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SYMPOSIUM REPORT

Kaj Ulrik Linderstrøm-Lang (1896-1959)

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Abstract

The Carlsberg Laboratory in Copenhagen has had a long tradition of outstanding science. At the time covered by this discussion, Kjeldahl, Sørensen, and Linderstrøm-Lang had been consecutive directors of the Chemical Laboratory for 83 years. Lang's inspired leadership began in the 1930s with a number of innovations (study of metabolism in single cells, titrations in non-aqueous solvents, relation of proteolysis to structure) but it was not until the early 1950s that Denmark had sufficiently recovered from the war for the laboratory to enter world science again. During World War II, Lang had been active in the Danish resistance movement.

After the war, a number of major advances were being made that would revolutionize the field of protein chemistry (Pauling and Corey's H-bonded structures, Sanger's sequencing techniques, chromatography, Watson and Crick structures, modern instrumentation). The time for the new field of the physical biochemistry of proteins had arrived. Lang, with his broad experience, adventurous spirit, and genius for innovation, created an environment that was ideal for the convergence of these disconnected advances into a uniform science. The emphasis was to be on quantitative measurements on proteins in solution with interpretations based on molecular structures.

During an all-too-brief period of time, Lang's laboratory attracted a large fraction of those who were destined to be the leaders of the next generation of protein chemists. At this time, the Carlsberg Laboratory was probably the most scientifically exciting environment for a protein chemist. The methods developed at that time—hydrogen exchange, limited proteolysis, optical rotatory dispersion, volume changes accompanying protein reactions, automatic titrations—are still all in common use and many of the visitors to the laboratory in that period and their students are still playing major roles in protein research.

Lang's other qualities should not be ignored. He was not only a great scientist but also a musician, raconteur, artist, and an exceptionally warm and compassionate human being.

Keywords: Carlsberg Laboratory; denaturation; gradient tube; hydrogen exchange; Linderstrøm-Lang; micromethods; proteolysis

In 1996, the centenary of the birth of Kaj Ulrik Linderstrøm-Lang was celebrated by a symposium at the August meeting of the Protein Society in San José, California. After an introduction by Charlotte Schellman, John Schellman and Walter Englander gave lectures on which this paper and the following one are based. On Lang's birthday in November, another symposium was held at the Danish Royal Academy of Sciences and Letters in connection with the awarding of the Linderstrøm-Lang medal to Kurt Wüthrich.

It is now 37 years since Lang's death in 1959. Yet, in spite of the notoriously short memory of modern science, his memory still endures in the minds of all who knew him and the lasting influence of his scientific work pervades a good deal of current biophysical science. The Science Citation Index lists about 250 references to his work in the past ten years. He was a major originator of ideas in both the physical chemical and biological aspects of biochemistry; he was, simultaneously, a master in developing ingenious and novel experimental techniques. In addition, his character, humor, diversity, artistic talents, and light-hearted approach to the deepest scientific matters inspired his students and associates and endeared him to all who knew him.

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A late-blooming scientist

Lang was born on November 29, 1896, in a suburb of Copenhagen into a family that had produced generations of educators. His father, Carl Frederik, was a teacher of German and Latin at the Frederiksberg Gymnasium and his mother, Ellen Hedwig, was the daughter of a banker. His childhood was spent in a graceful, cultured social atmosphere where art, music, and literature prevailed. These influences were to remain with him all of his life. As a boy he was interested in writing, music, art, and science, quite possibly in that order.

Lang entered the Technical University of Copenhagen as a chemistry major, although he regarded chemistry only as a way to make

Frontispiece, Kaj Ulrich Linderstrøm-Lang in 1951.

a living while deciding which of his artistic talents to pursue. He entered enthusiastically into student life and was very popular. He was editor of the chemistry majors' newspaper and demonstrated his wit and humor as the writer of the annual plays. In his last year, Lang combined his artistic and scientific aspects in a lecture entitled "Strindberg as a Chemist," which was well received. He obtained his degree in 1919 and left the university with literary hopes for the plays that he had written.

His first job, a temporary position at the Institute of Animal Husbandry in Copenhagen, had a major consequence. The head of his department, who formed a high opinion of Lang, had worked at the Carlsberg Laboratory and recommended him to its director, S.P.L. Sørensen. In August 1919, he became an assistant to Professor Sørensen in the Chemistry Section, where he remained for the rest of his life. Lang found himself working under a master experimentalist on problems that attracted world-wide attention, and his interest in a career in the arts was replaced by an overriding enthusiasm for science. He also came in contact with Niels Bjerrum, a major figure in physical chemistry, who exerted a strong influence on the physical aspects of his research and who became a life-long friend. With Sørensen's inspiration and guidance, within a few years he was turning out research that was comparable to that of his most mature and experienced periods.

