SOME FUNDAMENTALS OF PROTEIN CHEMISTRY

WITH SPECIAL REFERENCE TO ADDITIONAL SYNTHETIC SUBSTANCES FOUND BY THE FLOCCULATION TEST TO HAVE POTENTIAL THERAPEUTIC VALUE

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Chemistry of the living organism in recent years has been the subject of intensified study. Biological experimentation has passed from the crude stimulation of muscles by a drug to electronmicroscopy of the genes and cytoplasm of the cells. These are the substances which control the processes of life. It has been found that these fundamentals of life can be resolved into molecular structures. As a result of this information, it is known that the origin of cells begin, then, with a combination of properly arranged molecules to form the complete normal cell.

However, not all cellular development is normal. Any molecular deflection could lead to untold pathology, but physiological processes are constantly on the alert. Some are stimulated by acute infections. For instance nature may have designed the common cold or other acute infections as a physiological safety mechanism, manifested usually by a fever and accompanying symptoms. If severe, it creates a rapid catabolic reaction breaking down unessential cells and abnormally formed protein molecules. In this way it assists in eliminating from the system excess waste materials and possible disease-producing substances, with a consequent reorganization and reestablishment of more normal cellular construction. If the patient recovers completely, he should experience improved health following the attack. From this point of view any speculation about the prevention of the common cold and grippe may be considered hazardous for future health. The aim for health is, after all, normal physiology, and this is dependent on normal chemistry.

Physiology is the function of the living organism and its parts. Into each part goes a multiple complexity of cells. Into each cell go thousands of different proteins to make up that living cell. These proteins perform a great variety of different acts in exact sequence to cause the cell to live. They are giant molecules of great size, complexity and diversity.

Chemical understanding of living matter did not begin until 1828, when the first test-tube synthesis of an organic substance, urea, was made by Friedrich Wohler of Germany. A large number of comparatively simple organic molecules such as sugars, alcohols, soaps, fats, fruit acids, the coal and petroleum hydrocarbons, and so on, have been worked out by his successors; and during the past century all over the world scientists have become absorbed in organic chemistry. Thus, organic chemistry defined the structure of simple organic substances. It led to the classification and properties of more complicated organic substances and to the birth of new synthetic products, such as dyes, perfumes, drugs, fuels, etc.

But the more complicated related members of the organic group, such as proteins and the rest, could not be analysed by the same methods. That is, the chemists could not separate them by solution, melting, crystallization and the like. For instance, cellulose, the chief component of wood would not melt, but hardened and decomposed when heated. This was also true of wool, silk, starch, and rubber. Occasionally, organic chemists have accidentally produced large organic molecules, but when this has happened, they were always disappointed because the materials were always resinified, waxy, gluey or sticky and could not be purified to a clear crystalline substance.

So the chemistry of macromolecules was not seriously attempted until after 1920. There had been some attempts at chemical analysis of cellulose, rubber, starch, and proteins after 1880, but the only conclusion was that they were composed mostly of carbon, hydrogen, and oxygen. As investigators further studied the more complicated compounds, they decided that the main distinguishing features of these substances, which makes their properties so different from other organic materials, must be the size of their molecules. These big molecules became known as giant molecules, or high polymers (polymer, from the Greek, "many parts"). It was then deduced that they were made up of smaller molecules of certain units, known as building blocks and called monomers, "a building unit." These building blocks or units are of three different kinds—simple sugars in cellulose and starch, isoprene in rubber, and amino acids in proteins. A high polymer, then, is a complicated chemically-arranged combination of monomers.

While polymers, such as rubbers and plastics, have been made on a commercial basis from late in the nineteenth and early twentieth centuries, they were all made empirically. The principles governing the structure and behavior of polymers were still unknown. The chemists knew something of the "how" to make them, but did not know their chemical construction. In the late 1920's investigation moved rapidly ahead on a broad front. Polymerization processes were developed and refined. It was found that polymers followed a general pattern in their formation. Basically, the high polymers and co-polymers (co-polymers are polymeric chains in which more than one kind of monomeric unit occurs) are formed by the linking of monomers end-to-end in chains, sometimes several thousand units long. The chains, then, become grouped in two different ways: coiled to from a ball-shaped molecule, or straight in more or less rigid bundles. While the monomers hook themselves end to end to each other they take on different arrangements, such as atactic (groups arranging themselves at random), isotactic (all of the groups lie on the same side of the chain), or syndiotactic (the groups alternate from one side to the other in regular order).

