ADVANCES IN PROTEIN CHEMISTRY

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PREFACE

This volume begins with a biographical article on Kaj Ulrik Linderstrøm-Lang, which is not comparable to anything published previously in the *Advances*. It is unique because of the practically unique relation of Linderstrøm-Lang to protein chemistry and protein chemists. The publication of this obituary indeed recalls the fact that in the last year we have lost a number of distinguished contributors to protein chemistry. Notable among them was Jesse P. Greenstein, whose life and work have been described by Alton Meister [*Arch. Biochem. Biophys.*, **82**, i (1959)] and by J. T. Edsall [*Science*, **130**, 83 (1959)]. Among his many other contributions to protein chemistry Greenstein was the author of the chapter on "Nucleoproteins" in Volume I, and of the chapter on "Resolution of Racemic α-Amino Acids" in Volume IX of this series. The death of John G. Kirkwood in August, 1959, at the early age of 52, ended the career of one of the world's great physical chemists. He had made major theoretical contributions to protein chemistry by his development of the theory of thermodynamic and electrostatic properties of dipolar ions and of acid-base equilibria of polyvalent substances, and had made an important experimental contribution by the development of the technique of electrophoresis convection. Dr. Hsien Wu, who died in the same month, had made numerous contributions to protein chemistry, including a pioneer paper in 1931 in which he formulated for the first time some of the present conceptions of denaturation in relation to protein structure.

No extensive comment by us is needed on the seven reviews included in this volume. The opening contribution by Kauzmann deals with the perennially interesting problem of protein denaturation from the point of view of one who has a deep knowledge of modern physical chemistry and has made, in recent years, many of the most important experimental contributions to the subject. This article differs in its emphasis from most reviews on the subject which have appeared in the past in that it is concerned with the underlying structural features which are characteristic of the native protein molecule and with the changes that occur in this structure as the molecule becomes unfolded in the process of denaturation. The second paper, by Smithies, describes in detail the technique of zone electrophoresis in starch gels—a technique primarily developed by the author himself. This method has permitted the resolution of different protein components in complex mixtures to an extent never before achieved. The results already attained are most striking, and the subject has made important contributions to biochemical genetics, by the resolution of the different types of haptoglobins and transferrins. In the third paper Vaughan and Steinberg critically examine the available evidence concerning the degree of specificity with which the processes of protein biosynthesis operate to produce molecules with uniquely defined amino acid sequences. It is now ap-

parent that many protein preparations can be obtained which consist of molecules with such uniquely defined sequences, and the resolution of mixtures of proteins into such truly individual components is steadily progressing as the discriminating power of preparative techniques increases. The mechanisms involved in protein biosynthesis show very high discriminating power in selecting the correct amino acid to go into the correct place in the peptide chain sequence of a protein. The extent to which closely related amino acids can be discriminated in these processes is also critically examined in this article. Knowledge of the detailed structural chemistry of tobacco mosaic virus has progressed rapidly in recent years, and we now have a remarkably detailed picture of the relations between the protein and the nucleic acid component of the virus. The protein chemistry involved has been simplified to a degree that would have seemed impossible a few years ago by the discovery that the protein of the virus is built up of several thousand apparently identical subunits, each of relatively small molecular weight. The arrangement of these subunits in relation to one another and to the nucleic acid which they enclose has been largely clarified, and much of the amino acid sequence in the protein subunit is known. The present state of our knowledge of the chemistry of the virus is reviewed here by Fraenkel-Conrat and Ramachandran. Kekwick reviews the subject of the serum proteins of the fetus and the young of certain animals. What is known in this field is still rather fragmentary, but the field is important and a vast amount remains to be done. The author and editors hope that this contribution will serve to stimulate further work in this area. In the most comprehensive review of this volume, Cecil and McPhee survey the sulfur chemistry of proteins, with special reference to sulfhydryl and disulfide groups. Although several reviews related to this field have appeared in recent years, this contribution is distinct in character from any of the others, and gives, we believe, the most critical study yet available of the methods for determination of S—S and S—H groups and of the significance of the results for protein structure, with a very comprehensive bibliography. In the concluding article Sela and Katchalski, who gave a broad survey of the synthesis and chemical properties of poly-α-amino acids in Volume XIII, here review the biological properties of these substances, including their numerous uses in the study of enzymes, their effects on blood clotting and their interactions with many biochemical systems. The polyamino acids also furnish a remarkably varied group of reagents which can be attached to existing protein molecules in various ways, thus enhancing greatly the range of techniques available for protein modification.

