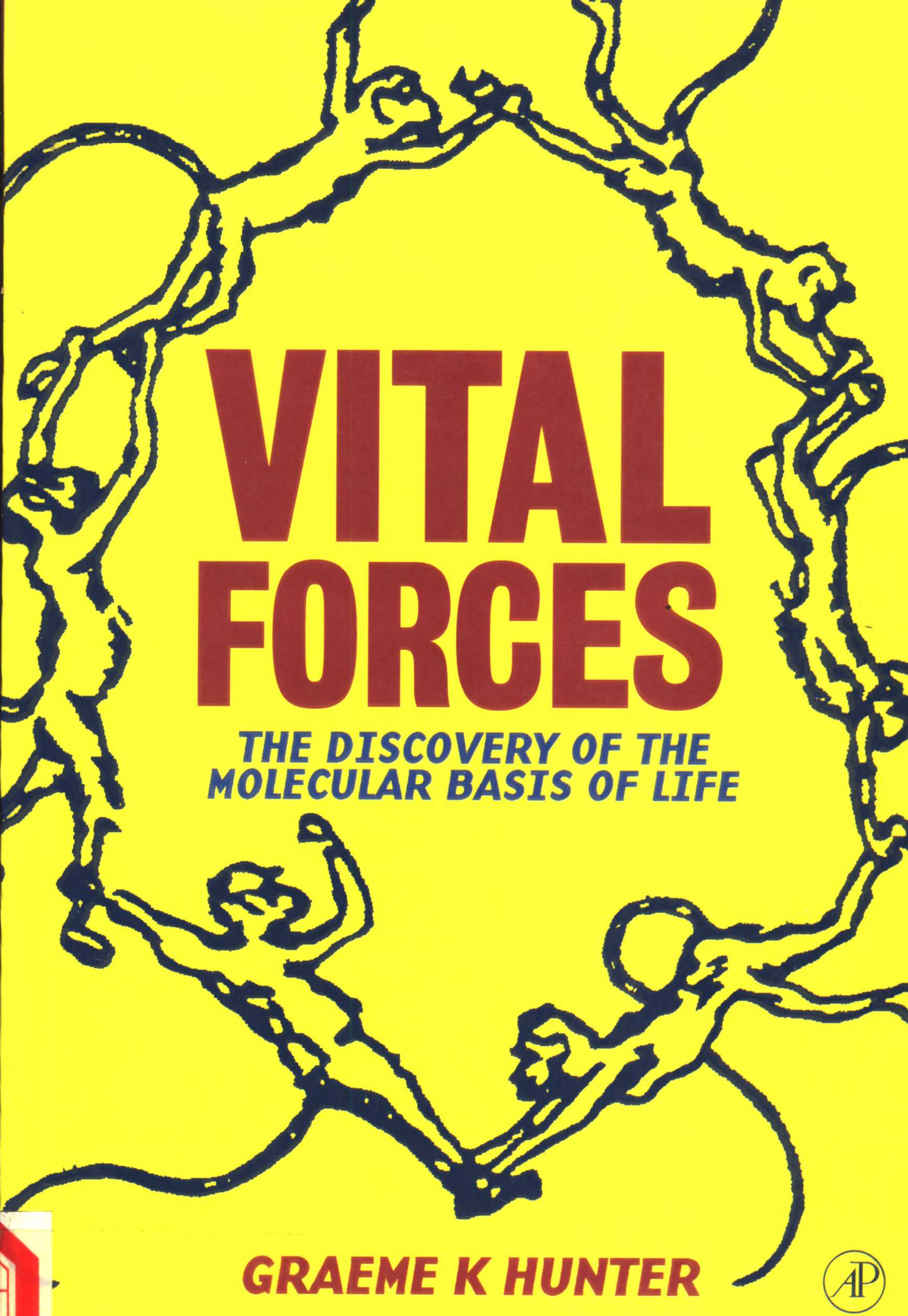

VITAL FORCES

THE DISCOVERY OF THE MOLECULAR BASIS OF LIFE

GRAEME K HUNTER

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To Miranda, Paola and Amelia

'The meanest living cell becomes a magic puzzle box full of elaborate and changing molecules, and far outstrips all chemical laboratories of man in the skill of organic synthesis performed with ease, expedition, and good judgement of balance.'