The Carlsberg Laboratory (Holter & Max Møller, 1976)

History

The setting for the career of Linderstrøm-Lang was the chemistry division of the Carlsberg Laboratory. The unusual character of this institution shaped the progress of the work that was done and the people who worked there and vice versa. In this section, we will digress from our main theme to outline the history and nature of this remarkable institution. It begins with the brewer Jacob Christian Jacobsen in the mid-nineteenth century. He was an outstanding brewer and a philanthropist with a prescient view of science. He wanted to emulate the scientific approach to fermentation with which Pasteur had transformed the wine and beer industries of France, and Jacobson entered into extensive correspondence with Pasteur and other major figures. In 1876, he formed the Carlsberg Laboratory which achieved enormous success. In 1883, the first director of the Physiological Department, E.C. Hansen, isolated a single yeast strain and introduced the system of brewing with pure yeast cultures, thereby avoiding the occurrence of aberrant brews. This discovery eventually changed the industry. As a result of his great financial success, Jacobsen later diverted a major fraction of the profits of the brewery to the Carlsberg Foundation, a non-profit organization for the support of the Carlsberg Laboratory and the arts and sciences in Denmark. The foundation supported the various divisions of the laboratory, a number of major historical and art museums, and provided fellowships in the arts and sciences. The Carlsberg Laboratory was independent of the brewery, which maintained its own laboratory. The board of directors has always involved Denmark's most eminent scientists, engineers, and mathematicians, as well as major figures in government, education, and business.

Jacobsen's enlightened and altruistic character is revealed in the laboratory motto printed on a border in the grand entry hall of the laboratory: "No result of the activities of the Institute that is of theoretical or practical importance may be kept secret." When Hansen proposed selling his yeast strain, *Saccharomyces carlsbergiensis*, to other breweries, Jacobsen vetoed the idea and made it freely accessible all over the world. It is still the dominant strain in the industry.

Research at the Carlsberg Laboratory has been divided among several sections or divisions (Chemistry, Physiology, Cytochemistry) but our main interest is in the Chemistry Section.

As can be seen from the list of directors of the Chemistry Section (Table 1), this tiny, brewery-supported laboratory achieved international distinction from the start and has continued to maintain its influence ever since.

The lab as a Mecca for quantitative biochemists

By the late 1930s, visitors in the Chemistry Section were becoming common, because, as Fritz Lipmann said, "the Chemistry Department began to be known not only for its scientific excellence but also for its joyful spirit." (Lipmann, 1980) Visitors during that period included David Glick, Fritz Lipmann, Chris Anfinsen, Oliver Lowry, R.D. Hotchkiss, and Milton Levy.

During World War II, the laboratory slowed down owing to the German occupation and a lack of supplies. But soon after the war

Table 1. *Carlsberg Laboratory chemistry section*

Directors	
Johan Kjeldahl	1876–1900
S.P.L. Sørensen	1901–1938
Kaj Linderstrøm-Lang	1938–1959
Martin Ottesen	1959–1987
Klaus Bock	1988–

a few visitors began to arrive again and by about 1950 word had spread that there was a laboratory in Denmark that provided an inspiring and exhilarating approach to the physical chemical aspects of protein science. During the following decade more and more visitors to the lab returned home confirming the rumors of great science and a light-hearted atmosphere.

Protein science was making enormous strides after the war. The Pauling-Corey structures provided detailed models for the interpretation and generation of experiments. The modern graduate student will find it hard to believe that prior to this the common model for a protein was a hydrated ellipsoid of revolution. Sanger had determined the sequence of both the A and B chains of insulin and was working on the position of the disulfide bridges. Lang's primary/secondary/tertiary classification was newly at hand to help in the structural perception of these kinds of information. Chromatography was well established and growing almost daily in power and versatility. Commercial instruments for the measurement of pH, spectra, sedimentation, electrophoresis, etc., were finally becoming available to the working biochemist. The journals were already beginning to grow in size owing to the sudden burst of progress that was conceptually deeper yet easier to accomplish.

The Carlsberg Laboratory very rapidly advanced to a position of world leadership in the new type of protein investigation. Great strides were also made in other places, e.g., the structural groups at CalTech and Cambridge and the polymer groups in England and Israel and at Harvard, but the work in Lang's department differed in being directly related to problems that had arisen in protein biochemistry. To a large extent, this must be credited to Lang's creativity and leadership but credit should also be given to the group of Danes and foreign visitors that he had brought together. Eventually, the laboratory attracted a large fraction of the best biophysical chemists from the United States and other countries. A list of the visitors to the laboratory from 1952 to 1960 looks like a roster of the "establishment" for the following twenty years (see Table 2). In this way, Lang's influence has permeated laboratories all over the world. Many of the leaders of today are the scientific grandchildren of Linderstrøm-Lang, whether they know it or not.

Life in the lab, Carlsberg style

From time to time there are discussions as to what factors create a productive laboratory. Certainly of primary importance is a leader who is scientifically creative. But the atmosphere or culture that the leader creates is also essential. Following Sørensen's example, Lang maintained a collegial laboratory spirit rather than being a dictator. Aside from the stimulating scientific atmosphere, including weekly seminars, the extremely convivial working atmosphere accounts for the enthusiasm with which old visitors recall their stays. The permanent staff were helpful and welcoming to visitors. While money for supplies and equipment was not plentiful, such shortages often led to ingenious improvisations or repairs. Most of the visitors' desks were in the two larger labs, so everyone was in close contact and frequent communication. Lang usually passed through the labs at least once a day and was always open to discussions. Essentially the entire laboratory met for lunch in the basement at tables carefully set with an array of Carlsberg products. Lively conversation flowed, with frequent joke-telling contests. Afternoon tea was served in the stately main hall; anyone with a birthday was expected to supply a special Danish layer cake and "Happy Birthday" was sung. Many visitors carried home the determination to create a similar spirit in their own laboratories.

