BIOGRAPHICAL MEMOIRS

Pehr Victor Edman, 14 April 1916 - 19 March 1977

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PEHR VICTOR EDMAN
14 April 1916 — 19 March 1977
Elected F.R.S. 1974,
BY S. MILES PARTRIDGE, F.R.S., AND BIRGER BLOMBÄCK

PEHR EDMAN died after a short illness at the age of 60 in Munich. His untimely death brought to an end a long and challenging undertaking which although not quite finished to his own satisfaction has brought about a revolution in protein chemistry. Edman’s work has provided chemists with a capability undreamt of a generation ago: the power to determine the amino acid sequence of long runs of peptide chain with speed and precision by an automated method. This has put into man’s grasp essential information for the study of the genetic replication of enzymes and structural proteins and has completed the graduation of protein chemistry from a branch of colloid science to a fundamental organ of molecular genetics.

It now seems scarcely necessary to say that the sequencing of proteins, combined with X-ray diffraction analysis, provides a powerful tool to aid the determination of their complete spatial configuration including that of the interface with solvent which carries most of the biological activity. Extension of knowledge of protein sequences is essential in the search for evolutionary relationships between different proteins. In addition, studies of numerous heritable diseases are now showing how aberrant enzymes and structural proteins produced by genetic errors affecting the primary sequence may give rise to the malfunctions observed and how this exact knowledge may sometimes be used to alleviate the condition.

Edman set out to solve, in a determined and single-minded way, the problem of rapid and accurate sequencing and has resisted all temptation to deviate from his set course even though this meant leaving to others the rewards of early application to many interesting biological and medical problems. In preparing this memoir the authors have sought some clue to Edman’s motivation in providing a highly refined tool for others to use and have found no solution other than his own personal integrity and the satisfaction of a task well done.

EARLY LIFE

Pehr Victor Edman was born in Stockholm, Sweden, on 14 April 1916. His father, Victor Edman, was a judge. On the paternal side the male members of
the family had for generations served as public officers in state agencies and the armed forces. The father was a serious man and a devoted Christian. The mother, Alba Edman, was of a more joyous nature, lively and neat. As a boy Pehr Edman attended the elementary public school in Stockholm and at the beginning of the thirties started his high school education at the ‘Norra Latin-skolan’ in Stockholm, which specialized in the humanities. He was very unhappy during his first year at high school. The reason for this appears to have been a sadistic teacher, who made life in school unbearable for the sensitive young boy. He became depressed and confused and put on weight. Finally his father decided to take him out of school. He now started in the ‘Norra Real-skolan’, where emphasis was put on mathematics and the natural sciences. From now on everything went well in school. He was lucky enough to get a biology teacher, Mr Ringselle, who took good care of him and who was instrumental in awakening his interest in the biological sciences. It was very likely at this time that Pehr Edman finally decided to become a physician, although he had earlier shown an interest in this profession. Important for his development as a scientist were certainly the summers he spent with Mr Ringselle out in the archipelago of Stockholm on an island named Singö, where Mr Ringselle had a house. Under the expert guidance of Mr Ringselle he went out botanizing and bird-watching. His intense interest in nature was certainly formed during these summers. Pehr Edman’s brother tells that he sometimes used to join Pehr on excursions in the fields. He recalls how Pehr could sit for hours watching some natural phenomenon—be it live or dead, organic or inorganic. Later on in life the scientist Pehr Edman would sit still for hours, in the same way, at his laboratory bench meticulously watching chemical reactions or physical phenomena. During these summers he developed a keen interest in fishing—mainly for pike and perch. Pehr Edman would often talk with warmth and appreciation about Mr Ringselle and this time of his life. Mr Ringselle, this stout, jovial and warm man, meant in these formative years of Pehr Edman’s life a great deal for his eventual development as a scientist. No doubt his natural gift of observing phenomena and making deductive abstractions was sharpened. Already as a boy he gave proof of qualities in his character which later became prominent—i.e. his logical mind and stringency of expression. His brother recalls the burst of laughter Pehr produced when reading an announcement a tenant in their house had put in the elevator: ‘Grey gloves found, can be obtained against description.’

In 1935 Pehr Edman passed his matriculation examination with an excellent record. This examination is a great step in the career of a young man and on the day of the examination the family and friends usually come to school with flowers and gifts to give to the graduates when they are through. Pehr Edman’s family had to wait in vain. He left the school through the back door and went straight home. This episode shows another quality in Pehr Edman’s character. He was a shy person and disliked pompous performances especially when he himself was the centre of attention. After the matriculation examination he wanted to study medicine and applied to the Karolinska Institute in
Stockholm, a medical school. He started his medical studies in 1935 and received a bachelor of medicine degree in 1938. He graduated as physician in 1946. During his studies at the Karolinska Institute he joined a Marxist political organization, Clarté. A number of Swedish intellectuals, politicians and artists were members. Among the politicians was Tage Erlander, who later on became prime minister of the Swedish government. Among the artists was a famous poet, Nils Ferlin, whose anarchistic ideas and whims quite often turned the meetings to pandemonium. During his studies Pehr Edman also met his first wife, Barbro Bergström, whom he married in the early forties.

