same year, the results of measurement of noradrenaline in organs and pharmacopoeia products were reported by both universities and companies (8-70, 8-71).

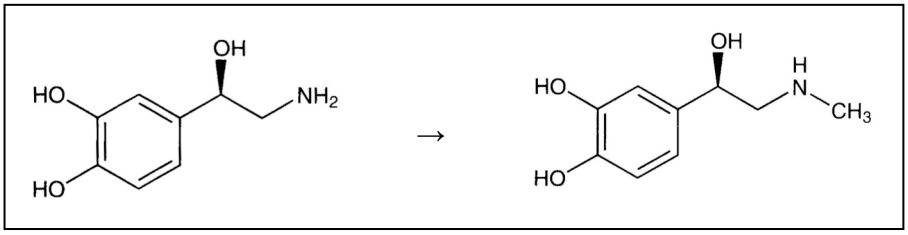


Figure 8-2. noradrenaline → adrenaline
(primary amine) → (secondary amine)
(NH_2 methylated *in vivo*)

6. More Nobel Prizes

Most of the research by von Euler described above was built around extremely detailed tests using animals, and was carried out at a research laboratory with absolutely none of the chemical isolation and analysis techniques such as chromatography and spectroscopy. In 1951, he wrote a detailed review paper in which he cited 243 research works (8-72). Von Euler was honored for these achievements with a Nobel Prize in 1970, which he received jointly with Julius Axelrod of the US and Bernard Katz of the UK.

In a paper he compiled in 1966, titled "Twenty Years of Noradrenaline," von Euler gives an extremely interesting and easy to understand overview (8-54). He starts by commenting humbly that the work of his research group was accepted only because of the outstanding work of the other two research groups mentioned earlier, i.e. W.B. Cannon's Harvard group and P. Holtz's Rostock group, and of many other researchers as well; von Euler was a very modest man.

Julius Axelrod, the joint prizewinner, was the child of Polish Jews that emigrated from Poland to the US. His Nobel Lecture entitled, "Noradrenaline: Fate and Control of its Biosynthesis," which he delivered on December 21, 1970, was a very well explained account. It was striking that he, an American researcher, did not use the name "norepinephrine," which appeared in the *US Pharmacopeia*, but instead announced the name "noradrenaline" to the world in his acceptance speech.

The third prizewinner, Bernard Katz, was born to a Jewish family in Leipzig, Germany, and at the age of 24 he fled from the Nazis, going into exile in the UK. He won the prize for his huge achievements in research into the mode of action of transmitters in synapses, and his Nobel Lecture was titled "On the Quantal Mechanism of Neural Transmitter Release." Katz was knighted in his later life.

The development of analytic methods gradually revealed some very interesting facts. I will give two examples of those here. The first of these is the data obtained by four researchers at the Department of Medicine and Biochemistry, College of Physicians and Surgeons, Columbia University and Presbyterian Hospital, New York, who were using paper chromatography (Table 8-3) (8-73). It is astonishing first of all to see that the composition of the *US Pharmacopeia* reference standard samples and commercially available *U.S.P.* epinephrine were not consistent. It is also surprising to realize that when this study was conducted in 1949, after World War II, there was still only this level of pharmaceutical technology and regulation. The research also showed that pheochromocytoma (a type of cancer) cells contain abundant noradrenaline.

Table 8-3. Abundance ratio of adrenal medulla principles in pharmaceutical products and cancer cells (8-73).

Exp. No.	Sample	Epinephrine %	Norepinephrine %
1	*U.S.P.* reference standard sample 1	81.5	18.5
2	*U.S.P.* reference standard sample 2	64	36
3	*U.S.P.* reference standard sample 3	84	16
4	Commercially available *U.S.P.* epinephrine sample 1	88	12
5	Commercially available *U.S.P.* epinephrine sample 2	100	0
6	Pheochromocytoma sample 1	47	53
7	Pheochromocytoma sample 2	12	88
8	Pheochromocytoma sample 3	10	90

*Each value represents at least 5 parallel determinations (concordant within 10%)

The second fact to emerge is that the ratio of the two active principles to each other varies greatly depending on the species of animal. This was shown by data from a literature review carried out by G. B. West of the Department of Pharmacology and Therapeutics, Queen's College, Dundee, Scotland in 1955, which are shown in Table 8-4 (8-74). In 1856, Vulpian found that the adrenaline color reaction occurred in the adrenal medulla of 14 animal species, and I simply assumed that the amount would be more or less the same in all mammals. West's findings were a revelation.

