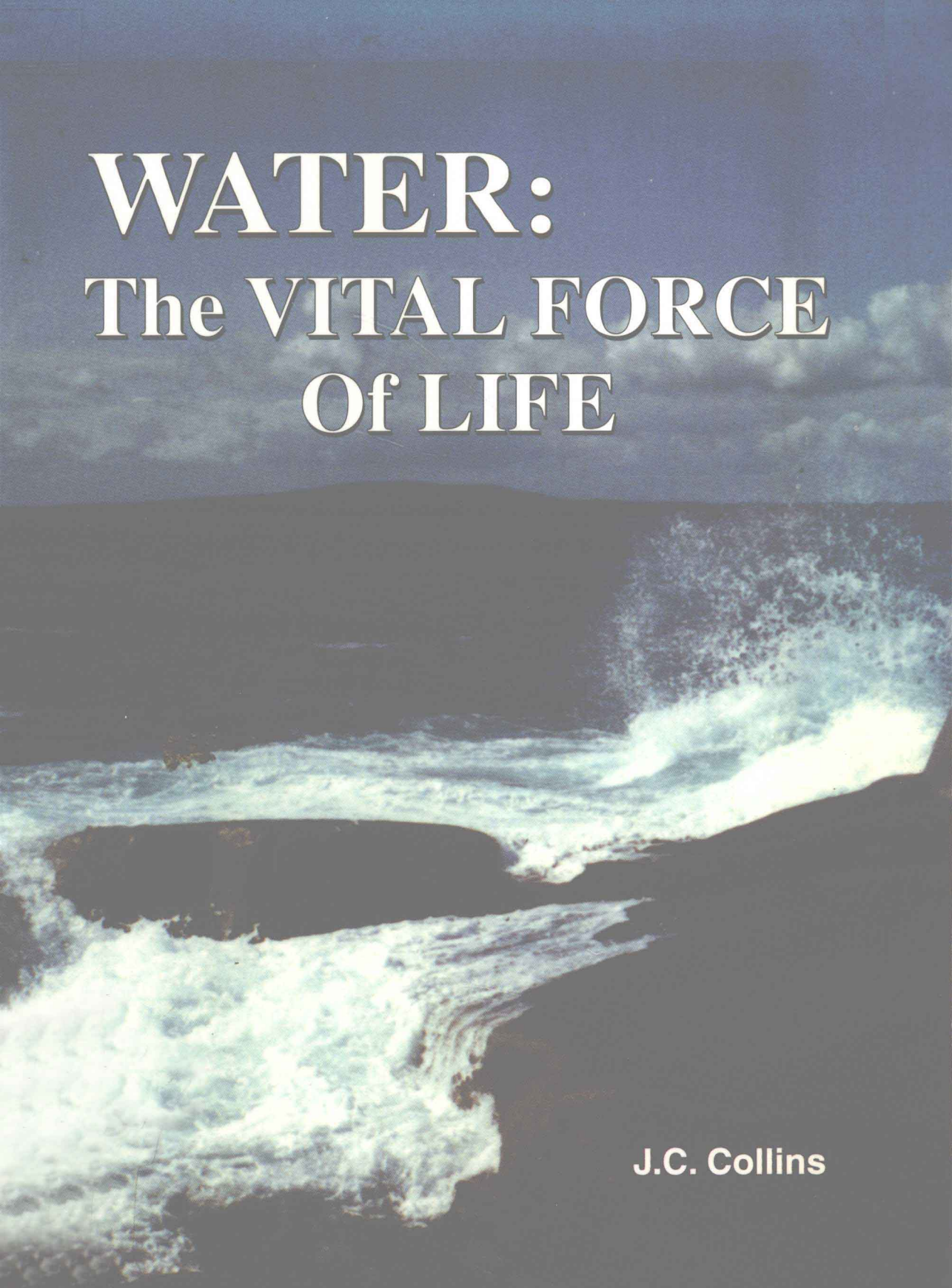
WATER:
The VITAL FORCE
Of LIFE
J.C. Collins