Concurrently with his studies in medicine he started his training in biochemistry with Professor Erik Jorpes. For a short period he also studied with Professor Hugo Theorell. It was in Professor Jorpes's department that he first became interested in protein chemistry. Professor Jorpes’s main interest at the time was the biochemistry of mucopolysaccharides, especially heparin. Erik Jorpes had also, with Einar Hammarsten, an interest in secretin. Pehr Edman took some part in the work on secretin together with Professor Gunnar Ågren. However, he soon started on a project of his own, which was the isolation and characterization of angiotensin. As starting material he used bovine blood, obtained in early morning hours from a nearby slaughterhouse. During this work he became acquainted with all of the preparatory and analytical tools used at that time in protein chemistry. One of these tools was column chromatography. Seeing the need for collection of fractions at short regular intervals he invented the first automatic fraction collector. He also developed solvent systems for partition chromatography of amino acids on paper sheets. His work on angiotensin resulted in a preparation which he considered to be chemically pure. He determined the amino acid composition of the compound and other chemical characteristics including molecular mass. This work resulted in a thesis which was presented at the Karolinska Institute in 1946, receiving the highest mark. Pehr Edman’s thesis work took place during World War II. For a long period of time he was drafted to serve as a physician in the armed forces. Knowing Pehr Edman’s dislike for military establishments, this must have been a rather dull time for him. However, he got some relief by the good fortune of being offered a horse for transportation between the military units. This gave him a good opportunity to practise horse riding which he enjoyed tremendously. After his dissertation, Pehr Edman applied for a 'docentship' (equivalent to lecturer) at the Karolinska Institute and this was granted. He also applied for and received a Rockefeller fellowship at the Rockefeller Institute in Princeton, which resulted in a one year stay with Dr Northrop and Dr Kunitz in their laboratory. Pehr Edman wanted to widen his experience and perspectives in protein chemistry and at that time the laboratory of Northrop and Kunitz was indeed one of the most prestigious places—a laboratory where most of the enzymes known at that time had been prepared and isolated in crystalline form.
The time spent at the Rockefeller Institute was in a way crucial in Pehr Edman’s scientific career since it was here that he made the first attempts towards stepwise degradation of proteins. However, the turning point in his thinking had occurred before that. During his work with angiotensin, it became clear to him that molecular mass and amino acid composition were not parameters that could give information explaining the biological activity of the protein. Obviously this must reside in the amino acid sequence of the protein. That this prediction was correct we can witness today. It is true that for the function of a protein the secondary and tertiary structures are equally as important as the primary structure. However, all evidence is now for the concept that the information necessary to give a protein a particular conformation is inherent in its primary structure. In fact, this latter view was already clearly expressed by Pehr Edman in the early sixties. At the Rockefeller Institute Pehr Edman studied reagents which would allow both carbamylation of the α-amino acid residue in proteins and subsequent rearrangement and release under mild conditions. Abderhalden and Brockmann had previously used phenylisocyanate for carbamylation. On acid hydrolysis the N-terminal amino acid was released as a hydantoin derivative. However, extensive cleavages of other peptide bonds also occurred during the hydrolysis. Edman considered that the use of phenylisothiocyanate would be more advantageous, since hydantoin formation with subsequent release of the N-terminal amino acid could occur under much milder conditions with this reagent. Towards the end of his stay at the Rockefeller Institute he had tried phenylisothiocyanate on model peptides and obtained evidence for the soundness of his idea.

Lund 1947–57

On his return to Sweden Pehr Edman applied for and was awarded a vacant associate professorship at the University of Lund. In Lund he continued to work on the use of phenylisothiocyanate for sequence analysis. In fact, from there on the problem of sequence analysis was his major scientific interest. He showed that coupling to amino groups of peptides and proteins occurred easily and that the thiocarbamylation N-terminal amino acid was swiftly rearranged and released as thiohydantoin in acid media under conditions when a secondary hydrolysis of peptide bonds did not occur to any appreciable extent. In order to make the method more generally applicable to sequence analysis, Pehr Edman made thorough investigations on the reaction mechanisms involved, as well as procedures for characterization of the thiohydantoins formed during the reaction. It now became evident that the product formed in anhydrous media was not a phenylthiohydantoin derivative but rather the isomeric phenylthiazolinone. This derivative is extremely labile and easily undergoes rearrangement to the corresponding thiohydantoin. The basic experimental work on the phenylisothiocyanate method was done between 1950 and 1956. It was now clear that three discrete reaction steps were involved, i.e. (1) coupling with
Pehr Victor Edman

phenylisothiocyanate, (2) cyclization to phenylthiazolinone under anhydrous conditions and (3) hydrolysis and conversion to phenylthiohydantoins. The following account by his mother reflects the importance Pehr Edman himself put into the discovery of the phenylisothiocyanate reaction: ‘One day Pehr came home to me and asked me to sit down with him because he had something interesting to tell me. He then told me that he had discovered a way to analyse proteins which had not been possible before and that this discovery would certainly be of great importance for biochemistry in the future.’*

Melbourne 1957–72

In 1957 Pehr Edman accepted an offer as Director of Research at St Vincent’s School of Medical Research in Melbourne where he remained for 15 years. The reasons for his leaving Sweden were rather complex. Pehr Edman emphasized the circumstance that he did not get enough support in Sweden because of lack of understanding for the work he was engaged in. There were, however, other factors which certainly played an important role in his decision to leave. One of them was the breakdown of his first marriage, ending in separation in 1957 and eventually divorce. Another reason may have been the personal friction in the biochemistry department where Peter Edman worked. As a result of all this Pehr Edman very likely felt himself rather isolated in Lund and there was a lack of scientific stimulation for him at the biochemistry department. The few persons he could enjoy discussing scientific matters with included Arvid Carlsson, professor of pharmacology at the University in Lund, Dr John Sjöquist, his close associate, and Dr Ikuo Yamashina, at the time a visiting scientist from Professor Jorpes’s laboratory.

Dr Ikuo Yamashina stayed in Pehr Edman’s laboratory at the end of 1956 and the beginning of 1957. He was very likely the first, outside Edman’s group in Lund, to learn the phenylisothiocyanate technique. When he returned to Professor Jorpes’s laboratory in Stockholm he enthusiastically told about how easily N-terminals in proteins could be determined with this method. At this time Birger and Margareta Blombäck, working in Professor Jorpes’s laboratory, had just finished their work on purification of fibrinogen from different species. Stimulated by Dr Yamashina’s account they decided to try the method on fibrinogens from different species. This turned out to be worth while since it showed for the first time that the molecule was composed of three pairs of polypeptide chains indicating a dimeric structure of the molecule. Pehr Edman occasionally came to Stockholm to see his mother during this period. During these visits he took time off to come to Professor Jorpes’s laboratory. These visits were always welcomed since they gave the young scientists an opportunity to discuss with Pehr Edman the problems which they had had with the method. He gave his advice in a friendly and competent way.