Table 8-4. The amount of adrenaline and noradrenaline in the suprarenal glands of various adult animals (8-74)

Species	Adrenaline (mg/g)	Noradrenaline (mg/g)	Noradrenaline %
Whale	0.15	1.50	91
Fowl	2.02	8.08	80
Dogfish	0.90	2.40	73
Lion	0.20	0.30	60
Cat	0.60	0.37	38
Sheep	0.50	0.25	33
Dog	1.16	0.40	26
Ox	1.20	0.42	26
Mouse	0.75	0.25	25
Man	0.52	0.10	16
Rat	0.91	0.10	9
Rabbit	0.48	trace	2
Guinea-pig	0.21	trace	2
Baboon	0.83	0	0

I am unaware of any record of how much noradrenaline was contained in the Solution Adrenalin Chloride that Parke, Davis & Co. produced by extraction from bovine adrenal glands, but going on the data in the above table for the ox, one can conjecture that there must have been sufficient quantity to have a significant effect on the activity of the product.

If adrenal glands from different animal species were used, the risk of different compositions would have made it impossible to guarantee efficacy or side effects, and even with glands from the same species, differences due to sex and age would have had to be taken into account.

This is the difficulty of animal-based medicines. Even after it became possible to prepare adrenaline cheaply through synthesis, Parke, Davis & Co. hesitated for a long time to make the change from a preparation of animal extracts to a synthetic product; this was presumably because the company would be unable to avoid deviation in the efficacy that it had guaranteed for many years through activity tests using preparations that contained noradrenaline (8-75).

While it is a digression, von Euler referred to a very interesting fact in relation to this. Dr. Goodall, who studied under von Euler at the Karolinska Institutet in Stockholm in around 1950, had a private plane and flew to Africa to collect and analyze the adrenal glands of various different animal species. The purpose of this was apparently to test Goodall's hypothesis that predators have relatively higher levels of noradrenaline and prey species have relatively higher levels of adrenaline, which strongly affects their decision to flee. Some researchers are born into great wealth (8-54).

The natural world is full of mysteries, and one such mystery is the existence of noradrenaline in plants. This discovery was made through joint research between L. J. Haynes and K. E. Magnus of the Department of Chemistry at the University College of The West Indies, Jamaica, and P. C. Feng from the Department of Pharmacology at the same university. They discovered that purslane (*Portulaca oleracea* L.; Figure 8-3), a species of plant that is believed in Jamaican folklore to have therapeutic effects for cardiovascular disease, contains abundant noradrenaline. They reported that the noradrenaline content may be 2.5 mg per 1 g fresh weight of plant (0.25%), which is more than the content of animal adrenal glands. They also reported the presence of noradrenaline in different banana and potato varieties (8-76).

Figure 8-3. *Portulaca oleracea* L contains noradrenaline in high concentrations. (photo taken by the author in Yokohama, Japan)

The first person to synthesize noradrenaline was Friedrich Stolz, the same German researcher of Hoechst A.G. who first synthesized adrenaline. However, he did not synthesize it out of any awareness that it was a compound with important physiological properties. During the course of his research, he was experimenting with a compound that could be reduced to form adrenaline—in other words, a precursor. He treated this with liquid ammonia, and wrote down that the compound that was created was a "free base." He then performed an elemental analysis twice and wrote the values he found against the theoretical values, and these results were published in a paper in 1904 (8-20). The molecular formula shown in the paper has one carbon atom in excess, and even my calculations show that there is clearly a mistake. The numerical values have the low level of accuracy of the time, but looking at the chemical reaction process, later researchers have recognized that the compound was the first synthesized noradrenaline (8-30, 8-54). Hoechst A. G. gave this the trademark "Arterenol," and there have been a few papers here and there that have used this name (e.g., 8-70, 8-71).

