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The Search for the Chemical Structure of DNA

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ABSTRACT—**The history of the chemistry of DNA has to a large extent been neglected in favor of the physical and genetic aspects. Yet the chemical studies on the nucleic acids spanned a period of more than 80 years, and were crucial to our current understanding of the structure and function of DNA. We have examined the published scientific record and interviewed several participants who made major contributions to the elucidation of the chemical structure of DNA. A partial analysis covering the period 1900-1955 is presented.**

This work is principally directed towards those who believe that deoxyribonucleic acid sprang upon the scene in the early 1950's and had no prior history. In fact, DNA was, of course, discovered in 1869 by Friederich Miescher, and a great deal of painstaking work was done in the intervening years. Unfortunately, in the space available I will only be able to present a small portion of the material covering this topic. I will concentrate on the elucidation of the structure of nucleotides, the monomer units from which nucleic acids are built, and the tetranucleotide hypothesis for the structure of DNA, and the evidence against it.

Mononucleotides and the Tetranucleotide Hypothesis

From the turn of the century until the 1940's DNA was generally considered to be a small molecule with a molecular weight of approximately 1,500, consisting of only four nucleotide units and having marginal biological importance. Thus, Walter Jones in the preface of the first book on nucleic acids said in 1914, "The nucleic acids constitute what is possibly the best understood field of Physiological Chemistry."[1] And P. A. Levene in the preface to "Nucleic Acids" in 1931 stated, "The chemistry of nucleic acids can be summed up very briefly. Indeed, a few graphic formulas which need not fill even a single printed page might suffice to express the entire store of our present-day knowledge on the subject."[2] Yet, subsequent work showed DNA to be one of the largest molecules, containing many thousands of nucleotide components, and to be of the utmost genetic significance. These profound changes culminated in 1953 in the proposal by Watson and Crick of a double-helical model for the structure of DNA, which has been described by the eminent geneticist, C. H. Waddington, as "certainly the greatest discovery in biology in this century."[3] It is our intention to describe the progress of this amazing reversal and attempt to analyze the underlying causes for it.

The credit for laying the foundation for the determination of the structure of the nucleic acids, by clarification of the structure of their hydrolysis products, must go chiefly to Phoebus Aaron Theodor Levene.[4,5] He was born in 1869, the same year that DNA was discovered by Friederich Miescher, and was one of the few Jewish students allowed to enter the Imperial Military Medical Academy in St. Petersburg. As a result of the growing persecution in Russia, his family emigrated to the United States in 1891, and he practiced medicine on the lower East Side of New York City for four years. However, his interest lay in fundamental medical research, and he enrolled as a special student in the Chemistry Department of the School of Mines of Columbia University. Although he never obtained a chemistry degree, he was described by the citation to the Willard Gibbs Medal of the American Chemical Society, which he was awarded in 1931, as the "outstanding American worker in the application of organic chemistry to biological problems."

He received his first appointment to the New York Pathological Institute in 1894 and in 1905 he joined the Rockefeller Institute. In 1909 he and his coworkers made their first important discoveries, the nature of the carbohydrate group in yeast nucleic acid and the order of linkage of the three components, base, sugar and phosphate in a nucleotide.

The ordering of the three chemical components of inosinic acid derived from meat extract had been speculated on by Haiser in 1895[6] when he had shown the presence of phosphorus. By a comparison of the products of mild alkaline and acid hydrolysis of inosinic acids, Levene and Jacobs were able to establish in 1909 the order phosphate-pentose-purine. Alkali gave phosphoric acid and inosine, while acid gave ribose phosphate and the base, hypoxanthine.[7] The term nucleoside was introduced in the same year (1909) by Levene and Jacobs to describe the purine-carbohydrate compounds, such

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as inosine, derived from nucleic acid hydrolysis, and the term nucleotide to describe the phosphate ester of a nucleoside such as inosinic acid.