* This quotation is a recollection of a conversation between Birger Blombäck and Pehr Edman’s mother during her visit to Australia in the spring of 1962.
Pehr Edman also enjoyed discussing scientific matters with Professor Erik Jorpes, his former teacher. He appreciated Jorpes and his work, which he admired perhaps because it was solid and reliable like his own. This relationship with Erik Jorpes, however, came to an abrupt end in 1963. The background may seem trivial but is worth mentioning since it shows important facets in Pehr Edman's personality—his craving for absolute honesty and his inability to compromise. The background was this: When Professor Jorpes was about to retire in 1963, Pehr Edman and Erik Jorpes had a discussion about the possible candidates to succeed him. A name was mentioned and Pehr Edman was very pleased that Erik Jorpes considered the particular man a strong candidate for the position. However, another candidate eventually got the chair. Later on Pehr Edman happened to read the appraisal on the candidates that Erik Jorpes had delivered to the nomination committee at the Karolinska Institutet. Finding that the candidate he and Erik Jorpes had discussed was not even considered among the first three top names enraged Pehr Edman. For him this was an almost unforgivable deceit. In a letter to Erik Jorpes at Christmas time 1963 he told him this and broke all further communications with him.

During his first years in Australia Pehr Edman did not take much initiative in creating scientific contacts with other laboratories. He may therefore have appeared shy and unsociable to many of his fellow scientists. It is probable that his inclination for withdrawal was a consequence of spells of depression, which he very likely had suffered earlier.

In Australia Pehr Edman finished the work on the manual three-stage degradation technique about 1960. When Birger and Margareta Blomback came to the laboratory as visiting scientists in 1961 they tried the three-stage technique on human fibrinopeptide A consisting of 16 amino acid residues. Starting with a few micromoles of peptides they were able to make a complete stepwise degradation with excellent yields up to the very last residues. These results impressed Pehr Edman very much. He now became convinced that longer peptides could be degraded in good repetitive yields too. This being the case, the need for automation was evident. Pehr Edman became at this time convinced that automation was absolutely necessary in order to carry out, within a reasonable time, the formidable task which sequence determination of protein molecules required. At the end of 1961 he started to analyse different possibilities to solve the automation problem. The problem was to find a single physical process which could accommodate the various operations in the manual procedure. Guided by his great knowledge, imagination and intuition, he almost at the very start of this endeavour conceived the idea of the spinning cylindrical cup, in which all reaction media were spread out as thin films on the vessel wall. He considered that the rotating film containing the protein would be well suited for extraction with a solvent as this is continuously fed in and glides over the surface of the first film and is subsequently removed in the upper part of the cup. Although the principle for the 'sequenator' was simple and brilliant, a number of technical problems had to be solved before the automation of all 30 individual operations was finally realized. Pehr Edman and
his associate Geoffrey Begg started experimental work with rotating glass cups in January 1962. Pehr Edman would sit watching in strobe light the properties of films—monofilms and composite films—formed in the cup under different physical conditions. After finding a cup that was convenient for the purpose they continued step by step to experiment on the remaining operations necessary for automation. In 1964, after two years of hard work he reported, at a meeting of the International Committee on Thrombosis and Haemostasis at Gleneagles in Scotland, that automatic stepwise degradation of proteins could be done. Pehr Edman concluded his presentation with the following: ‘Our experience of the performance of this apparatus is as yet limited. However, trial runs have shown that it is capable of an output of at least 15 amino acids in 24 hours. Furthermore, the high repetitive yield of 97% indicates that the determination of extended sequences will be possible. These expectations are supported by the results of sequence determinations on intact proteins currently being made in our laboratory. However, more work is required before a full assessment is possible.’ In 1967 the work on the automated sequence analysis was finished and published.

In 1966 Pehr Edman met Agnes Henschen, whom he married in 1968. This encounter changed Pehr Edman’s personal life in a more happy direction. He appeared now more relaxed and at peace with himself and the years to follow were certainly among the happiest in his life. In the early seventies Pehr Edman began to contemplate leaving Australia for Europe, where he thought the chances to pursue his scientific work would be better. He accepted an offer to be Director of the Department of Protein Chemistry I of the Max Planck Institut für Biochemie in Munich. He and Agnes Henschen moved there in 1972. During the last years of his life he continued to work on the sequenator with the main objective of improving the yields. He was of the opinion that high repetitive yields are crucial in stepwise sequencing and that all efforts must be directed to this aim. The actual yield of 98% had made possible 60 sequence steps but he believed 99% repetitive yield was possible, which would allow additional sequence steps to be performed.

Munich 1972–77

After settling down in Munich Pehr Edman and his family visited Sweden every summer where his wife had a summer house in the archipelago of Stockholm. There he had the opportunity to exercise the hobbies of his young days—fishing and bird-watching and to enjoy being close to nature. For the whole of his life nature meant a lot for Pehr Edman. During his years in Australia he took every opportunity to make long excursions to remote places for hiking, fishing and bird-watching. He loved the seashores of Australia and was an eager swimmer and surfer. Bird-watching was no doubt his favourite hobby and he was almost as knowledgeable in ornithology as he was in chemistry.

Pehr Edman was widely read in his profession, and he used to spend much
time every day reading scientific journals. He respected and admired thoroughness in scientific work, but, above all, he was sensitive to all sorts of new ideas in science. When he himself had an idea he scrutinized with extreme care the possibilities of proving it in practice. He designed his experiments with the same thoughtfulness. Pehr Edman despised fortuitous experiments for the simple reason that if successful they would be difficult to reproduce. There was a great deal of perseverance and single mindedness in his personality. If he had an idea which he believed must be proved experimentally, there seemed no obstacle that could not be surmounted. This quality of personality was certainly an asset in his scientific work. Though Pehr Edman's most important work was concerned with stepwise degradation of proteins, he worked also in other areas in biochemistry, i.e. on the N-O acyl shift and the cleavage reaction with CNBr. He made at an early stage interesting experiments on coupling of proteins to insoluble matrices. He observed as early as the thirties that some proteins such as fibrinogen had a lower solubility in the cold and consequently could be frozen out of solution.

Pehr Edman preferred to work in a modest setting with only a few people around him. In his group in Lund Dr John Sjöquist worked on a new method for amino acid analysis using their phenylhydantoin derivatives and Dr Lars Josephsson was engaged in studies of the N-O acyl shift. In Melbourne his group consisted also of a small number of people: Dr Frank Morgan and Hugh Nial worked mainly on applications of the phenylisothiocyanate degradation technique. Dr Ilse studied the mechanism of the reaction. His closest associate during his work on the sequenator was Geoffrey Begg, who was of indispensable help in solving the technological problems.