Noradrenaline is optically active, as is to be expected in the natural world, and laevorotatory *l*-noradrenaline is the physiologically active isomer. Stolz synthesized a dextro and laevorotatory mixture (called a "racemate"), and this was separated into dextro and laevorotatory forms by B. F. Tullar and his colleagues at the Sterling-Winthrop Research Institute, an American pharmaceutical company, 40 years after Flächer's optical resolution of adrenaline (8-77, 8-78). Some 10 years later, the three-dimensional structure of active noradrenaline was clarified by P. Pratesi and associates at the Istituto di Chimica Farmaceutica, Università di Pavia, Italy (8-79).

In the discussion above, we have followed the struggles of many physiologists and chemists after the crystallization of adrenaline. After von Euler showed the importance of the action of noradrenaline, the field of research expanded exponentially, and it saw astounding development during the 20 years between 1940 and 1960.

J. Malmejac of the Faculty of Medicine of the University of Paris gives an overview of this development in a long review paper titled "Activity of the Adrenal medulla and its Regulation," which was published in English in an American journal in 1964 and which cites 365 different works, including 76 of the author's own (8-80).

7. New medicines developed by chemical structure modification

Finally, we come to the invention of the fourth man to win a Nobel Prize in 1988 after Dale, Loewi, and von Euler. This was the Scottish chemist and physiologist Sir James Whyte

Black (1924-2010), who was honored for work that is monumental in the history of the quest for β-blockers (beta-adrenoreceptor antagonists) (8-81).

Until Black's research, all work in this field had been groping in the dark. Simply put, his achievement was the invention of a miracle drug for *angina pectoris*, which is a difficult problem in the field of cardiology. Black developed a new drug by modifying the structure of adrenaline, and won a Nobel Prize in Physiology or Medicine in 1988. While it gets a little technical, I have shown a group of chemical structures side-by-side, as it may be of interest to readers to have a visual image of the concepts that medical and pharmaceutical researchers pursue and the process of trial and error they use.

As the chemical structures in Figure 8-4 show, by just a small structural modification (technically, by inserting CH_2O), Black arrived at propranolol (V) from pronethanol (IV), which he had previously invented. Pronethanol had a fatal flaw as a drug candidate—it was carcinogenic. Black's new invention, propranolol, successfully eliminated the carcinogenic properties to yield a safe, new drug.

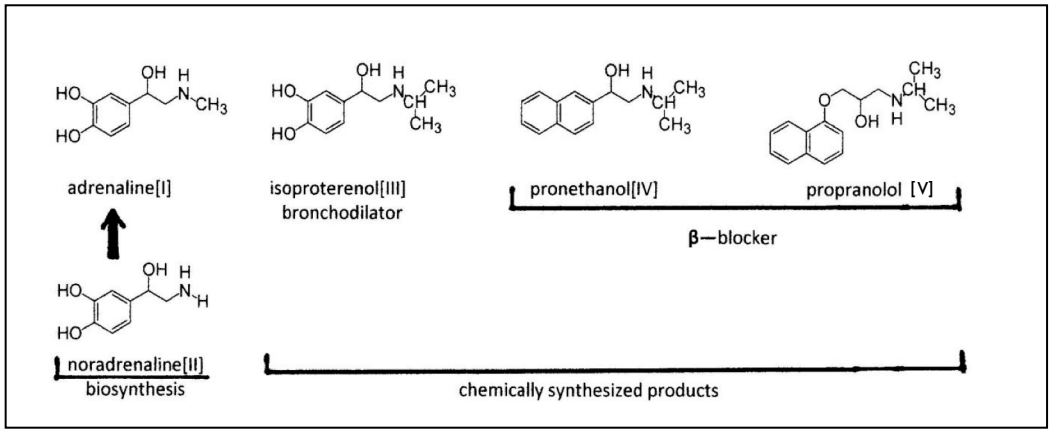


Figure 8-4. Invention of new medicines by chemical structure modification.