Once more we wish to express our appreciation to the staff of the Academic Press for their excellent help and cooperation in the preparation of this volume and in the important though tedious task of preparing the index.

October, 1959

M. L. Anson, C. B. Anfinsen, Jr., Kenneth Bailey,
John T. Edsall

KAJ ULRIK LINDERSTRØM-LANG

1896–1959

By John T. Edsall

Kaj Ulrik Linderstrøm-Lang, who died in Copenhagen on May 25, 1959, held a unique place in the development of protein chemistry during the last thirty years. This was not only because his own scientific work was of the first importance; even more significant, perhaps, was the inspiring influence which he exerted on his colleagues throughout the world and especially on the numerous investigators who came to work at the Carlsberg Laboratory. Because of this, it seemed to the Editors that the present volume would be incomplete without some record of the man and his achievements.

Linderstrøm-Lang was born in Frederiksberg, Copenhagen, November 29, 1896, the son of Dr. Phil. C. F. Linderstrøm-Lang; his father was a teacher of Latin and Greek. During his schooldays his interests ranged widely; he wrote poetry and dramas; nevertheless he pursued and completed a course of study at the Danmarks tekniske Højskole, receiving a degree in chemical engineering in 1919. He then became assistant to Professor S. P. L. Sørensen at the Carlsberg Laboratory, which had been known since the time of Kjeldahl as a center for fundamental protein research. During his first year as an assistant Linderstrøm-Lang carried out many thousands of Kjeldahl nitrogen analyses, and received his initial exposure to the patient, rigorous, and scrupulous standards of scientific work that were characteristic of Sørensen's laboratory. Twenty years later, after Sørensen's death in 1939, Linderstrøm-Lang, who had succeeded him as director, gave an admirable description of the career of his great teacher and of his influence on those who worked with him in the laboratory. Sørensen, with his profound knowledge of chemistry, and his fundamental contributions to the measurement and concept of pH and to the study of enzymes and proteins, tended to avoid theoretical speculation and aimed always to design and execute experiments with the utmost care, and with attention to every detail. In his great series of studies on ovalbumin, published in 1917, he had established decisive evidence that this typical protein was a definite molecule, with a molecular weight that could be determined by osmotic pressure measurements, and that the equilibrium between crystalline and dissolved ovalbumin could be defined in terms of the phase rule of Gibbs, in systems in

which pH, salt concentration, and temperature were all controlled. This represented a great advance, at a time when many influential scientists regarded proteins as ill-defined colloidal aggregates, reacting with other ions and molecules chiefly by physical adsorption, in a manner little related to their chemical structure. Linderstrøm-Lang arrived in the Laboratory at this crucial period, when the awareness of new worlds to be conquered in the field of protein chemistry was dawning for a small but enthusiastic group of investigators. From the beginning Lang showed a high mathematical aptitude, a thorough acquaintance with the development of modern physical chemistry, and a more daring and speculative approach to many problems than Sørensen. The rigorous discipline of Sørensen's laboratory had obviously a profound influence on his development, and helped to shape that combination of imagination with rigorous care and skill which was characteristic of his work throughout his career. Another teacher who inspired him was the great and versatile Niels Bjerrum, whose work on the dipolar ionic structure of the amino acids and on the ionization of dicarboxylic acids in relation to their structure was in process of development at this time.

In 1924 Linderstrøm-Lang published an important paper on the nature of the salting-out effect, in which he studied the influence of salts on the solubility of hydroquinone, quinone, and succinic and boric acids. These studies evidently grew out of his early work with Sørensen on the potential of the quinhydrone electrode and its variation with the salt concentration of the medium. They served indeed to clarify the observed effects, but Linderstrøm-Lang in his discussion went far beyond this immediate problem and considered the data in terms of the sizes and hydrations of the ions involved, in the light of the theories of Debye and others.