The human mind likes order. A scientist analyzing data will always search for a relationship to clarify the problem before him. Quantitative relationships between the two purine and two pyrimidine bases which had been found to be present in nucleic acids were reported as far back as 1893 by Kossel and Neumann.[8] Steudel in 1906[9] and Levene and Mandel in 1908[10] concluded that they each occurred in equi-molecular proportions in thymus nucleic acid, and Levene came to the same conclusion in 1909[11] for yeast nucleic acid. This was confirmed by later workers, such as Jones in 1914.[1] At that time such evaluations could be expected to provide no more than crude results. Yet from 1909 until the 1940's it was almost a dogma that the four bases were present in equal proportions in the nucleic acids. This led to the formulation of what has become known as the tetranucleotide hypothesis for the structure of nucleic acids. This term seems to have originated with Kossel and Neumann who believed that each purine and pyrimidine was present in a separate chemical entity in the nucleus. Thus, they stated in 1893, "It is highly probable that four nucleic acids exist of which each contains only one of the nucleic acid bases."[8] Levene appears to have adapted this idea to describe one nucleic acid containing equal quantities of the four bases, although in his published works he never fully committed himself to this—it remained a hypothesis.

It was necessary to ascertain how the mononucleotide units were chemically linked together in the proposed tetranucleotide moiety. In a paper published in 1912 Levene and Jacobs[12] reported products which they identified as thymidine and and cytidine di-phosphoric acids. Levene identified 2-deoxyribose in 1929 as the carbohydrate in thymus nucleic acid (DNA),[13] 20 years after identifying that in yeast nucleic acid (RNA) as ribose.[7] Then in 1935 he and Tipson proved that thymidine has a furanoside (5-membered) ring structure. They could then conclude "Thus it is evident that in deoxyribose nucleic acid the positions of the phosphoric acid radicals are carbon atoms (3) and (5) of the deoxyribose."[14] This was the first time this significant fact was specifically noted.

While Lord Todd and his co-workers are rightly credited with establishing the position of the internucleotide bond in the 1950's, it is not generally realized that Levene had proposed the correct answer in the early 1930's. In view of the fact that Levene was friendly with O. T. Avery, a colleague at the Rockefeller Institute and the discoverer, with Maclyn McCarty and Colin MacLeod, of the role of DNA in biological transformation,[15] the question arises—could Levene have influenced this work? McCarty answered this question thus, "I do not believe that there was any direct relationship between Levene's work on nucleic acids and Avery's interest in the phenomenon of pneumococcal transformation. After Griffith's description of the phenomenon in 1928, his findings were confirmed quite early in Avery's laboratory by Martin Dawson. From the beginning Avery was convinced of the potential biological importance of the phenomenon, and his goal for many years was discovering the chemical nature of the substance responsible for transformation. Work in this direction, though intermittent, began in 1935 after cell-free extracts became available. I believe that there were no preconceived ideas concerning the involvement of nucleic acids. However, by about 1940 it was known that the crude cell-free extracts with transforming activity contained both RNA and DNA as well as other macromolecular constituents. I was told by the late Colin MacLeod that when he and Avery consulted Dr. Levene about the possibility that nucleic acids might be involved in the biological activity, he discouraged them by citing the essential invariability of nucleic acids on the basis of the tetranucleotide theory of their structure. This notion that "nucleic acids are all alike" was repeated to us subsequently by others."[16]

The widely held belief in the fundamental vital nature of the proteins in the life process conspired to bring about this situation. However, the tetranucleotide hypothesis provided Levene with a vehicle for refining the structural knowledge of nucleotides. Other workers in the field provided no more than variations on the same theme. One must conclude that what proved a barrier to further progress in elucidating the structure of DNA was not simply the tetra-nucleotide hypothesis itself, but rather a lack of insight by the workers in this difficult and unfashionable field, coupled with lack of suitable techniques at that time to carefully study the structure of intact DNA rather than its degradation products.

However, to prove the correct structure of a natural product it is necessary that it be chemically synthesized. Emil Fisher first attempted the chemical synthesis of a nucleotide as early as 1914.[17] He used as reagent phosphorylchloride to attach a phosphate group to a nucleoside, a chemical process termed phosphorylation. However, the yields of desired product using this reagent were very low, as a result of many side reactions. The introduction of a mild and efficient phosphorylation agent by Alexander Todd and co-workers led to a significant breakthrough in the chemical synthesis of nucleotides for which he was later awarded the Nobel Prize. Alexander Robertus Todd obtained his D.Phil degree in the laboratory of Robert Robinson in Oxford in 1933. He then went to Edinburgh to work with George Barger on vitamin B¹, which led him to work on the related co-enzymes, many of which were found at that time to be pyrophosphates. In 1936 Todd went to the Lister Institute in London where he replaced J. M. Gulland, at that time the most prominent British nucleic acid chemist, who was appointed Head of the Chemistry Department at Nottingham. In 1938, at the age of 30, Todd became Head of the Chemistry Department of Manchester University, where he started several lines of research, on cannibis and related drugs, on purines and nucleosides, and on methods of phosphorylation.

