1 The Discovery of DNA

Contrary to popular belief, the discovery of the chemical structure and biological function of deoxyribonucleic acid (DNA) did not occur within the past several years and was not accomplished by a small, select group of scientists. In fact DNA was discovered more than one hundred years ago; but only comparatively recently have we begun to appreciate the significance of that discovery. Solving the problems of DNA was similar to the tedious, painstaking work involved in assembling the many isolated pieces of a large jigsaw puzzle. A great number of scientists working in a variety of fields contributed to the final outcome, but few ever received anything more significant than the personal satisfaction of having been a participant. The most recent steps appear particularly exciting because they were made at a time when a sufficiently large portion of the puzzle had been completed to suggest a tantalizing view of the solution. The puzzle began, however, with the discovery of DNA in 1869.

This discovery, one of the most significant scientific accomplishments of the nineteenth century, was made by accident and was not reported until more than two years later. This unusually long delay was caused by the reluctance of an older and better known scientist to publish novel observations made in his own laboratory until he had personally confirmed them. Felix Hoppe-Seyler, then forty-four years old, was at the height of his scientific career. Friedrich Miescher, the discoverer of DNA, was only twenty-five and unknown. Miescher had not become involved in physiological-chemical research entirely by chance. Both his development and training had been closely supervised by his father Johann F. Miescher and his uncle Wilhelm His, each of whom was a well-known physician and scientist.

The elder Miescher was born on March 2, 1811, in Walkringen in the Swiss canton of Bern. As a young man he rejected his parents' advice to enter the family linen business and instead decided to study medicine. He began his studies in Bern, after which he moved to Berlin and joined Johannes Müller as one of his earliest students. Among Müller's other students who would also become famous biologists were Rudolph Virchow and Theodor Schwann. Miescher's reputation was assured after his novel studies on bone and bone inflammation. In 1834, to increase his social standing and prestige, Johann Miescher purchased for the sum of 1600 Swiss francs the right to become a citizen of Burgdorf. From 1837 to 1844, he held the post of professor

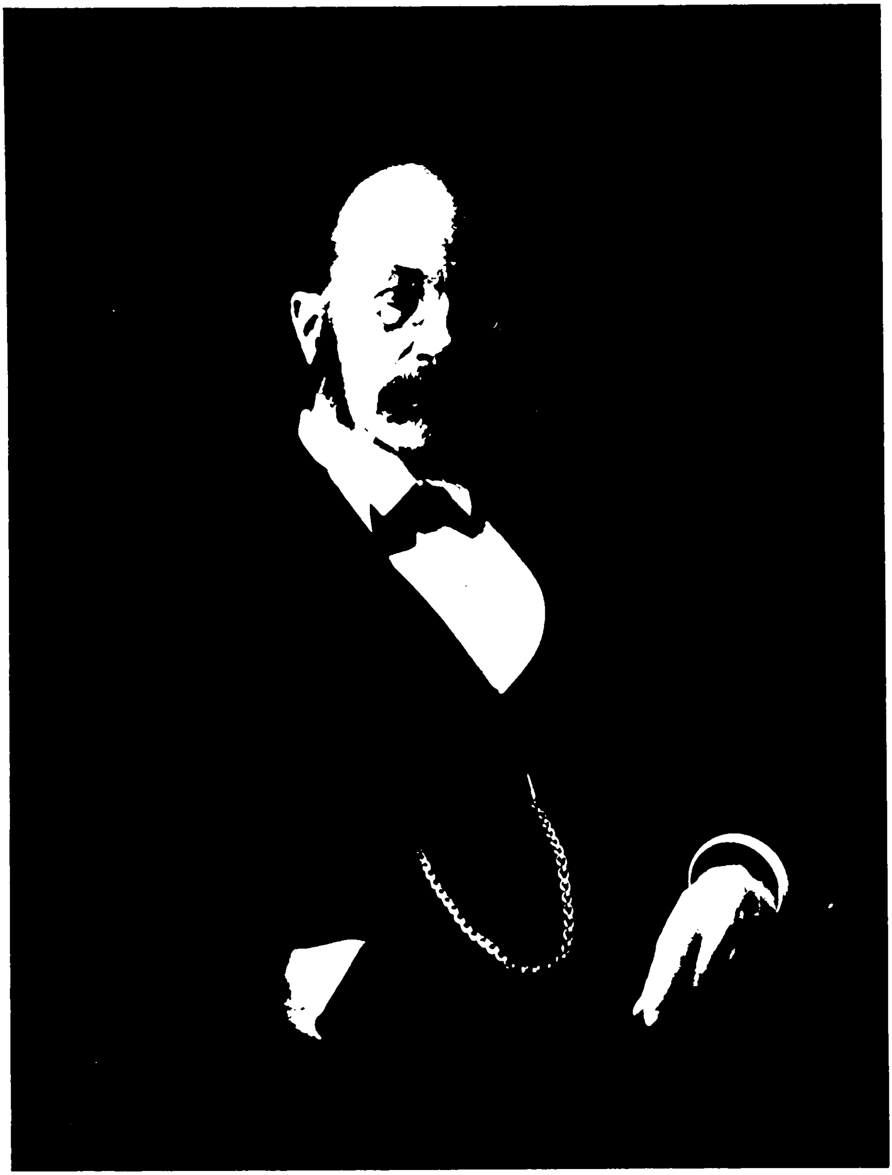

Figure 1.1 Wilhelm His (courtesy of the National Library of Medicine).

Figure 1.2 Friedrich Miescher (courtesy of the National Library of Medicine).

of anatomy and physiology at the medical school in Basel, Switzerland. In 1843, he married Charlotte Antonie His, a citizen of Basel, thereby simultaneously gaining both a wife and the right to become an honorary citizen of Basel without any additional payments.

The His family was descended from Peter Ochs of Basel, an acknowledged statesman and historian of French ancestry. At the beginning of the nineteenth century strong anti-French sentiment was prevalent in Switzerland, and Ochs, recognizing that his family had been associated with French interests, changed his name to His. Charlotte's brother Wilhelm was a noted embryologist and histologist who held the position of professor of anatomy and physiology in Basel from 1857 to 1872 (figure 1.1).