In the same year (1924) he published a paper of fundamental importance, "On the Ionization of Proteins." In this, only a year after the appearance of the original papers of Debye and Hückel, he applied their theory of electrolyte solutions to polyvalent acids and ampholytes, and showed how the form of the titration curve should vary with the ionic strength, for solutions of spherical macromolecules which could be regarded as polyvalent acids, in which a set of acidic groups could all be taken as intrinsically equivalent, but subject to electrostatic interactions which varied with the ionic strength. This paper laid the foundation for a great number of studies on the titration curves of proteins which have been carried out over the last thirty-five years.

Two years later (1926) a paper appeared by Sørensen, Linderstrøm-Lang, and Lund in which these fundamental ideas were applied in detail to the experimental data obtained on egg albumin in Sørensen's laboratory, involving titrations in the pH range 4 to 5.2, over a wide range of ionic

strengths of ammonium chloride. It was shown that as the ionic strength is increased the titration curves become steeper—that is, the buffer capacity increases—due to the shielding of the electrostatic interactions between the groups in the macromolecule by the ions of the added electrolyte. In this paper also the important distinction between the isoelectric and the isoionic pH values of protein solutions was first formulated. The isoelectric point may be defined as the pH value at which the mean net charge of the protein, arising from *all* bound ions, including H^+ and OH^-, is zero. The isoionic point may be defined by considering a protein in its state of maximum positive charge Z^0 (in proton units) when all potential cationic groups in the molecule are charged, and then removing a mean number of protons ($\bar{h}$) per molecule equal to Z^0, by addition of hydroxyl ion. When this has been done, the protein is by definition isoionic. If the protein combines with no ions other than H^+ and OH^-, the isoelectric and isoionic points coincide; but if it combines with other ions as well, the two may be quite different. To determine the isoionic point one needs isoionic protein, which may be prepared by electrodialysis or (better) by treatment with a mixed bed ion exchange resin. Two experimental methods were proposed for determination of the iosionic point; according to one, it is the pH of the protein solution which does not change on the addition of more isoionic protein; according to the other, it is the pH of a solution of the isoionic protein in water, or in a solution of another solute which does not produce H^+ or OH^- ions when dissolved in water alone. These definitions are taken from the most recent discussion, which is given in a review by Linderstrøm-Lang and Nielsen (1959) to which we refer further below.

Lang perceived, apparently before anyone else, that the titration of a protein, or indeed of any polyelectrolyte, involved the formation of a statistical equilibrium between many individual ions of the polyelectrolyte. The mean net charge, at any point in the titration, represented the average value of this distribution, in which the individual macromolecular ions were constantly giving up protons to the medium at acidic sites, and taking them up again at the conjugate basic sites. He had clearly perceived the general nature of the situation in 1924, in his paper on the ionization of proteins; some fifteen years later he formulated an important consequence of this general concept—namely that the slope of the titration curve of a polyelectrolyte, i.e. the buffer value, was a direct measure of the standard deviation of net charge among the molecules. If $\bar{Z}$ is the mean net charge, and $\overline{Z^2}$ its mean square value, then the Linderstrøm-Lang equation may be written:

$$-0.434\,\frac{d\bar{Z}}{d\ \mathrm{pH}} = \frac{d\bar{Z}}{d\ \ln(\mathrm{H}^+)} = \overline{Z^2} - (\bar{Z})^2$$

Alternatively it may be written in terms of $\bar{h}$, the mean number of protons

removed from the most acidic form of the polyelectrolyte (which contains n acidic protons) by making use of the relation $\bar{Z} = n - \bar{h}$. The derivation is simple and rigorous.

I well remember the seminar in E. J. Cohn's laboratory at which Lang presented the derivation of this equation and its implications, during his stay at Harvard as Dunham lecturer in 1939. Dr. Cohn and I asked his permission to incorporate the equation in the book on "Proteins, Amino Acids and Peptides" which we were then writing. Actually it was in our book that the equation first appeared, in 1943, at a time when Denmark was occupied by the Nazis, Lang was active in the resistance movement, and all communication with America was cut off.