**Awards**

In his professional life Pehr Edman was well known throughout the world. He received the following honours for his achievements in science: Britannica Australia Award, the Berzelius Gold Medal, the Gold Medal of the Swedish Academy of Engineering, the Linderström–Lang Medal. Pehr Edman was a Fellow of the Australian Academy of Science, Fellow of the Royal Society of London and a scientific Member of the Max Planck Society.

**Edman as a Person**

Pehr Edman had broad interests outside science. Of the arts he appreciated music most. He could sit for hours and listen to his favourite records. He enjoyed the company of friends, who mostly were from circles outside the scientific field. Only his friends could fully appreciate the greatness of his genius. They recognized in him a man full of generosity, warmth, humour and sympathy. His thoughts turned not merely to science but to culture, society and politics as well. Pehr Edman had a vast knowledge in many areas. His mind
was logical and he was stringent in expression. The integrity on which his opinions were based was admirable and respected and in these opinions he was rock-firm, almost to the extent of stubbornness; but he could change views if well founded reasons were presented. Pehr Edman was not an individual who thoughtlessly followed fashions in scientific or other kinds of thinking for he was too analytical and had too strong a desire to reach for causes on fundamental levels. There was in his personality a certain aloofness, which people who did not know him may mistakenly have taken for snobbishness. His friends enjoyed more than animated discussions with him: an integral part of being together with friends was food; and he was a fine cook. It may have been crispy duck or simply Swedish meatballs. The dishes prepared by Pehr Edman were always cooked in a masterly way—thoughtfully and meticulously.

In trying to describe Pehr Edman’s character, words like incorruptible and uncompromising come to mind as do qualities of sincerity and loyalty to his friends. In Pehr Edman’s language ‘yes’ and ‘no’ stood for fundamentally opposite meanings. He had courage and dared to express, without hesitation and without political or opportunistic consideration, opinions which he felt were morally right. The uncompromising quality of his personality made him distrust politicians with few exceptions. At the core of his personality was a sincere humanism. Therefore he was, on the whole, against violence and oppression and he was a sworn enemy of militarism in the world.

Finally, there was in Pehr Edman’s personality an encompassing trait of purism which may be common to many scientists of his calibre—his teacher Erik Jorpes had this quality and J. J. Berzelius, the first professor of chemistry at the Karolinska Institute, had it. This trait may perhaps to some extent be linked with Scandinavian culture since one recognizes it in so many works by Scandinavian novelists, poets and playwrights, e.g. in characters in Ibsen’s and Strindberg’s plays. Pehr Edman’s urge for purity and perfection in life may therefore partly have been a cultural heritage. After all his father was a judge—a profession symbolizing a cornerstone of society. Whatever its origin, indigenous or through influence, this quality was very likely instrumental when he joined the Marxists as a young man in the thirties, when he chose the self-imposed expatriation in the fifties and it was probably a strong driving force in his scientific accomplishments. For a purist nothing except the whole is good enough. He is beset by one idea: to reach perfection and impeccability. We believe that this quality in Pehr Edman’s personality was a pre-requisite for his motivation for spending so much time and effort on perfection of the phenylisothiocyanate method.

In February 1977 when leaving a scientific lecture in Munich Pehr Edman suddenly fell down unconscious. After a few weeks of illness he died on 19 March. The disease was caused by a tumour of the brain, which had not given any symptoms before he was struck by unconsciousness.

Pehr Edman leaves his widow, Dr Agnes Henschen, and their children, Karl and Helena. From the first marriage he leaves two children, Martin and Gudrun.
The renaissance of protein chemistry since the mid-century; Edman's paper on sequence determination in 1949

What has been called the modern renaissance of protein chemistry perhaps could be said to have started almost at the turn of the century with Tswett's (1906) demonstration of the chromatographic column (1). However, Tswett's discovery was almost completely neglected and 35 years were to pass before this elegant application of the principle of multi-stage counter-current distribution was to reach recognition as a practical method of solving the complex technical problems of amino acid analysis faced by the protein chemist.

What in Tswett’s hands was an art was systematized and put on a quantitative basis by Tiselius (2) and by Claesson (3). Early chromatographic separations had made use of molecular adsorbants such as charcoal or alumina which are only marginally useful for separation of mixtures of the 18 or 20 aliphatic amino acids which commonly faced protein chemists and a genuine upsurge in technical capability did not really begin until 1941 when Martin & Synge (4) demonstrated that solutes such as amino acids are distributed selectively between two liquid phases, one of which could be immobilized by mixing it with an absorbent powder such as paper pulp, kieselguhr or silica gel. It was later shown (5) that this 'partition chromatography' could be conducted in a filter paper sheet in a moist chamber by allowing the wick action of the filter paper to propel the solvent phase relative to the stationary water phase held within the fibres. When the technique of paper chromatography was first introduced by Consden et al. in 1944 it was regarded by many protein chemists as an amusing toy, but it soon proved to be so rapid and convenient for qualitative identification of mixtures of amino acid that within a few years it was adopted everywhere, and has since had a major influence on the course of protein chemistry.

However, it soon became obvious that the first necessity for the chemical study of proteins is the availability of an accurate and rapid method of amino acid analysis, and for this purpose a number of groups became interested in the use of synthetic ion exchange resins as reversible adsorbents of amino acids. Moore & Stein found that when the sodium form of a strongly acidic cation exchange resin is used and adsorption of an amino acid takes place from a large excess of sodium buffer, the concentration of the adsorbed ion is small compared with the buffer ion concentration and adsorption of the solute is directly proportional to its concentration.

This situation is ideal for quantitative analysis by elution chromatography and Moore & Stein were able to show that symmetrical peaks and high recoveries of amino acids were obtainable by eluting cation exchange columns with buffers or with high concentrations of mineral acids. In their 1951 paper Moore & Stein (6) were able to demonstrate the separation and quantitative determination of the products of acid hydrolysis of about 6 mg of a protein on a 100 cm column for the acidic and neutral amino acids and on a 15 cm column run at 25 °C for the basic amino acids. With synthetic mixtures simulating a
protein hydrolysate the estimates from the peak areas were correct to about
±3% and the whole operation could be completed in about two weeks. Today
the same information can be obtained in a few hours on a much smaller sample
but before the advent of chromatography, the amino acid analysis of a protein
was an operation which required at least two years’ work by an experienced
and skilful chemist.