The first full characterization of the reagent dibenzylphosphorochloridate and description of its use as an efficient phosphorylating agent appeared in two short papers submitted in February 1945, and published together in the Journal of the Chemical Society.[18 19] The first of the two was by B. C. Saunders and co-workers, and the second by Todd and co-workers. During the war years, Bernard Saunders in Cambridge had actually been doing secret research on nerve gases.[20] These contain a phosphorus fluorine bond, in place of the phosphorus chlorine bond found in the relatively innocuous phosphorylating agent. Saunders and his co-workers considered it safer to work with the unstable P-Cl compounds than with the highly toxic P-F analogs.

Todd had been appointed Professor of Chemistry and Chairman of the Department at Cambridge in 1944. World War II was still in progress, and the Western allies crossed into Germany in early February that year. With the end of the war in sight apparently February was judged to be a safe time to begin publishing these findings. Thus, the research on the nerve gases had an important, positive, scientific by-product in the understanding of the nucleic acids. Not an unparalleled event in the history of warfare.

Todd's work was predominantly involved with the synthesis of nucleotides and co-enzymes and in his early years in this field he rarely speculated on the structure of the nucleic acids themselves. However, one of the most important later contributions to come from his work was the clarification of the hydrolytic differences between RNA and DNA which had mystified chemists for so long. In 1949 C. E. Carter and W. Cohn described the separation of "yeast" adenylic acid into two forms by ion-exchange chromatography.[21] It was naturally thought that these were the 2′and 3′-phosphates of adenosine. Levene and Tipson in 1935 appear to have been the first to specifically relate the difference in hydrolytic properties of the two kinds of nucleic acid to their different chemical structures.[22] They suggested that the alkaline lability of RNA, compared to the stability of DNA, resulted from the presence of the 2′-hydroxyl group in the ribose of the former, which could assist in the hydrolysis of RNA. Todd and Daniel M. Brown brought all the evidence together in an influential paper in 1952.[23]

The first successful chemical synthesis of a dinucleotide as found in nucleic acid was finally accomplished in 1955 by Michelson and Todd,[24] and confirmed the chemical structure of DNA which had been proposed.

Todd and Levene, the two who contributed most to the understanding of nucleic acids in half a century of research, met only once, in the elevator at the Rockefeller Institute—a revealing fact about the degree of communication in science at that time. Todd said, "I went to see Herbert Gasser who was then director of the Rockefeller Institute in 1938. We were going out to lunch—my wife, myself and Gasser—and Gasser got into the elevator and this little old man was in the elevator and just said 'Hello,' and Gasser said 'This is Levene,' and he got out, and that was the only time I ever saw him, and I never said anything more to him than 'good morning'."[25]

The Molecular Size of DNA

A major obstacle to the chemical characterization of DNA was the question of its true molecular size. In the 1920's to 1930's it was generally believed that substances which manifested high molecular weights were "colloids;" that is they were thought to consist of aggregates of small molecules held together by partial or ionic bonds. The opposing point of view, argued most ably by J. H. Staudinger was that proteins were truly very large molecules, which were termed "macromolecules" or "polymers," in which the individual components were joined together by actual chemical linkages, or covalent bonds. There were naturally suggestions that, in their native state, the nucleic acids were colloidal aggregates of tetranucleotides.

In 1924 Einar Hammarsten at the Karolinska Institute in Stockholm, Sweden set out to study the colloidal properties of thymus nucleic acid. In doing so he essentially re-discovered the careful biochemical preparation of DNA.[26] Some 50 years earlier Miescher had worked very carefully in primitive cold-rooms making preparations of nucleic acids. But with the advent of interest in the nucleic acids by classical organic chemists such as Kossel and Levene much of the art in the biochemical techniques was ignored. Since these chemists were studying the degradation products it was considered permissible to use harsh conditions during the preparation of DNA. It is not surprising that values obtained for the molecular weight of DNA in the years 1900-1938 were usually low and variable. It was an unfortunate coincidence that they averaged around 1,500, just the value expected for a single tetranucleotide.