Lang's studies on acid-base equilibria served as the starting point for many later works—for example, on the experimental side, we may mention particularly the studies of R. K. Cannan and of C. Tanford and their collaborators; on the theoretical side the work of J. G. Kirkwood, and his generalization of the Debye-Hückel theory to apply to dipolar and multipolar ions, were of particular importance. Linderstrøm-Lang followed all these developments with close attention. In a much later paper (1953) "On the Activity Coefficient of Large Multipolar Ions," he extended the theoretical analysis to a number of cases not treated by Kirkwood, and in particular dealt with the very difficult mathematical problems presented by rod-shaped and ellipsoidal ions in media of varying ionic strength. Fortunately an admirable and comprehensive summary was given in the article on "Acid-Base Equilibria of Proteins" by Linderstrøm-Lang and S. O. Nielsen, published in the book "Electrophoresis" (1959) edited by Milan Bier. In this, which was perhaps his last major contribution to science, Lang gave a brilliant and searching survey of the whole field, with a theoretical discussion in terms of electrostatics and statistical mechanics containing many original thoughts and interpretations, and a penetrating evaluation of many experimental data.

In 1926–27 he worked for some months in the laboratory of Professor Willstätter in Munich. This period was of great importance in his life, since it marked the beginning of his intense concern with the chemistry and mode of action of enzymes, in particular the proteolytic enzymes. Two studies of trypsin and of its interaction with enterokinase, published in collaboration with E. Waldschmidt-Leitz, were the direct result of the work in Munich, but they represented only the beginning of the long series of studies on proteolytic enzymes which Linderstrøm-Lang continued for the next thirty years.

In 1927 he returned to the Carlsberg Laboratory and two years later completed his doctoral thesis, which dealt with purification and fractionation of casein. The work involved was published in a series of three papers (the

first in collaboration with S. Kodama) in the *Comptes rendus des travaux du laboratoire Carlsberg*, the last in 1929. It is perhaps difficult, especially for the younger protein chemists of the present day, to appreciate the uncertainty and confusion that existed thirty years ago when attempts were made to analyze the available data on such complex protein mixtures as casein. Electrophoretic analysis had scarcely begun to be employed, and ultracentrifugal analysis was a new tool, not yet extensively used. Some believed casein to be a pure protein; others held it to be a mixture. Most of the evidence was indecisive. Linderstrøm-Lang's studies clearly showed that casein was indeed a mixture of several different components, the separation of which proved to be very difficult. A number of widely different fractions were obtained and analyzed in various ways, and it was shown that their recombination in suitable proportions yielded a mixture virtually indistinguishable from the original casein. Although subsequent progress in this field has rendered these studies now largely of historical interest, they represented important progress at the time.

Shortly after this, in 1931–32, Lang went to the United States as a Rockefeller Research Fellow; at Pasadena he studied general biology with Thomas Hunt Morgan, who made a powerful impression on him, and worked in chemistry and biochemistry with Linus Pauling and Henry Borsook. This experience in California was an important event in his life, and established close relations between him and many American friends, which were to continue and grow in the years to come.

Just before this time, in 1930, Dr. Heinz Holter came to the Carlsberg Laboratory and, in a lasting collaboration of major importance, over approximately a decade, Linderstrøm-Lang and Holter developed an astonishingly wide array of sensitive and precise ultramicro methods for the study of the distribution of a great variety of enzymes and other constituents of cells and tissues. These methods have been widely adopted throughout the world in laboratories concerned with the problems of histochemistry, and their influence has been enormous. Since we are here concerned primarily with Linderstrøm-Lang's contributions to the chemistry of proteins we pass over these beautiful and important studies with this brief mention, referring only to his Harvey Lecture (1938–39) on "Distribution of Enzymes in Tissue and Cells." This furnishes an admirable brief survey of these extensive results, written toward the end of this particular period in his career.

We may note, however, 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; and the converse is also true. The fusion of these two very different types

of ability in the same individual was indeed extraordinary. We may note, however, that in the development of techniques Linderstrøm-Lang displayed his outstanding skill in applying simple but basic physical principles for practical analytical use. The Cartesian diver technique for the measurement of metabolism in single microsections of tissue, or of individual cells, is one outstanding example. Another method, which he used brilliantly for other purposes later on, involved the use of density gradient columns for the very precise determination of the density of minute quantities of material.