Friedrich Miescher (figure 1.2) was born in Basel on August 3, 1844. About this time his father accepted a position as professor of pathologic anatomy and hospital physician in Bern, but the family returned to Basel in 1850 where he continued to work as both a physician and a teacher of pathological anatomy. Friedrich was the eldest of five brothers born between 1844 and 1851. Comments made by students who knew the family, as well as by Wilhelm His, indicate that Friedrich grew up in an extraordinarily stimulating atmosphere. The Miescher family was highly respected in Basel and apparently enjoyed a wide circle of friends. His wrote:

> More than half of Miescher's immediate contemporaries became outstanding even in the early years, some still as students and some as the result of their professional activities. But the most talented of these from the very beginning was F. Miescher and he was recognized as such by his fellow students. At the time he was shy to some degree and had some difficulty in communication, partly caused by a hardness of hearing which he acquired in youth. This however, did not prevent him from being the focal point of a youthful circle of friends which circulated around him. . . .[1]

Friedrich decided to follow his father into medicine after the latter refused to allow him to follow his initial desire to become a priest. The greater part of Miescher's studies was carried out at Basel Medical School, which he attended starting in 1863. During the summer of 1865, Miescher spent one semester in Göttingen, where the university records indicate that his courses included practical chemistry with F. Wöhler, as well as studies in microscopy, general pathology, and related medical courses.[2] On his return to Basel, Miescher contracted a severe case of typhus, which required several months of convalescence until autumn 1866.

In spring 1868, Miescher completed his studies with what was recognized as a brilliant doctoral dissertation. Shortly before this, Miescher debated with his father and uncle on his plans for the future. This was of particular concern because hearing difficulties prevented him from considering many branches of medicine: "My hardness of hearing eliminates me from those medical functions in which auscultation and percussion are necessary and important in examining the disease and its causes. This includes the greater part of cases that fall under the scope of the general practitioner." Miescher further eliminated the specialties of surgery, obstetrics, gynecology, and laryngoscopy for this reason, as well as from a consideration that these specialties are "altogether contrary to my talents and more so to my inclinations." But he had already concluded that "I have used my period as a student to prepare myself for the medical profession. I shall have to base my living on the practice of this profession, as I see no alternative." What he saw as possible specialties fell in the realm of ophthalmology and otology. He wrote,

The crown of these specialties is ophthalmology. The scientific grounding based on a thorough anatomical and physiological basis which is necessitated by this specialty with accuracy of diagnosis based on a uniquely direct observation of the pathological tissue alterations and the excellent successes of treatment make ophthalmology one of the most rewarding and satisfactory medical activities, even in my eyes.[3]

But, he continued,

No matter how much I had this objective in mind, however, I do not wish to deny that during my studies my gaze was directed toward another side. A decisive factor in my choice of a profession was my interest in natural science which dates back to my earlier school years, even though it did not get much stimulation from the teaching I received. . . . It was only with the lectures on physiology that the entire splendor of the research on organic matters became apparent. . . . It seemed to me that it was here that work was being done most directly on the tasks which in my opinion are essential in science. . . .

It seemed to me that this was the task upon which I wished to collaborate in some manner, and that such efforts would produce a satisfactory background for the future.

. . . I already had cause to regret that I have had so little experience with the essential auxiliary sciences in physiology, namely chemistry and physics: one because of lack of facilities in the institutes, and the other because of a lack of mathematical training. For this reason, the actual narrower understanding of physiological facts still remained obscure to me in some points.

I was soon brought back from the idea of a purely scientific or academic career, if I ever entertained it at all, by a recognition of the limits of my talents. The possibility of basing my prospects of my material existence upon my future work as a scientific personality was one which I never considered.

I believe, however, that a medical activity which centers around a narrowly limited specialty would allow a certain amount of time to engage in scientific work.[3]

This letter from Friedrich to his father was subsequently passed to Wilhelm His for his views and opinion. His wrote in reply,

. . . I believe that he overestimates the importance of special training in the same way that I myself did at one time. For example, I see absolutely no reason to doubt his ability to work successfully in general physiology, or to conduct chemical research or even to work effectively as a general practitioner, because he appears to lack some of the necessary training for which acute hearing is not an essential necessity. . . . In view of the considerable mental talents which Fritz possesses, I am convinced that he will achieve success and satisfaction in any direction in which he will go with enthusiasm and courage. The first year of his activity may not give the full feeling of familiarity in the area in question. But if not in the first then in subsequent years that will come, for I believe it to be entirely impossible that anyone who works in a certain field earnestly and with energy will not finally achieve his own particular importance in the field in question. Self-confidence, however, is necessary for all things—not the confidence that one cannot ever make a mistake, but the confidence in oneself that by continuous work one will contribute one's very best. No one can be equal to everyone else in all things, but everyone can, through certain aspects of his activity or through the particular combination of his activities, distinguish himself from others. This is what the value of the individual is based on. However, this feeling of individual worth and individual ability, to which Fritz is entitled far more than many others, is one which he has to seek to acquire and make others aware of also. The fight for a career repeats itself continuously in science and in life in general. If we do not step forward with the consciousness that we are as good as anybody else, then we cannot hope to find that recognition in others since we do not feel it ourselves.[4]

His proposed that young Miescher follow his inclinations into physiological research by returning to Göttingen for more chemistry courses and then traveling to Berlin to undertake actual physiological research with either Friedrich Wilhelm Kühne or Emil Du Bois-Reymond. In any case, he cautioned, if Friedrich entered a narrow area of specialty this early in his career it might be a hazard; it would be better, instead, to develop first a good general medical foundation and then, if necessary, a specialty.

Miescher chose to follow his uncle's advice but went to the University of Tübingen, in southern Germany closer to Basel, instead of returning to Göttingen. In 1865, the University of Tübingen became the first university in Germany to create a faculty of natural science. Felix Hoppe-Seyler had established a laboratory of physiological chemistry there and had rapidly developed a reputation as a pioneer in the newly founded field of tissue chemistry. Miescher, after carrying out a few preliminary experiments in Basel, arrived in Tübingen in 1868 with definite plans for studying the chemistry of the cell. Certainly His's advice to Miescher "that he choose the direction of histochemistry, since I had recognized again and again in my own histologic works that the final questions in the development of tissue could only be solved on a chemical foundation" was of decisive importance.[5]

Miescher's arrival at Tübingen coincided with an important period in the development of thought on the origins and functions of the cell. Only a short time before, the concept of spontaneous generation had dominated biologists' thinking. This theory held that living organisms arose by an unknown transformation of lifeless matter. By 1868, sufficient evidence had accumulated to discredit this idea. In France, an elegant series of experiments by Louis Pasteur helped pave the way, once and for all, toward the realization that dormant but living material carried in the air—not some unknown vital force—was responsible for what appeared to be the spontaneous generation of life from nonliving material. Joseph Lister in England showed that surgical infections could be prevented if surgeons used sterile techniques. Infections did not occur spontaneously as most physicians thought; the use of contaminated instruments was the cause. These and similar studies redirected attention to the cell and its components as both the basis of organization of living things and the source from which new cells developed. In 1858, Rudolph Virchow published studies supporting his idea that the causes of diseases are to be found within cells and therefore have a specific organic basis. It was also Virchow who developed the concept that cells arise only from other cells. In 1861, Max Shultze enunciated the modern idea of the cell by emphasizing the importance of protoplasm surrounding a nucleus. In 1866, Ernst Haeckel stirred further interest in the nucleus by suggesting that it contained the factors necessary for the transmission of heredity.