Table 2. *Some scientists who have worked in the chemistry section of the Carlsberg Laboratory*[a]

Visiting Scientists	Danish Scientists
C.B. Anfinsen	L. Allen
R.L. Baldwin	W. Andersen
E.E. Benson	B. Foltmann
H. Berg	H. Holter
A. Berger	Aa. Hvidt
C. Bigelow	C. F. Jacobsen
J. Foster	G. Johansen
E. Fredericq	A. Johansen
E. Frieden	H. Kalckar
D. Glick	L. Korsgaard-Christensen
W.F. Harrington	K. Max-Møller
J.I. Harris	B. Meedom
G. Hevesy	S.O. Nielsen
M. Hoagland	M. Ottesen
R. Hotchkiss	P. Schach
R. Hubbard	Aa. Vaslow
W. Kauzmann	E. Zeuthen
D. Kupke	
J. Leonis	
M. Levy	
F. Lipmann	
O.H. Lowry	
R. Lumry	
F.W. Richards	
C.G. Schellman	
J.A. Schellman	
H.A. Scheraga	
W. Schroeder	
D. Steinberg	
J. Strominger	
H. Tuppy	
L. Vandendriessche	
F. Vaslow	
R. Warner	
D.B. Wetlaufer	
P. Wilcox	
P. Zamecnik	

[a]There have been many other visitors during Lang's directorship. We have only included those who might be familiar to readers of *Protein Science*.

Lang's scientific career

Lang's publication list contains 154 papers in scientific journals. (Holter, 1960) We will review a small number of exceptional papers from his early work and then summarize several areas of his later work. The variety of subject matter caused John Edsall to comment, "We may note the extraordinary versatility that was displayed in all this range of accomplishment. Those investigators who combine power and skill in abstract mathematical analysis seldom show high aptitude for the development of refined and sensitive biochemical techniques." (Edsall, 1959)

Solution physical chemistry

Lang worked directly with Sørensen as his assistant for the first three years at the Carlsberg Laboratory. Sørensen's combination of long-range scientific vision and meticulous care with experimental verification evidently made a huge impression on Lang, since he

emerged from this period as a dedicated scientist. Many of his later papers show the Sørensen touch, containing large quantities of data with careful and imaginative controls of reagent purity and experimental procedure. His very first independent papers were already outstanding. He was also aided by his regular contact with Niels Bjerrum. This was particularly important for the physical studies that are described below.

"On the salting-out effect," (Linderstrøm-Lang, 1923)

Lang's first paper, published in 1921, dealt with the effects of salts on the newly discovered quinhydrone electrode. Sørensen and Sørensen's wife were coauthors. The potential was found to vary slightly with salt concentration.

This result led to Lang's first independent investigation, which dealt with the salting-out effect. He made use of the Setchenow relation, $\ln(S/S_0) = -kC$, where S_0 is the solubility of a substance before the addition of salt (or other reagent), S is its solubility in the presence of salt at concentration C, and k is a constant at a given temperature. Lang correctly interpreted the relationship of the factor kC to the activity coefficient, $kC = \ln(\gamma/\gamma_0)$ where γ_0 is the activity coefficient before the addition of salt (assumed equal to unity) and γ is the activity in the presence of salt.

The paper contains masses of data on four organic compounds, twelve salts, and a number of temperatures. Values for k, activity coefficients, and ΔH of solution (from the variation of $\ln \gamma$ with temperature) were determined; Setchenow's equation and the Hofmeister series were tested and mainly verified for a number of systems. Probably the most important aspect of the paper is the introduction of the thermodynamic activity into the discussion of biochemical systems at a time when it was generally ignored by many physical chemists. Furthermore, Lang demonstrated that an activity coefficient is not merely a measure of the non-ideality of a solute, but an important thermodynamic aspect of a solution, which can be used to control chemical systems, in this case by perturbing the solubility. Nowadays we would call kC the free energy of transfer of the solute from a solution without the salt to a solution containing the salt.

"The Ionization of Proteins," (Linderstrøm-Lang, 1924)

This is a truly amazing paper. At the time of its publication the Debye-Hückel theory was not yet a year old. Yet Linderstrøm-Lang used the Debye-Hückel formalism to calculate the effect of total protein charge on the apparent pK of titratable groups of proteins. To the standard free energy of protonation of a group a term is added for the electrostatic work of increasing the protein charge by one positive unit. This is negative or positive depending on whether the total protein charge is negative or positive. As a result, the pKs of the -COOH, $-NH_3^+$, histidine, etc. are spread out into a spectrum of pK values by the varying total charge produced by the titration. The theory is also suitable for the binding of small ions.

Lang's formalism was actively used for more than forty years in many types of calculations and has only been supplanted in recent years by the use of the detailed structures for proteins and the widespread availability of computers. These permit one to sum the fields of individual charged groups. The Lang theory is still the first approximation and remains in use for many problems where the molecular structure is not known.

