Hans T. Clarke

PREFATORY CHAPTER

IMPRESSIONS OF AN ORGANIC CHEMIST IN BIOCHEMISTRY

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When during the last century the early gropings were made towards the clarification of biochemical problems, these were mainly carried out by investigators whose prime interest lay in the rapidly expanding areas of organic chemistry. Their orientation was in many instances influenced by training in medicine, in which the need for wider knowledge of the processes underlying physiological and pathological phenomena had become obvious to them. As in every other branch of science, the initial phases necessarily consisted in observation and description. Early impetus to investigations of this character was given by Mulder, Chevreul, Liebig, Wöhler and Pflüger, but in the rudimentary state of organic chemical knowledge then existing, such stimulation was necessarily more general than specific in direction.

For many years progress towards biochemistry was slow, in spite of the autocatalytic development of organic chemistry, for the ramification of that discipline developed more along the lines of synthetic reactions than of the study of compounds of biological origin. A decisive change, however, was initiated towards the end of the last century by the masterly researches of Emil Fischer on the chemical structure of purines and sugars. These set the pattern for the next approach to fundamental biochemistry. Fischer's ensuing attack on the chemistry of proteins, skillful though it was, proved less fruitful than arduous. The procedure which he developed for the determination of the component amino acids of proteins yielded only approximately quantitative values and required many months of intensive labor for the analysis of a single protein; his synthetic approach, based on recognition of the peptide linkage of the components, yielded polypeptides which bore little physical resemblance to proteins.

This was the situation around 1907, when, as a prospective organic chemist studying in London under Sir William Ramsay and J. Norman Collie and taking physiology as a minor subject under E. H. Starling, I was first exposed to physiological chemistry. The instruction in this subject, which had not yet attained the status of a discipline, was given by R. H. Aders Plimmer. It included the isolation of a few crystalline proteins, carbohydrates, and amino acids; the preparation of lecithin and kephalin; and the estimation of some urinary components by procedures which had recently been developed by Folin. These exercises did not greatly appeal to me; I found them intellec-

tually far less rewarding than the rich fare offered by Collie and Smiles in their courses on organic chemistry.

In the years 1911 to 1913, I had the privilege of working in Emil Fischer's laboratory in Berlin as a research guest supported by an "1851 Exhibition" scholarship from London. According to rumor, Fischer had once taken into his group a Briton who had not worked assiduously, and he had consequently decided never again to accept a "lazy Englishman" as a collaborator. Although I worked on problems of my own, Fischer visited me almost daily in the laboratory and discussed my experiments, and many other subjects, with the greatest friendliness. It was also my good fortune there to associate with several men who later became intimate friends, particularly Roger Adams, Max Bergmann, Harold W. Dudley, and Karl Freudenberg, as well as my cousin B. Helferich, who was then Fischer's private assistant.

On my arrival in Berlin, Helferich advised me, much to my surprise, not to ask the other members of the laboratory what they were doing. This was so contrary to British tradition that I was interested to find out the reason; it appeared that most of the chemists who were working on topics of their own were retained as consultants by one or another of the German chemical manufacturing firms, which had priority on any patentable discoveries made by the individuals concerned. This system appeared to me, as it still does, as being at variance with the prime function of an academic laboratory.

After three profitable and enjoyable semesters in Berlin I spent a summer at Queen's University, Belfast, in
Stewart, and then returned to University College, London. In the early summer of 1914 I went to Rochester, New York, at the behest of George Eastman of Eastman Kodak Company, for which concern my father was in charge of technical developments in Europe. As the company at that time had no organic chemist on its staff, Mr. Eastman had occasionally sought my advice on organic chemical matters. While I was in Rochester, C.E.K. Mees, the director of the recently established research laboratory of Kodak, offered me a full-time appointment in a new section for organic chemical research and this I accepted. Owing to the outbreak of the first World War, the importation of German chemicals had been cut off, and my first tasks were to work out processes for the manufacture of photographic developing agents, and then of the laboratory preparation of sensitizers. In this last-named effort I was, after a few years, joined by L. G. S. Brooker, who has since carried forward the work independently with masterly skill.