These important histochemical studies did not deflect him from his fundamental concern with problems of protein structure and function. One paper which remains of outstanding interest in this respect is that by Linderstrøm-Lang and C. F. Jacobsen (1940) on "The Properties of 2-Methylthiazoline and their Relation to the Protein Problem." This simple compound served in some respects as a model for the "masked" sulfhydryl groups of proteins, for it contained no titratable sulfhydryl group and yet could readily undergo hydrolysis under suitable conditions to form *N*-acetyl-β-mercaptoethylamine, whereas under other conditions hydrolysis at a different point of the thiazoline ring led to the isomeric *S*-acetyl derivative. Under certain conditions the reactions were reversible, and their rates were found to be influenced by urea and by guanidine hydrochloride. 2-Methylthiazoline was also found to undergo a most unusual reaction with ammonia in which the nitrogen of the latter could apparently be incorporated into the thiazoline. The analogies between the reactions of 2-methylthiazoline and those found in proteins, in which sulfhydryl groups "appear" on denaturation and "disappear" on its reversal, are striking. This work has stimulated much further research, including a number of studies which are proceeding at the present time, and although current interpretations of the data tend to differ in some respects from those suggested by Linderstrøm-Lang and Jacobsen, the interest and importance of their work remains undiminished.

In 1941, also with Jacobsen, Linderstrøm-Lang initiated a series of studies of major importance on the volume changes accompanying the enzymatic breakdown of proteins. The hydrolysis of a peptide linkage, with the resultant formation of a negatively charged carboxyl and a positively charged ammonium group, was already known to be accompanied by a contraction of the order of 20 cc per mole of linkage hydrolyzed, due to the electrostriction of the solvent by the charged groups. With a very delicate and precise dilatometric method it was possible to follow the volume change in a protein solution during enzymatic hydrolysis, as a function of the number of peptide bonds hydrolyzed. It was observed, notably in the hydrolysis of β-lactoglobulin by trypsin or chymotrypsin, that the hydrolysis of the

first bonds attacked by the enzyme led to a very much greater contraction—sometimes as great as 100 cc per mole of bonds broken—than that accompanying the later stages of hydrolysis. This total "extra" contraction, in the case of β-lactoglobulin, was most marked at low temperature, near 0°, where it was of the order of 700 cc per mole, beyond what would have been expected from the simple electrostriction effect; it was due almost entirely to processes occurring in the earliest stages of the action of the proteolytic enzyme. It appeared clear, from this and from other work, that these initial processes must involve some far-reaching rearrangement in the structure of the protein molecule. Associated with this was the previously known fact, which the researches in the Carlsberg Laboratory confirmed and greatly extended, that many proteins in their native state were resistant to the action of certain proteolytic enzymes but became readily susceptible to attack after denaturation. Linderstrøm-Lang formulated the possible rearrangements in a scheme which in its simplest form could be written:

$$\text{Native Protein} \rightleftharpoons \text{Denatured Protein} \xrightarrow{\text{Enzyme}} \text{Split Products}$$

Thus, if the native and denatured protein are in a reversible equilibrium, the attack of the enzyme on the latter will constantly shift the equilibrium in the direction of the denatured protein and the hydrolysis will proceed. The possibility was also considered that the enzyme can act as a "denaturase," directly catalyzing the conversion of the native to the denatured form. Another possibility is that a few peptide bonds, of critical importance, are first attacked, the protein molecule then unfolding and becoming accessible to further hydrolysis. More elaborate schemes were considered by Linderstrøm-Lang and presented in 1949 at the Cold Spring Harbor Symposium on Quantitative Biology (published in 1950). Later at the Ninth Solvay Congress in 1953 he presented a comprehensive analysis of a number of possible reaction sequences that could be involved in the attack of proteolytic enzymes on their substrates, and worked out many of the consequences mathematically in great detail, while at the same time pointing out the profound incompleteness of our present knowledge of the subject.

A major consequence of the studies of the formation of modified proteins by the action of enzymes was the observation of the formation of plakalbumin from ovalbumin, first reported in detail by Linderstrøm-Lang and M. Ottesen in 1949. This arose from the chance observation that certain preparations of ovalbumin, which proved to have been contaminated with bacteria, crystallized in plates rather than in the usual characteristic small needles. It was apparent from the first reported studies that a small peptide is split off from ovalbumin in the conversion to plakalbumin.