"The Volumetric Determination of Amino Nitrogen," (Linderstrøm-Lang, 1927)

This third paper on solution physical chemistry makes use of the ideas in the previous two. The problem to be solved was to find a direct titrimetric procedure to follow reactions such as proteolysis. The difficulty arises because the splitting of a peptide bond generates both an acid and a base group and cannot be followed by acid/base titration methods. In particular, the extant methods of measurement of the amino group involved irreversible and often incomplete chemical reactions such as the Folin procedure. Lang's solution was simple: Perform the titration with acid in 90% acetone, using naphthyl red as an indicator. This result has the earmarks of an empirical study optimizing several variables in an Edisonian manner but it was nothing of the sort. He classified acid-base titrations into five classes depending on the charge of the acid and base forms. He considered the effect of adding a low dielectric constant organic solvent to each of the five classes. This harkened back to his early work and depended on the different changes in activity coefficient experienced by charged molecules of different states of charge. The conclusion was that a high concentration of alcohol or acetone would bring the pK of amine groups or amino acids into the right range. He then applied the same arguments to the titration of the indicator and concluded that naphthyl red would have a responsive change of color at the endpoint of the titration in acetone.

This was a masterful treatment of the theory of acid-base titrations. It was the first paper that brought international attention to Lang's work. This was not because of the elegance of the theory and reasoning, but because biochemists were in desperate need of a quick and reliable method of titrating amine groups in a wide variety of applications.

Micromethods

During the 1930s, Linderstrøm-Lang's research turned to new directions. This occurred when he joined his interests with those of Heinz Holter, his long-term collaborator and friend. The target of their projects was to observe biochemical processes and their kinetics at the cellular level. The motivation came from the fact that in vitro experiments in the early thirties could only be done with impure preparations or cellular (or tissue) extracts, when often the operative enzymes had never been isolated. As a result, experiments at the cellular level were more secure in their interpretation than in vitro experiments. In 1931, Lang stated his belief: "It is only in the study of typical secretion enzymes outside the cells (e.g., in the alimentary canal) that experiments 'in vitro' may be considered with any certainty to reproduce the processes taking place in the organism" (Linderstrøm-Lang & Holter, 1931). It is interesting that this point of view is reflected in the modern interpretation of experiments in dilute solutions compared to in vivo conditions. See the volume of *Biophysical Chemistry* (Minton, 1995) devoted to this problem.

The problem was that the sensitivity of the methods for detecting biochemical reactions had to be improved by five orders of magnitude. Lang and Holter published a long series of papers on this project from about 1930 until after the Second World War.

"The Estimation of Small Cleavages Caused by Enzymes," (Linderstrøm-Lang & Holter, 1931)

This paper brought conventional titrimetric methods to the limit of their sensitivity. It made use of miniature versions of macroscopic apparatus, together with ingenious ideas for the transfer of reagents, magnetic stirrers, micrometer syringes, capillary pipettes, and burettes. One sees in this apparatus one of the earliest explorations of the techniques that would be used much later in auto-

mated analytical chemistry. It was first used to study peptidase activity and reduced the error of a titration to about 1.5 nanomoles. This was not enough to study single cells but was sufficient to study a small number of cells that were histologically homogeneous. It had obvious uses for any situation in which the sample size was small and was soon made available commercially.

"Studies on Enzymatic Histochemistry XXIX. Dilatometric Micro-Estimation of Peptidase Activity," (Linderstrøm-Lang & Lanz, 1938)

This is the paper that first described the gradient tube. The method essentially solved the single-cell problem for reactions that were associated with an appreciable change in volume such as the proteases and fat-splitting enzymes that were under study at the Carlsberg Laboratory at the time. The idea was simple and ingenious. Two fluids, immiscible in water and of slightly different density, are prepared. The lighter of the fluids is carefully overlaid above the heavier in a tube with a long cylindrical section in the middle (Fig. 1). The center section is gently stirred and after a day or so it is found that there is a linear density gradient in the long central section. Typically, the density changes only by 0.001 $g \cdot mL^{-1}$ per cm. Small drops (about 0.1 μL) of a solution to be analyzed are added to the column and fall until their density matches that of their surroundings. This converts the measurement of density to the measurement of position in the tube, which can be done with great accuracy with a cathetometer. A series of KCl solutions of known density are run alongside the unknowns for calibration. We omit details concerning saturation, calibration, rejuvenation of the column, etc., which are covered in the original paper.

With this procedure complicated chemical or titrimetric techniques are replaced by a simple, non-invasive physical measurement As illustrated in the figure it also permitted the study of kinetics, provided that the reaction was slow compared to the equilibration time in the column.

This technique increased the sensitivity by a factor of 50 relative to the 1931 paper described above, and could measure the peptidase activity of single cells. It was also used in isotope experiments to determine the amount of deuterium in water. (Linderstrøm-Lang et al., 1938) One of us used the method to measure the partial specific volume of proteins many years ago with very satisfactory results on extremely small samples of protein. The procedure was also the basis of Lang's method for the hydrogen exchange of proteins, which will be discussed later.