Propranolol is a β-blocker, and as well as the curative effect on *angina pectoris*, it acts to lessen the impact of bad memories if taken immediately after recollection of a trauma. It is also taken by performers to lessen the effects of stage fright. Black was awarded the Nobel Prize in Physiology or Medicine in 1988 together with Gertrude B. Elion and George H. Hitchings for their discoveries of important principles for drug treatment [Note 8-5].

Note 8-5.
Black studied medicine at the physiology department of the University of St. Andrews in Scotland before beginning his research. He worked for a time as a teacher at the University of Malaya in Kuala Lumpur, and then returned to Scotland, where he was instrumental in establishing the Physiology Department at the University of Glasgow. Black was a highly talented man, who worked for ICI (ICI Pharmaceuticals),

SKF (Smith, Kline and French), and Wellcome (Wellcome Research Laboratories), three major pharmaceutical companies, before accepting a post as professor of pharmacology at the University of London and King's College.

8. Later information on the two American scientists

(1) Aldrich

Aldrich (see Figure 4-4 in Chapter 4) made a glorious name for himself as a chemist by isolating crystals of adrenaline, somewhat later than Takamine but independently, and by deducing the correct molecular formula. But what did he work on after that? I searched the scientific literature to find out.

Some of his subsequent research was in fields other than constituents of the living body. In 1911, he carried out research into methods of manufacturing a drug named brometone [$(CH3)_2C(OH)CBr_3$] (8-82); in 1912, he carried out an analysis of the iodine content of cattle thyroid (8-83); and in 1915, he worked on the trial manufacture of an experimental apparatus (8-84). However, his main work was with hormones. First, in 1912 Aldrich published a report on the development of the anterior pituitary (adenohypophysis) in puppies (8-85), and then from 1915 he published preliminary research reports relating to the posterior pituitary (8-86).

Later, in 1928, he published his findings as one of the five members of the pituitary hormone project of Parke, Davis & Co. This was a groundbreaking work that separated and identified the two physiologically active substances secreted from the posterior pituitary: vasopressin (the blood pressure-raising constituent) and oxytocin (the mammalian neurohypophysical hormone). These two substances are both peptides comprising nine amino acids (Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2) and have a single ring structure. They also have the same terminal structure which determines their reactivity. Given the separation technology that was available at the time, it was no easy task for the group to arrive at their results (8-87).

(2) Houghton

Dr. Houghton, who directed the research group of Parke, Davis & Co. that tested the activity of the adrenal principles, was born in New York in 1867, and entered the University of Michigan at the age of 22. He acquired his PhD in medicinal chemistry and medical science in 1893, and was welcomed into Parke, Davis & Co. two years later.

On March 24, 1904, the German medical scientist Paul Ehrlich, who was visiting the US,

was invited to give a lecture at Parke, Davis & Co. Ehrlich was a towering presence who had established the chemistry of immunization; he later received the Nobel Prize in Physiology or Medicine in 1908 and had been respected as "a researcher for life" for perfecting the technology to produce diphtheria therapeutic serum. Parke, Davis & Co. was the first company in the US to start production and sales of diphtheria therapeutic serum, and Houghton, who was involved in this project, was probably on good terms with Ehrlich.

Ehrlich had been a colleague of the Japanese bacteriologist Shibasaburo Kitasato during the decade that was later known as the "golden 1880s"; the two had established the theory of the antigen (toxin)-antibody reaction at the same time in the Koch laboratory in Berlin. Houghton may perhaps have met Kitasato when the latter visited the US in 1904 at Takamine's invitation. Takamine acted as Kitasato's guide in the US, and there is a photograph still in existence that shows them sailing on Lake Michigan in a yacht owned by a director of Parke, Davis & Co. (8-88).

Houghton took up residence in 1905 in a mansion at 680 Longfellow in the Boston-Edison district of Detroit, where the great automobile pioneer Henry Ford lived. Boston-Edison, which is in the heart of the city, was home to a great many celebrities. The mansion is renovated following colonial time architectural style. Today it is designated as an historical landmark. Houghton passed away in 1937 (8-89).