In the summer of 1918, Mees charged me with the organization of a laboratory for the preparation of research organic chemicals to meet the urgent needs of universities, whose stocks had become depleted owing to the impossibility of securing supplies from Germany. As at that time almost all of the relatively few American-trained organic chemists were actively employed in government service, the laboratory was staffed by young women, all

recent college graduates who had majored in chemistry. These girls displayed immense enthusiasm, cooperativeness, and application, but in general were not well adapted to preparative work on a large laboratory scale; accidents were alarmingly frequent and it proved impossible to assign more than two preparations to each girl for simultaneous operation. After the first year, therefore, replacements and additions to the group were made with men. Among these was a youngster named Warren Sperry, who some ten years later again became associated with me at the Columbia-Presbyterian Medical Centre. During the past thirty years the work of the laboratory of Synthetic Organic Chemistry in Eastman Kodak Company has been directed by W. W. Hartman under whose supervision an impressive list of available organic compounds has been built up.

Around 1926 I undertook an additional assignment for the initiation of a laboratory for the exploitation of cellulose esters for photographic film. The experimental work in this field was carried out from the outset, and has subsequently been directed with great acumen, by C. J. Malm.

In these various laboratories the staffs were from time to time reinforced by young academic organic chemists who joined on a temporary basis. Of these I recall with special pleasure and admiration W. E. Bachmann and C. R. Noller, both of whom subsequently made notable contributions to organic chemistry.

In 1928, on the suggestion of H. D. Dakin, I was invited by Columbia University to direct the Department of Biological Chemistry in the College of Physicians and Surgeons. The Faculty of Medicine, in view of the trend in biochemistry, recognized the desirability of departure from the classical approach of physiological chemistry to the problems of medicine. I gladly accepted the challenge and have been happy ever since, in spite of a keen and constant recognition of my inadequacies in the field.

When I entered Columbia, the medical school had just moved to its present location on West 168 Street. The departmental laboratory, though new, was not well equipped with modern facilities; fortunately, a liberal grant from the Chemical Foundation made possible the supplementation of laboratory installations and the purchase of a truly adequate series of chemical journals for the general library. A highly satisfactory feature of the available departmental space was a large laboratory for graduate instruction. It has always seemed to me important that graduate students be located in close contiguity, for they can learn more from one another than from their formal instructors. For the same reason I have continually encouraged the greatest possible diversification in the subject matter of departmental researches.

On arriving in New York I found, on the biochemical staff, colleagues who proved to be towers of strength in the instruction of medical students: Edgar G. Miller, Jr., an incomparable lecturer; G. L. Foster, indefatigable

in the teaching laboratory; and Maxwell Karshan, who throughout the years has taken charge of the course for dental students. This group was soon joined by Crawford Failey, a physical chemist interested in biochemical problems, and Oskar Wintersteiner, a former student of Pregl, who on coming to the United States had collaborated first with P. A. Levene and then with J. J. Abel. The department also had the privilege of close association with Michael Heidelberger who was located in the Department of Medicine; he was then working on the chemistry of bacterial polysaccharides and subsequently developed his fruitful theories on the quantitative relationships in immunochemical reactions.

In the early thirties the junior staff was joined by Robert M. Herbst and Marianne Goettsch. Herbst, who had recently graduated from Yale, was primarily an organic chemist; after some years with us he left to go into industrial research but soon returned to academic life. Miss Goettsch, on the other hand, was an expert in nutritional biochemistry; after completing her formal training with us she ably represented her specialty in the instruction of our medical students. In 1942 she transferred to the Department of Biochemistry of the School of Tropical Medicine in San Juan, then affiliated with Columbia University, where she is now a professor in the University of Puerto Rico. Subsequent additions to our teaching staff have largely been recruited from the roll of graduates of Columbia University; these include David Rittenberg, now the head of the department, David Shemin, David Sprinson, and Stephen Zamenhof.