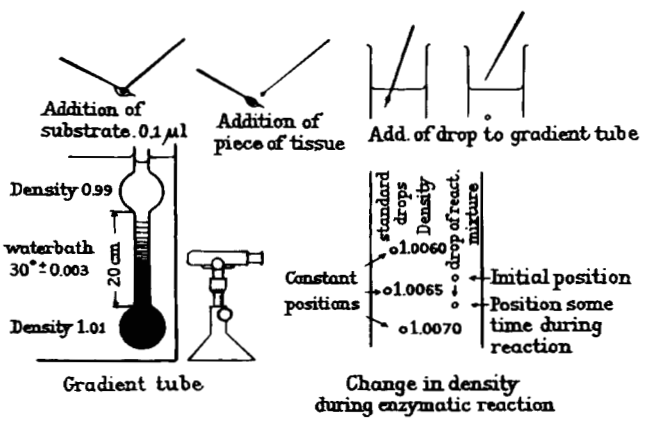

Fig. 1. A kinetic experiment with the gradient tube. The drops on the left side of the drawing on the lower right are the stationary standard drops.

The work aroused Lang's interest in the origin of volume changes in chemical reactions. In particular, he found that the volume change associated with the proteolysis of an intact, globular protein was not the sum of the peptidase reactions of the individual peptide bonds. This influenced his ideas on the nature and mechanism of proteolysis and the denaturation reaction, topics which will be brought up later.

"Micromethod for Determination of Choline Esterase Activity," (Linderstrøm-Lang & Glick, 1938)

This paper makes use of the exquisite sensitivity of the Cartesian diver to slight changes in buoyancy. We will omit details. It was proposed for the detection of reactions or cellular processes that generate or absorb gases. In this preliminary paper, it was used for the determination of choline esterase, a task that would normally be performed on a macro scale with a Warburg apparatus. Its ultimate purpose was to measure respiratory and other processes in single cells. It was later used extensively by Holter and his associates for investigations of single-cell organisms, amoebae in particular. Lang later wrote a monumental but intimidating paper on the theory of the apparatus. (Linderstrøm-Lang, 1943) At the same time Holter published a more user-friendly guide to the technique. (Holter, 1943) Lang's comment on his own paper is typical: "Some of the sparrows could have been shot with smaller cannons."

Proteolysis and denaturation

Lang published more than fifty papers on various aspects of proteolysis and it is impossible to summarize his contributions by citing a few papers in depth as in the previous sections. His interest in proteolytic enzymes was aroused initially by a two-month visit to Willstätter's laboratory in Munich in 1926, one of his rare leaves of absence from the Carlsberg Laboratory in over forty years. His early work on the subject dealt with peptidase specificity and he was able to establish, against considerable opposition, that the specificity depended on the nature of the amino acids near the split and not on the length of the peptide's chain, as was then thought. In particular, Lang disproved the concept of a peptidase specific for dipeptides. His reputation grew considerably as it gradually became established that sequence is the determining factor in peptidase specificity. Some details are in Holter (1960).

Because of his previous work, it was natural for Lang to choose a proteolytic system to test his micromethods, in particular the amine titrations and the gradient tube. He discovered that the volume change in the proteolytic breakdown of intact proteins was not proportional to the number of peptide bonds broken (Linderstrøm-Lang & Jacobsen, 1941; Linderstrøm-Lang, 1952). The quantity $\Delta V/\Delta n$, where V is the volume and n is the number of peptide bonds hydrolyzed, begins at high negative values (about -40 mL/mole) and only late in the reaction settles down to about -20 mL per mole, which is characteristic of structureless polypeptides. Lang assumed that this initial process was associated with an early structural change in the protein, which exposed internal peptide bonds to the environment. It was known that the initial proteolysis of intact proteins was in general very slow compared to structureless polypeptides and also that certain side-chain groups (-SH, tyrosine, amino, and carboxyl groups are "buried" and inaccessible in many proteins). He at first assumed that the initial reaction was a spontaneous unfolding (or denaturation) of the entire protein molecule with the kinetic scheme:

$$N \overset{1}{\longleftrightarrow} U \overset{2}{\longrightarrow} \text{amino acids}$$

where process 1 is an equilibrium unfolding with a very low equilibrium constant and 2 is the irreversible breakdown of the exposed peptide bonds. The basis for the first step was the conclusion of Anson and Mirsky (Anson & Mirsky, 1931) that the "denaturation" of proteins is a reversible, spontaneous process.

After World War II, Lang revised his ideas to incorporate the activation of zymogens and the formation of plakalbumin (see below) into a more general scheme, which included limited proteolysis as a possible initial step. He also published a very powerful analysis of various kinetic schemes so that kinetic theory could help distinguish amongst the many mechanisms (Linderstrøm-Lang, 1953). His final conclusions, based also on his later experience with hydrogen exchange, were as follows: The initial break (or nick) may lead to a product that is highly stable (zymogens, plakalbumin, ribonuclease S, etc,), or has intermediate stability, sufficient to retard the proteolytic action significantly, or has low stability so that very fast unfolding occurs, followed by fast proteolysis. Reversible folding and unfolding take place in all stages but do not necessarily involve the entire molecule. This differs little, if at all, from current opinion.