Of course, unresolved by all of these efforts was the most fundamental question of all: What causes a cell to live? Many believed that the movement and interaction of the components making up the nucleus and the protoplasm gave rise to life within the cell. This hypothesis implied that individual components could not be isolated and examined since doing so would alter their life-giving properties. On the other hand, there were pioneers such as Felix Hoppe-Seyler (figure 1.3) who were convinced that the study of the chemical and physical properties of individual components of the cell was possible and would eventually lead to a deeper understanding of the molecular forces regulating cell life. Hoppe-Seyler's father had died when he was nine years old, and upon being adopted by his guardian and brother-in-law, Dr. Seyler, he added that name. Hoppe-Seyler was one of the first to crystallize hemoglobin, the protein responsible for the red color of blood, although this may have been accomplished as early as 1840.[6] He was the first, however, to describe the characteristic interaction between hemoglobin and oxygen. Hoppe-Seyler's interests focused on the chemistry of the blood. At this time it was known that cells found in pus closely resembled the white lymphoid cells found in the blood. Hoppe-Seyler believed that an understanding of the chemistry of these cells might lead to a better view of why pus formed during infection. These interests, coupled with Miescher's desire to study tissue chemistry, evolved into an almost ideal collaboration of efforts.

It is not generally known that Miescher initially chose to study the lymph cell.[7-9] In a letter dated February 26, 1869, he described his

Figure 1.3 Felix Hoppe-Seyler (courtesy of the National Library of Medicine).

initial, tentative steps into physiological-chemical research: "In full agreement with Hoppe, I set myself the task of seeking information on the composition of lymphoid cells. I was fascinated by the thought of tracing the most generally valid conditions of cell life from the simplest and independent forms of animal cells. The nature and quantity of this study material in itself imposed certain limitations on my work. The cells in question could be obtained from lymph glands only with great effort and in small quantities. On the other hand it was possible to obtain fresh pus daily even though in small quantities."[10] He obtained the discarded bandages from a nearby clinic and washed the pus cells from them. The success that Miescher achieved in these studies was due in great measure to the selection of pus cells as a sufficiently simple animal cell model for experimentation (figure 1.4). Today a study of pus cells would not be practical, for infections are relatively rare; in 1869, when the use of antiseptic techniques during surgery had not gained widespread acceptance and infections were quite common, human pus was available in plentiful quantities.

Miescher encountered his initial technical problem in the first experiment with pus cells. How were the cells to be removed from the bandages and separated from the accompanying pus fluid or serum? While Hoppe-Seyler encouraged Miescher to tackle this work, he also was quick to point out the complete absence of methods of study for these questions, and Miescher's very early difficulties clearly confirmed these views. The first salt solutions they tried caused the cells to swell so badly that they became an unmanageable mass. Eventually the use of a sodium sulfate solution, known as Glauber's salt solution, allowed Miescher to isolate the cells more readily. The

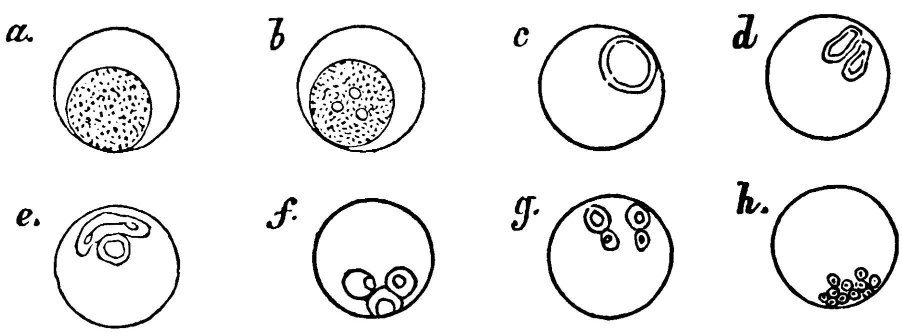

Figure 1.4 Engraving of pus cells showng various stages of disintegration of the nucleus, done in 1867.

cells were well preserved, although the presence of cotton fibers from the bandages obstructed the study of certain cell substances. Nor was there sufficient cell material for studying cell metabolites that were present in small quantities.

Miescher's studies were motivated by an interest in learning what materials form tissues in pus cells. His goal was to identify and characterize a group of substances termed *proteins* (from the Greek *proteios,* meaning "of the first importance"). Proteins, which had been discovered about thirty years earlier by Gerardus Johannes Mulder, were considered the most significant materials in cells at this time. Miescher wrote, "First of all an attempt was made to determine whether it was possible to obtain substances from protoplasm alone, that is to say separately from the nuclear substances, without appreciable alteration in one or the other. Our hopes were pinned on the effects of salts. The most diverse earth and alkali salts were each tested in three or four concentrations and under constant microscopic control, an extremely time-consuming task."[11] Miescher found that immersing the cells in these different salt solutions produced considerable differences in their behavior, with swelling, dissolving, or shrinking noted under the microscope for the entire cell as well as the nucleus, a readily separable and identifiable cellular component. In one such experiment, Miescher made the key observation that was to lead to the discovery of DNA: "In the experiment with weakly alkaline fluids, precipitates were obtained from the solutions by neutralization that were not soluble in water, acetic acid, in very dilute hydrochloric acid, or in sodium chloride solution and consequently cannot belong among any of the protein substances known hitherto."[11] Earlier studies by others had suggested that pus cells contained a particular protein termed *myosin,* which had previously been isolated from muscle tissue. Careful testing and comparing of the behavior, in different solutions, of muscle myosin to the "protein" that Miescher had isolated immediately convinced him that they were different. But where did the pus cell material come from? Was it derived from the nucleus or from the protoplasm? Examining the cells under the microscope, he noted that weakly alkaline solutions caused the nucleus to swell and eventually break open. Miescher stated, "According to this fact, known to some degree by histologists, the substance could belong to the nuclei and therefore fascinated me. The most rational approach was to prepare pure nuclei."[12] The possibility that this material could be derived from the nucleus was already an important biological observation. It suggested that the nucleus might have a unique chemical composition at a time when most investigators believed that there was nothing unique about the nucleus and that it was a relatively unimportant cell structure.