In a study bearing on the size of the DNA molecule reported in 1934 Torbjorn Caspersson gave the results of some filtration experiments.[27] Miescher had found in 1871 that nucleic acid was retained by a filter, and others had noted high viscosities for nucleic acid preparations which were early indications of a high molecular weight. Casperson was a student of Hammarsten and used DNA prepared by the method which the latter had described 10 years previously. Caspersson concluded "the astonishing fact that the complexes of nucleic acids must be larger than the protein molecules." Three further studies reported in 1938 applied physical methods to the determination of the actual size of DNA by measuring its molecular weight. Caspersson and Hammarsten supplied DNA to R. Signer in Berne, Switzerland, to measure the molecular weight using flow birefringence.[28] They reported a value of 500,000 to 1 million for the molecular weight of DNA.

A second paper in 1938 indicating a similar molecular weight for DNA was by W. T. Astubry and F. O. Bell using X-ray fiber diffraction,[29] and a third paper, also published in 1938 showing that DNA was a large molecule, was by Levene and Gerhard Schmidt.[30] They used ultracentrifugation, a technique which had been developed by T. Svedberg in the 1920's and had been applied by him and others to show that proteins were true macromolecules with molecular weights on the order of thousands. In the late 1930's an ultracentrifuge was still a rare piece of equipment, even in the U.S.A. E. G. Pickels built an improved model in 1937 at the Rockefeller Institute. Thus in 1938 Levene and Schmidt were able to measure the molecular weight of native DNA at between 200,000 and 1 million by ultracentrifugation, and showed that the results depended on the means of preparation used.

In their study, Levene and Schmidt also observed a non-sedimenting nucleic acid material of low molecular weight, which they concluded, "It is not improbable that it represents a single tetranucleotide."[30] This implied that native DNA was a polymer of tetranucleotides, an idea which was propounded by several authors. Gulland discussed this idea before the Chemical Society of London in 1943, and made a revealing comment, " . . . the conception of a molecule composed of polymerized tetranucleotides has grown from a mental superposition of the later demonstrations of high molecular weights on the older ideas of a simple molecule containing one each of the four appropriate nucleotides; had the true molecular sizes been realized earlier it is doubtful whether the conception would have gained such firm hold as is apparently the case." He then suggested that the ratios of the four bases might be "statistical," but then surprisingly recommended the concept of a polytetranucleotide as a "practical working hypothesis."[31]

Physiochemical Characterization of DNA

In an attempt to clarify several questions Gulland and co-workers studied the titration characteristics of DNA. In an important contribution in 1947[32] they showed that the ratio of primary to secondary phosphoryl groups in carefully prepared DNA samples had a minimum value of approximately 16:1. This is inconsistent with a simple tetranucleotide, although still much too low a ratio to account for the highly polymeric nature of DNA as then known. But, more important in its implications for DNA structure were their findings on the amino and enolic hydroxyl groups of the purines and pyrimidines. These also exhibit characteristic ionization constants. In following the titration of DNA in both acid and alkaline directions, Gulland, *et al.* noted the significant fact that these transitions were not completely reversible. Changes in other properties, such as viscosity, had previously been noted on titration of DNA, and had been explained in terms of a chemical de-polymerization. Gulland *et al.* favored an explanation for this hysteresis in terms of the exposure of amino and hydroxyl groups from the base, which, they concluded, had been hidden in the original structure. This, they attributed to hydrogen bonds—weak bonds between oxygen and nitrogen atoms mediated by hydrogen atoms. They noted that their evidence did not enable them to distinguish whether the hydrogen bonds united different parts of the same chain or different chains of DNA.[33] The presence of hydrogen bonds had been suggested as being important for maintaining protein structures on the basis of similar phenomenon in acid-base titration. Unfortunately, Gulland did not live to follow up the questions posed by these results, nor to achieve the recognition this significant contribution warranted, for he was killed in a train derailment in 1947, the very year of its publication.