The availability of accurate amino acid analysis combined with the evidence
from the work of Svedberg (7) and Tiselius (8) that purified proteins migrated
as single boundaries in a centrifugal or electrical field brought about a changing
view about proteins which was eventually accepted by chemists and physicists
alike. Traditionally proteins were regarded as biocolloids and few supposed that
any protein preparation contained identical molecules; after 1950 the realization
dawned that, unlike most carbohydrates, proteins such as haemoglobin con­tained
molecules which were identical replicates, had exact molecular masses
and amino acid compositions, and identical packing of the long polypeptide
chains. This new view of proteins gained final acceptance when crystalline
preparations of various proteins including some enzymes became available and
it was found that these crystals gave rise to X-ray diffraction patterns which
could be interpreted on the same basis as the crystals of inorganic compounds
and organic substances of low molecular weight.

Although the analysis of the amino acid composition of a protein became
little more than a matter of routine, the chemistry of the primary structure was
still not defined because the sequence of the amino acid in the polypeptide was
unknown. Indeed, some chemists, making calculations about the number of
possible permutations of sequence in a polypeptide chain some 500 residues
long, expressed the view that this was information that would for ever be
beyond human reach.

It is not easily possible to be sure if in these early days of the renaissance of
protein chemistry Edman had already conceived the ambition to complete the
chemistry of peptides by determining amino acid sequences. However, 1948
finds him devising the first of what later became many designs of chromato­
graphic fraction collectors with escapement mechanisms operated by a clock.
That of Edman (1948) consisted of a weight-driven circular rack which was
prevented from moving by a spiral-cut ratchet engaging a rod. The rod was
driven continuously outward by a clock and the ratchet was so designed that
equal time intervals elapsed for each notch to be passed.

The first successful attack on the problem of sequence determination in pro­
teins was that mounted by F. Sanger and published during the period 1945-51.
As usual with all great scientific enterprises the basic idea was simple. All that is
necessary to determine the composition and sequence of a dipeptide is a semi­
quantitative amino acid analysis and a method of determining which amino acid
is N-terminal or which C-terminal. Thus the basic requirement for sequencing
is some specific and, hopefully, simple way of recognizing a terminal amino acid.

Sanger was the first to realize that even with a quite complex peptide—
say one containing five or six residues—the unique sequence could be
established by locating the terminal in the starting molecule and in each of the smaller peptides derived from it by random partial hydrolysis. With this bold concept in mind he devised a method of labelling the N-terminal end of a peptide or polypeptide chain by reacting it with 1:2:4-fluorodinitrobenzene (FDNB) to form the dinitrophenyl derivative of the N-terminal amino group (9). These dinitrophenyl (DNP) derivatives were substantially stable to acid hydrolysis and their bright yellow colour facilitated qualitative analysis and quantitative colorimetric estimation. Thus, by use of paper chromatography and column chromatography on silica gel both the amino acid composition and the N-terminal amino acid could be recognized with any small peptide and its sequence could be worked out by partial hydrolysis and by fitting the overlapping sequences.

By these simple means Sanger was able to elucidate the order of the amino acid residues in proximity to the free N-terminal amino groups and the lysyl ε-amino group in the A and B chains released from insulin by oxidation with performic acid (10, 11). Two years later these researches resulted in elucidation of the complete sequence of the 30 amino acid residues in the B chain of insulin by Sanger & Tuppy (12, 13). This was indeed a milestone on the road of protein chemistry and was the final convincing evidence required to demonstrate unequivocably a protein carrying a unique primary sequence.

At this point we can return to consider the development of Pehr Edman’s researches which were certainly influenced by the frequent publications appearing from Sanger’s laboratory. In 1949 Edman’s method for the determination of the amino acid sequence in peptides was ready for publication and displayed a concept of sequencing which was basically different from Sanger’s, and which, as time was to show, had finally a greater potential for development and for automation.

It is interesting that 10 years before Sanger introduced the FDNB method of end group analysis, Jensen & Evans (14) had been able to isolate the phenylhydantoin of phenylalanine from a hydrolysate of insulin that had been treated with phenylisocyanate, thus demonstrating that some free amino groups of insulin are present on phenylalanine residues.

Edman considered the use of phenylisocyanate for labelling the N-terminal amino acid but soon decided to take advantage of the much greater chemical reactivity of isothiocyanates. What he had in mind was to attempt a stepwise degradation starting from the N-terminal end, with removal and identification of one amino acid at a time. This immediately raised the problem of how to break a terminal peptide bond and leave all the others unaffected. The approach he had in mind was to offer the —CO group of the peptide bond adjacent to the N-terminal a more favourable reaction partner than the NH₂— group of the next amino acid in sequence. It was here that the use of phenylisothiocyanate seemed to offer good prospects (figure 1, reaction 2).

This expectation was dramatically fulfilled and the peptide bond was split in a few seconds at room temperature and in an anhydrous acid medium. From the work of Abderhalden & Brockmann (15) on phenylisocyanate, Edman
had expected the formation of the phenylthiohydantoin (figure 1, formula III) of the N-terminal amino acid together with the peptide shortened by one residue. He was, therefore, not too surprised when this was what he isolated. It was only some years later that the suspicion arose that this was not the whole story. It was discovered that the first compound to form during the cleavage reaction was not the phenylthiohydantoin but a 2-anilino-5-thiazolinone (figure 1, formula II).

The thiazolinone readily rearranges to the isomeric phenylthiohydantoin (III). This reaction had not previously been described and its extreme facility is explained by the nucleophilic attack of the thioketonic sulphur in reaction (2). Its generality has subsequently been demonstrated in many other stepwise reactions using other reagents but where the key reaction is always the formation of a thiazolinone. The reaction has also proved a general one for peptide bound N-terminal amino acids and is the basis for nearly all the protein sequencing work carried out at the present time.

Unlike the approach adopted by F. Sanger which depended upon the initial cleavage of the protein into rather small peptides, Edman’s approach of successively removing and identifying the N-terminal amino acid from a long polypeptide demanded that each step should be as quantitative and as free from side reactions as possible. This stepwise degradation must come to a halt.
as soon as the product of side reactions reach concentrations comparable with those of the linear degradation.