Among the many benefits which accrued to Columbia University from the racial policy adopted by the Germans under the Third Reich was the arrival in our laboratory of various European-trained biochemists, notably Erwin Chargaff, Zacharias Dische, Karl Meyer, Rudolf Schoenheimer, and Heinrich Waelsch. Erwin Brand, who joined our group during the same period, reached this country somewhat earlier. The scientific achievements subsequently made by these men are so well known that their enumeration is unnecessary.

Visiting biochemists or organic chemists who spent a year or more in the laboratory and thereby contributed to the stimulation and informal training of our students included Sune Bergström, R. J. Block, W. J. Darby, R. Gordon Gould, Max Huffman, R. C. Lewis, Gwei Djen Lu, M. S. Newman, E. S. West, W. W. Westerfeld, and R. R. Williams. Among others, some who worked in different departments but held academic titles in ours and took part in the instruction of medical or graduate students were L. S. Dietrich, Samuel Graff, Seymour Lieberman, Irving London, David Nachmansohn, Warren Sperry, and Irwin B. Wilson.

The circumstances under which Schoenheimer's most notable associate came into biochemistry may be of interest. When Harold Urey had prepared a sufficient quantity of heavy water to release some of it for work in labora-

tories other than his own, he secured from the Rockefeller Foundation funds for its exploitation in biological fields, and our department was one of the beneficiaries. With a supply of deuterium hydroxide came the opportunity to take in one of his assistants versed in the appropriate techniques. When David Rittenberg joined the laboratory in that capacity he was invited to circulate among the members of the group with a view to establishing a profitable collaboration. From his discussions with Schoenheimer came the idea of tracing the metabolic fate of a fat labeled with heavy hydrogen. The result of the very first experiment, which indicated that ingested fats were immediately deposited unchanged in the tissues, gave all of us an unforgettable thrill. It was not long before the novel concept of the dynamic state of body constituents had developed from the ensuing studies, and this was soon confirmed, in the area of protein metabolism, by experiments in which heavy nitrogen was employed as a tracer. The preparation of the labeled compounds, in which isotopic hydrogen and nitrogen were the limiting factors, involved many technical considerations unnecessary in the practice of classical organic chemistry.

The gratification afforded to the head of a department through the researches of his associates, great as it is, is equalled and perhaps surpassed by that derived from the success of his students. In both respects I have much cause for thankfulness. Of the 94 students who received their doctoral degrees in biochemistry from Columbia University during the period 1931 to 1956, many have made their mark as investigators and several are departmental chairmen in other universities. I cannot forgo the satisfaction of listing the names of those who have attained the national recognition implicit in election to the American Society of Biological Chemists or one of the other organizations which constitute the Federation of American Societies for Experimental Biology. These are Anthony A. Albanese (1940), Marjorie Anchel (1939), Herbert S. Anker (1945), Fred W. Barnes (1944), Aaron Bendich (1946), Konrad Bloch (1939), Ernest Borek (1939), George E. Boxer (1945), Seymour S. Cohen (1941), Thomas B. Coolidge (1938), James D. Dutcher (1940), David Elson (1952), Lewis L. Engel (1936), Bernard F. Erlanger (1951), Earl A. Evans (1937), Martin Flavin (1951), Joseph S. Fruton (1934), Marianne Goettsch (1931), Samuel Gurin (1934), C. H. Werner Hirs (1949), Henry D. Hoberman (1943), Elvin A. Kabat (1938), Alfred Linker (1954), Boris Magasanik (1948), Paul H. Maurer (1950), Manfred M. Mayer (1946), Abraham Mazur (1939), John W. Palmer (1933), William H. Pearlman (1940), Sarah Ratner (1937), Marianna Richards (1938; now Mrs. Max Bovarnick), Richard W. Schayer (1949), David Shemin (1939), David B. Sprinson (1946), William H. Stein (1939); DeWitt Stetten (1940), Marjorie R. Stetten (1945), Herbert E. Stokinger (1938), Gilbert C. H. Stone (1933), Paul K. Stumpf (1946), Norman Weissman (1941), and Stephen Zamenhof (1950). The figures in parentheses indi-

cate the official years of graduation. In addition to the above, two of our graduates, not enrolled in the Federation, are now leaders of productive research groups: Max Bovarnick (1939) and Samuel Graff (1932).