WATER: THE VITAL FORCE OF LIFE

A Primer on Water in The Living Cell

J. C. Collins

MOLECULAR PRESENTATIONS
KINDERHOOK, NEW YORK 12106

Dr. Collins received his degrees in chemistry from Wayne University in Michigan and the University of Wisconsin. After research employment at General Motors Research, E. I. Dupont and Sterling Drug, he accepted a position as Chairman of the Chemistry Department and Associate Professor at Illinois Wesleyan University. In 1967, he returned to Sterling Drug to direct drug research at Sterling Winthrop Research Institute until 1987 when he retired to devote full time to his driving interest in the role of water in natural molecular shape and function. He has a number of publications and patents to his credit and has had a synthetic organic reaction technique named after him. However, natural molecular shape and cellular hydration have been his primary interests for many years. In this summary of his studies, he provides a coherent explanation of how the dynamic properties of water regulate the motions and interactions of vital molecules in living cells.

All illustrations were developed on Apple™ Macintosh™ computers using PostScript™ in Adobe Illustrator™. Coordinates used for structural analyses and the preparation of drawings of crystalline proteins were obtained from the published literature. Proteins were rotated for spatial analysis using a program developed by the author. Custom physical model-building was performed primarily with Framework™ molecular model parts (Prentice Hall, Englewood Cliffs, NJ 07632).

First edition published 2000.

Library of Congress Catalog Card Number 00-90325

ISBN 0-9629719-2-8

This work is dedicated to the late
Professor William S. Johnson
The University of Wisconsin
Stanford University
and to
Professor Carl Djerassi
Wayne State University
Stanford University

CONTENTS

Preface

For over forty years, I have had the profession, the passion and the obsession of constructing three-dimensional models of the molecular components of living cells and of drugs that effect their function. Initially, models were constructed in preferred conformations using average bond lengths and angles. As more accurate structural information became available, they were modified to satisfy the new data.

At first, they were made with wax atoms in miniature sizes so that hundreds could be stored (in the cold) and periodically examined to compare dimensions and measure distances between polar atoms on their surfaces. Later, molecular model kits of various types were purchased and thousands of polystyrene balls. Finally, bulk quantities of *Framework Molecular Model* ® parts were purchased to construct custom models of proteins and membranes with as many as 9,000 atoms, once again to gain more experience in viewing, first hand, the many ways these molecules can form associations and binding arrangements.

Hypothetical binding sites were developed for drug and regulator molecules based on their three-dimensional models. As details regarding the actual binding sites became available, they were constructed and examined for interactions and binding dimensions.

As more and more molecular models of physiologically-active substances were examined, it became clear that certain distances between polar oxygen and nitrogen atoms on the ends of these molecules were multiples of about 2.3 angstroms - it was as if a ruler with units of this length had been used to "design" them. For a number of years, I wondered what this unit-dimension represented until one day an article on water attracted my attention. It included an illustration of a linear segment of water molecules hydrogen-bonded together showing the distance between every-other water molecule as 4.3 to 4.7 angstroms.

Further study revealed that both X-ray diffraction data and molecular orbital calculations supported the thesis that short, linear segments of water molecules with those unit lengths do, in fact, appear to form in liquid water adjacent to surfaces as transient, ordered units.

Once I had seen these correlations between dimensions in the natural molecules and water and continued to see them as more and more molecules were analyzed, I wondered about the significance and why such correlations had not been reported. On further searching, I found that a few had been observed but that no broad analyses had been performed. Numerous proposals for the nature of water in living tissues were presented in a New York Academy of Sciences Conference in 1973, but none explained the dimensional correlations I had observed.

The other question that perplexed me when I began giving oral presentations on the subject was: Why is it so difficult for people, including chemists and biologists, to visualize the three-dimensional structures of molecules? The answer came from a number of colleagues who said: "It is difficult for us to visualize spatial structures because we usually see them in two dimensions - on blackboards, in texts and articles. If you want us to understand your ideas, you will have to present them in book form so the structures can be studied and we can think about your concepts and interpretations."

Thus, in 1987 I retired, took courses in computer graphics and publishing and wrote the book, *The Matrix of Life.* The purpose of that first edition was to distribute copies to leading scientists in chemistry, biochemistry and cell physiology for a peer review of the concepts of *Transient Linear Hydration.*

In this formal presentation, I am providing a much broader view of the nature of water in the living cell. Hopefully, you will be able to visualize the structures, grasp the ideas and come away with a more thorough understanding of how living cells perform their vital functions and how natural molecules and water interact symbiotically to assure optimal efficiency and control.