The quantitative values for the ratio of amino and hydroxyl groups present in DNA, which were determined by Gulland *et al.*, from titrations, were also not in accord with the exactly equivalent stoichiometry of the bases required by the tetranucleotide hypothesis. It had been accepted for several decades that the four bases were present in nucleic acids in equimolecular proportions. From 1948-1952 Erwin Chargaff, an Austrian emigré working at Columbia University, published a series of papers in which he and his co-workers proved this belief to be unfounded. They described in detail a sensitive and accurate technique for the determination of the purine and pyrimidine components in nucleic acid hydrolysates. Chargaff and Vischer[34] and Rollin Hotchkiss[35] at Rockefeller Institute utilized paper chromatography to separate small amounts of a mixture of purines and pyrimidines into its components, and UV spectroscopy to determine the proportions. They were able to show that the ratios of purines and pyrimidines in DNA from different species varied greatly.[36] Although discrepancies from exact stoichiometry of the four bases had been reported in the literature since its inception, these had been conveniently ignored in favor of the imaginative simplicity of the tetranucleotide hypothesis.

During the course of his work Chargaff noted some other quantitative relationships between the base ratios, which with the accumulation of reliable data and the work of others[37] gradually became compelling. Thus, in 1948 he said "A comparison of the molar proportions reveals certain striking, but perhaps meaningless, regularities."[38] And in 1951, "as the number of examples of such regularity increases, the question will become pertinent whether it is merely accidental or whether it is an expression of certain structural principles that are shared by many deoxypentose nucleic acids despite far-reaching differences in their individual composition and the absence of a recognizable periodicity in their nucleotide sequence."[39] The regularities referred to where the findings that the amount of adenine equalled the amount of thymine, and guanine that of cytosine. Later Chargaff stated, "It will surprise many readers . . . to learn that the first announcement of base-pairing was made in 1950."[40] There is, of course, a major distinction between unitary base ratios of unknown origin and specific base-pairing as advanced later by Watson and Crick.[41] Further, according to James Watson's personal account after he had 'discovered' specific hydrogen-bonded purine-pyrimidine base-pairing, "Chargaff's rules" then suddenly stood out as a consequence of a double-helical structure for DNA."[42] Nevertheless, the quantitative studies of Chargaff and his co-workers represented the last nails being driven into the coffin of the tetranucleotide hypothesis for the structure of the nucleic acids.

As a result of these studies over a period of more than 50 years, DNA was known to be a high molecular weight polymer with phosphate groups linking deoxyribonucleosides between the 3′ and 5′ positions of the sugar groups. The sequence of bases was unknown, although some quantitative regularities in base composition had been noted. While the detailed chemical structure of DNA had been determined, its molecular geometry remained a mystery. In the elucidation of this mystery molecular biology was, of course, to become the operative phrase.

REFERENCES

Based on a talk (by J.S.C.) at the Ninth Joint Atlantic Seminar in the History of Biology, Boston, April, 1973, and the Washington Society for the History of Medicine, October, 1973, and derived from "DNA: A Critical History of the First Century," to be published by MIT Press. We wish to acknowledge the support of the American Academy of Arts and Sciences in the gathering of information for this project, and express our sincerest thanks to John Edsall, Chairman of their Committee on the History of Biochemistry and Molecular Biology, for his help and personal interest in this work.