Admission of applicants to graduate studies in biochemistry at Columbia was, during my time there, always based on personal interview rather than on college grades. In these interviews, which frequently lasted an hour or more, an attempt was made to evaluate not only knowledge of and laboratory experience in the various branches of pure chemistry, but the way in which a candidate was able to coordinate such background information as he had acquired in college. After admission, prospective graduate students were frequently required to defer their entrance into the elementary course in biochemistry while they broadened their acquaintance with organic and physical chemistry, both theoretical and practical, at the graduate level. They also were often directed to take courses in biology as preparation for the study of human physiology, mandatory in the program. Such requirements were as a rule far from welcome to students eager to get on with their lifework, but their value usually became apparent to students in the later stages of their training, and was often frankly acknowledged.

It now seems appropriate to outline some of the ways in which organic chemistry has contributed to the development of biochemistry during the past half century. In my opinion two of the most influential factors have been of an essentially practical nature. The first was the elaboration by Pregl of methods of microanalysis and the manipulation of organic compounds on a milligram scale. The second is the more recent addition of chromatographic and countercurrent distribution procedures to the armamentarium of the biochemist. These modern techniques have made possible the emergence of physiological chemistry from a subject which, as admitted by Halliburton in 1904, was formerly regarded by chemists with scarcely veiled contempt, into an exact science. Without the help of these procedures, many more years would undoubtedly have elapsed between the recognition of the existence of enzymes, hormones and vitamins, and the present-day accurate knowledge of their chemical nature.

The exponential growth of biochemical knowledge is also largely ascribable to the perfection of methods for the isolation and synthesis of organic compounds of natural origin, since exact information concerning the chemical constitution of these compounds is essential for the elucidation of the chemical reactions which underlie vital processes.

I should like to illustrate these generalities by specific reference to some of the contributions, made in various laboratories during the past 50 years by organic chemists, or by biochemists with the aid of the methods of organic chemistry, which have particularly impressed me by reason of their practical utility, their technical elegance, or their influence in the expansion of biochemical concepts.

The part played by organic chemistry in the acquisition of knowledge of the chemistry of vitamins has of course been decisive; most of the work in this field, however, has been along well-recognized lines which added little of fundamental novelty to theoretical chemistry. Only exceptional cases will, therefore, be discussed here.

An early instance is that of the chemical changes induced by irradiation of certain steroids. The discovery by Hess in 1925 that cholesterol prepared in the usual manner develops antirachitic potency when exposed to ultraviolet light stimulated Windaus to survey the photochemical sensitivity of a wide variety of sterols, of which ergosterol was found to yield by far the highest degree of physiological activity. Careful study of the isomerization induced by light disclosed the successive migration of double bonds which culminated in the opening of Ring B, a result that could not have been foreseen. Calciferol, the antirachitic compound derived from ergosterol, was crystallized in 1931 by Bourdillon and by Windaus, working independently, but was found to differ from natural vitamin D in being less effective, per rat unit, towards avian rickets. This discrepancy was not observed, however, with the corresponding product from 7-dehydrocholesterol, crystallized in 1936 by Windaus and simultaneously isolated from tunny liver oil by Brockmann.

While these impressive researches were proceeding, investigations were being carried out by Kuhn and by Karrer on the constitution of riboflavin and its irradiation products. Here, again, the photochemically induced cleavage of the ribityl group, at position 1 in acid solution and position 2 in alkaline solution, could not have been predicted on the basis of existing knowledge. On the other hand, the chemistry of thiamine, the constitution of which was also unraveled during the middle thirties, offered little fundamentally new information. Pyrimidines and thiazoles were familiar classes of organic compound, though the latter had not previously been encountered in natural products. Rupture of the linkage between the two rings by oxidation was accomplished in 1934 by Windaus, but the concomitant degradation of the sulfur-containing constituent hindered the recognition of its exact chemical nature. In the following year, however, R. R. Williams discovered that this portion of the molecule could be split off essentially unchanged by the action of bisulfite, and this observation facilitated the determination of the constitution of the vitamin and its ensuing synthesis.