But, before you begin reading, let me tell you what the book is not. It is not a scientific publication with footnotes and literature credits for every structure and idea presented. It is not a textbook of chemistry, biochemistry or cell physiology, although these subjects are included.

It includes as few scientific terms as possible to minimize the space required for definitions. It is presented with the understanding that the only three-dimensional model of a natural molecule most readers have ever seen in detail is the double helix of DNA.

It is intended to be a *Primer on Biomolecular Hydration* to illustrate how water integrates the motions and interactions of vital molecules to give us "life."

It is presented in three ways: 1) The Prologue is a brief historical background of concepts of spatial atomic bonding and basic principles of hydration. 2) The captions on illustrations in the text present the basic ideas and how they apply to all the major classes of molecules that compose living cells. And 3) The dialogue goes into further detail and describes how water may have been involved in the formative development of these molecules.

For those who become interested and wish to delve more deeply into background information, a list of important works and authors are included at the end. Unfortunately, so many individuals have made significant contributions to the life sciences, that it is impossible to include credits to all of them.

To some, personal thanks must be given. Foremost, to Professor William S. Johnson to whom this work is dedicated. He gave me my first real glimpse of molecules – of their intricate design. He gave me an appreciation for the smallest detail in structure that might provide a clue as to how the substance might be synthesized and how nature might have done it as well. He passed on his love for chemistry to countless students and collaborators who were fortunate enough to work with him.

Second, my thanks go to Professor Carl Djerassi to whom this work is also dedicated. He gave me my first taste of research and directed me to chemistry as a career and set forth the example of boundless enthusiasm and productivity. Third, to Dr. Sydney Archer who gave me my first glimpse of how the molecules of life interact with each other to make us well or make us ill - of the world of drug molecules and how they effect cell function.

It was those interactions that prompted me in 1960 to set off on a quest to identify critical dimensions in regulator molecules - hormones, neurotransmitters and intracellular messengers. Over five hundred molecular models of natural substances and synthetic drugs were constructed and analyzed in a search for common structural and spatial features.

It was during that search in 1967 that the initial realization came that these regulator molecules appeared to be mimicking various combinations of water molecules bonded together and that all biological molecules might be doing this in varying degrees; that, in a sense, they might be spatial analogs of the environment in which they evolved. On expanding the model-building, a broader, more comprehensive picture emerged, but one which held to the same basic principles.

What began as a search for dimensional similarities between natural and drug molecules appears to have yielded some of the basic principles of hydration that govern the interactions of all molecules within living tissues.

Before we begin our adventure together, I must also thank my many colleagues at Sterling Drug who encouraged me to continue the venture: Dr. Frederick Nachod who felt I should promote my ideas on hydration more aggressively; Drs. Guy Diana, Roman Lorenz, Denis Bailey and Bob Clarke who, even though they did not always share my views, offered encouragement; Professor Ronald Breslow who, in the early years, offered valuable advice; Dr. Ted Miller who encouraged me to put the ideas down in book form and Mrs Betty Bruso who prepared a number of interim manuscripts for review by experts in a variety of disciplines. Thanks as well to Dr. Stanley Moore and Mrs. Mindy Potts who assisted in reviewing manuscripts. Last, but not least, my thanks go most sincerely to my wife, Betty, and the family who put up with my nightly vigils and molecular drawings and models all over the house.

If you find the reading and viewing of what follows rewarding, your thanks should go to all those whose love of molecules and nature made this work possible.

J. C. Collins
January, 2000

WATER:
THE VITAL FORCE OF LIFE

The Prologue

WATER: THE FORM-BUILDING FORCE

Within water, all life emerged – without it, no life, as we know it, is possible. It was within aqueous space that all of molecules of life first formed and within that same space that they function today. If we are to understand how they move and interact in such a miraculous fashion, we must begin with the environment that gave them birth.

The concept that water holds some of the basic secrets of life is not new - antiquities recorded that living forms emerged spontaneously from it and unceasing efforts have been devoted to the task of defining the properties that make it such a unique, life-forming, life-sustaining medium.

Postulates have been advanced that liquid water contains regions of ice to provide order. Some suppose that water within the cell has some "vital force" or "cardinal principles" not possessed by normal water; that it is these principles that permit it to exhibit its unique life-giving properties.