To attempt a better separation of this unknown material from the large quantity of contaminating protoplasmic proteins, Miescher sought to develop techniques for the separation of pure nuclei from the remainder of the cell. Such a separation had not been carried out successfully before. Miescher first tried treating the pus cells, after removing them from the bandages, with dilute acid. He described his work in these words:

> The complete extraction of cells with hydrochloric acid (and also with acetic acid) is very difficult. Several weeks go by until everything goes into solution. The liquids do not settle and they filter very poorly; in brief, I was able to make no progress. I therefore sought to prepare the substance directly from the cell, and to separate it from the other substances that go over into weakly alkaline solutions. But here I found myself in a quagmire, for there is nothing more difficult than the sharp separation of protein substances. I am well aware that the definition of these substances is very diverse and subject to dispute, and it is precisely the curse of these amorphous substances that one has no guarantee of the purity of their preparation. That is why genuine chemists avoid them so much.[13]

Miescher noted that if the acid-treated cells were shaken vigorously with ether and water, the incompletely degraded cells remained at the boundary line between the two immiscible fluids, while a fine powder sedimented to the bottom of the flask. Examination of this precipitate, obtained by filtering of the fluid, indicated the presence of nuclei. The amount of nuclei obtained, however, was unsatisfactory, and Miescher searched for a more efficient method. Returning once again to a previous observation, he hypothesized that certain fluids containing a protein-digesting enzyme termed *pepsin* might be useful for breaking up pus cells, which were mainly protein in nature, and might permit a separation of the protoplasm from the nuclear elements. Thus, he first washed the cells with warm alcohol to remove the fatty materials that would interfere with the subsequent analysis. Then he prepared clearly filtered extracts of swine stomach, which were known to be a good source of pepsin. The cells were treated with this solution for several hours. During this period a pulverized, grayish sediment separated from the clear yellow solution. Under the microscope the sediment was revealed to be pure nuclei. When these isolated nuclei were treated in the same manner as the original pus cell, that is with weakly alkaline solutions followed by acidification of the extract, the same precipitate was detected as first observed on the whole cell. This clearly indicated that the precipitated material had indeed come from the nuclear fraction of the cell.

In late August 1869, Miescher reported finding this same material not only in pus cells but also in yeast, kidney, liver, testicular, and nucleated red blood cells. He concluded that this material did not behave like any of the known classes of proteins. If it was not a protein, then what else could it be? He termed this new substance *nuclein,* which would later be identified as DNA. To distinguish nuclein chemically from other known cell substances, he undertook to determine its elementary composition. This involved determining the relative proportions of hydrogen, carbon, oxygen, and nitrogen present in the substance. Miescher was fortunate to have isolated this material in Hoppe-Seyler's laboratory at this time, for his colleague had recently announced that lecithin, another cell component, was unique in that it contained phosphorus in addition to the four elements normally associated with organic cell materials. Thus, there was probably considerable interest in the Tübingen laboratory in analyzing newly discovered substances, such as nuclein, for phosphorus. The chemical analyses indicated not only that phosphorus was present but that the ratio of phosphorus to nitrogen was unique. If the analyses for phosphorus had not been made, the discovery of nuclein might not have received the same amount of attention.

What did Miescher believe was the importance of nuclein in the cell? He wrote: "I cannot close my mind to the thought that the essential function of the P [phosphorus] is uncovered here."[14] Miescher considered nuclein to be nothing more than a storehouse of phosphorus for the cell. He envisioned nuclein breaking down to release its phosphorus content whenever the element was needed by the cell.

In evaluating the circumstances leading to the discovery of DNA, we must realize that Miescher's initial concepts bear little resemblance to our present knowledge of this substance. These modern concepts, however, have slowly evolved from the initial foundations that Miescher laid. Miescher's surprising but very important observations, his thorough training under the supervision of his father and uncle, both noted physician-scientists in their own right, and his pragmatic decision to join Hoppe-Seyler contributed toward his success.

In autumn 1869, Miescher left Tübingen and traveled to Leipzig to join Carl Ludwig's Physiological Institute. On December 23, 1869, he wrote to his parents, "On my table there lies a sealed and addressed package of my manuscript which I have already taken the necessary measures to send by parcel post. I am now sending it to Hoppe-Seyler at Tübingen. In other words, the first step into publication has been taken provided Hoppe-Seyler does not refuse."[15] While Hoppe-Seyler ultimately did not decline to publish Miescher's novel observations, a protracted delay in publication did ensue. Hoppe-Seyler did not reply to the letter accompanying Miescher's manuscript until late February 1870. In his letter, Hoppe-Seyler stated doubts about the correctness of Miescher's work based on a preliminary experiment that he had recently performed; further he noted that the next volume of his *Medical-Chemical Journal* would not be published until May. He advised sending a copy of the manuscript to *Pflüger's Archives* or the *Berliner Centralblatt für Medizinerwissenschaft* if rapid publication of the work was essential.

Miescher replied shortly after receiving this letter. He noted that since the publication of the next issue of Hoppe-Seyler's journal was to be delayed only a month or two there was no urgency for sending his manuscript elsewhere. He rejected the idea of sending his observations to the *Centralblatt* because he was opposed for various reasons to the concept of preliminary publications. However, he specifically asked Hoppe-Seyler to put either the date of writing the manuscript (October 1869) or the date of its receipt at the end of the published paper. This would insure that Miescher received the proper credit if someone else completed similar studies on nuclein after October 1869 but managed to have it published elsewhere before his article appeared. That scientists compete to be the first to make a discovery was as true in 1869 as it is today.

Miescher went to Leipzig to study with Ludwig with no preconceived experimental program. Ludwig assigned him the problem of studying the nerve pathways that conduct pain to the spinal cord. In addition, Miescher carried out a collaborative study with an American visitor, Bowditsch, and with Worm Müller on oxygen absorption by hemoglobin. Miescher's letters during this period give us a view of how the laboratory was operated: "I am gradually becoming convinced that in many of the works on blood-gas published here the ideas are those of Ludwig and the technical work, when necessitating manual dexterity, are the merit of the servant Salvenmoser."[16]

In July 1870, Miescher returned to Basel from Leipzig. His work still had not been published. He wrote to Hoppe-Seyler expressing his concern and specifically noting that no advanced notice of the impending publication of the *Medical-Chemical Journal* had been given. Fearing still another delay beyond the late summer, Miescher indicated an interest in sending a brief abstract of the work to the *Basel Natural Science Reports* because he had just become a member of the society. He received no reply, and sent still another letter in August in which, not surprisingly, one may note an increasing sense of urgency. Miescher was to be inaugurated as a lecturer at the Basel Medical School before the start of the new year, an event that would require the presentation of his Tübingen work. He needed either the publication proofs or the original manuscript because he had not retained a complete rough draft of the paper.