1. Jones W: *Nucleic Acids/Their Chemical Properties and Physiological Conduct*, Longmans Green & Co., London, 1914
2. Levene PA, and Bass LW: *Nucleic Acids*, Chemical Catalog Co., New York, 1931
3. Waddington CH: "Some European Contributions to the Pre-history of Molecular Biology," Nature *221*, 318-321 1969
4. Van Slyke DD and Jacobs WA: "Phoebus Aaron Theodor Levene. 1869-1940," Biograph Memoir. Nat. Acad. Sci. *23*, 75-126, 1944
5. Tipson RS: "Phoebus Aaron Theodor Levene, 1869-1940," Adv. Carbohyd. Chem. *12*, 1-12, 1951
6. Haiser, F: "Zur Kenntnis der Inosinsaure," Montash. fur Chemie *16*, 190-206, 1895. Inosinic acid had been discovered by J. von Liebig in 1847 and had been named from the Greek *inos* meaning meat.
7. Levene PA, Jacobs WA: "Uber Inosinsaure," Ber. der Deutsch Chem. Gessel. *42*, 1198-1203, 1909
8. Kossel A, Neumann A: "Uber das Thymin, ein Spaltungproduct der Nucleinsaure," Ber. der Deutsch Chem. Gessel. *26*, 2753-2756, 1893
9. Steudel H: "Die Zussamensetzung der Nucleinsauren aus Thymus und das Heringsmilch," Hoppe-Seyler's Z. Physiol. Chem. *49*, 406-409, 1906
10. Levene PA, Mandel JA: "Zur Herkunft der Cytosine bei der Hydrolyse der tierischen Nucleinsauren," Biochem. Zeit. *10*, 233-239, 1908
11. Levene PA: "Uber die Hefenucleinsaure," Biochem. Zeit. *17*, 120-131, 1909
12. Levene PA, Jacobs WA: "On the Structure of Thymus Nucleic Acid', J. Biol. Chem. *12*, 411-420, 1912
13. Levene PA, London ES: "The Structure of Thymonucleic Acid," J. Biol. Chem. *83*, 793-802, 1929
14. Levene PA, Tipson RS: "The Ring Structure of Thymidine," J. Biol. Chem. *109*, 623-630, 1935
15. Avery OT, MacLeod CM, McCarty M: "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III," J. Exptal. Med. *79*, 137-158, 1944
16. McCarty M: Letter dated March 27, 1973
17. Fischer E: "Uber Phosphorsaureester des Methylglucoside und Theophyllinglucosids," Ber der Deutsch Chem. Gessell. *47*, 3193-3205, 1914
18. McCombie H, Saunders BC, Stacey GJ: "Esters Containing Phosphorus. Part I," J. Chem. Soc. 380-382, 1945
19. Atherton FR, Openshaw HT, Todd AR: "Studies on Phosphorylation. Part I. Dibenzylchlorophosphonate as a Phosphorylating Agent,"

J. Chem. Soc. 382-385, 1945. Phosphorochloridate is now the accepted nomenclature for P(O)Cl compounds.

20. Saunders BC, Stacey GJ: "Esters Containing Phosphorus. Part IV. Diisopropyl Fluorophosphonate," J. Chem. Soc. 695-699, 1948

21. Carter CF, Cohn WE: "Separation of Three Naturally Occurring Adenine Ribonucleotides by Paper Chromatography and Ion-Exchange," Fed. Proc. *8*, 190, 1949; W. E. Cohn, "The Anion Exchange Separation of Ribonucleotides, "J. Amer. Chem. Soc. *72*, 1471-1478, 1950

22. Levene PA, Tipson RS: "The Partial Synthesis of Ribose Nucleotides. II. Muscle Inosinic Acid," J. Biol. Chem. *111*, 313-323, 1935

23. Brown DM, Todd AR: "Nucleotides. Part X. Some Observations on the Structure and Chemical Behavior of the Nucleic Acids," J. Chem. Soc. 52-58, 1952

24. Michelson AM, Todd AR: "Nucleotides. Part XXXII. Synthesis of a Dithymidine Dinucleotide Containing a 3′, 5′-Internucleotide Linkage," J. Chem. Soc. 2632-2638, 1955. The first synthesis of a dinucleoside phosphate, i.e. a phosphate with two nucleoside ester groups, was carried out in 1948 by Gulland and Smith (J. M. Gulland and H. Smith, "The Constitution of Yeast Ribonucleic Acid. Part XIII. The Synthesis of Diuridine-2′, 2′ Phosphate," J. Chem. Soc. 1502-1534, 1948). As a result of the position of the blocking agent a 5′,5′ linked product was obtained. In 1952 Elmore and Todd prepared a similar product by a different route (D. T. Elmore and A. R. Todd, "Nucleotides. Part XVIII. The Synthesis and Properties of Adenosine-5′ Uridine-5′ Phosphate," J. Chem. Soc., 3681-3686, 1952). Neither of these substances was susceptible to alkaline or enzymatic hydrolysis indicating that they were not the naturally occurring combination of nucleotides. The thymidine dinucleotide prepared by Michelson and Todd in 1955 was 3'.5' linked and behaved as the natural products.

25. Lord Todd, Interview, July, 1972.

26. Hammarsten E: "Zur Kenntnis der Biologischen Bedeutung der Nucleinsaurerverbindungen," Biochem. Zeit. *144*, 383-466, 1924

27. Caspersson T: "Druckfilterung von Thymonucleinsaure," Biochem. Zeit. *270*, 161-163, 1934

28. Signer R: "The Molecular Weight of Polystyrenes and the Shape of the Molecules in Solution," Trans. Faraday Soc. *32*, 296-307, 1936

29. Astbury WT, Bell FO: "X-ray Study of Thymonucleic Acid," Nature *141*, 747, 1938. They obtained this value from a combination of the diffraction spacing of 3.34 A and the solution properties of a length to thickness ratio of ca. 300. Further discussion of this and other X-ray work on DNA will be presented elsewhere.