The principal enzyme involved in the transformations originally observed was found to arise from *Bacillus subtilis*; it was later obtained in crystalline form at the Carlsberg Laboratory and named subtilisin. Studies with E. Fredericq showed that the solubility of plakalbumin in concentrated salt solutions was consistently higher than that of ovalbumin, while osmotic pressure measurements with Güntelberg showed that the molecular weights of the two proteins were very nearly indistinguishable. Later studies by Ottesen, by C. A. Villee, by D. Steinberg and others, led to further clarification of the processes involved. The most recent paper is that by Ottesen (1958) who used pure subtilisin to effect the transformation and showed that the principal peptide released possessed the structure Glu·Ala·Gly·Val·Asp·Ala·Ala. The smaller peptides Ala·Ala and Glu·Ala·Gly·Val·Asp, obviously derived from the same sequence, were also obtained. By working at pH 8 it was shown that the reaction occurs in two steps, the first involving the opening of a single peptide bond in the ovalbumin molecule, without release of any amino acid residues.

In his last major sequence of researches, Linderstrøm-Lang was concerned with the general aspects of protein stability and structure. Already in his Lane Medical Lectures on "Proteins and Enzymes" (1952) at Stanford University he had formulated the general concepts of protein structure on three levels—the primary structure, representing the sequence of amino acid residues in the peptide chains; the secondary structure, representing repeating patterns of order in the spatial arrangement of the residues of the peptide chains, stabilized in general by hydrogen bonds; and the tertiary structure, involving the more individual folds and twists, characteristic of the structure of a particular protein in its native state, partially imposed by cross-linkages such as disulfide bonds, and presumably stabilized by interactions between the side chains of the amino acid residues. This terminology has been widely adopted by protein chemists and others, for it gave explicit expression to ideas of which many investigators had been vaguely aware, but had not succeeded in formulating.

In his Lane Medical Lectures, also, Lang gave a thoughtful statement of the problems of protein biosynthesis, including the factors determining the free energy of formation of peptide bonds, the need for "high energy" intermediates to furnish the free energy necessary to make the process go, with even some suggestions concerning the possible importance of amino acids in protein biosynthesis, inspired in large part by some very interesting work which Chantrenne had carried out in the Carlsberg Laboratory in 1948. This discussion by Lang just preceded the great outburst of activity in this field, which is now in progress, and shows remarkable insight into the directions of research which were to become most significant.

Linderstrøm-Lang's last series of researches was concerned with the study

of the factors stabilizing the secondary and tertiary structures of proteins by observation of rates of hydrogen and deuterium exchange between proteins and the surrounding aqueous medium. The principles of the method were simple, and the techniques showed his usual skill. The method was described in detail in papers with I. M. Krause, A. Hvidt and others (1954 and 1955). It was readily shown that in solutions of simple peptides all the hydrogens bound to oxygen and nitrogen were readily exchanged. In insulin, the first protein to which the method was applied, the potentially exchangeable hydrogens were found to fall into several groups. Approximately two-thirds of the 90 exchangeable hydrogen atoms were exchanged almost instantaneously at any temperature and in all solvents studied. The remaining hydrogens could be classified in three groups with different rates of exchange, the most resistant group of 8 hydrogens showing practically no exchange at room temperature or below, but a slow exchange at 38°. Addition of urea to the solvent greatly increased the exchange rates, as would be expected from the probable mechanism of its denaturing action on proteins. On the other hand, the hydrogens in Sanger's A-chain of oxidized insulin showed practically instantaneous exchange, like simple peptides.

Similar studies were carried out on ribonuclease and on β-lactoglobulin, and again revealed the presence of a number of potentially exchangeable hydrogens which nevertheless exchanged only at a very slow rate, or indeed practically not at all, at low temperatures in water solution. Rise of temperature, or addition of high concentrations of urea, increased the exchange rates very greatly for many of these hydrogen atoms, but especially in β-lactoglobulin it was noted that there is a very resistant "core" of atoms which scarcely exchange at all, even under conditions which would favor a considerable unfolding of the structure of the native molecule.

A study with A. Berger (1957) of poly-DL-alanine, which exists as an α-helix in aqueous solution, revealed that even in this simple molecule a substantial fraction of the peptide hydrogens exchanged only very slowly, the rate being sensitive to temperature and pH. This was in striking contrast to most of the straight chain peptides studied, which consisted only of L-residues, and in which generally all of the peptide hydrogens exchanged rapidly. (An exception was the B-chain of insulin, studied by Leach and Scheraga, 1958; but here it seemed probable that the formation of aggregates inhibited the exchange). It was suggested that the D- and L-alanyl residues, more or less randomly distributed along the chain, would protrude in different directions from the core of the helix, a considerable fraction of them forming close pairs over the gap between two turns of the helix. Hence in this case the stabilization may be considered as arising from the formation of a hydrophobic bond between the methyl groups.