Max Delbrück, *A Physicist Looks at Biology* (1949)

List of Plates

Plate section appears between pages 204 and 205.

Preface

This is the story of a scientific revolution. It began around 1770, when the French chemist Antoine Lavoisier commenced the work that would demonstrate the common nature of living processes and chemical reactions, and ended around 1970, when the solving of the genetic code made it possible, in general terms, to describe the molecular interactions that underlie all forms of life. From the origin of chemistry to the advent of genetic engineering took only two centuries: Justus Liebig performed some of the first chemical analyses of organic molecules; his great-grandson, Max Delbrück, helped to determine the mechanism of gene replication. This 200-year period defines what I will refer to as the 'biochemical revolution'.

A revolution must be against something. The scientific revolution of the sixteenth and seventeenth centuries overthrew Ptolemaic astronomy and Aristotelean mechanics. The chemical revolution of the late eighteenth century overthrew the phlogiston theory of combustion and the four-element theory of matter. The biochemical revolution of 1770–1970 overthrew the vitalistic belief that the characteristic features of living organisms were manifestations of a special force operating only in living organisms and known variously as *pneuma*, *archeus*, *Lebenskraft*, *élan vital*, entelechy, 'biotonic laws', etc. By discrediting vitalism, the biochemical revolution achieved for biology what the scientific revolution had achieved for physics.

It is not difficult to see why belief in a vital force was so common throughout most of human history. After all, only living things exhibit such quintessentially vital properties as growth, reproduction, assimilation, sensibility and consciousness. Surely the laws that explained an overflowing bathtub or a falling apple could not also explain the nest-building behavior of a bird, far less the cathedral-building behavior of humankind. The 'natural' idea that separate laws governed the animate and inanimate worlds appears first to have been threatened by the chemical revolution: Lavoisier's rejection of the phlogiston theory was based on an analogy between respiration and combustion; his law of the conservation of matter was derived from the study of alcoholic fermentation; his chemical analyses revealed the common elemental compositions of plant and animal tissues.

In the 1830s, further cracks appeared in the foundations of vitalism when it became clear that the 'ferments' (enzymes) of living organisms had the same effects on reactions as inorganic catalysts. Like the findings of Lavoisier, this indicated analogy, not identity, between the living and non-living. Around the same time, however, the German chemist Friedrich Wöhler threw a bridgehead across the philosophical chasm by achieving a feat long thought to be impossible – the chemical synthesis of an organic molecule. By mid-century, the hope was openly stated that all the vital phenomena could be explained by physics and chemistry.

As more and more of the animate world fell to physical and chemical explanation, vitalism began its long retreat. It was never a rout, however; the adherents of vitalism periodically regrouped around a new 'special' form of matter – protoplasm, 'living protein' and, most importantly, colloids. Nor should it be supposed that the debate about the nature of life was one in which the vitalists were always in the wrong: Theodor Schwann's cell theory, Liebig's theory of fermentation and John Northrop's view of bacteriophage as autocatalytic enzymes are instances in which materialism was taken to excess.

Vitalism's last stand came in the quixotical quest by the physicist Max Delbrück, inspired by Niels Bohr, to find the 'paradox' at the heart of genetics. The discovery of the structure of the gene, its mode of replication and control over protein synthesis, completed by 1970, finally made untenable the belief that biological chemistry was fundamentally different from the ordinary kind. Delbrück himself had abandoned the quest in 1953, coincidentally at almost exactly the time the structure of DNA was solved, but another decade and a half would be required to work out the mechanism of gene expression.