30. Schmidt G, Levene PA: "The Effect of Nucleophosphatase on 'Native' and Depolymerized Thymonucleic Acid," Science *88*, 172-173, 1938

31. Gulland JM: "Some Aspects of the Chemistry of Nucleotides," J. Chem. Soc. 208-217, 1943

32. Gulland JM, Jordan DO, Taylor HFW: "Deoxypentose Nucleic Acids. II. Electromeric Titration of the Acidic and Basic Groups of the Deoxypentose Nucleic Acid of Calf Thymus," J. Chem. Soc. 1131-1141, 1947

33. Creeth JM, Gulland JM, Jordan DO: "Deoxypentose Nucleic Acids. III. Viscosity and Streaming Birefringence of the Sodium Salt of the Deoxypentose Nucleic Acid of Calf Thymus," J. Chem. Soc. 1141-1145, 1947. In D. O. Jordan, *Chemistry of Nucleic Acids*, Butterworths, London, 1960, p. 169, it is stated "That the bonds were intermolecular and not intramolecular was determined by the viscosity data of Creeth, Gulland and Jordan," but the original reference states the opposite conclusion.

34. Vischer E, Chargaff E: "The Separation and Characterization of Purines in Minute Amounts of Nucleic Acid Hydrolysates, "J. Biol. Chem. *168*, 781-782, 1947; ibid "The Separation and Quantitative Estimation of Purines and Pyrimidines in Minute Amounts," J. Biol. Chem. *176*, 703-714, 1948; ibid, "The Composition of the Pentose Nucleic Acids of Yeast and Pancreas," J. Biol. Chem. *176*, 715-734, 1948

35. Hotchkiss RD: "The Quantitative Separation of Purines, Pyrimidines and Nucleosides by Paper Chromatography," J. Biol. Chem. *175*, 315-332, 1948. This paper included the first published report of a "rare" base termed epicytosine, later shown to be 5-methyl cytosine.

36. Chargaff E: "Chemical Specificity of Nucleic Acids and Mechanism of their Enzymatic Degradation," Experientia *6*, 201-209, 1950

37. Daly M, Allfrey VG, Mirsky AE, "Purine and Pyrimidine Content of Some Deoxypentose Nucleic Acids," J. Gen. Physiol. *33*, 497-510, 1950; G. R. Wyatt, "The Purine and Pyrimidine Composition of Deoxypentose Nucleic Acids," Biochem. J. *48*, 584-590, 1951; A Marshak and H. J. Vogel, "Microdetermination of Purines and Pyrimidines in Biological Materials," J. Biol. Chem. *189*, 597-605, 1951

38. Vischer E, Zamenhof S, Chargaff E: "Microbial Nucleic Acids: The Deoxypentose Nucleic Acid of Avian Tubercle Bacilli and Yeast," J. Biol. Chem. *177*, 429-438, 1949

39. Chargaff E, Lipschitz R, Green C, Hodes ME: "The Composition of the Deoxyribonucleic Acid of Salmon Sperm," J. Biol. Chem. *192*, 223-230, 1951; see also E. Chargaff, "Structure and Function of Nucleic Acids as Cell Constituents," Fed. Proc. *10*, 654-659, 1951

40. Chargaff E: *Essays on Nucleic Acids*, Elsevier, New York, 1963. See also "Preface to a Grammar of Biology," Science *172*, 637-642, 1971

41. Watson JD, Crick, FHC: "A Structure for Deoxyribose Nucleic Acid," Nature *171*, 737-738, 1953

42. Watson, JD: "*The Double Helix*, Athenaeum, New York, 1968, p. 125. Notwithstanding this quotation it is clear that Watson and Crick were well aware of Chargaff's "rules" well before this time (Feb., 1953). The relationship of the calculations of John Griffiths on base-pairing, Chargaff's visit to Cambridge (June, 1952) and the "electric" effect on Crick of first learning of the complementarity relationships have been described in great detail by Robert Olby in "Francis Crick, DNA and the Central Dogma," Daedalus *99*, 938-987, 1970