A very large part of Edman's work since the original description of the method (16) was given in 1950 consisted in identifying and eliminating side reactions. Thus it was discovered that the thiocarbamyl group in reaction (2) easily lost sulphur by oxidation. The resulting carbamyl group was unreactive and the degradation came to an end. This side reaction could be eliminated by performing the reaction in a nitrogen atmosphere. Further, reaction conditions for coupling, cleavage and conversion that were equally suitable for all amino acids had to be found. Thus it appeared that proline was much more slowly released than other amino acids during the cleavage reaction (2) which was normally carried out at 55 °C in heptafluorobutyric acid. Also the position of proline in the sequence was important, since the rate of release of proline could vary considerably depending upon its near neighbours.

IDENTIFICATION OF AMINO ACIDS AND PTH-AMINO ACIDS

With a few exceptions the chemical stability of PTH-amino acids is excellent, and in general they are easily crystallizable compounds with high melting points. The less stable compounds are those with an —OH or —SH group on the β-carbon of the amino acid chain and these show in varying degrees a tendency for β-elimination. In PTH-cystine and PTH-cysteine the tendency for β-elimination is so strong that the derivatives are not useful for identification purposes. On the other hand, the PTH derivatives of S-alkylated cysteine are more stable as is also PTH-cysteic acid. The tendency for β-elimination becomes important in the identification of the susceptible compounds and it should be borne in mind that β-elimination also occurs in the corresponding thiazolinones.

The PTH-derivatives of asparagine and glutamine are unstable to the extent that the amide group is hydrolysed by acids and alkalis. In sequence determination these PTH-amino acids are found to be contaminated by the corresponding acids. PTH-tryptophan is also susceptible to the action of strong acids, but fortunately the acid conditions of the degradation procedure do not seem to be sufficiently vigorous to affect the tryptophan PTH derivative appreciably. Finally, solutions of PTH-amino acids show a tendency for photodecomposition, which becomes apparent after prolonged exposure to daylight. Solutions must therefore be protected during storage.

All PTH-amino acids show strong absorption in the ultraviolet with a maximum around 268 nm and a minimum around 245 nm. The ratio $A_{269}/A_{289}$ lies around 0.4 and is a useful index in assessing the purity of a PTH-amino acid preparation.

Two different principles have been used in the identification of the amino acid residue removed in a degradation cycle. The direct method relies on the positive identification of the residue split off. The indirect method depends for the identification on a difference in the peptide before and after a degradation cycle.
The PTH-amino acids tend to be poorly soluble in water and readily soluble in organic solvents and may be separated and identified by chromatography. For this purpose paper chromatography, thin layer chromatography and liquid–liquid and gas–liquid partition chromatography systems have been proposed. Paper and thin layer chromatographic techniques have been most commonly used by Edman and have been worked out in detail. These techniques are not well suited for the identification of some PTH-amino acids with strongly ionized groups in the side chain such as PTH-arginine, PTH-histidine and PTH-cysteic acid but here spot reactions and paper electrophoretic techniques may be used.

The indirect method of identifying the amino acid split off by a difference in analytical composition of the peptide before and after a degradation cycle has been used extensively by Hirs et al. (17), in their classical elucidation of the first enzyme structure. It has also been used in a rather different way by Gray & Hartley (18) who determined the new N-terminal amino acid by use of the sensitive dansyl technique after each degradation cycle. This requires complete hydrolysis of a small portion of the peptide after each cycle but the method permits sequence determination on very small amounts of peptide because of the great sensitivity of fluorescence measurements.

Edman clearly favoured the direct identification method for the following reasons:

1. The chief criticism of the indirect method relates to the fact that it requires complete hydrolysis of the peptide. Therefore the difference in composition before and after hydrolysis relates only to the hydrolysate of the peptide. Thus amino acid residues like asparagine, glutamine and tryptophan cannot be identified.

2. Many proteins contain modified amino acid residues, e.g. by a covalently bound carbohydrate moiety. If these groups are removed by hydrolysis in concentrated acid as they usually are, they cannot be located by the indirect method. They may even escape detection because there is nothing in the method to indicate their presence.

Edman maintained that the weaknesses enumerated above are inherent in the indirect method and cannot be made good by any improvement in technique. He therefore concentrated his effort on improvements in the direct method. Since the value of a sequence technique depends largely on the length of the sequence which may be determined, the important factor is a high repetitive yield, i.e. the yield of amino acid calculated from one degradation cycle to the next. Losses of a small percentage severely limit the practical length of the degradation. This is another reason why it is undesirable to remove samples from the peptide stock for hydrolysis. Perhaps the most telling reason against the use of the indirect method for automation purposes is the length of time required actually to carry out acid hydrolysis. This process is clearly not suitable for rapid automated sequencing techniques and, taking these objections together, Edman decided not to attempt further development of the indirect technique.
The concentration of effort only on the direct technique stimulated improvements in the methods for rapid identification of the PTH-amino acids. This has finally resulted in a whole arsenal of procedures including gas chromatography, mass spectroscopy and recently also high performance liquid chromatography. These methods have been reviewed by Edman & Henschen (1975).

THE PROTEIN SEQUENATOR

By 1962 the stage was set for automation of the degradation. Standard reaction conditions applicable to all amino acids were already known, and side reactions had largely been eliminated. Thus a high repetitive yield was possible. The importance of the repetitive yield from one degradation cycle to the next was repeatedly emphasized by Edman who illustrated the point with a simple calculation which showed that repetitive yields of 97%, 98% and 99% make possible 30, 60 and 120 degradation cycles, respectively.

It remained to find a suitable technical device in which to perform the reactions of the degradation cycle. Edman and his colleague Begg provided a characteristically ingenious solution to this problem. In order to establish a large surface and the equivalent of rapid stirring they chose to spread out the media in thin films inside a spinning cup (figure 2, Edman & Begg (19)).

The film is very suited for carrying out extractions, dryings and other procedures and the whole degradation cycle may be programmed. The automated instrument was called a sequenator. This instrument allows the degradation of long runs of polypeptide, in favourable cases up to 50 and 60 amino acids. The speed was about 15 amino acids a day in contrast to the one or two amino acids per day possible with the manual technique.