9. A ring connecting Nagai, Takamine, and Wooyenaka

We have already seen how Wooyenaka, who was the first person to obtain adrenaline crystals, thus energizing hormone science, joined a non-regular course at the Pharmaceutical Department of Tokyo Imperial University. There, he diligently studied the chemistry of natural substances under Professor Nagai. Nagai became well known around the world for his research into the alkaloid ephedrine, which was the active principle of *mahuang* (*Ephedra sinica*), a Chinese medicine. The research had been completed and a report published in an academic journal by the time Wooyenaka joined Nagai's course, so Wooyenaka did not have any direct involvement in this job. However, it is likely that he learned about the extraction and purification of ephedrine and carried it out himself, as part of the course. Even if that were not the case, he would have listened carefully to the details at every opportunity, as an example of handling natural products. Later in his life, Wooyenaka stated that he remembered the teaching of Professor Nagai as being the key to his own success (see Chapter 5, section 10).

The results obtained by Wooyenaka led to rapid development of the study and application

of hormones. One outcome was a report in 1903 by two New York doctors, Jesse Bullowa and David Kaplan, that subcutaneous injection of adrenaline chloride was very effective against asthma. This led to the spread of adrenaline injections as a treatment for asthma (8-90, 8-91). In fact, the statement of efficacy of adrenaline chloride solution from 1905, in the initial stage of sales in the US, states that its primary indication is "for asthma."

We have already seen in Chapter 3 how in 1897, the American Solis-Cohen brought about a dramatic recovery in a 22-year-old woman who was suffering respiratory difficulty by administering Burroughs & Wellcome's Supra-renal Tablets, and he published a paper on the efficacy of adrenal substance against asthma. The news that the product of Parke, Davis & Co., which was of consistent quality, was effective must have been welcomed by respiratory doctors.

Some 10 years after adrenaline was reported to be highly effective for asthma, two researchers began a full study of the medicinal action of ephedrine, which has a similar chemical structure [Figure 8-5]. These were Hajime Amatsu and Seiko Kubota, assistants at the Medicinal Science Laboratories of Kyoto Medical College, Kyoto, Japan (8-92, 8-93). They published two research reports demonstrating that this substance was effective against asthma in experimental animals, but unfortunately their findings were never applied to clinical trials on humans in Japan, and because their reports were written in Japanese, the content was never disseminated beyond the shores of Japan.

Ten years later, in 1924, K. K. Chen and Carl F. Schmidt at the Laboratory of Pharmacology of the Union Medical College, Peking, China, published a report of the great efficacy of ephedrine against asthma in English. They were credited with this discovery (8-94), even though Chen cited the work of Amatsu and Kubota in the report. It was unfortunate for Amatsu and Kubota that scientific research in Japan was still undeveloped and Japanese was a minor language.

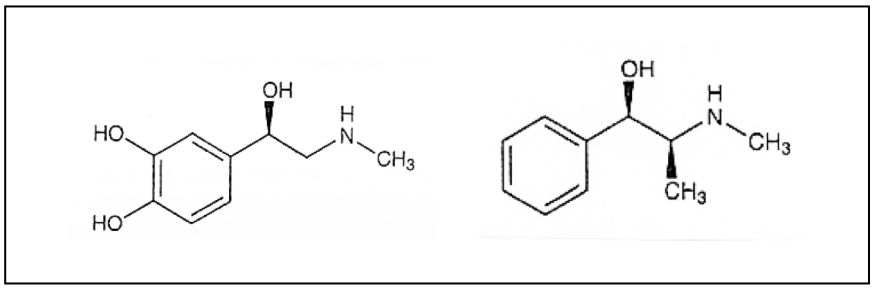


Figure 8-5. **Left:** Takamine and Wooyenaka crystallized an adrenal hormone "adrenaline"
Right: Nagayoshi Nagai extracted a plant alkaloid "ephedrine"

Leaving that aside, Nagai's methods for natural product research, in particular ephedrine, led to the success of Wooyenaka, who had crossed the ocean to New York and taken on the task of the crystallization of adrenaline in Takamine's laboratory.

The news that adrenaline showed great efficacy against asthma made its way back to Japan, where two young medical researchers in Kyoto discovered the efficacy of ephedrine on bronchial smooth muscle—thus Nagai's methods traveled full circle. The researchers were members of the Tokyo Chemical Society, which Nagai and Takamine had developed from 1888 onward as president and permanent member of the board, respectively.