Though Lang's first thoughts on the nature of proteolysis were based on the idea of an initial, reversible unfolding of the entire molecule followed by rapid breakdown, his work following volume changes had suggested intermediates as an alternative. This possibility stimulated his interest in the process of limited proteolysis, i.e., proteolysis that either stops at well-defined early stages of proteolytic breakdown, or slows down sufficiently so that intermediates can be detected or isolated. He had followed closely the work of Northrop, Kunitz, and Herriott on the formation of the enzymes trypsin, chymotrypsin, and pepsin from their zymogen precursors, which are glowing examples of limited proteolysis although they may not have been considered in this light in the early days. In addition, by 1940 the specificity of proteolytic enzymes was beginning to be understood and it was clear that side-chain specificity sharply limited the number of sites at which a splitting could occur. This had obvious inferences for limited proteolysis.

In the early 1940s, Lang had proposed to C.F. Jacobsen that he take up a study of the limited proteolysis of chymotrypsin as a basis for his doctorate degree. In Denmark this is a higher degree than a Ph.D. in the U.S., for the candidate must demonstrate both experience and independence. It is usually obtained about ten years after formal training has been completed. Jacobsen proceeded with an elegant study of the activation of chymotrypsinogen by trypsin, discovering two forms which preceded α-chymotrypsin on the kinetic pathway and which were more active than α-chymotrypsin itself (Jacobsen, 1947). Jacobsen concentrated on the physical aspects of the activation process by following kinetics, volume changes and optical rotation as well as activity, in order to test for partial denaturation reactions of the kind postulated for proteolysis. The detailed mechanism in terms of primary structure (position of cleaved bonds, sequence of the small peptides released) was later established by Neurath and Desnuelles and their coworkers making use of postwar methods of sequence determination.

The step into a more general type of limited proteolysis was serendipitous. In the late 1940s, Martin Ottesen, later Lang's successor as director of the laboratory, was working up and recrystallizing old preparations of ovalbumin. He noted that the crystals that formed were not the standard fine needles, but relatively large plates. He and Lang decided that the situation was worth investigating. They eliminated one possible mechanism after the other, and concluded that the new form must arise from bacterial contamination. This was tested by exposing solutions of ovalbumin to the air at room temperature, with positive results. Since the most common bacterium contaminating the laboratory was *Bacillus subtilis*, they obtained a proteolytic bacterial extract of that organism from the Nordisk Insulin Company and purified one of the main components, which turned out to be the protease subtilisin. (Lang called it "Subtle as sin"!) This enzyme readily converted ordinary ovalbumin into 'plakalbumin,' their name for the new form of ovalbumin.

There were actually two forms of plakalbumin, I and II, generated in succession by the liberation of a dipeptide and a tetrapeptide. The story of Ottesen's work is discussed at length in the fourth "Stanford Lecture" (Linderstrøm-Lang, 1952). This paper shows how an apparently insignificant observation, followed by thorough and imaginative work, can lead to penetrating results in a variety of fields.

This study lent credence to the idea that proteolysis could begin with the formation of initial intermediates possessing almost intact structures, which were stable or had at least transitory stability. This fit in with the data on volume determination as a function of breakdown, and was Lang's favored mechanism.

The above studies, together with the attractions of Lang's laboratory, made the Carlsberg Laboratory an ideal place for visitors to take up studies of limited proteolysis and the next steps were taken by Chris Anfinsen and Fred Richards, who arrived in the laboratory in 1954. Anfinsen had a wonderfully large supply of pure ribonuclease, which he distributed liberally among all projects that could make use of it. The sequencing of this enzyme was well underway and it was an especially attractive protein to work with. The ultimate results of their studies were of the greatest importance and have made a history of their own with which most readers are probably acquainted. See Richards' account of his stay at the Carlsberg Laboratory (Richards, 1992).

Hydrogen exchange

Lang's work on the relationship of denaturation and proteolysis had gradually caused him to view protein structure from a more dynamic viewpoint. He had focused for some time on the mechanism by which inaccessible peptide bonds become accessible at the beginning and during the course of enzymatic proteolysis.

The helical and sheet structures for polypeptides proposed by Pauling and Corey in 1951 permitted him to put his thoughts into a more concrete form. He wanted to seek a probe for the presence of these structures in globular proteins. As mentioned before, he had already made use of the gradient tube back in 1938 (Linderstrøm-Lang et al., 1938) to measure the deuterium content of water containing various D/H ratios, and he knew that hydrogen-deuterium exchange took place rapidly when the hydrogen was bonded to a strongly electronegative atom like N or O. He reasoned that in proteins the peptide NH protons that were tied down by the hydrogen bonds of secondary structures would exchange very slowly, if at all. He had already introduced the concepts of the primary, secondary, and tertiary structure of proteins in the third Lane Lecture (Linderstrøm-Lang, 1952) to aid in classifying such mechanisms. The method he developed was based on the gradient tube and involved quenching the reaction at $-60\,^{\circ}C$ and cryosublimating the solvent for the analysis of density. His conjecture that burial and participation in secondary structures would slow down the exchange of peptide hydrogens was verified, and the study of

protein hydrogen exchange remains as one his most enduring innovations, though with a gradual evolution of techniques. The difficult cryosublimation technique was replaced first by infrared spectroscopy (Nielsen, 1960), then for a long period by Englander's method using tritium exchange and gel filtration (Englander, 1963), and finally by magnetic resonance with assigned proton spectra. Lang certainly would be entranced by the high-tech NMR procedures of today. (During a 1955 lecture on NMR to the Danish Academy of Sciences, Richard Ogg was asked what the cost of such an apparatus might be. On learning that it might be of the order of $50,000, one of the members declared "Then Denmark will never have one!" Nonetheless, today there are several in Danish laboratories including the Carlsberg lab.)