The 200-year period of the biochemical revolution can be conveniently broken up into four periods in which different names were given to the chemical analysis of life. The first of these periods, from about 1780 to 1850, was that of 'animal chemistry', characterized by the elemental analysis of organic compounds. Progress in animal chemistry was made possible by the identification of chemical elements, the development and refinement of techniques for determining the elemental compositions of compounds, and the application of these techniques to a wide range of animal and vegetable tissues. The second period, that of 'physiological chemistry' (roughly 1850 to 1900), was characterized by the development of theories, particularly valence theories, describing the ways in which atoms were arranged in compounds, and the application of these theories to small organic molecules. By the end of the century, structural formulas had been determined for many classes of biological molecules, including the amino acids, simple carbohydrates and nucleic acid bases. The third period, that of 'biochemistry', about 1900 to 1940, was characterized by the analysis of the interconversions of simple organic molecules within living cells. However, the rediscovery of Gregor Mendel's laws of inheritance at the very beginning of the century, and the identification of chromosomes as the bearers of the hereditary elements shortly thereafter, brought the phenomena of inheritance and embryonic development within the ambit of biochemistry. This led to such important developments as the recognition that genes are composed of deoxyribonucleic acid (DNA) and that they function by directing the production of enzymes. The final stage of the biochemical revolution, the period of 'molecular biology' (about 1940 to 1970), was characterized by the structural analysis of complex organic molecules, in particular by X-ray diffraction techniques. The realization that proteins and nucleic acids were gigantic polymers suggested that many important properties of these molecules would be defined by their three-dimensional structures, and were therefore not amenable to 'biochemical' analysis. By 1970, the structures of at least some nucleic acids and proteins had been determined.

Even this brief summary of its main stages makes clear that the biochemical revolution of 1770–1970 encompasses elements of organic chemistry, physiology, genetics and physics. It would be a gargantuan task to write a definitive history of all the interwoven strands that resulted in our contemporary view of the molecular nature of life, and it is not attempted here. Rather, what I have tried to do is derive the historical origins of our understanding of the central mechanisms of transmission and expression of hereditary information. As it turns out, these mechanisms are essentially represented by the structure and function of proteins and nucleic acids: the nucleic acids acting as the bearers of hereditary properties; the proteins, generally speaking, by acting as catalysts for specific biological reactions.

Although it was not possible to talk about the chemical nature of the hereditary material or the mechanism of action of enzymes until, say, 1870, it was only because of earlier breakthroughs that such speculations were then possible. The discovery of the molecular basis of life therefore includes nineteenth-century attempts to determine the structures of organic molecules, which proved that life has a chemical basis, and to define the nature of fermentation, which showed that life chemistry was directed along certain channels by the action of specific organic catalysts.

In the course of the research for this book, there emerged two major themes that underlie the history of the biochemical revolution. The first is the distinction between genetic information and the structures formed when that information is expressed. In various forms, this concept arose from several scientific disciplines in a number of different countries. In the late nineteenth century, the French physiologist Claude Bernard attempted to explain the vital phenomena by distinguishing between the 'legislative' and 'executive' forces of living systems; the British 'student of heredity' Francis Galton concluded from his observations on familial resemblances that humans have both 'latent' and 'patent' characteristics; and the German zoologist August Weismann proposed that multicellular organisms arose by a division of labour between reproductive 'germ-plasm' and structural 'somatoplasm'. By the time the Danish botanist Wilhelm Johannsen formalized these distinction by introducing into genetics the terms 'genotype' and 'phenotype', there was already strong evidence to suggest that genes function by producing enzymes. Only in 1945, however, was this formally proposed, in the 'one gene–one enzyme' hypothesis of George Beadle. Enzymes therefore represented Johannsen's 'phenotype'. Around the same time that the one gene–one enzyme hypothesis clarified the nature of the phenotype came the first (modern) suggestion that the 'active ingredient' of genes could be DNA rather than protein. By 1953, when the double-helical structure of DNA was proposed by James Watson and Francis Crick, it was generally accepted that hereditary information was carried by nucleic acids. However, the final twist in this theme came in 1965, when John Kendrew, by restating the distinction between legislative and executive as 'information and conformation', incorporated the recognition that, whereas nucleic acids are linear information strands, proteins are three-dimensional, stereospecifically interacting molecules.