While this is something of an aside, the biography of Friedrich Stolz, the head of the Chemical Department at Hoechst A. G. who succeeded in the total synthesis of adrenaline, states that he devoted his later life to chemical research into ephedrine (8-27). Stolz outlived Takamine and Nagai, but right up until the end they were bound by a curious thread of fate.

10. Adrenaline still in use today

I have condensed the results of the diverse research into adrenaline during the period of over a century since Wooyenaka first obtained the crystals, and I would like to give a brief account of how the physiological action of adrenaline is used in medical treatment today.

Our bodies do not stop producing adrenaline even for an instant, and it continually demonstrates its action as an essential hormone. At the same time, adrenaline that has been extracted (or today, adrenaline that has been chemically synthesized) acts differently within the body. Its action is effective for hemostasis and against asthma and food or pollen allergies, so adrenaline has a double role.

In the US there have recently been many cases in which young children suddenly have lost their lives as a result of severe allergy (anaphylaxis) to some foods, particularly peanuts. Because of this, the EpiPen (a pen-like device that delivers an injection of epinephrine) is kept on hand at schools and teachers are trained to be able to give prompt administration (injection) of adrenaline without any mistakes. Japan has learned from the system in the US, and similar systems are starting to go into place there as well.

(1) Physiological activities

Adrenaline and noradrenaline both act directly on the heart muscle to increase contraction and raise the pulse rate. However, while they are similar in this respect, they are not entirely

the same. Adrenaline acts strongly to increase glucose levels in the blood by glycogenolysis, whereas noradrenaline has marked blood vessel contraction action.

Adrenaline and noradrenaline are both classified into the group known as catechol amines. Body tissues have special parts that act when they receive hormones—these are called "receptors."

There are two types of receptor for catechol amines: α- and β-receptors. The α-receptors receive both adrenaline and noradrenaline, while the β-receptors receive adrenaline.

When α-receptors act, there is blood vessel contraction. When Oliver and Schäfer were astounded to see the column of mercury in the sphygmomanometer rise in 1893, they were witnessing the α-receptors acting to make vascular smooth muscle contract so that the blood pressure rose sharply.

When β-receptors act, there are effects such as vasodilation, accelerated cardiac output, and bronchial tree dilation. The α- and β-receptors have been studied in detail, and it has been shown that α-receptors can be further classified into α_1 and α_2, and β-receptors into β_1, β_2, and β_3. The different actions of each of these have been clarified.

(2) Adrenaline products listed on Pharmacopoeia (combination drugs are excluded)

(a) US Pharmacopeia

(i) Epinephrine Inhalation Aerosol
(ii) Epinephrine Injection
(iii) Epinephrine Inhalation Solution
(iv) Epinephrine Nasal Solution
(v) Epinephrine Ophthalmic Solution
(vi) Epinephrine Bitartrate
(vii) Epinephrine Bitartrate Inhalation Aerosol
(viii) Epinephrine Bitartrate Ophthalmic Solution
(ix) Epinephrine Bitartrate for Ophthalmic Solution
(x) Epinephrine Borate Ophthalmic Solution

(b) Japanese Pharmacopoeia

(i) Adrenaline Solution
(ii) Adrenaline Injection

11. Other contributions by Jokichi Takamine

Takamine granted Matasaku Shiobara, who at that stage was a manager of a pharmaceutical company, a monopoly to market Taka-Diastase in Japan.

In 1913, Takamine established Sankyo Co., Ltd. together with Shiobara in order to develop the business. While still living in the US Takamine took the position of company president, and he gave his undivided attention to new product development.

However, his extensive knowledge and rich experience did not stop there.

(1) Toyota Motor Corporation

In his later years, Takamine was said to be the best-known Japanese person in America, and many of the Japanese people who crossed the Pacific Ocean to the new continent called to visit him at his mansion in Manhattan.

One of these was Sakichi Toyoda, the founder of Toyoda Automatic Loom Works, who was known as the "King of Inventors." At a time when combined businesses in Japan were stagnating and there seemed to be no hope for the future, he had left Japan in despair. He landed in Seattle and crossed America by train, eventually arriving in New York. It was 1910, and he was 43 years old.