![Figure 2. Schematic presentation of the operation of the sequenator cup. An aqueous solution (white) is being extracted by an organic solvent (black).](image-url)
The instrument was designed to contain reservoirs to hold all the reagents required for the reaction cycle together with receivers for effluents and means for controlling reaction temperature. A system of feed tubes and automated valves was provided and programmed to supply the reagents and extraction solvents to the spinning cup in the correct order at preset time intervals. The process embraced the formation of the phenylthiocarbamyl derivation of the protein and splitting off the N-terminal amino acid as thiozolinone. The degradation cycle proceeded at a rate of 15.4 cycles in 24 hours and with a yield in the individual cycle of 98%. The thiazolinones of each N-terminal amino acid were automatically stored in a fraction collector accommodating 50 tubes and they were converted to the corresponding phenylthiohydantoins in a separate operation for later identification by thin layer chromatography.

The process was applied to the whole molecule of apomyoglobin from the humpback whale and it was possible to establish the sequence of the first 60 amino acids from the N-terminal end. About 0.25 μmol of the protein was required for this operation.

Automation has changed the strategy of sequence determination. It is no longer necessary to begin by cleaving the protein backbone into many small peptides since long direct sequences are possible. It is then ideal to work with large and few fragments. At the fragmentation high cleavage yields are of the greatest importance, otherwise the heterogeneity in the cleavage mixture will cause considerable difficulty in the isolation of fragments. As pointed out by Agnes Henschen-Edman (20) it should be taken into account that with incomplete cleavage two cleavage points will give rise to six components, three will give 10, four will give 15 and so forth, thus resulting in increasing difficulty of separating pure cleavage products.

**The strategy and tactics of protein sequence determination as exemplified by work in Edman’s laboratory**

Since the appearance of Edman’s first paper on the isothiocyanate degradation in 1949 and up to the time of his lecture delivered at the Carlsberg Laboratory Centennial in 1976, more than 80 000 amino acids had been put into sequence by various laboratories throughout the world. As Edman commented, ‘this figure may seem large but it is dwarfed when compared with the maximum coding capacity in a mammalian genome of 2 billion residues. Most likely only a very small fraction of this capacity is actually used for the coding of protein structures. Even so the number is likely to be large, and this means that the road before us will be long. At present there are several hundred sequencing devices in operation. This will undoubtedly speed up the data accumulation, and it may reasonably be expected that in the next few years the 200 000 mark will be reached.’

Edman’s own chief concern up to the time of his death was in the refinement of the methodology and in the necessity for the establishment of adequate arrangements for data storage, data retrieval and data processing. Needless to say, his laboratory in Munich was endowed with a wealth of hard-won experience.
in sequencing and at the time of his death was engaged in, among other projects, the elucidation of the primary structure of fibrinogen. This work Pehr Edman regarded as exclusively the project of his wife Agnes Henschen-Edman. However, to quote her own words in December 1977, 'It would for certain, not have been possible to proceed with the investigation in such a way without Pehr Edman's insight and his organization of the department. The γ-chain sequence was finished and published half a year ago. The complete β-chain sequence has now appeared in print after just a few months of work. My husband also gave the idea to a workshop on fibrinogen here in Martinsried. It was sad and strange, but perhaps appropriate, that the workshop took place on the day of his death.'

Agnes Henschen-Edman's lecture (20), prepared for the 2nd Solid Phase Symposium, Montpellier, September 1977, gives a very good insight into the strategy and tactics in protein sequence determination current in Pehr Edman's laboratory at Martinsried at the time of his death, and some passages warrant quotation in full.

'The first instance when N-terminal analysis might be introduced is already during the isolation of the protein. Once the N-terminal of the chosen protein is known, N-terminal analysis can be a most convenient method for monitoring the progress of purification. It might be much easier to use than, e.g., a biological activity test. It is, of course, very important to use high quality reagents and general caution during the purification procedure, as N-terminals may get blocked and chemical heterogeneity may be introduced already at this stage. Any new heterogeneity in size or charge of the protein molecule or modification of its side chains will substantially increase the difficulties at most subsequent fractionation steps and also at many fragmentation reactions. A few examples may suffice. Blocking of amino groups or loss of amide groups will change the charge and thus change the behaviour in ion-exchange chromatography and electrophoresis. Furthermore, trypsin will not cleave at lysine residues with modified ε-amino groups. Cyanogen bromide will not cleave at methionine residues in the sulfoxide form. N-terminal glutamine in the native protein cyclizing to pyroglutamic acid during the purification represents a special, not yet solved problem.

'As a purity test N-terminal sequence analysis is in many respects superior to physico-chemical analysis, like ultracentrifugation and gel electrophoresis. At the evaluation of sequencing results no assumptions are needed. The result is independent of aggregation or quaternary structure, many foreign substances will not interfere, and, above all, the result is more informative. The probability that a certain sequence will show up by chance is about one in twenty for each step in the sequence. That a contaminating protein by chance will have the same first four amino acid residues as the protein to be purified is then about one in 160 000. This means that even a short sequence will give an excellent characterization of the protein.'
'A further application of the N-terminal analysis is for molecular weight determination. The subunit weight is calculated from the quantitative yield of the N-terminal amino acid(s). Again the result is more informative and often complementary to those obtained by physico-chemical measurements. The number of identical or non-identical subunits can be deduced. The N-terminal amino acids in human fibrinogen and fibrin may serve as an example. Fibrinogen contains per molecular weight of 340 000 two moles of alanine and two of tyrosine, fibrin contains four moles of glycine and two of tyrosine (21). The interpretation was that both proteins consist of three pairs of non-identical peptide chains, two of which are cleaved proteolytically at the fibrinogen–fibrin conversion, and that one pair of chains in fibrinogen has a blocked N-terminal.'

Another instance in which N-terminal sequencing was used was for monitoring the isolation of non-identical subunits. The three subunits (Henschen & Edman (22)) of mercaptan-reduced, carboxymethylated fibrin were separated on CM-cellulose, all the chromatographic fractions being identified by their N-terminal yields. For chromatography, buffers containing 8 M urea have to be used and urea is known to form cyanate which blocks amino groups. Earlier, N-terminal yields of only about 20% were obtained after chromatography in such buffers. However, when Tris was introduced as the positive ion of the buffers the yields increased to 90–95%. This is because Tris acts as a scavenger for cyanate and thus protects the protein amino groups. The acetic acid used was free of aldehydes which also could have blocked amino groups.