The second major theme of the biochemical revolution is the concept of the aperiodic polymer, or macromolecule composed of non-repeating subunits. This was first explicitly stated by Albrecht Kossel around the time that Johannsen proposed the terms 'genotype' and 'phenotype'. Like the concept of phenotype and genotype, Kossel's idea that complex biological molecules are composed of different arrangements of *Bausteine* (building blocks) also had its roots in the nineteenth century. The earliest glimmer of this idea can perhaps be discerned in Justus Liebig's 1846 disproof of the theory that all proteins contained an identical 'radical' to which various amounts of phosphorus and/or sulfur were attached. By the 1870s it had become clear that proteins consisted largely or entirely of subunits called amino acids, and that proteins from different sources contained different amounts of the various amino acids. When Franz Hofmeister and Emil Fischer proposed the polypeptide structure of proteins in 1902, the way was clear for Kossel to suggest that proteins were 'mosaics' or 'railroad trains' of amino acids. The importance of aperiodic polymers in genetics was only clearly stated in 1944, when the physicist Erwin Schrödinger proposed in his book *What Is Life?* that the gene was an 'aperiodic crystal'. Schrödinger's book attracted to the study of the gene both Erwin Chargaff, who demonstrated in 1950 that DNA, like protein, was an aperiodic polymer, and Francis Crick, who saw more clearly than anyone else the importance of aperiodic polymers in encoding hereditary information.

The year 1970 represents a watershed in the development of biological science because the breaking of the genetic code made possible a comprehensive description of the molecular mechanisms of life. The solving of the genetic code also represented a fusion of the two concepts discussed above. By 1953, it was clear that the genotype corresponded approximately to DNA, and the phenotype approximately to protein, and that both of these molecules were aperiodic polymers. The expression of phenotype from genotype therefore represents a translation of nucleotide sequence in DNA into amino acid sequence in protein. It took another dozen years to decipher the correspondence between what Crick called 'the two great polymer languages'. At that point, the aperiodic polymer and the distinction between genotype and phenotype became textbook information rather than research-guiding concepts.

In writing any historical work, certain stylistic choices have to be made. One of these concerns the amount of space to be allocated to the lives of the historical figures involved rather than the events in which they participated. In contemporary history of science, the biographical aspect is often minimized. No doubt this is a reaction against a regrettable earlier tradition of personality cults and hagiography. Taken to an extreme, however, the current historiographic fashion may create the impression that scientists are the helpless agents of social forces, and therefore their motivations and biases are irrelevant. In fact, scientific research, like any creative activity, is an intensely personal matter. Its protagonists identify closely with their findings, as exemplified by the *ad hominem* reactions of individuals such as Justus Liebig, Louis Pasteur and Linus Pauling to criticisms of their work. Such visceral reactions have, no doubt, affected the progression of scientific thought throughout history. The antagonism between the American chemists Phoebus Levene and Walter Jones may have been in part responsible for the slow development of nucleic acid chemistry in the early part of the twentieth century. Similarly, Max Delbrück's antipathy for biochemist John Northrop may well have contributed to his distaste for the reductionist approach of biochemistry. The poor relationship between the British crystallographers Lawrence Bragg and William Astbury may have resulted in John Randall, rather than Astbury, being awarded a biophysics unit and thereby hastened (or perhaps delayed!) the discovery of the double helix. Similar examples of personal factors impinging upon scientific 'progress' will be encountered throughout this book. In science, one might say, the personal is epistemological.

For this reason, I believe it important that history of science should recognize the human side of scientific research, and I have therefore attempted to describe the elucidation of the molecular basis of life in large part through the life stories of the scientists involved. In that sense, the present work owes more to Suetonius than to Tacitus. Clearly, two hundred years of science involve a large number of individuals. In order to keep the *dramatis personae* within manageable limits, I have concentrated upon the major figures involved. For the sake of narrative coherence, therefore, many peripheral events have been omitted.