Takamine, then 56, welcomed him kindly. Toyoda was feeling lost, and Takamine patiently spoke of the path he himself had followed. In his autobiography, Toyoda records what Takamine told him: "Many profitable inventions often end in failure and are consigned to oblivion. Society is at fault, but the inventor himself is also responsible. I believe that for an invention to be successful, the inventor must never be separated from it. It is the inventor's responsibility to apply the invention. This means that the inventor cannot part from the invention until there is the prospect that the invention will be fine even if he lets society use it.

Before an egg becomes a bird, it may die or be incapacitated if someone else gets hold of it. The inventor has the responsibility to carefully watch over it until it grows fine, strong wings, so that wherever it is set free it has the prospect of being able to fly safely. That is the way to perfect an invention." Listening closely to Takamine, Toyoda felt his confidence returned.

After returning to Japan, he pushed forward with his business. Having seen the huge growth of motorization in the US, he believed the future lay with the automobile industry. Although he did not live long enough to achieve his desire, he continually insisted to his

family that the automobile would be the next big thing. It was from Sakichi's dream that today's Toyota Motor Corporation developed (8-95, 8-96).

(2) RIKEN

Another seed that Takamine sowed was the Rikagaku Kenkyusho (the Institute of Physical and Chemical Research, usually known as RIKEN) in Japan, which looks set to bring great benefits to humanity in the near future.

Takamine developed the concept and put forward the proposal for a new research institute, and with the support of his acquaintances in the business world such as Eiichi Shibusawa and Takashi Masuda, RIKEN was established over a century ago, in 1917 (8-97).

Dr. Masayo Takahashi of RIKEN has been carrying out joint research with Prof. Dr. Shinya Yamanaka, who won the 2012 Nobel Prize in Physiology or Medicine, into tissue therapy for age-related macular degeneration (AMD) using induced pluripotent stem (iPS) cells. She recently announced the start of clinical tests. The Japanese government gave its approval in July 2013, and around the world, patients of this as yet incurable disease are hoping for their success as soon as possible.

12. Takamine as an unofficial ambassador

The year 2012 marked the 100th anniversary of the planting of the famous Japanese cherry trees that flower every year along the banks of the Potomac River in Washington and the Hudson River in New York. The gift of the cherry trees came about as a result of the love of travel writer Eliza R. Scidmore and First Lady Helen Taft for Japan's cherry blossom, the efforts of the Japanese Consul General Kokichi Mizuno, and an offer for donation by Jokichi Takamine. On the Japanese side, Foreign Minister Jutaro Komura took a leading role in facilitating the donation (8-98).

Figure 8-6 shows a photograph of a meeting of the Japanese delegation for the peace negotiations of the Russo-Japanese War, taken at Metropolitan Club, New York, in 1905. The delegation was about to leave for Portsmouth, New Hampshire, carrying the responsibility for Japan's future. Jokichi Takamine joined in the meeting as the only non-government person to give encouragement to the delegation.

Figure 8-6. Japanese delegation for peace negotiation with Russia at the end of the Russo-Japanese War in 1905. Sitting from right to left in the front row: Jokichi Takamine, Koichiro Tachibana, Kogoro Takahira, Kentaro Kaneko, Jutaro Komura (Courtesy of Masayoshi Matsumura).

One member of the delegation, Kentaro Kaneko, had attended Harvard University at the same time as the then U.S. President Theodore Roosevelt, and he was instructed to tour America to explain the Japanese position in the negotiations. As he was not accompanied by his wife, Takamine's wife, Caroline, accompanied Kaneko in her place to his lectures in different parts of the country. Kaneko later recalled that he could not possibly have fulfilled his responsibility without the help and cooperation of Dr. and Mrs. Takamine.

Takamine, then living in America, was fully aware that the task of the chief delegate, Jutaro Komura, was to fight for the destiny of Japan, and he spared no effort in giving support to his home country.