An outstanding example of skillful and imaginative application of organic chemical methods by a small group was afforded in 1941–1943 by du Vigneaud's work on the isolation, structure, and synthesis of biotin. This gem of research forms a striking contrast, relative to organization, with the recently completed determination of the constitution of Vitamin B_{12}, a task which ultimately enlisted the efforts of a galaxy of experts in many fields and in various laboratories. A glance at the structural formula shown on

page 400 of the *Annual Review of Biochemistry* for 1956 should give justification for the opinion that here we have the most magnificent of all the achievements in the field of the chemistry of natural products.

In recent years the structure of antibiotic compounds of microbiological origin has attracted much attention from organic chemists. Reference will be made below to antibiotics of polypeptide nature; many, however, have proved to be compounds of smaller molecular dimensions, and some of these have been shown to contain chemical groupings previously encountered only in synthetic products. Penicillin contains thiazolidine and β-lactam rings and also an amino acid group with D configuration; nemotin is a lactone of a straight-chain hydroxy acid containing one allenic and two conjugated acetylenic groups; agrocybin is the amide of a triacetylenic hydroxy acid; chloromycetin (chloramphenicol) contains a *p*-nitrophenyl and a dichloroacetyl group; azaserine is an ester of diazoacetic acid; elaiomycin is an aliphatic azoxy compound. A compound which is not strictly an antibiotic but inhibits a sarcoma and was isolated from a streptococcal culture has turned out to be 6-diazo-5-oxo-L-norleucine. The therapeutically important antibiotics terramycin and aureomycin consist of highly substituted, partially hydrogenated derivatives of 2,3-benzanthracene. Streptomycin, a compound even more complicated in structure, contains three glycosidically linked units, two of which are of strikingly unusual character. The determination of the structures of these three "mycins" required intense labor by various large teams, mainly in industrial research laboratories, and represents organic chemical skill of the highest order.

As stated above, massive frontal attacks have not always been necessary for the solution of important problems in organic biochemistry. An example of fruitful research by a relatively small group is afforded by the isolation, identification and synthesis, in K. P. Link's laboratory, of the hemorrhagic agent in spoilt sweet clover hay. This achievement not only shed light on a matter of grave import to veterinary medicine but furnished an early example of the principle of biochemical antagonism, subsequently elaborated by Woolley. A parallel instance is the recognition of the chemical nature of the toxic factor produced in flour by the action of nitrogen trichloride. This product, the sulfoximine of methionine, represents a previously unknown type of compound which has widened the horizon of the organic chemist.

The chemistry of porphyrins exemplifies the development of an initially rather restricted field in a few laboratories. In this area the name of Hans Fischer stood supreme for many years; on the solid foundation constructed by him, Shemin has recently based a masterly explanation of the mechanism of the biosynthesis of heme from simple metabolites.

The chemistry of the steroid hormones, painstakingly elaborated by many groups during the past thirty years, beautifully exemplifies the con-

tributions of organic chemistry to biology and medicine. The constitution of the ovarian hormone estrone, isolated in crystalline form by Doisy and by Butenandt in 1929, was established in 1932 by Butenandt, who shortly thereafter isolated a male sex hormone and determined its constitution, confirmed two years later by Ruzicka. The analogy of the structures of the sex hormones to those just previously accepted for cholesterol and the bile acids suggested the possibility of a biochemical relationship; this was established in 1945 by Bloch's demonstration of the conversion in a human subject of deuterated cholesterol into deuteriopregnandiol. Absolute confirmation, according to the strict canons of organic chemistry, of the constitution of a sex hormone was supplied by Bachmann's total synthesis of equilenin in 1940, and by Johnson's synthesis of estrone in 1952.