In most cases, I have found myself in agreement with previous writers concerning the importance of individual scientific contributors to the history and prehistory of biochemistry and molecular biology. In some instances, however, I have been forced to conclude that the significance of a particular scientist or group of scientists has been overrated or underrated. In the former category are, for example, Delbrück, whose ability to inspire his fellow scientists seems to have been matched only by his unfortunate tendency to endorse erroneous theories, and Liebig, whose views on vitalism, fermentation and animal chemistry surely do not justify his traditional status as a 'father of biochemistry'. On the other hand, many workers emerge from this account with their reputations significantly enhanced. Among these are Torbjörn Caspersson, a crucial figure in the recognition of the genetic roles of the nucleic acids; Maurice Huggins, whose contributions to protein chemistry have been unfairly attributed to Linus Pauling; and Phoebus Levene, the traditional scapegoat for the failure to recognize DNA as the genetic material, but in fact a giant of nucleic acid chemistry.

Historians have long been aware of the fallacy of depicting the present as an inevitable consequence of the past. In the history of science, this Whiggish tendency may be influenced by the fact that the development of science, unlike the development of human civilization, is, in one sense of the word, progressive. One could make a good argument that Periclean Athens represented a higher form of civilization than the present-day United States of America, but it would be far harder to justify the view that the scientific world-view of a century ago was closer to physical reality than that of today. Even those philosophers of science who adopt the professional position that science is a purely cultural artifact with no basis in external reality do not scorn the use of synthetic drugs or electronic devices based upon the findings of that science.

In the history of science, therefore, the risk is not so much the glorification of the present as the oversimplification of the past. Even a cursory reading of the older scientific literature reveals a plethora of failed theories, forgotten controversies and obsolete terminology. Consider the following quote, from a 1902 paper by Leopold Spiegel: 'According to the side chain theory these enzymes act on added substances after having become attached to the haptophoric groups directly or by means of an amboceptor.' I imagine that no scientist working today would recognize the terms 'side chain theory', 'haptophoric groups' and 'amboceptors'. Similarly, the biochemist Malcolm Dixon wrote in 1974 of his early days in science: 'Some of us worked on things like gamma-glucose, pnein, physin and thio-X whose very names are now forgotten.' Indeed, the older scientific literature echoes with many such names, as alien and yet strangely familiar as the minor principalities of medieval Europe: inogen and biogen, chyme and enchyme, *Abwehrferment* and *Atmungsferment*, chromonucleic acid and plasmonucleic acid . . .

The paradox here is that any attempt to make sense of the past represents an oversimplification; but any attempt to present the past in all its complexity would not be history, not even mere 'chronicle writing'. I have attempted to steer a middle course by presenting, wherever possible, the major competing theories and the evidence upon which these were based. In the early twentieth century, for example, there coexisted at least three major concepts of protein structure: the polypeptide, colloid and cyclol theories. As described below, the polypeptide theory endured and its alternatives were discarded. However, by no means all scientific disputes are resolved by knock-outs. The nineteenth-century debate between Liebig and Pasteur over the nature of fermentation is a good example of the Kantian idea of thesis and antithesis resulting in a synthesis, as the eventual solution lay somewhere between the two positions. In other instances, biochemical thought advanced by a series of incremental stages. A good example of this is the series of 'factor theories' of inheritance in the late nineteenth century. The ideas of Charles Darwin, August Weismann and Hugo de Vries represent a progressive refinement of theory to conform more closely to experimental observation.

From consideration of controversies like these, two points relating to scientific method become clear: scientific theories are always based on incomplete information, and are therefore more inductive than deductive; and, as a corollary of this, most incorrect scientific theories are based on excellent reasoning. For example, compare Max Bergmann's periodicity hypothesis of protein structure (see Chapter 8) with Francis Crick's 'central dogma' (see Chapter 13). Based on the incomplete evidence available at the time, both theories were perfectly feasible. When it became possible to determine the amino acid sequences of proteins, Bergmann's hypothesis was discredited; when the mechanism of protein synthesis was worked out, Crick's hypothesis was supported. The essence of science is to make generalizations (hypotheses) from particulars (observations); in the case of all non-trivial hypotheses, however, it is more likely than not that a counter-example will subsequently invalidate the generalization.