Matthias Schleiden, who, in 1838, first proposed that cells are the fundamental units of life, also proposed that life processes are manifestations of a "form-building force" which permeate the whole of nature. If that "force" is resident in any one ingredient in the cellular world, then it must be in the water. But how can such a simple liquid as water provide a "form-building force"?

For some time, it has been known that water is unique in its effects on the three-dimensional shapes of natural molecules. For example, the cylindrical, ordered structure of double-helix B-DNA, which was first proposed by Watson and Crick in 1953 and which rapidly became the symbol of modern, molecular biology, exhibits its unique regular, helical form only when surrounded by water. If the strands are dehydrated, they, like most other polymeric constituents of living tissue, lose their orderly structure and adopt more random shapes and forms.

In spite of the fact that water is critical to the spatial forms and functions of natural molecules, it seems to have been ignored in the field of structural, molecular biology. Natural molecules are never pictured with water surrounding them. Water is accepted as a vital component and critical to function, and yet, no comprehensive concepts have been advanced to explain how it performs these functions.

In a recent book, *The RNA World,* evidence is presented to suggest that living forms have existed on the earth for more than 3.85 billion years; this leaves only about 600 million years for the evolution of the molecules which composed those early life forms. Since this is an extremely short period of time for these unique and complex molecules to evolve and assemble, the authors refer to their spontaneous development as a "near miracle."

In fact, if it had not been for the ordering and regulating properties of the aqueous environment in which they developed, it would have been an "absolute miracle." What is truly amazing, is that the natural sciences continually seem to ignore the fact that water holds the answers to many of the questions regarding the formation, motions and interactions of naturally-occurring molecules.

As Nobel Prize winner Albert Szent-Györgyi expressed it: "Water is the *mater* and *matrix*, the mother and medium, of life." If we wish to comprehend the nature of life, its molecular roots - how it moves and flows in such an orderly and spontaneous fashion to sustain and reproduce itself - we must begin with water.

WHAT IS WATER?

We all know that water is composed of small molecules with two atoms of hydrogen and one of oxygen. We also know that its molecules are flying around at great speed, occasionally taking off from the surface of the liquid to become gaseous water vapor.

But, before we get into a detailed, scientific description of liquid water and how it most likely performs its functions, it is important to keep a simple anthropomorphic analogy in mind. Water is to a vital molecule like a mother is to her child: a child to whom she has given birth. The child is full of energy, it moves its arms and legs in uncoordinated fashion - its motions uncontrolled and unconditioned. But, the mother is not concerned - she knows her child - she permits it to move in whatever way it wishes. But, she continually brings it back to positions of comfort

and control. She holds the child gently in her arms so that its body follows the contours of her own; she provides for comfort, security and control.

Water is the mother of life. She knows her children - she formed them in her own image. And yet, they are free: free to develop into new forms, free to evolve into more versatile, useful forms. Water continually provides the energy and means to freedom, but it also continuously brings components of living tissue back into control, back into harmony with all systems involved. The question we hope to address within these few pages is this: How does water permit freedom, while, at the same time, maintain order?

Genetics defines what molecules are produced; water defines how they move and associate with each other.

SPONTANEOUS SHAPE AND FORM

Living cells function at such high levels of efficiency and order, that there must be some global principles of spatial regulation operating within them to limit the options of motion and form.

For many years, it was felt that living cells contain some sort of shaping or patterning codes to direct the folding of polypeptide, polysaccharide and polynucleotide strands into their unique, functional forms. However, it is now quite clear that all the information needed to direct these molecules into their functional shapes is resident in the molecules themselves: in their associations with each other and the surrounding aqueous medium.

It is the structural units that compose these polymeric strands, operating in concert with water, that determines the shapes they adopt. In fact, the wrapping of these vital molecules into their unique, functional forms does not require the cell at all: wrapping occurs spontaneously whenever they are maintained in an aqueous environment which simulates that within the living cell.

It appears that the molecules themselves are the "code" and water the guiding, spatial matrix in which that code is expressed. As long as the environment and temperature are maintained as they are within the cell, even the most complex components appear to assemble themselves into the same unique, functional forms that exist within living cells.

In some cases, specialized, "chaperone" molecules assist newly-formed strands from getting tangled and guide them into preferred shapes, but it is the molecules themselves that "know" what shapes they can adopt in water. If concentrations of salts (positive and negative ions) surrounding these molecules are varied to simulate the changes that occur within living cells, the shapes of molecules change in the same fashion as they would within the cell.