The entire matter was further complicated by the outbreak of war during summer 1870, interfering with the publication of everything but the daily news. Miescher's letters were not answered until October. Hoppe-Seyler explained the delay: "I have just received your second letter and hasten to reply to you this time since now I am certain that you are in Basel. I had scarcely received the earlier letter in which you reported your departure when the war broke out, and I was afraid that my letter might not reach you."[17] He returned Miescher's manuscript along with one of his own for Miescher's comments. Together with these two works, Hoppe-Seyler revealed, additional nuclein studies would be published that had been carried out after Miescher's departure from Tübingen and completed during the protracted delay before publication. One concerned the nuclein obtained from nucleated blood cells, and the other dealt with nuclein or a nucleinlike substance from milk. Hoppe-Seyler also offered to publish Miescher's comments on the manuscript he had sent. Miescher replied on October 20, 1870. Another letter from Hoppe-Seyler to Miescher written on October 31, 1870, resolved the final details. Unfortunately, Miescher's follow-up remarks were not published because of their length and because they contained no new information. Hoppe-Seyler further stated, "The fact that I have checked your studies on nuclein from certain aspects will not surprise anybody since aside from the considerable interest which the discovery itself offers, I have a certain responsibility for works carried out under me and appearing in these volumes."[18]

Hoppe-Seyler clearly intended to continue the nuclein studies, and he indicated a particular interest in the cleavage products and relationships between nuclein and lecithin. While he agreed to break off further work in this direction other than studies on nuclein in yeast and lower plants, he made it very clear that if no publications on nuclein were forthcoming from Miescher during the coming year he would resume the studies himself.[18] Finally, in 1871, Miescher's initial observations on nuclein were published. Hoppe-Seyler was not the only investigator to pursue studies on nuclein. Wilhelm His recalled, "When chemists, due to their personal contact with Miescher, learned of the significance of the new substance, several immediately started working on it. . . . Miescher's early laboratory associate Worm Müller also tried his hand at nuclein, although with only moderate success, and in Basel, Jules Piccard . . . used the material given to him."[19] Albrecht Kossel, another student of Hoppe-Seyler, also made many notable contributions to the study of nuclein and nuclear proteins.

Shortly after his return to Basel, Miescher resumed his physiological-chemical studies. His first experiments were carried out on the yolk of the chicken egg, experiments started during his autumn vacation in 1869. The purpose of the work was to confirm Wilhelm His's view that small spheres present in the egg yolk were preformed cells containing nuclei that give rise to embryonic tissue after fertilization. Originally many investigators had believed that these spheres were drops of fat or lipid. This view had to be altered when it was found that the drops were insoluble in ether and boiling alcohol and thus did not show the characteristic behavior expected of lipids. Because the presumed nuclei of these yolk spheres stained with dyes in the same way as other nuclei, His proposed the preformed cell theory, an idea that failed to gain many adherents. Miescher reasoned that since all nuclei from the many diverse tissues examined contained nuclein, the demonstration of nuclein in the spherules would strongly favor His's arguments. Miescher eventually obtained a nucleinlike precipitate when he treated the yolk with the same chemical reagents that he had used in the pus cell studies. The newly isolated nuclein differed from that of pus cells by a significantly higher phosphorus content. Later in the 1870s when the morphological characterization of genuine cell nuclei and their behavior in cell division became clearer, His's idea could no longer be maintained. Somewhat later, after the publication of this work, Miescher revised his own views and declared that egg yolk nuclein differed from true nuclein and was most likely some combination of protein and phosphoric acid. Later studies confirmed this theory.

Miescher's studies on the egg yolk, under the influence of His who remained with him in Basel until late in 1872, led him next into exploring the relationship of nuclein to the process of embryological development. Wilhelm His had been working on the development of the bonefish embryo and had also been studying the development of eggs from the ovary. The egg material, which was so much more readily available than pus, was obtained from salmon.

Fishermen had long known that while salmon remained in fresh water they failed to eat. Located on the Rhine River near the juncture of the German, French, and Swiss borders, Basel occupied a fortunate position. Salmon entering from the sea traveled from Holland into the upper regions of the Rhine to spawn. During the summer and autumn the sexual organs of the salmon increased in size; in the case of females the ovary weight might increase up to 25 percent of the total body weight. While Miescher was attracted to salmon eggs as a source of nuclein material, he recognized that salmon sperm would perhaps be even better for his studies. It was already known through morphological studies that the spermatozoa heads consisted mainly of nuclei, whereas in the egg the nucleus appeared only as a small portion of the total egg mass. Thus, the sperm might be an excellent source for obtaining nuclein.

Miescher started studies on the egg and sperm in the autumn of 1871, and he was able to report his results to the Basel Society for Biological Research the next spring. He found, in addition to nuclein, a new substance in the nuclei that he termed *protamine.* During the crystallization of protamine, Miescher noted that when it was warmed with nitric acid a yellow solution formed which changed to bright red when alkali was added. This reaction was characteristic of another class of chemical substances, termed the *xanthine bases,* and was used as a test for them. This test had been developed earlier by Adolf

Strecker. Miescher was probably thoroughly familiar with the test because he had taken a general chemistry laboratory course with Strecker at Tübingen before entering Hoppe-Seyler's laboratory. The results of these experiments led Miescher to suspect that the xanthines had been derived from the protamine.

Although Miescher believed that pus, egg, and sperm nuclein were different from one another, he was confused about where the xanthine substances were derived. In a letter to an associate in 1872 he wrote, "The xanthine alkaloid is especially suspicious here [in the sperm]. It is absent in the hen's egg, whereas there are the same materials or analogous bodies in the egg as in the sperm."[20] Miescher asked an associate, J. Piccard, whom he provided with some laboratory space, to investigate the source of the xanthine bases. Piccard, by using the established acid extractions and precipitations, arrived at the following conclusions: the xanthine bases were present not solely as protamine derivatives, as Miescher had surmised, but as "preexisting in addition to it in the salmon sperm."[21] Unfortunately he incorrectly concluded, "The composition of the salmon sperm as reported by Miescher must be revised in such a way that [the xanthine bases] must be distributed in part in the proteins and in part in the nuclein."[21] Thus, both Piccard and Miescher initially failed to recognize that the nucleic acid component of the sperm, and not the protein, was responsible for the release of the xanthine bases. This significant distinction would be made later by Albrecht Kossel and would serve as a basis for distinguishing proteins from nucleic acids.

