VOLUME 1

Chemistry of the Amino Acids

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To Our Wives and Children

Preface

Few products of natural origin are as versatile in their behavior and properties as are the amino acids, and few have such a variety of biological duties to perform. They are at once: water-soluble and amphoteric electrolytes, with the ability to form acid salts and basic salts and thus act as buffers over at least two ranges of *p*H; dipolar ions of high electric moment with a considerable capacity to increase the dielectric constant of the medium in which they are dissolved; compounds with reactive groups capable of a wide range of chemical alterations leading readily to a great variety of degradation, synthetic, and transformation products, such as esters, amides, amines, anhydrides, polymers, polypeptides, diketopiperazines, hydroxy acids, halogenated acids, keto acids, acylated acids, mercaptans, shorter- or longer-chained acids, and pyrrolidine and piperidine ring forms; indispensable components of the diet of all animals including man; participants in crucial, metabolic reactions on which life depends and substrates for a variety of specific enzymes *in vitro;* binders of metals of many kinds; absorbers of ultraviolet and infrared radiation within specific ranges of wavelength; possessors with one exception of optical rotatory power related to the configuration of asymmetric centers; and essential constituents of protein molecules whose biological and chemical specificities are determined in part by the number, distribution, and spatial interrelations of the amino acids of which they are composed. They reveal at once uniformity and diversity; uniformity, in that with rare exceptions they are α-amino acids with all the physical consequences which flow from this fact, and, for those that are constituents of proteins and hence of living tissues, the same optical configuration at the α-carbon atom is common to all; diversity, because each possesses a different side chain which confers upon it unique properties distinguishing it physically, chemically, and biologically from the others. In this duality, the array of the amino acids is a partial reflection of the larger biological world which is always the same and always different.

Let it be admitted that we have been entranced by the spectacle of these many and diverse phenomena. In attempting to transfer these pleasurable emotions to paper, and to portray accurately the work of so many able investigators, we were well aware of the magnitude and difficulty of the task which we set for ourselves, and of the impossibility of perfection. With filial piety and in justice to those who have built this field, as well as to convey a sense of perspective, we have stressed where we could the historical development of each subject. The literature of science is generally curt and colorless, except when enlivened at times by the statement of controversial opinion; on occasion an expression of notable and graceful generosity or of far-seeing imagination finds its way into this literature, and such examples have been quoted where pertinent in the pages to follow. The topics covered in this work have generally been those wherein a certain stability has been attained and are thereby suitable for comprehensive treatment—not that progress has ceased in such topics, but rather that nothing has so far emerged to render any substantial portion of them obsolete or questionable.

Emphasis has been placed on the organic chemistry and the physical chemistry of the amino acids, and on the role these compounds play in the nutrition of a variety of living forms. The pertinent literature has been covered to the close of 1958. The design of the treatise embraces both the intellectual development of the subject and its corollary, experiment; and a large number of illustrative experimental procedures, many of them either tested or developed in our own Laboratory, have been interpolated in the text. The subject of the metabolism of individual amino acids has been omitted because of its brilliant coverage by our former colleague, Dr. Alton Meister, in his recent book on the Biochemistry of the Amino Acids.

Volumes 1 and 2 have been designed to embrace the study of the amino acids as a class of chemical compounds, including aspects more or less general to these compounds. Volume 3, on the other hand, takes into consideration the history, reactions, syntheses, physical characteristics, and optical resolution of each individual amino acid. Only the α-amino acids and their derivatives have been treated in detail in these volumes. A comparable treatment of the ω-amino acids would require more space than appears desirable at this time.

We have read thousands of papers in preparation for this treatise, and despite our best efforts it is likely that some reports equal in significance to those cited may have inadvertently eluded our notice. Much of the modern Russian scientific literature is still accessible with difficulty, and it is not improbable that some areas of value in this literature have also been overlooked. On the other hand, it has been deemed necessary to omit consideration of many reports of doubtful interest. It requires skill and constant

vigilance to keep afloat in the flood of contemporary scientific reports, and although we can lay claim to little of the former quality and only to a modicum of the latter, we take a certain assurance in having rarely referred to any work without the original before us at the moment.