The isolation of the hormones of the adrenal cortex offered even greater technical difficulties because of their large number and chemical similarities, but these were ultimately overcome by the work of independent groups led by Kendall, Reichstein, and Wintersteiner. Largely as a result of the chemical study of the sex hormones, the determination of the constitution of the cortical steroids proved less formidable. The synthesis of cortisone, though rendered difficult by reason of the unusual chemical characteristics of the hydroxyl group at position 11, was effected in 1952 by Sarett and his associates in the Merck laboratories. The constitution of aldosterone, which displays several unique chemical features, was determined in 1954 in a collaborative effort by Reichstein and a British group, the work being carried out with consummate skill on a total of 57 mg. of material. Its synthesis, by methods resembling those employed by Sarett, was completed by Wettstein in the following year.

The isolation of amino acids from protein hydrolysates and from other natural sources has required not only great skill in organic chemical manipulation, especially when previously unknown members of the class were involved, but an extensive knowledge of organic reactions in connection with their quantitative estimation and the determination of their constitution by degradative and synthetic procedures. Outstanding instances are the discovery of tryptophan by Hopkins & Cole; the isolation of thyroxine by Kendall and the demonstration of its constitution by Harington; the isolation of methionine by Mueller, its synthesis by Barger, and the demonstration of its biochemical relationship to cystine by du Vigneaud and others; the discovery of djenkolic acid by Van Veen and its synthesis by du Vigneaud; the isolation of canavanine by Kitagawa; the isolation of hydroxylysine by Van Slyke and its synthesis by Sheehan; the discovery of threonine by Rose and its synthesis by Carter; and the demonstration by Gross & Pitt-Rivers of the presence of tri-iodothyronine in plasma.

The quantitative separation of amino acids, essential for the establishment of a sound basis for an approach to the urgent problem of protein con-

stitution, has enlisted the attention of many skilled organic chemists subsequent to the early attempts by Emil Fischer. Help towards such efforts was afforded by the ingenious gasometric methods devised by Van Slyke, by Dakin's procedure for partial separation by extraction with butanol, and by Bergmann's development of the use of specific precipitants. Real progress, however, became possible only after the aid of specific microbiological mutants had been enlisted by Beadle and developed by Tatum & Bonner in 1944. However, the use of these was soon largely abandoned in favor of chromatographic procedures, first exploited by Martin & Synge and elaborated by Stein & Moore, Sanger, and others.

For many years the class of peptides was represented almost exclusively by synthetic compounds. The preparative procedures of Emil Fischer, which culminated in the production of an octadecapeptide, were rendered far more versatile by the introduction in 1932 of Max Bergmann's carbobenzyloxy method. No one but a keen organic chemist, alive to the possibilities of hydrogenolysis, would have conceived the idea of taking advantage of the lability of the benzyl linkage. Improved methods for the formation of peptides from optically active amino acids soon followed as a result of Bergmann's work. As is usual in such cases, most of these were developed by application of reactions previously discovered by others in purely organic chemical studies, such as the use of azides and mixed anhydrides in place of the classical acid halides, and of the condensing ability of highly reactive compounds like tetraethyl pyrophosphate. Another example is the application by Sheehan of Khorana's ingenious use of carbodi-imides, whereby not only peptides but β-lactams can be produced in either organic solvents or aqueous media. A major achievement by this procedure is Sheehan's practical synthesis of penicillins, a goal which the concerted work of many laboratories in this country and Britain failed to reach during the second World War.

With the exception of carnosine and anserine, which are not typical peptides, no naturally occurring compound of this class was known prior to the discovery of glutathione by Hopkins in 1921. The constitution of this tripeptide proved surprisingly difficult to solve, and was not rigorously established until 1935, when its synthesis was accomplished by Harington on the basis of evidence contributed independently by Hopkins, Hunter, Kendall, and Nicolet.