Miescher's letters to Hoppe-Seyler two years later reveal his continuing preoccupation with the chemical products resulting from the degradation of nuclein. He wrote, "When your letter arrived I was just involved in the preparation of the experimental decomposition [of nuclein] with HCl according to the method of Hlasiwetz for which the protein-free sperm preparation is particularly suitable."[22]

During this same period Miescher also studied, in collaboration with Hoppe-Seyler, a group of globulinlike proteins combined with nuclein, which had been termed *ichthin.* A similar substance, emydin, had been isolated earlier by Edmond Frémy and Achille Valenciennes.[22] Miescher's elementary analysis indicated that the two substances were different from each other but constituted a distinct group of phosphorus-containing substances. Later work by R. Altmann showed that nuclein could be separated into two distinct substances: nucleic acid, which contained phosphorus, and protein, which did not.[23]

The erroneous conclusions were due to the inability of these early investigators to separate nucleic acids completely from protein. This fundamental problem was one of the most significant factors determining the slow rate of progress in distinguishing the nucleic acids present in nuclein from the smaller quantities of protein that were also present. The ability of the nucleic acids to bind protein tightly made the separation particularly difficult. Even Hoppe-Seyler had failed to recognize the fundamental differences between these two classes of cell components and concluded that "in many ways the nucleins are related to the proteins."[24]

Miescher's working conditions during this period were a severe handicap to the progress of his investigations. The institute in Basel had limited funds for research and insufficient space for laboratories. There was one advantage: he could work independently for the first time. He wrote to a friend about this period: "In the past two years I have looked back longingly on the fleshpots of the palatial Tübingen laboratories [figure 1.5] for I have had no laboratory at all, and have scarcely dared move, since the small room is more than overfilled by students and the professor of chemistry works in it as well. You can imagine what it means to be prevented from working energetically on matters which may never again in my life be so readily accessible to my hands due to miserable external circumstances."[25] As the result of these conditions, Miescher was forced to carry out the elementary analyses on nuclein in a corridor that was also available to other university staff members. For assistance he received the services of an aide who devoted 25 percent of this time to Miescher and the remaining 75 percent to anatomists, physiologists, and pathological anatomists. Fortunately, this situation soon came to an end.

On February 27, 1872, Wilhelm His, in a letter to the president of the University of Basel, announced his decision to accept an appointment as professor of anatomy in Leipzig beginning August 1. He recommended Miescher as his successor. Letters were then sent to Hoppe-Seyler and Ludwig asking for their comments on Miescher's suitability for this position. On May 19, 1872, Hoppe-Seyler replied from Strasbourg: ". . . it will be a very great pleasure if I am able to do something in the interests of arranging for F. Miescher to receive

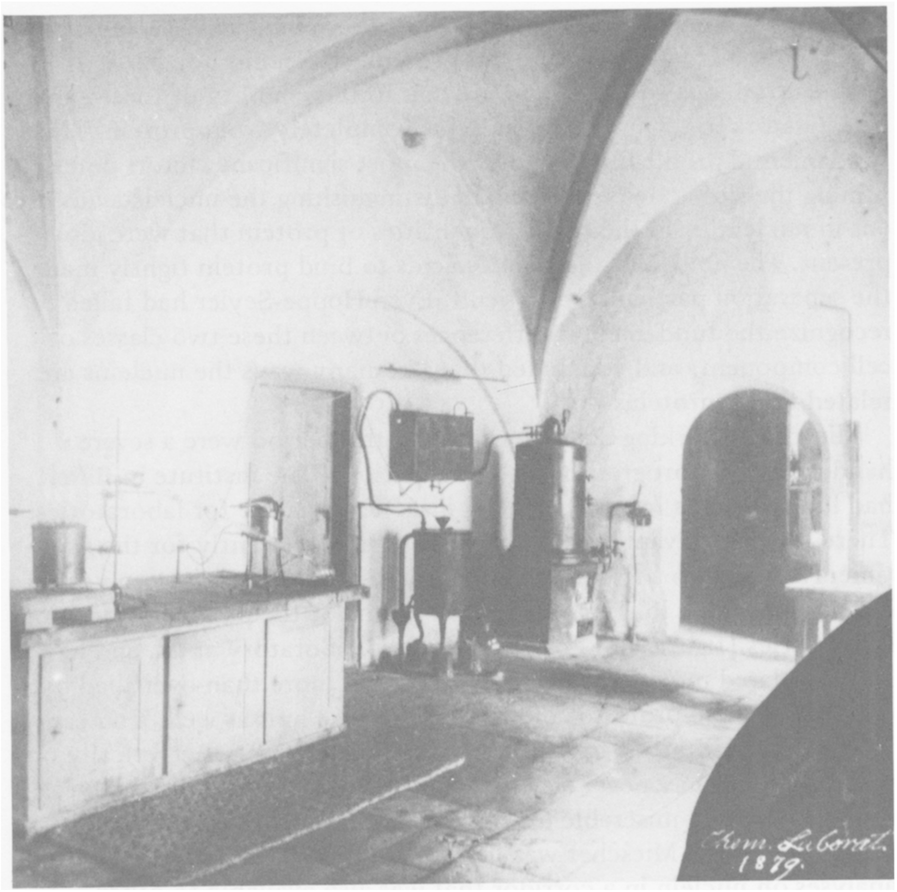

Figure 1.5 The laboratory at Tübingen where Miescher isolated nuclein (courtesy of the University of Tübingen Library, Tübingen, Federal Republic of Germany).

well-deserved recognition and a worthy position to continue his work in a post as Professor of Physiology. Miescher worked long enough in our Tübingen laboratory and was in so much contact with us almost daily that I feel confident that I can give a true estimate of him."[26] Ludwig concurred: "The direction which Dr. Miescher gave to his studies and the achievements which are the result of this course of education in my opinion qualify him to be a Professor of Physiology; as a matter of fact I took it for granted that, when required, one would make full use of his outstanding abilities."[27] Minutes from meetings at the university indicate that Miescher, who by summer 1872 had already taken over His's teaching duties for that semester in physiology, was to be appointed professor of physiology on November 1 with an honorarium of 3,000 Swiss francs. Wilhelm His had held the combined position of professor of physiology and anatomy. The rapid expansion of knowledge in both fields could no longer be handled by one person. Miescher thus began as professor of physiology, a position he held from 1872 until his death in 1895. He followed in the footsteps of his father, who had held the position from 1837 to 1844, and his uncle, who had held it from 1857 to 1872.