In drawing on the accumulated experience of several years in the writing of this treatise, it is only proper to express our appreciation to our colleagues, past and present, who have labored with us and contributed so much to the various programs of the Laboratory, namely, Drs. Shiro Akabori, Carl G. Baker, Leo Benoiton, Louis Berlinguet, Sanford M. Birnbaum (to whom we are additionally indebted for help on various phases of this treatise), Leah Bloch-Frankenthal, Charles E. Carter, Harold W. Chalkley, Jerome Cornfield, Mariano de Mingo, Jean P. DuRuisseau, Irene Z. Eiger, Maurice Errera, Paul J. Fodor, William S. Fones, Shoucheng J. Fu, James B. Gilbert, Jose M. Gonçalves, Robert E. Greenfield, Piero Gullino, Douglas Hamer, Nobuo Izumiya, Gerson Kegeles, Robert J. Koegel (to whom we are additionally indebted for the infrared spectra given in volume 3), Guy Letellier, Leon Levintow, Robin Marshall, Alton Meister, Kimiyo Michi, Vithal B. Mitbander, Toru Miyaji, Thomas Moore, Jo Nordmann, Einosuke Ohmura, Theodore Otani, M. Clyde Otey, W. K. Paik (to whom we are additionally indebted for the translation of a large number of papers from the Japanese chemical literature), Jekishan R. Parikh, Elbert A. Peterson, Vincent E. Price, Krishnarau R. Rao, Miloslav Rechcigl, Donald S. Robinson, Irene Rosenfeld, Joseph Shack, Herbert A. Sober, Matthys Staehelin, Gerassimos Stelakatos, Takashi Sugimura, Roy Wade, Ellinor Weiss, Elizabeth Work, and Leonidas Zervas. It is also a pleasure to thank our administrative assistant, Mrs. Betty Ann Mitchell, and her staff (especially Mrs. Anna Elizabeth Stotler), for their devotion and skill in handling the numerous and varied duties involved in the office of the Laboratory. To the Director, and Associate Director, of the National Cancer Institute, Drs. J. R. Heller, Jr., and G. B. Mider, respectively, we are especially grateful for establishing a milieu conducive to scientific effort. To Dr. Hubert B. Vickery of the Connecticut Agricultural Experiment Station, who generously devoted a considerable amount of his time to reading these volumes in their entirety, and whose constructive criticism and invaluable suggestions contributed greatly to the readability, accuracy, and proper historical perspective of their contents, we express our heartfelt gratitude and warmest thanks.

In venturing upon a pleasant but thorny task, we have been consoled by the unknown author of the Second Maccabees, whose delightful description of his mutual problems and aspirations may well close *this* long prologue:

> For considering the multitude of books . . . and the multitude of the matter, we have taken care for those indeed that are willing to read, that it might be a

pleasure of mind; and for the studious that they may more easily commit to memory; and that all that read may receive profit. As to ourselves indeed, in undertaking this work . . . we have in hand no easy task . . . but as they that prepare a feast, and seek to satisfy the will of others, for the sake of many we willingly undergo the labour. For as the master builder of a new house must have care of the whole building, but he that taketh care to paint it, must seek out fit things for the adorning of it, so must it be judged for us. To stand upon every point . . . and to be curious in particulars, belongeth to the first author of the story. But to use brevity, and avoid much labouring of the work, is to be granted to him that will make an abridgement. . . . If we have done well, and as is fitting the story, it is that which we desired, but if slenderly and meanly, it is that which we could attain unto. For as it is hurtful to drink always wine or always water, but pleasant to use sometimes the one, and sometimes the other, so if the speech be *always* nicely framed, it will not be grateful to the readers. Here then we will begin the narration. Let this be enough of a preface, for it is a foolish thing to make a long prologue, and to be short in the story itself.