For some years thereafter, little attention was devoted to peptides from natural sources, until in 1943 Dubos & Hotchkiss prepared in crystalline form the antibiotic polypeptides gramicidin and tyrocidin, produced by certain bacilli. This discovery was followed by the isolation, in various laboratories, of several other products of similar character. It is interesting, and perhaps significant, that these antibiotics yield on hydrolysis some of their constituent amino acids in their D form. In several instances their constitu-

tions have been established; tyrocidine, for instance, was shown by Craig to be a cyclic decapeptide in which two of the members of the ring are D-phenylalanyl groups.

The methods by which the sequence of the components of such peptides was determined command the intense admiration of the organic chemist. They involve the isolation of multitudes of simpler peptides from partial hydrolysates, by Craig's countercurrent distribution or by chromatography, and the identification of their terminal amino groups by Sanger's procedure involving labeling with dinitrofluorobenzene. Impressive, also, is the intellectual feat of assembling the scraps of information thus obtained into a picture from which the sequential constitution can be deduced.

Attempts to devise more direct and less laborious procedures have not been lacking. The most promising of these seems to be that of a stepwise degradation, proposed in 1949 by Edman, based upon the rapidity with which phenylhydantoins (or phenylthiohydantoins) are detached by acid from the corresponding ureides, with production of the next lower member of the peptide chain. Unfortunately, simultaneous hydrolysis of peptide bonds occurs to an extent which, though negligible in the first step, cumulatively complicates the isolation of significant products.

Undoubtedly the outstanding success in the field of polypeptides is that attained with the hormones of the posterior pituitary. After years of relatively sterile effort by various groups, the isolation in crystalline form of pure oxytocin and of two varieties of vasopressin was successfully accomplished in 1953 by du Vigneaud and by Fromageot, working simultaneously and independently. Both groups then determined the structures of their products, with entire concordance. In an astonishingly short time the Cornell group then confirmed the constitution of all three hormones by synthesis, an achievement of first rank in the annals of organic biochemistry. In this work, extensive use was made of the recently developed method of peptide formation by means of tetraethyl pyrophosphite. A fortunate circumstance was the fact, previously established by du Vigneaud, that in these closely related cyclopolypeptides the disulfide bridges which close the ring can be restored unchanged after reduction.

Considerable progress has also been made during the past few years in the determination of the structure of bacitracin A, the principal component of a mixture of antibiotic polypeptides elaborated by a rare bacillus which had been isolated from a wound in a patient named Tracy. In addition to yielding several D-amino acids on hydrolysis it exhibits other unusual structural features, the most challenging of which is the presence of a masked sulfhydryl group, uncovered when the antibiotic activity is destroyed by mild acid hydrolysis. Craig in the United States and Abraham in England, who have carried out most of the work on this cyclopolypeptide, have adduced evidence that the sulfur atom is present in a thiazoline ring, apparently

formed between D-isoleucine and L-cysteine residues. Abraham and his group have recently shown that the ϵ-amino group of lysine is involved in the closure of a ring containing six amino acid residues and that its α-amino group forms the point of attachment of a side-chain.

It is not surprising that polypeptides should have been subjected to elaborate structural studies, for they furnish comparatively simple models for attack on the constitution of proteins. A most impressive exploration in this vast and intricate area has recently been completed by Sanger in the case of insulin.

The history of insulin chemistry presents many episodes of special interest to the organic chemist. The recognition of its protein nature by Dudley in 1923 was confirmed by Abel after he had crystallized it in 1926, the same year, incidentally, as that in which the first crystalline enzyme, urease, was prepared by Sumner. In 1927, du Vigneaud, working in Abel's laboratory, demonstrated the presence of labile disulfide groups in insulin and showed that on reduction of these linkages the physiological activity thereby lost was not restored on reoxidation (an effect strikingly contrasted by his later findings with oxytocin). Intensive search in various laboratories for a prosthetic group proved fruitless, and subsequent quantitative studies confirmed the view that insulin contains nothing but amino acid groups. Molecular weight determinations by physical methods suggested that the protein consists of associable units of magnitude about 12,000. In 1952 Craig tested the validity of this figure in an ingenious approach, made possible by a combination of Sanger's procedure, developed in 1945, for the labeling of amino groups in insulin with the dinitrophenyl radical and his own method for the separation of mixtures by means of countercurrent distribution in systems of incompletely miscible liquids. In principle, the dinitrophenyl content of the individual fraction containing only one such added group should constitute a measure of the molecular weight. This method was first rehearsed with the crystalline antibiotic polypeptides gramicidin S and polypeptin, and gave results consistent with data secured by quantitative amino acid analysis. When applied to partially dinitrophenylated insulin it yielded a molecular weight value of 6500. A similar figure was obtained from a fraction consisting of disubstitution products.