In spite of the fact that an incredible volume of information has been assembled, liquid water is so ubiquitous in living tissues and its interactions with molecules so rapid and dynamic, that it has been extremely difficult to develop a coherent interpretation of how it exercises spatial control. The prevailing view within the sciences is that water simply bonds to molecules in unique ways, that it has no uniform ordering properties and that we should not bother to look for them.

RANDOMNESS AND ORDER

One of the unique qualities of life is that of self-assembly. In contrast to the inert world around us, the molecules of life assemble and multiply themselves. Common experience tells us that, over time, everything tends to degrade and fall apart. If we build a house or manufacture an automobile, it must be maintained or it will deteriorate, eventually returning to the molecular dust from which it came.

We say that "nature takes its toll." Stated in terms of the second law of thermodynamics: "spontaneous processes move from order to increased randomness and freedom." The paradox is that "nature," in moving from dust to extremely complex, highly-integrated organisms, like ourselves, has moved from disorder to order, from small molecules to large, from states of low integration, to organisms that are so highly organized that they are aware of their own existence.

Biological evolution and life itself appear to reverse the direction of spontaneity toward self-assembly and integration. It is this self-sustaining, self-assembly property, perhaps more than any other, that differentiates the animate from the inert.

Of course, it is the energy of the sun that drives this process of assembly. Energy absorbed by plants powers the conversion of carbon dioxide and water to sugars and sugars to polysaccharides

like starch and cellulose. Random, smaller molecules are combined by electronic bonding to give larger, more integrated forms.

Energy from the sun, in tying more and more atoms together to form complex molecules, reduces their options to move freely in space. Atomic and molecular freedom give way to molecular order. In physical terms, the "entropy" (freedom) has been decreased – solar energy has moved it toward order – the opposite direction from freedom, disorder and spontaneity.

As the sun powers the formation of electronic bonds between atoms and pulls them into more ordered associations, energy is stored within the bonds. When wood burns in air, a portion of that electronic bonding energy is liberated in the form of heat (molecules move at higher speeds), while another portion is used to provide rotational spin to the smaller molecules that are formed. Both processes move the atomic components of wood spontaneously in the direction of freedom to drive the combustion.

On the other hand, when sugar molecules are "burned" within a cell, they are taken apart piece by piece. At each stage, small losses in energy and increases in freedom drive the formation of new bonds between atoms to attach other molecules together: to attach charged groups (particularly negatively-charged phosphates) to the surfaces of proteins to change their shape and associations - to move charged ions from one region of the cell to another.

Thus, it is the systematic and orderly release of bond energy and the increase in freedom of the atoms of fuel molecules, like glucose and oxygen, that build new molecules and move an arm and a leg. But, it is the persistent and dominating order of environmental water that provides the spatial discipline and directed force for self-assembly and integrated function between the multitude of molecules involved.

WHERE ARE WE IN OUR QUEST?

Where are we in our quest to translate the systems of life into molecular terms? If we pause to look at where we are and how we arrived at the present state of our knowledge, I think we will be able to see why the question of the role of water in regulating molecular shape and function has not been resolved.

Since the beginning of scientific studies on natural, biological forms, the physical and chemical goal has been to identify individual molecular constituents and define their roles within the cell. To accomplish this, each individual constituent had to be separated from all others, purified and dried. The objective was to obtain each component as a single, pure entity which could be analyzed in detail for its properties as a pure substance.

Based on spatial molecular theory, as originally proposed by van't Hoff and Le Bel in 1874, molecules were considered to be three-dimensional entities with constituent atoms bonded to each other at particular angles in space. At the time, there was no experimental way to validate their hypotheses or determine the precise location of atoms within molecules. The only test of the proposed, hypothetical models was to use them and see if they provided rational interpretations of the properties of the substances. Acceptance of the basic principles permitted development of chemical formulas for pure substances that could be used systematically to record changes in atom-bonding arrangements as one substance was converted into another.

Symbols like H_2O, permitted the classification of a vast number of the smaller, molecular constituents of plant and animal cells. In addition, the formulas permitted chemists to develop rational syntheses of molecules that had never existed before and to begin to understand how changes in structure effect physical and chemical properties. The fact that they did not know precisely where the atoms were in space did not deter them from achieving absolutely amazing progress and the assembly of an incredible body of knowledge about natural and synthetic substances.