We will say no more about the H-exchange method itself since it is the topic of the accompanying paper from the Lang Symposium by Walter Englander, who has probably done more than anyone to establish and maintain H-exchange as a viable and important procedure in protein chemistry after the heroic and pioneering work of Lang, Hvidt, and Nielsen.

The H-exchange work both confirmed and broadened Lang's concept of proteins as dynamic systems. About this time, one of the authors of this paper (J.A.S.) was calculating the fluctuations of the α-helix, and assigning probabilities to the fraying of first, second, third, etc., residues from the ends and to breaks in the middle. Lang immediately adopted this view as a way of looking at proteins as an equilibrium distribution of all possible structures with probabilities depending on the free energy increase required for their formation. Here was a way of accounting for the broad distribution of exchange rates which are observed for proteins. This idea was brought to experimental fruition later by Aase Hvidt who used hydrogen exchange to measure the "free energies of exposure" of classes of hydrogens in ribonuclease (see page 348 of Hvidt & Nielsen, 1966). The current and detailed observations of protection factors could be converted into the same kind of information or vice versa. This way of thinking also provided a rational basis for the understanding of proteolysis and limited proteolysis.

By 1955, the atmosphere of the Carlsberg Laboratory was pervaded by the dynamic nature of protein structures, long before the notion of conformational changes became generally fashionable. It was referred to as protein "motility" (Linderstrøm-Lang & Schellman, 1959), though the term has never caught on. The development of this picture, which permits rational thought about so many aspects of protein behavior, may well have been Lang's final source of satisfaction in science.

Linderstrøm-Lang, the man

After Lang's death, Fritz Lipmann wrote, "He was one of the most gifted, generous and lovable men I have ever met, and is remembered, indeed worshipped, by those who came in contact with him, particularly those who had the experience of working in his laboratory." (Lipmann, 1980)

Your first encounter with Linderstrøm-Lang would have been an interesting and pleasant experience. You were naturally aware of his eminence in science and also that he was reputed to be a good fellow. Conversation flowed easily and naturally. He did not try to impress you and you would learn later that it would not have been a good idea to try to impress him overtly. After quite ordinary comments and questions on your trip to Denmark, your lodgings and personal plans, and a brief query and discussion about mutual acquaintances, you would be shown around the lab, your first experience of walking about in a cloud of cigar smoke. Thereafter the conversation had a tendency to take unexpected and usually humorous turns over a wide range of subject matter. Finally, you were sent on your way with well wishes to get yourself established. You were struck with the amiability and easy naturalness of the man and you knew that you were going to get tremendous pleasure out of working in his laboratory.

These first impressions were not wrong. Later, as you got to know him better, you discovered his passion for science, the amazing breadth of his interests and abilities, his generosity, his constant humor and his unparalleled talents as a host.

He was an immensely popular man. It was a rare week when there were not one or more visitors to the laboratory from all over the world who came by to see him, usually bringing cigars or other small gifts.

His breadth in science was demonstrated by a wide diversity of interests. He worked in theoretical and experimental physical chemistry, enzymology, biology, histochemistry, and instrumentation, and did not feel himself above a number of applied problems that were brought to him by others. Examples included nitrogen exchange of sunflower leaves, diffusion and precipitation of phosphate in the Gomori test, and peptidase activity in the roots of barley. We have compiled a partial list of his scientific innovations in Table 3.

As mentioned earlier, Lang as a youth had a stronger interest in the arts than in science. Music was constantly with him. We have heard that, in his earlier days, chamber music sessions—with Lang playing the violin—were a regular feature of his parties. During our time, he usually wandered around amongst his guests during the later stages of a party, playing and leading the singing of traditional Scandinavian songs and melodies. Bellman, Sjöberg, and humorous folksongs were all favorites. The effect of this was to spread warmth and conviviality. Figure 2 is the famous photo of Lang playing the viola with cigar accompaniment at a party. The picture also shows the scientist at work. Lang had essentially no experience with the viola and, while playing, he was experimenting with the manner in which the brain and nervous system could quickly adjust to the larger spacings between the notes.

Creative writing had at one time been his main interest and he fulfilled this ambition after his university days in a secondary way by contributing humorous and op-ed types of articles to Danish newspapers and journals. His articles on New York taxi drivers, the thermodynamics of the fly, science and industry, etc., may be found in his selected works (Holter et al., 1962) and the book on the Carlsberg Laboratory (Holter & Max Møller, 1976).

Table 3. *Lang's innovations*

Acetone titrations
Capillary pipettes → Constriction pipettes (M. Levy)
Gradient tubes
Cartesian divers
Cryostatic microtome sectioning
Volume change as an experimental probe of hydrolysis and unfolding
Single-cell kinetic measurements
Constant pH titrations → pH-stat (Jacobsen & Leonis)
Hydrogen-exchange as a probe of protein structure and motility
Primary, secondary, tertiary structures
Motility

Fig. 2. Lang as a wandering minstrel at a party in 1953 given by newly arrived Americans in C. Green's (later Schellman's) apartment.