There are two methods in the formation of polymers. One is by condensation, the other by addition. The first procedure is rather slow and tends to stop before the molecules have attained a truly giant size. The second method can produce giant molecules of almost unlimited size. They add one monomer at a time and rapidly build a chain, which in theory can go on growing indefinitely as long as the supply of building blocks holds out. These do not have built-in chemical hooks, as the condensing monomers do; instead, the hooks have to be generated. There are various ways of generating such a hook ; each requires a catalyst or enzyme. For example, a single active catalyst can polymerize monomers at the rate of 250,000 per hour. Giant molecules can be synthesized more rapidly than it is possible for a living organism to produce them. Also, however, these manufactured complexes bear no exact basic resemblance to a living organism. A living creature builds its high polymers precisely tailored according to a set design by means of a catalyst or enzyme. Nevertheless, it has also been observed that a molecule growing from a catalytic complex on the surface of a crystal is like a hair growing from its root ; that is, the monomers are added at the root. So in this way it may be said, there is some resemblance to living tissue.

Living cells consist principally of protein giant molecules. Thousands of different proteins go into the make-up of a living cell. They perform thousands of different acts in the exact sequence that causes the cell to live. It is an exquisitely subtle and enormously involved process. It is known that these are giant molecules of great size, complexity, and variety, and each appears designed with high specificity for its particular task.

Proteins, like synthetic high polymers, are chains of repeating units. The units are peptide groups, made up of the monomers called amino acids. There are over 25 amino acids, each with a distinguishing cluster of atoms as a side group around a certain identical group. These groups link to form the peptide units and in turn the peptides link to form the polypeptide chain. Proteins, then, are polypeptides of elaborate and very specific construction. Each kind of protein has a unique number and sequence of side groups which give it a particular size and chemical identity. Another distinction of proteins is that they are apparently folded in a unique configuration which they seem to maintain, as long as they evidence biological activity.

There are also patterns in which proteins or polypeptide chains become twisted to form a helix. Collagen, the protein of skin, is said to come from three polypeptide helixes twisted around one another. Also nucleic acids, the substance of genetic chemistry, have been shown to have the structure of a double helix. It has been demonstrated that the collagen molecule is extremely long and thin and is the most asymmetric molecule yet isolated. When a solution of collagen is just slightly warmed, the rodlets of the collagen are irreversibly broken down. The solution will gel, and the product is gelatin. The reason the dissolution cannot be reversed was made clear when it was found that the molecules in the warmed up solution had a weight of about one third that of collagen. It appeared that the big molecule of collagen had broken down into three polypeptide chains. Collagen of the skin is irreversibly broken down in a first degree burn.

We now pass from proteins to nucleic acids. Proteins may be classed as the principal materials of life and the nucleic acids as the blueprints of life. There are two kinds of nucleic acids: desoxyribonucleic acid and ribonucleic acid. The first, or desoxyribonucleic acid, is always found in the nucleus of the cell; the second, or ribonucleic acid, lies mainly in the cytoplasm outside the nucleus. They are known as DNA (desoxyribonucleic acid) and RNA (ribonucleic acid). Chemically, they are very similar. Each consists of a long chain of phosphate and sugar molecules with small side groups, called bases, attached to the sugars.

In DNA the sugar is desoxyribose; in RNA it is a slightly different molecule called ribose. One of the four bases is different in each. DNA contains adenine, guanine, cytosine and thymine. RNA contains, adenine, guanine, cytosine and uracil. The bases do not follow a regular order and it is believed that the sequence in each case has a particular meaning and determines the function of the molecule.

Electron-microscopic studies have shown that DNA is actually a double molecule with one chain twisted around the other in helical fashion. While there is less known about RNA it is thought to be somewhat similar in design, although X-rays suggest a more irregular structure.

There are many reasons to think that DNA is either the genetic material of life (what used to be called the genes) or an important part of it. DNA is always associated with chromosomes and not with any other part of the cell. Each set of chromosomes seems to have a fixed amount of DNA.

Many experiments that led to these findings were done on bean seedlings. The advantage of bean cells for such experiments is that their chromosomes are very large, so that under a microscope it is possible to see their various parts and to catch them in the act of dividing.

Chromosomes live and are stable in a particular chemical environment in the nucleus of the cell. It has been found that a change in this immediate environment may alter not only the structural relationship of the molecules but also their biochemical and genetic activity. It has also been found that the DNA can be dissolved out of the nucleus of the cell by a salt solution. This is a very weak chemical treatment, yet it effectively breaks the bonds that link the DNA molecules to protein molecules and to one another. The fact that these bonds can be broken so easily is a strong argument against highly concentrated drug therapy.

The microsome is a combination of protein and ribonucleic acid, RNA. It is a small particle found in the cytoplasm of the living cell. It is theorized that the nucleic acids control the making of each organism's characteristic living substances—its proteins. DNA bears the master plans, carrying them on from generation to generation. RNA constitutes the working copies used in the actual synthesis of the proteins. This production is controlled in a rather straightforward way by the sequence of bases in each nucleic acid, which determines in what particular order amino acids will be assembled in the polypeptide chains that make up the protein. The order is all-important, for it governs the character and the function of the protein.

As a broad outline, this hypothesis seems correct. It makes sense of an enormous body of biochemical facts, and it has the characteristics of soundness which we associate with simplicity and generality.