It was not until the advent of X-ray crystallography in 1912, that there was an experimental way of determining the precise location and arrangement of atoms within molecules. But, it was not until 1931, when Linus Pauling published his thesis, *The Nature of the Chemical Bond,* that molecular theory was refined to accomodate crystallographic data on bond angles and lengths. It was that momentous contribution, that awarded Professor Pauling his first Nobel Prize and ushered in the use of accurate three-dimensional models to represent molecules.

During the decades that followed, the fields of synthetic organic chemistry and biochemistry literally exploded with studies on thousands of naturally-occurring substances to deter-

mine precise, spatial structures and synthetic pathways by which they are produced within living tissues. From this gigantic effort came the realization of just how extremely selective and conservative living cells are in their production of structural and spatial, molecular forms.

Whereas a given synthetic pathway in a cell might produce one or two spatial forms, a chemist in the laboratory could produce hundreds of structures with the same basic chemical formula but with atoms attached in many different ways. Furthermore, the cell, at each synthetic step along the way, produced only the single structural intermediate required for the next step. A chemist in the laboratory, using the best synthetic methods available, might be able to obtain a 60 or 70% yield of a desired chemical intermediate. Even then, it might be a complex mixture of substances with the same formula, requiring complex methods of separation before the next step in the synthesis could be performed.

During this same prolific period, it was discovered that chemical reactions in the cell are accomplished in clefts in the surface of large polypeptide (protein) molecules. Reacting molecules bind in these clefts in such a way that the bonds between particular atoms are broken and new ones are formed. These "enzymes," as they are called, bind the atoms of molecules in precisely the proper, spatial relationships to take them apart and put them together. Often, the molecules are twisted in the binding sites to accomplish the reactions.

As one might expect, these "catalytic" enzymes are extremely specific in binding only particular molecules: they bind only those forms with the proper external spatial shape and distribution of atoms on their surfaces. If a molecule with a different, internal arrangement of atoms but similar, external shape is presented to an enzyme, it might bind in the same cleft with the same affinity, but will not undergo a reaction because the internal atoms are in different locations. It will prevent the enzyme from performing its normal function of converting one molecule to another.

Thus, living cells not only produce the molecules they need using enzymes, they also produce molecules that block other enzymes. By mimicking the external features of other molecules, these agents block normal metabolism. Employing this mechanism, antibiotics produced by one micro-organism kill other micro-organisms: they block the synthesis of vital molecular components. Many hormones and medicinal drugs exert their pharmacological effects in the body by slowing down or blocking particular physiological processes to relieve pain or inflammation or to restrict cell function in specific ways.

One approach used in the medicinal sciences today to find new, more selective drugs to treat specific disorders is to identify the precise enzymatic or regulator systems involved and then attempt to block them with synthetic molecules: to bring them back into control. As a consequence of these detailed studies on drugs and hormones, we now know how extremely critical the three-dimensional spatial form of a molecule is for it to bind selectively to a given enzyme or functional protein - to change the shape of that protein - to turn its functional properties on or off.

Since enzymes are so specific and hold reacting atoms in such precise, spatial relationships, they permit reactions to proceed thousands or millions of times more rapidly than they would if the enzyme were not present. This permits cells to produce molecular parts extremely rapidly at normal temperatures.

At one time, it was felt that enzymes perform reactions at maximum speed. However, we now know that many enzymatic reactions are catalyzed more rapidly in solvents other than water or in water containing other solvents. In fact, water appears to constrain enzymes in some way: to "control" reaction rates. Furthermore, it was discovered that water, in some way, also controls which molecules bind to the reaction sites. If water around enzymes is denatured by the addition of other solvents, molecules with a much broader range of spatial structures can be bound. Thus, surface water, in some way, regulates not only reaction rates but the shapes of the binding sites themselves.

But, water not only influences the shapes of sites where molecules are produced, it also is intimately involved in guiding the molecules that are produced into their final shapes. This is particularly true of the long filamentous polypeptides which are synthesized in the cell in specific sequences using about twenty different amino acid (peptide) units. Polypeptides are like long sentences composed from an alphabet of