Lang's scientific style is best exemplified in his Lane Medical Lectures (Linderstrøm-Lang, 1952), where he is uninhibited by a standard format. It is direct, engaging, clear, and very simple. Heinz Holter, who knew him better than anyone, stated that this apparent simplicity was the product of a great deal of work. Our own experience is that the introduction to the 1959 article in *The Enzymes* (Linderstrøm-Lang & Schellman, 1959) was dictated orally to J.A.S. and needed very little correction thereafter. Writing presumably became more spontaneous later in life.

An excellent example of his scientific humor is to be found in "The Thermodynamic Activity of the Male Housefly" (Holter et al., 1962). Some idea of the flavor of this article can be gleaned from the Schroedinger equation for the housefly

$$H\Psi = E\Psi.$$

and from the P vs T diagram for houseflies where it is shown that the fly pressure increases up to about 60 °C, above which there is a mysterious and precipitous drop to zero.

Lang's artistic side was expressed in his paintings and caricatures (and in his science as well!). The paintings were done during rather strange but infrequent interludes in which he dropped everything else and went into intellectual isolation for periods of a few months. The subjects were family members, street scenes, and coworkers, including S.P.L. Sørensen, and were to be found in his home and that of close relatives. The best of the paintings have been looked at with approval by well-known Danish painters. In painting, as in science, Lang could not resist experimentation and the result was a variety of styles. The authors have been looking at them off and on for more than 40 years and their attraction has not worn off.

The caricatures were spontaneous expressions of his humor and camaraderie. Each year before the lab Christmas party new members or visitors to the laboratory were called into his office. A brief appraisal followed by rapid strokes of a crayon produced a usually comic caricature. These made fun without being offensive and were also often experimental: his secretary Aase was drawn to resemble Ikhnaton's daughter, Hevesy and Olsen were paired as a Don Quixote and Sancho Panza, etc. The authors were portrayed as a pair, linked together by disulfide bonds: J.A.S. with pockets hanging out, the comic strip emblem of poverty, C.G.S. with a large bag with a dollar sign on it. This represented both a marriage and the fact that, at that time, J.A.S.'s fellowship ran out while hers did not. Ninety-one of these caricatures are preserved in the archives of the Royal Danish Library.

Lang's humor was an inherent part of his general sociability. He produced a steady stream of humorous anecdotes at the lunch table. He also loved parties and the role of genial host suited him well at the Langs' dinner or lawn parties. He was a natural master of ceremonies, even at parties given by others, and particularly enjoyed the long ritual of the snaps songs.

Nonetheless Lang's life was not all cheer and accomplishment. Early in his life he had periods of uncertainty and doubts and he was strongly affected by Hitler's ascendancy and the Second World War. He was very active in the Danish resistance movement and had a successful record in helping slip Jewish refugees and key scientific and political figures out of the country. It was he who personally conducted the Bohrs and many others to rendezvous with small fishing vessels that transported them to Sweden on their way to other parts of the world.

This was dangerous work and had a tragic outcome. The fiancé of one of Lang's daughters was a known member of the resistance movement. At one point both he and Lang were arrested. Lang was released after two weeks because of a lack of compelling evidence but the young man was executed. This was a bitter blow for Lang, who later was very reluctant to discuss his wartime experiences.

Because of his great science, leadership ability, and genial disposition, Lang was well known and admired in the world of the biosciences as well as outside. As a result, he collected many honors (see Holter, 1960). He became the youngest member of the Royal Danish Academy and later was similarly honored by a dozen or so royal and national academies, including those of the United States and the former Soviet Union, and the American Philosophical Society. He received seven honorary degrees and a number of prizes and medals as well as becoming a Knight of the Order of the Dannebrog in Denmark. These honors and prizes were very rarely mentioned by Lang's friends and associates, and never by Lang himself unless it made up part of a good story. He was the type of man who was not elevated by honors, so they were not an important part of his perceived character. Around the lab he was known as "the man who cannot say no in ten different languages."

We "old Carlsbergers" are all very grateful that we have had the benefit of his inspiration and charm. From him we learned that science can be exciting, humane and amusing.

Acknowledgements and References for Further Reading.

Our own reading and memories would not have been adequate to produce this essay and we made free use of earlier writings on Lang and the Carlsberg Laboratory, most especially the detailed and sensitive biography by Heinz Holter (Holter, 1960). The history of the Carlsberg foundation and the laboratories as well as many biographical sketches may be found in the very interesting centenary volume "The Carlsberg Laboratory, 1876/1976," which also contains the Holter biography (Holter & Max Møller, 1976). Many of Lang's best papers are to be found in the "Selected Papers" (Holter et al., 1962). Other sources were the writings of John Edsall (Edsall, 1959), David Glick (Glick, 1976), Rollin Hotchkiss (Hotchkiss, 1976), Herman Kalckar (Kalckar, 1960), Fritz Lipmann (Lipmann, 1980), Oliver Lowry (Lowry, 1990), Hans Neurath (Neurath, 1960), Martin Ottesen (Ottesen, 1959), Fred Richards (Richards, 1992), and L. Vandendriessche (Vandendriessche, 1976).

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