In his earliest (1945) work on dinitrophenylated insulin, Sanger showed that the hydrolysate from a fully substituted product contained only three labeled amino acids, namely glycine, phenylalanine, and lysine (substituted in the ϵ-position), in amounts corresponding to two of each on the basis of a molecular weight of 12,000. Four years later, Sanger published the important observation that insulin is split into two polypeptides when the disulfide groups are oxidized to sulfonic acid groups by performic acid. One of these, Fraction A, proved to contain glycine in the amino terminal position; on the basis of its apparent molecular weight, about 2500, only one glycine

residue was present. The other components, all of which were quantitatively estimated, included four cysteic acid groups, at least one of the sulfur atoms of which must have been involved in the original linkage of the two peptide chains. The other polypeptide, Fraction B, was found to have a molecular weight about twice as great and to contain phenylalanine in the N-terminal position.

Sanger and his group then heroically undertook the task of determining the amino acid sequences in the two polypeptides by subjecting them to partial hydrolysis by means of acids and proteolytic enzymes, separating and identifying the resulting simpler peptides, and fitting the results together as in a jigsaw puzzle. In a phenomenally short time they completely solved this problem insofar as the sequence of constituent amino acids was concerned. The position of the amide groups was then cleared up by means of determinations of the ionophoretic mobilities and amide contents of peptides from enzymic hydrolysates of the two fractions from the oxidized insulin. Establishment of the constitution of the hormone itself, which involved determination of the position of the disulfide linkages, was complicated by disulfide interchanges during hydrolysis. A study of this reaction with simple models showed that in neutral or alkaline solution it is catalyzed by thiols and inhibited by thiol-binding agents, whereas in acid solution it is inhibited by thiols. With this information, suitable conditions could be selected for the partial hydrolysis of insulin; with the aid of ionophoretic separation of the resulting peptides the complete structure was determined. Chain A contains 21, chain B 30, amino acids in sequence, the two chains being doubly connected by disulfide links at points 6 and 20 in chain A to points 7 and 19, respectively, in chain B. These chains are actually cyclic in form owing to the presence of internal disulfide groups, which in the case of chain A is of the same size as that in oxytocin. Finally, Sanger and his colleagues have shown that insulins from cattle, swine, and sheep differ in amino acid composition at points 8, 9, and 10, but that the constitution of chain B is the same in the three species. The molecular weights of these structures conform with the findings of Craig.

With these remarkable achievements, a long-standing primary goal of organic biochemistry—the constitution of a protein—has now been reached. As much admirable work, along analogous lines, is being conducted in other laboratories with other proteins, a new era in protein chemistry has undoubtedly been entered.

The topics above outlined represent merely a few examples, arbitrarily selected, of the achievements of organic chemistry in the solution of problems posed by biochemistry. No reference has been made to the many notable contributions, such as the demonstration by Westheimer & Vennesland of the stereospecificity of DPN-linked dehydrogenation reactions, which have been effected with the aid of techniques peculiar to the biochemical

laboratory. It is surely obvious that the scope of research by the biochemist is greatly widened by a command of the theory and practice of organic chemistry. A significant proportion of the work signalized in these Annual Reviews has emanated from biochemical laboratories directed by scientists with extensive experience in the organic field.

Similar considerations apply to the increasingly important role played by physical chemistry in biochemical research. Biochemists in charge of the training of graduate students would therefore, in my earnest opinion, do well to discourage too early specialization and to insist on the acquisition of a thorough grounding in the fundamental branches of chemistry prior to embarkation on the intensive study of biochemistry.