The result of this new appointment was better equipment and more working space. Unfortunately, Miescher found his new duties required a considerable amount of time away from the laboratory. In particular, he had to prepare lectures. Miescher seemed to be compulsive in his desire to do things as well as possible, and as a result no matter how long he took to organize the lectures, he was never satisfied. In addition to reading, Miescher had to construct apparatus, prepare samples and microscopic materials for demonstrations, and organize the entire work so that it developed logically and could be readily understood by the students. By 1875, he had arranged things so that each student was assigned a group of experiments on which he had to report his results and conduct a discussion. In this way, Miescher felt that the students would teach him. The experiments dealt with the speed of impulse conduction through nervous tissue, muscular movement, and many other areas of physiological experimentation. Despite these efforts and the considerable attention that his teaching duties received, Miescher does not appear to have been highly regarded as a teacher. Impressions from former students indicate that Miescher's restlessness and discomfort with his audience were readily apparent. He frequently appeared preoccupied with other matters. He failed to make himself clear to his audience because he presupposed a level of interest and knowledge that many of them apparently did not have. Only those who were already advanced students of physiology regarded Miescher as a stimulating teacher and lecturer.

In December 1877, Miescher became engaged to Marie Ann Rusch of Basel, and they were married on March 21, 1878. Their first child, a daughter, was born in July the following year. Two years later a son was added to the family, and another daugher was born in April 1885. Unfortunately, the first two children died early while the last eventually became insane.

The family later moved to a four-story house located at 21 Augustinergasse close by the Münsterplatz, a very broad square dominated by the Münster cathedral. The rear of the house faced the Rhine River from which Miescher obtained the salmon that provided him with sufficient materials for years of continuous experimentation; the house is still in use today. The Münsterplatz itself is bordered by a number of very large homes and a large park on the left with rows of trees carefully laid out in symmetric rows; at the far corner of the park close by the cathedral is a very large, elaborate fountain built in 1784.

Miescher's work on the nuclein and protamine of salmon spermatozoa led him to investigate several related questions. He tried to correlate different morphological sections of the spermatozoa heads with a specific chemical composition. In 1872 and 1873, he studied the semen of other organisms, including the bullfrog and the carp. He found that in the spermatozoa of these species, as in the immature salmon testis, protamine and other similarly related bases were absent.

A central part of Miescher's research activity focused on the considerable metabolic changes that occurred in the body of the salmon during the development of the sexual organs. The large increase in the latter, coupled to the large decrease in other body organs, suggested an extensive migration of substances from one tissue to another. Starting in autumn 1875, and during the course of the next several years, Miescher measured thousands of salmon and weighed them and their individual organs (including the muscle, liver, spleen, blood, and gonads) during different stages of development. He found that for as long as fifteen months the salmon neither ate nor even secreted the fluids necessary for digestion. Only after spawning was complete did the salmon revive and seek food. It was this search, in part, that drove them downstream toward the sea. Development of the sexual organs began in late spring, progressing through the summer and reaching a peak during September and October. Significant biochemical changes took place during this period as the salmon cells produced spermatozoa. After studying the gross physical changes in these organs, Miescher developed a more refined chemical approach. He asked what chemical components were being lost from one tissue and gained by another and, most significantly, what happened to nuclein and the materials involved in its production. Miescher concluded that the first essential factor was the degree of respiration experienced by a tissue. While the most important source of materials for tissue growth came from the muscle, not all muscles were involved. Degeneration of body muscle occurred while muscles of the fins, for example, showed no change. The fin muscles were known to have a larger blood content; thus, they were endowed with conditions more favorable to respiration. With sufficient respiration there was a deposition of substance rather than a liquefaction or loss. The blood served to transport oxygen to the tissues while simultaneously removing the products of degradation and oxidation. The gonads also were well perfused with blood, thus enhancing the conditions necessary for their increase. Miescher concluded that the reduction in blood to the body muscle coincided with a drop in blood pressure during the summer; most likely the drop in pressure occurred as the result of the swelling of the spleen, which serves as a reservoir for blood and retained blood withdrawn from the general circulation. Miescher noted that the absolute and relative protein content of the body muscle decreased in precisely the same way that it increased in the ovary. He made a similar observation for phosphoric acid, an important constituent from the production of nuclein. A portion of his time, therefore, was spent determining the forms in which these materials were transported between one tissue and another. In addition, the storage capacity of the liver for both protein and sugars was also noted.

During the last years of his life, Miescher continued to explore the chemical composition of the sperm in relationship to its morphology. He succeeded in separating the head of the sperm from the tail by continuous centrifugation. He found that the tail contained an abundance of lecithin and a peculiar protein substance similar to mucin. Furthermore, he noted that inside the nuclein shell of the heads there existed a substance free of phosphorus and sulfur but containing iron bound to organic groups; he termed this substance *karyogen.* He found it to contain more than 30 percent nitrogen, and with the binding of iron it resembled hematin. An associate, however, failed to confirm this observation, but he noted that "if the spermatozoa heads did contain something special, whether a live formation or some foreign substance, the mass of this substance compared to the heads can only be extremely small."[28] Miescher had already concluded that the mechanism of fertilization was the joining of two sexual stages that had developed along different lines. The egg contained well-developed cytoplasm but lacked a complete nucleus, which was supplied by the sperm. Based on observations made by Wilhelm His, Miescher believed that the formation of the egg yolk occurred by the entry of intact leucocytes carrying the raw materials supplied by other tissues into the egg. Here the materials condensed into larger formations coupled in an etherlike linkage. He speculated that, following fertilization, previously absent or dormant enzymatic activities developed, which broke down these condensations to release their component parts, analogous to the process of cleavage and digestion of food materials.