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In Memoriam *

The scientific community lost one of its most gifted investigators when, during the early morning hours of February 12, 1959, Dr. Jesse P. Greenstein suddenly succumbed to a massive cerebral hemorrhage suffered two days earlier. Thus was terminated the career of one of the century's most brilliant and colorful chemists, whose many contributions enriched the field of biochemistry and whose extraordinary productivity continued unabated until his death. Over the past two decades, Dr. Greenstein's Laboratory at the National Cancer Institute had become an outstanding center of research on amino acids and peptides, on nutrition, and on cancer. His numerous comparative studies on the enzyme patterns of normal and neoplastic tissues were among the first to bring modern biochemistry to bear on the cancer problem, and his masterful synthesis of the then current knowledge in the cancer field, published as his monograph, *Biochemistry of Cancer,* in 1947 (and extensively revised in 1954), constituted a milestone in cancer research. His equally numerous investigations on amino acids, peptides, and nucleoproteins enhanced our knowledge of biochemistry, nutrition, enzymology, and organic and physical chemistry. Particularly noteworthy was his development of general enzymic methods for the resolution of amino acids, which permitted ready access to the optically pure isomers of these substances on the large scale, and which will undoubtedly lead to a greatly increased use of these formerly rare materials in investigations of animal and human nutrition; the importance of these optically pure amino acids is evidenced by the virtually staggering number of requests for samples received from scores of investigators

* Taken in the main from a biographical sketch of Jesse P. Greenstein which was written in collaboration with Drs. C. G. Baker, S. M. Birnbaum, R. E. Greenfield, V. E. Price, and H. A. Sober (*The Jesse P. Greenstein Memorial Issue, J. Natl. Cancer Inst.,* **24,** vii (1960)).

throughout the world—requests that were honored by Dr. Greenstein in his characteristically generous fashion. The indefatigable energy and selfless devotion with which he pursued his scientific researches, and the high quality and permanent value of his results, have earned him the lasting respect and admiration of all who knew him.

Probably the most characteristic traits of Jesse Greenstein were his deep respect for the individual and his intense devotion and dedication to his chosen field. His mind was a massive vault filled with scientific knowledge, yet he was humble when he viewed the living miracles of the world about him while possessing a never-ending curiosity to unlock the mystery of their elusively concealed secrets. To his contemporaries he was a living dynamo, primed with a source of energy that permitted him to work at a vigorous pace for sixteen to eighteen hours a day, seven days a week, and the only waste product of which was the smoke which emerged from a seemingly endless chain of cigars. This boundless energy, coupled with a firm yet understanding and jovial nature, served as a continual source of inspiration to his younger scientific associates, whose eager compulsion to follow his lead from example often led them to scientific achievements of which they had believed themselves incapable. Of his scientific findings he imparted freely, in a written style that was elegant in its simplicity. Either alone or in collaboration with his scientific colleagues, he published some 300 original papers and his now classical book on *Biochemistry of Cancer*. Yet, despite this prodigious output of work, he was modest and unassuming about his own accomplishments while displaying an overt pride in the achievements of his younger collaborators, in whom he took a sincere personal interest, on whom he would freely lavish praise, and to whom he served as a leader in the finest sense. And despite his vigorous and rigorous scientific activity, he was invariably friendly and good humored and was always certain to give a warm and generous welcome to the visitors who frequented his Laboratory in a never-ending stream. His door was always open, too, to a constant flow of scientific associates who sought his counsel and advice, and those who were privileged to work closely with him remember the many pleasant conversations often held in the early morning hours, conducted while he was actively performing an organic synthesis or inducing the crystallization of a new compound, while a reaction mixture or two were refluxing in the background. Because of his personal characteristics—his high moral courage, his great knowledge of and insight into science, his insistence on excellence, his patient good humor—and because of his extraordinary scientific accomplishments, he attracted to his Laboratory a stream of capable young scientists—from England, France, Japan, Germany, Canada, Israel, China, Korea, Greece, Italy, Belgium, Switzerland, India, and Brazil—eager to

follow his lead. And indeed, it is the scientific legacy he left to these young scientists—in whom he imbued his zeal, his enthusiasm for science, his high standards, and his penchant for hard work—that may be ranked among the greatest of his contributions. The present treatise, which was begun in 1955 and very nearly complete at the time of his death, represents his last major writing effort.

The hiatus created by the death of an individual such as Jesse P. Greenstein can never be adequately filled, and for this we are much the poorer, but for the very reason that he was with us, we have been left a good deal richer and perhaps a little bit wiser.

MILTON WINITZ

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