Agnes Henschen-Edman emphasized the importance of using a highly selective procedure for cleaving the isolated protein subunits into a few fragments of fairly large size. As has already been pointed out, high cleavage yields are of the greatest importance as otherwise the heterogeneity of the cleavage mixture will cause considerable difficulty in isolating the fragments. The selectivity of the cleavage reaction is of equal importance. Many highly specific proteolytic enzymes have been described, but have not found extensive use so far. Two of these, thrombin and plasmin, belong to the blood coagulation system, and a growing group of specific proteolytic enzymes is being isolated from snake venoms. A few selective chemical cleavages have also been described, the most well known being the cyanogen bromide cleavage.

The cleavage reaction may, to great advantage, be studied with N-terminal analysis. A qualitative determination will give information about the number and kind of new N-terminal amino acids or how many different kinds of bonds have been split. A quantitative result can be used for calculating the number of bonds cleaved or the cleavage yield. Furthermore, the time course of the cleavage and the optimal cleavage conditions may conveniently be deduced from these determinations.

To quote again directly from Agnes Henschen-Edman’s lecture at Montpellier:

‘The fragmentation, isolation of fragments and subsequent sequencing
will be illustrated by our own recent work on one of the chains in fibrinogen, the \(\gamma\)-chain. The molecular weight of the chain indicated that it contained about 400 amino acid residues. Thus it was far too large for direct sequencing. The amino acid composition showed that cleaving the chain at methionyl or arginyl bonds should produce a convenient number of fragments having a suitable size. Furthermore, cleavages at these two residues are known to be specific and almost complete.

'First, the \(\gamma\)-chain was split at the eight methionyl bonds (23) with cyanogen bromide [CB\(\downarrow\) in figure 3], and nine fragments were expected to be formed. Six new N-terminals were found in the cleavage mixture. The fragments were then isolated by Sephadex chromatography and CM-cellulose chromatography and characterized by the N-terminal sequence and amino acid composition. The finding that three of the fragments had the same N-terminal amino acid, i.e. lysine, explained why only six new N-terminals were found in the original cleavage mixture.'

It is obvious from this description how all separation procedures were monitored by N-terminal analysis. This was done to reduce the great danger of losing a fragment, which otherwise might easily happen, especially when the fragment is small or is devoid of ultraviolet absorption. With this 'N-terminal book-keeping', all N-terminal amino acids once present in the original cleavage mixture or in a fraction of it have to be accounted for by the N-terminal amino acids of the isolated fragments.

In Edman's laboratory it was found convenient to name the isolated fragments after their N-terminal sequences in one-letter abbreviation (IUPAC-IUB Commission on Biochemical Nomenclature (24)). Such a name gives an almost unambiguous definition of the fraction. The name is easy to remember and independent of how the fragment was isolated.

Out of the nine cyanogen bromide fragments from the \(\gamma\)-chain of fibrinogen one fragment, YVAT-M, can be recognized as the N-terminal by its sequence. Another fragment, KIIP-, is recognized as the C-terminal by the absence of a methionine (represented by a homoserine) residue in the amino acid composition. It now remained to establish the true order of the seven internal fragments. For this purpose eight overlap sequences were needed.

In order to find these overlaps the \(\gamma\)-chain was fragmented in a different way. The 10 arginyl bonds (Henschen & Lottspeich (25)) were selectively cleaved by trypsin after blocking the \(\varepsilon\)-amino groups of the lysine residues by citraconylation (CT\(\downarrow\) in figure 3). The cleavage should give rise to 11 fragments. Seven new N-terminal amino acids were found in the digest. However, after fractionation on Sephadex and CM-cellulose chromatography the fragments were characterized by N-terminal sequence and amino acid composition and it was obvious that in fact 11 fragments had been formed. Five of these fragments contained all methionine overlaps.

By using this and similar approaches (26) it was possible to place all fragments, obtained by cleaving the \(\gamma\)-chain at the methionyl bonds, in their original order.
Figure 3. Amino acid sequence of human fibrin \( \gamma \)-chain. Cyanogen bromide cleavage points are marked by \( \text{CB}\uparrow \), trypsin cleavage points after citraconylation by \( \text{CT}\uparrow \).
in the chain (figure 3). About 70% of the total γ-chain sequence was obtained by direct sequencing of these fragments. Remaining sections were sequenced after supplementary cleavage by various methods.

The descriptions given by Pehr Edman and Agnes Henschen-Edman and her collaborators in the laboratory at Martinsreid give a good visualization of the remarkable feat required to assign the order of 410 amino acid residues in the γ-chain of fibrinogen as shown in figure 3. But to what biological end does the knowledge of the primary sequences in proteins lead us? From the very first it was clear that the amino acid sequence in a protein does not immediately lead to understanding of its biological function. The active centres of enzymes and the sites of immune affinity on proteins generally concern the three-dimensional description of the molecule as much as the primary sequence; although the tertiary structure is a consequence of the primary structure. One of the most potentially powerful applications of sequence studies lies in the search for evolutionary relationships between different proteins; but this becomes valuable only in proportion to the number of sequences available for comparison. It is to this fact that we can attribute Edman’s concern that the opportunity to set up adequate computer storage facilities should not be lost. There are now several hundred automatic sequencing devices in operation and in the next few years we can expect a rapid acceleration in the rate at which sequence data become available for comparison. At present the Atlas of Protein Sequencing provides a facility through its computer service, but this is done on an insecure year to year financial basis and Edman expressed his doubt if this important function is sufficiently safeguarded. In his words ‘we may in time expect the unravelling of a new systema naturalis among the biomolecules. It would be tragic if this development would be endangered through our own negligence.’

It might be right to conclude with an observation of Pehr Edman’s which is perhaps self-revealing. ‘People with no experience of structural work sometimes tell me that they believe it is (a) tedious, (b) with automation a routine. This is wide of the mark. It may have been tedious at the time when all sequencing had to be done by hand, but automation has done away with most of that. The belief that the work has become a routine is even more untrue. In fact, the solution of a large structure taxes the investigator’s resources of skill and knowledge to the utmost, and luck is not an unessential factor. The comparison to the solution of a super-crossword puzzle is perhaps apt. Anyone, who has experienced the elation in the laboratory when a fragment known to be missing has been found, or a tantalizing inconsistency has been resolved, would know what I mean.’

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