In autumn 1876, partly as a result of his salmon studies, the government asked Miescher to prepare a report on the nutrition of inmates of the Basel penal institution. It took more than a year to prepare the report, and Miescher considered it to be one of the most tiresome and thankless tasks of his entire life. He soon received similar requests from other penal institutions, teaching institutions, public societies, and others until it became too much for him, and he wrote, "I am turning green. Now I am being eaten up by goats. Inquiries into Swiss Folk nutrition, cookbooks for workmen, diet sheets for the state exhibition, controversies with the milk company. In brief, I am on the way to becoming the watchman over the stomachs of all three million of my compatriots."[28]

In 1878, Miescher reported the elementary composition of salmon sperm nuclein to be $C_{29}H_{49}N_9P_3O_{22}$. In the same year, Oscar Loew and Karl von Nägeli suggested that yeast nuclein was nothing more than a mixture of inorganic phosphate salts with protein. Nicholas Lubavin made a similar claim, reporting that cow's milk produced free phosphate and a protein when treated with boiling water. Georg Salomon thought that treatment of fibrin, a blood protein, with an extract of pancreas tissue produced a product associated with nuclein. Russell Chittenden examined another protein, albumin, and obtained results consistent with the possibility that nuclein derivatives could be obtained from protein. It was not until 1889 that Richard Altmann succeeded in obtaining nuclein free of protein and first suggested a name for the phosphorus-containing acidic component: nucleic acid.[23] It was then possible to show the characteristic differences in properties between the protein-free nucleic acids and the proteins themselves. In this way, Altmann showed that other substances, such as inorganic metaphosphoric acid, which had been proposed as the precursor of nuclein on the basis of similar activity in precipitating protein, were not related to the nucleic acids. Wilhelm His later recalled, "Miescher was fully aware of the acid character of the substance prepared by him, and the rechristening of nuclein proposed by Altmann under the name of nucleic acid was nothing new to him."[19]

Shortly after completing the work on the salmon, Miescher became involved with details for the construction of a new anatomical-physiological institute. In 1883, construction of the Vesalianum was completed, and in 1885 the First International Physiological Congress was held in Basel. For the festschrift, Miescher contributed a critical paper on the role of carbon dioxide as a regulatory factor in respiration.[29] The building still stands today, although it is well hidden on a side street near the Institute for Microbiology and Hygiene. A small bust in a niche on the left at the top of the stairs in the Vesalianum serves to remind visitors of the institute's relationship to Friedrich Miescher. On the right is a plaque listing the professors of physiology and anatomy.

Several of Miescher's papers were very important contributions to science; others contained errors and have long been forgotten. The failures did not arise because he lacked ability or desire to devote himself unselfishly to the very demanding work. On the contrary, Miescher was considered a brilliant investigator by many of his students and associates. He worked even harder toward the end of his life than most young men work at the start of their careers.

Throughout his professional career, Miescher sought to correlate his chemical studies with the structure or morphology of the cell. This correlation was then applied toward an understanding of the physiology of the entire organism. Thus, in his initial studies with Hoppe-Seyler, he determined by microscopic examination whether the chemical treatment that produced an unexpected chemical precipitate had resulted in disruption of the nucleus. The underlying purpose of the entire study was to understand better the transition of lymph cells into pus cells during infection. The same experimental style was reflected in his subsequent works, particularly on the tissue alterations in salmon.

Isolation of nuclein had to be carried out quickly in rooms kept at low temperatures because of the instability of the tissue components. Miescher described a typical working day as follows: "When nucleic acid is to be prepared, I go at five o'clock in the morning to the laboratory and work in an unheated room. No solution can stand for more than five minutes, no precipitate more than one hour before being placed under absolute alcohol. Often it goes until late in the night. Only in this way do I finally get products of constant phosphorus composition."[30] Miescher's belief in hard work bordered on the obsessive. A student, F. Suter, later recalled that when Miescher failed to appear for his wedding at the appointed hour, a search party went off to look for him. They found him quietly working in his laboratory. Why did he work so hard? In answer to this question he once wrote, "Should one ask anybody who is undertaking a major project in science, in the heat of the fight, what drives and pushes him so relentlessly, he will never think of an external goal; it is the passion of the hunter and soldier . . . the stimulus of the fight with its setbacks."[30]

Relentless work habits under such severe conditions gradually took their toll of Miescher's health, and he spent his last years as a patient in a sanatorium for tuberculosis in Davos. On May 23, 1895, Miescher wrote to the university that he had been informed by his physician that although he might recover gradually, he could make no commitments as to when he could resume his university responsibilities. He asked to be relieved of his position as professor of physiology as of October 1. An earlier proposal that a young lecturer temporarily replace him for six months or a year had been turned down on the recommendation of other faculty members. A visit to Davos by a university representative was followed by an administrative decision to raise Miescher's pension from 1840 to 2000 Swiss francs. On June 15, Miescher was officially retired from the position of professor of physiology, and in his honor the city of Basel sent a document praising him. In characteristic Swiss fashion Miescher replied,

I am not aware of having accomplished, besides my usual discharge of duties in my profession, anything more special than those things done by so many academic and nonacademic citizens of Basel in accordance with the tradition of our community. . . . I assume with this token of recognition the high government wishes to give pleasure to a seriously ill citizen and I would appreciate it if you would convey my deepest and warmest thanks to the authorities. [31]

Miescher's search for perfection in his studies prevented him from publishing the bulk of his observations. During his illness thoughts about his work often came into his mind involuntarily, and he eventually attempted to summarize them in preparation for publication. Unfortunately, the writing of even a few pages, in his seriously weakened condition, left him exhausted, and he was unable to continue. He died on August 26, 1895. He was only fifty-one years old. Although twenty-six had passed since he had first discovered nuclein, both its biological function and chemical structure were still uncertain. Few investigators, including Miescher himself, believed that nuclein was the chemical basis of heredity. Some who had no idea of the function and importance of DNA mistakenly regarded it as a drug suitable for treating patients suffering from tuberculosis, tonsillitis, anemia, diphtheria, and other serious diseases.[32] Miescher himself clung to the belief that proteins were the most important materials to be found in the cell.

Miescher's contributions were difficult to assess at the time of his death. Eulogizing Miescher during memorial services, an associate, Professor F. Wille, said,

If he did not reach the highest peaks of achievement, that was due solely to certain weakening and obstructing factors in his organization. Thus, even if we did not lose in him a teacher and investigator whose words and works were pioneering and decisive for the development of his science and science in general . . . still Friedrich Miescher, thanks to his strong interests and his relentless drive to do research, his competence and knowledge in his own field and his general knowledge, his sharp critical faculties, and his correct recognition of all that is involved in scientific research, was a well-known and able scholar.[33]

In contrast, Carl Ludwig, his former teacher, had written shortly before his death,

Of course, it is easier to preach patience than to practice it, and from my own experience I know what it is to give up well-loved, hopeful work. Sad as it is, there remains for you the satisfaction of having completed immortal studies in which the main point has been the knowledge of the nucleus; and so, as men work on the cell in the course of the following centuries, your name will be gratefully remembered as the pioneer of this field.[34]