These studies were of course only a part of the broader study of protein structure and its relation to enzyme activity in which the Carlsberg Laboratory maintained its leading place among the laboratories of the world. By his extraordinary scientific gifts and his inspiring personal qualities, Linderstrøm-Lang attracted to his laboratory a brilliant group of investigators, not only from Denmark but from many other countries. Thus a large number of investigations from the laboratory, to which his name is not attached, nevertheless reflect the influence of his thinking and his power to create an atmosphere in the laboratory which inspired others to original achievements. The study of C. F. Jacobsen (1947) on the activation of chymotrypsinogen represented one of the most thorough studies ever carried out on the conversion of a zymogen into a closely related group of active enzymes; two of the latter were identified in this work for the first time. The intensive study of L. Korsgaard Christensen (1953) on the denaturation of β-lactoglobulin, with observations on ovalbumin, was notable, among other things, for its use of the change in optical rotation which occurred on denaturation as a criterion of the unfolding of the native protein structure. Since then the Carlsberg Laboratory indeed has played a major rôle in optical rotation studies as related to protein structure. In 1955, J. A. Schellman produced the first systematic theory of the factors determining the relative stability of helical structures and of open random coils in peptide solutions. During this period and since, a whole series of important studies on the structure and physical properties of ribonuclease, in relation to its enzyme activity, emanated from the Carlsberg Laboratory. Much of the general outlook that emerged from all this and other work, which we pass over here for lack of space, is formulated in a comprehensive chapter on "Protein Structure and Enzyme Activity" by Linderstrøm-Lang and Schellman in Vol. I of "The Enzymes" (1959), edited by Boyer, Lardy, and Myrbäck.

The numerous honors which Linderstrøm-Lang received represented only the natural recognition that came to so outstanding a scientific leader. He was a foreign member of the Royal Society of London, the National Academy of Sciences of the U.S.A., the Academy of Sciences of the U.S.S.R., the Royal Swedish Academy of Sciences, the American Philosophical Society, the American Academy of Arts and Sciences, and of other academies and learned societies. At the time of the First International Congress of Biochemistry in 1949 he received the honorary degree of Doctor of Science from the University of Cambridge, and numerous other similar marks of recognition. He was President of the Danish Academy of Technical Sciences (1956) and of the International Union of Biochemistry (1958).

All these and other honors were external tokens of the special regard in which he was held. The breadth and power of his intellect, his skill in

devising experiments, his ardent devotion to his work, made him outstanding. Yet the enumeration of such qualities gives a totally inadequate idea of the impression he produced. His range of talents was immense. He was an accomplished painter in his earlier days, a gifted writer, a musician, a delightful conversationalist and raconteur, who was a center of attraction in almost any social group; one felt that, if he had not chosen to devote his energies primarily to science, he might have become outstanding in one of the arts. In science he could pass, with apparent lightness and ease, back and forth between detailed and minute experimental procedures and abstract mathematical theorems. There was a strong element of gaiety and fun in his approach to science, and to life in general; I remember well how he and Mrs. Lang welcomed me to Copenhagen in 1949 when I came to stay with them for a week and to give a lecture. After a pleasant informal supper at home they took me at once to the park at Tivoli, where we were all sent along roller coasters, lost in mirrored mazes, bounced about in cars in random motion, so that altogether I was given a most lively introduction to Copenhagen.

The gaiety was indeed an indispensable element of his personality; it was one important aspect, but only one, of a many-sided, sensitive and courageous character, with a broad range of human sympathy and understanding. It went with an arduous self-discipline, a steadfast devotion to fundamental purposes, and a thoughtfulness that went far beyond science and embraced a deep concern for the problems of mankind at large. Moreover his outgoing and generous nature served to encourage and inspire those of lesser talents than himself, and to bring out the best of which they were capable. In his capacity to arouse admiration and affection among his colleagues, and to inspire them to further scientific advances, he held a special place among us and no one can replace him.

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