Takamine and Komura had both learned English from the same teacher in their younger days—this was Guido Verbeck, the teacher of English at the *Chienkan* School in Nagasaki (8-99). Takamine had already returned to Kaga Domain by the time Komura enrolled at the school, so the two were never classmates.

Strangely enough, however, they were brought together 36 years later at the Russo-Japanese peace conference, where the fate of Japan was to be decided. They must surely have shared memories of their youth in Nagasaki, and Takamine would have given heartfelt encouragement to his junior from the *Chienkan* School by wishing the delegation well as it left for the negotiations.

13. Here lie the Japanese hormone hunters

In the early morning of July 22, 1922, Jokichi Takamine passed away at the age of 67 after

a full and eventful life. He had fought against illness for two years, but the medical treatment proved ineffective. At the end of his life, Takamine was cared for by his wife and two sons; his younger sister, Junko Takehashi; and Keizo Wooyenaka.

Takamine always said that he wished to spend his final years in his home country of Japan. However, Eiichi Shibusawa and Takashi Masuda, the two leaders of the financial world to whom Takamine felt himself obliged, begged him to stay in America to work for Japan-America friendship and diplomacy. Takamine's last years were tinged with sadness, as he finally had no choice but to give up all hope of returning to his homeland.

Three days later, on July 25, a majestic Catholic funeral service was held for Takamine at Saint Patrick's Cathedral in New York. Some 600 mourners, including many Japanese and American dignitaries, packed the cathedral.

His widow, Caroline, spoke at the funeral. She thanked the mourners for attending, and then went on to talk of her deceased husband. The records say that the widow's tear-choked words resonated strongly in the hearts of those who heard her; "Jokichi loved Japan until the very end, he missed Japan, he yearned for Japan. I ask you all to speed Jokichi on his way to heaven by singing together the Japanese national anthem."

A memorial ceremony was later held at the Nippon Club, and several hundred people made flower offerings.

Apparently, an anonymous American woman laid a bunch of flowers accompanied by a note; the note simply gave thanks for the joy of life that had been returned by the drug Adrenalin, and hoped that Dr. Takamine's soul would rest in eternal peace.

The name Adrenalin had become well known among the American people, and its effectiveness was very highly regarded (8-100, 8-101).

The day after Takamine's death, *the New York Times* published an obituary. As the headings accurately reflect the esteem in which Takamine was held, I will include them here:

> "JOKICHI TAKAMINE, NOTED CHEMIST, DIES"
> "Japanese Who Discovered Adrenaline and Takadiastase Had Been Ill Two Years"
> "FOUNDED THE NIPPON CLUB"
> "He Was Widely Known for His Work for Friendly Relations between Japan and United States"

Jokichi Takamine lies with his beloved wife Caroline in Woodlawn Cemetery in a suburb of New York, the city where he was so successful [Figure 8-7]. Some of his hair was buried in

Aoyama Cemetery in Tokyo. His friend Jutaro Komura also lies in the same cemetery.

Figure 8-7. Sepulcher of the Takamine Family in Woodlawn Cemetery, suburb of New York City (photo taken by the author).

Keizo Wooyenaka lived for many more years. In his later life he was an auditor for Sankyo Co., Ltd. for seven years from 1928, and after his retirement he continued his pursuit of knowledge through reading, acquiring the German language, and other activities, until he breathed his last at his home in Nakano Hikawacho, Tokyo, on January 11, 1960 at the age of 84.

He lies in Kodaira Cemetery on the outskirts of Tokyo with his wife, Yaeno, who shared his hardships, and their beloved children [Figure 8-8].

Figure 8-8. Grave of the Wooyenaka Family in Kodaira Cemetery, Tokyo (photo taken by the author)

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Through the sheer hard work of the "Hormone Hunters," it became possible to obtain a specific adrenal active principle in crystalline form. Until then, researchers around the world had only been able to conduct experiments that gave no more than frustrating glimpses of their target—now, they could make vigorous advances toward verifying the hypotheses they had pictured in their minds.

Animal physiology and pharmacology entered a completely new era, and for pharmaceutical manufacturers the way was open to a future in which they could dream of a vast range of new products.

Those who contributed to this, many of whom went on to explore new paths, left behind magnificent achievements for posterity.

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