Clearly, then, success in scientific research requires an element of luck. It would be absurd to conclude, however, that *only* luck is required. Some breakthroughs in the biochemical revolution involved a clear, even obvious, research plan, and a massive commitment of effort. Such instances include Levene's thirty-year odyssey in search of the chemical structure of DNA and Max Perutz's equally lengthy X-ray diffraction analysis of hemoglobin. In a similar conceptual vein, although on a smaller logistical scale, are James Sumner's crystallization of urease and George Beadle's *Neurospora* program.

In many cases, however, the defining feature of the successful scientist appears to be an intuitive sense of which alternative is likely to be correct. A good example of this is Lavoisier, who abandoned the phlogiston theory of combustion as soon as it came into conflict with experimental observations, while contemporaries such as Joseph Priestley and Henry Cavendish clung stubbornly to it. In some cases, scientific intuition can be caught in the act. Schrödinger's 'aperiodic crystal' and Pauling's 'conditions under which complementariness and identity might coincide' must have seemed oxymorons at the time. How can a crystal be aperiodic? How can identical molecules be complementary? In retrospect, however, it is clear that both Schrödinger and Pauling had partial insights into new forms of chemistry. Perhaps the best example from the biochemical revolution of a scientist operating on the intuitive level is Francis Crick. His studies on the genetic code reveal an uncanny ability to predict what experimentation would later demonstrate – almost as if he were in tune with nature to the extent that he could sense which mechanisms were 'biological' and which were not.

If a common factor among scientific revolutionaries can be discerned from the history of the biochemical revolution, it is that they are often newcomers to the field that they revolutionize. Lavoisier, Amedeo Avogadro and Mathias

Schleiden were lawyers who turned to science; Pasteur, Jacobus van't Hoff and Joseph Le Bel were in their twenties when they made their great contributions to stereochemistry; Delbrück, Schrödinger and Crick were imported into biology from physics.

Due acknowledgment of the achievements of scientific genius should not obscure the existence of scientific incompetence. The history of the biochemical revolution is littered with sloppy experimentation and lazy or self-serving reasoning. These include the preposterous metabolic schemes of Liebig's 'animal chemistry', the failure of professional botanists to match the rigorous experimental design of the amateur Gregor Mendel, the poorly controlled osmotic experiments that suggested a low molecular weight for proteins, and the speculative excesses of Astbury and Dorothy Wrinch.

However, the scientific method proved robust enough to eventually reject these experimental and conceptual failures. Nothing in science can be proved; nor do I believe, with all due respect to the philosopher of science Karl Popper, that anything can be disproved. The strength of science is that it is based on the reproducibility of observation. To be generally accepted, therefore, a scientific theory must be based on observations that can be replicated by anyone who has the appropriate equipment to repeat the experiment. It is this self-correcting mechanism, this constant reference to the external physical reality, that separates science from the humanities – and thereby distinguishes the history of science from other historical disciplines.

The identification of the pneumococcal transforming principle demonstrates in miniature many of the characteristics of scientific enquiry. It involved the chance observation that bacteria could be converted from one serotype to another; many failures in trying to produce transformation *in vitro* and in attempting to purify the transforming 'principle'; an eventual success involving careful experimentation and the use of newly developed techniques; a mean-spirited vendetta by a former collaborator; and a long struggle to convince the many skeptics.

The elucidation of the chemical basis of life, to the point that individual genes can now be altered at will, represents one of the most significant scientific achievements of all time. To the historian of science and the historically minded scientist alike, the origins of the biochemical revolution are therefore of great interest. This book is the story of the men and women, the theories and the experiments, the successes and the failures, that produced the modern conception of life as a molecular process.

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Vital Forces is a work of historical synthesis, not one of primary scholarship. The source materials consulted in the preparation of this book were all published ones: biographical writings, historical studies and the scientific literature itself. For this reason, I am indebted to those historians and scientists who have previously written on the history of biological chemistry. Historical and biographical works of particular interest or relevance are listed at the end of the text.

Finally, I wish to thank in advance any readers who do me the service of drawing my attention to any errors.

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Contents