**LAYING THE GROUNDWORK: LEVENE INVESTIGATES THE STRUCTURE OF DNA**

Meanwhile, even as Miescher's name fell into obscurity by the twentieth century, other scientists continued to investigate the chemical nature of the molecule formerly known as nuclein. One of these other scientists was Russian biochemist Phoebus Levene. A physician turned chemist, Levene was a prolific researcher, publishing more than 700 papers on the chemistry of biological molecules over the course of his career. Levene is credited with many firsts. For instance, he was the first to discover the order of the three major components of a single nucleotide (phosphate-sugar-base); the first to discover the carbohydrate component of RNA (ribose); the first to discover the carbohydrate component of DNA (deoxyribose); and the first to correctly identify the way RNA and DNA molecules are put together.

During the early years of Levene's career, neither Levene nor any other scientist of the time knew how the individual nucleotide components of DNA were arranged in space; discovery of the sugar-phosphate backbone of the DNA molecule was still years away. The large number of molecular groups made available for binding by each nucleotide component meant that there were numerous alternate ways that the components could combine. Several scientists put forth suggestions for how this might occur, but it was Levene's "polynucleotide" model that proved to be the correct one. Based upon years of work using hydrolysis to break down and analyze yeast nucleic acids, Levene proposed that nucleic acids were composed of a series of nucleotides, and that each nucleotide was in turn composed of just one of four nitrogen-containing bases, a sugar molecule, and a phosphate group. Levene made his initial proposal in 1919, discrediting other suggestions that had been put forth about the structure of nucleic acids. In Levene's own words, "New facts and new evidence may cause its alteration, but there is no doubt as to the polynucleotide structure of the yeast nucleic acid" (1919).

Indeed, many new facts and much new evidence soon emerged and caused alterations to Levene's proposal. One key discovery during this period involved the way in which nucleotides are ordered. Levene proposed what he called a tetranucleotide structure, in which the nucleotides were always linked in the same order (i.e., G-C-T-A-G-C-T-A and so on). However, scientists eventually realized that Levene's proposed tetranucleotide structure was overly simplistic and that the order of nucleotides along a stretch of DNA (or RNA) is, in fact, highly variable. Despite this realization, Levene's proposed polynucleotide structure was accurate in many regards. For example, we now know that DNA is in fact composed of a series of nucleotides and that each nucleotide has three components: a phosphate group; either a ribose (in the case of RNA) or a deoxyribose (in the case of DNA) sugar; and a single nitrogen-containing base. We also know that there are two basic categories of nitrogenous bases: the purines (adenine [A] and guanine [G]), each with two fused rings, and the pyrimidines (cytosine [C], thymine [T], and uracil [U]), each with a single ring. Furthermore, it is now widely accepted that RNA contains only A, G, C, and U (no T), whereas DNA contains only A, G, C, and T (no U)

**Strengthening the Foundation: Chargaff Formulates His "Rules"**

Erwin Chargaff was one of a handful of scientists who expanded on Levene's work by uncovering additional details of the structure of DNA, thus further paving the way for Watson and Crick. Chargaff, an Austrian biochemist, had read the [famous 1944 paper by Oswald Avery and his colleagues](https://www.nature.com/scitable/topicpage/Isolating-Hereditary-Material-Frederick-Griffith-Oswald-Avery-336) at Rockefeller University, which demonstrated that hereditary units, or genes, are composed of DNA. This paper had a profound impact on Chargaff, inspiring him to launch a research program that revolved around the chemistry of nucleic acids. Of Avery's work, Chargaff (1971) wrote the following:

*"This discovery, almost abruptly, appeared to foreshadow a chemistry of heredity and, moreover, made probable the nucleic acid character of the*gene*... Avery gave us the first text of a new language, or rather he showed us where to look for it. I resolved to search for this text."*

As his first step in this search, Chargaff set out to see whether there were any differences in DNA among different species. After developing a new paper chromatography method for separating and identifying small amounts of organic material, Chargaff reached two major conclusions (Chargaff, 1950). First, he noted that the nucleotide composition of DNA varies among species. In other words, the same nucleotides do not repeat in the same order, as proposed by Levene. Second, Chargaff concluded that almost all DNA--no matter what organism or tissue type it comes from--maintains certain properties, even as its composition varies. In particular, the amount of adenine (A) is usually similar to the amount of thymine (T), and the amount of guanine (G) usually approximates the amount of cytosine (C). In other words, the total amount of purines (A + G) and the total amount of pyrimidines (C + T) are usually nearly equal. (This second major conclusion is now known as "Chargaff's rule.") Chargaff's research was vital to the later work of Watson and Crick, but Chargaff himself could not imagine the explanation of these relationships--specifically, that A bound to T and C bound to G within the molecular structure of DNA.

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