In considering the function of RNA, it is found mainly in combination with protein in particles in the cytoplasm of the cell. These particles are believed to contain the templates on which specific proteins are molded. In this way the newly formed protein is dictated by the RNA which is a part of it.

The hemoglobin of horse's blood is not exactly the same as that of human blood, and the insulin molecule, also, differs slightly in the various animal species. It has been discovered that sickle cell hemoglobin (a fatal disease) is exactly like normal hemoglobin, as far as can be shown, except for a single alteration: in normal hemoglobin, with many properly-arranged amino acid units, about 300 in all, there are two glutamic acid units; in sickle cell hemoglobin, one of the glutamic acid units has been displaced by a valine unit. It is interesting that a change in one amino acid out of approximately 300 results in the fatal sickle cell condition. It is even more interesting to see that a gene, or the DNA, can control such a small change. Genes not only work powerfully but they can also work delicately.

From every point of view biology is getting nearer and nearer to the molecular level. Here, in the realm of heredity, we are dealing with polymers, and reducing the decisive controls of life to a matter of the precise order in which monomers are arranged in a giant molecule. Proteins and nucleic acids are two kinds of natural high polymers or giant molecules. They are organized in the cells and tissues of living organisms and can be considered monomers of larger structures. These macromolecules then organize themselves into the precisely specialized structures of cells and tissues. It is apparently a spontaneous process and depends on the specific properties built into the molecule.

These molecules, called the giant monomers of tissue construction, may be polymerized where they are made or they may be transported in inactive form to another part of the organism and there be activated and polymerized as the occasion demands. For example, the soluble protein, fibrinogen, is always present in the blood, but it is polymerized into the insoluble fibrin of a blood clot wherever bleeding occurs.

The function of natural high polymers varies according to their properties. The protein, keratin, is the principal substance of hair, horn and fingernail, and has great tensile strength. The protein, collagen, is found in the skin and tendon, and also in bone, teeth, and loose connective tissue. Elastin is a springy protein of ligaments, connective tissue, and elastic fibers. These polymers are more or less passive. Others, such as muscle protein, are active and change under chemical or other stimulating influence. Contraction is a property of a great many biological structures besides muscle, such as the oscillating tail of the sperm cell or the flowing pseudopods of the amoeba, and all of them may have a common molecular mechanism.

There are other high polymers of enormous lengths as compared to width. They are the fibrous protein of nerve cells, called neurofibrils. They are very long and less than 30 or 40 angstroms wide (the angstrom unit is a hundred-millionth of a centimeter).

Another class of proteins are the antibodies. These are body proteins resulting from the reaction of a particular foreign molecule to an organism's cells. They are so specialized that they react with just one counterpart and no other substance.

Chemistry of living matter involves many substances, and the specificity of reactions sets the molecules of biochemistry apart from those of ordinary chemistry. Inorganic chemicals react by classes, as an acid reacts with a base, regardless of composition or structure. A biological molecule, on the other hand, has a built-in capacity to recognize and react with a particular molecular structure, which makes possible the great variety of chemical reactions that constitute the processes of life. Chemical specificity of great complexity is one of the most important studies in biology, for it holds the answers to such vital questions as why a certain virus infects only certain tissues of an organism, or why certain drugs show the same selectivity.

In previous publications, work on several classes of substances used in the flocculation test has been reported. Various substances that were reported, in addition to the homœopathic remedies, were the amino acids, the fatty acids, food allergens, some animal allergens, vitamins, and many of the steroids, both plant and animal, including cortisone and cholesterol.

Subsequently, it has been possible to obtain the following in decimal strength of from 1-100 to 1-1,000,000: desoxyri bonucleic acid, ribonucleic acid, nucleic acid yeast, nucleic acid thymus, desoxyribose, ribose, adenine, cytosine, guanine, thymine, and uracil. Another preparation which has been used in the test is serotonin which has a chemical name of 5-hydroxy trytamine creatinine sulphate. It is a substance found in the blood, the brain, and certain tumours, and was discovered only ten years ago.

All of these preparations have been found active in the flocculation test. That is, they have all reacted positively at various times and at other times there has been no reaction. It is upon this principle that the efficacy of any substance used in the flocculation test is judged as to its pharmaceutical value. If the reaction is always positive, the substance is not acceptable for prescribing. If the results are always negative, there is no reason for prescribing it on the basis of the flocculation test.

It is still too early fully to evaluate the last mentioned substances. There have been promising results in conditions of abnormal tissue, such as skin eruptions, various tumours and some collagen diseases, but so far in the analysis it has not been possible to reach any definite conclusions. Reactions do frequently appear in the more chronic type of cases, although it is not unusual to get positive reactions in acute conditions. There have been found a greater number of reactions with the sugars and bases of the nucleic acid complexes than with the DNA and RNA themselves. This may be interpreted as meaning that the various molecular parts are more often impaired than the finished giant molecule, just as the amino acids, which are the monomers of the protein molecules, have shown greater activity in the test than their whole molecular substances.

For tissue construction to be perfect, every cell must be perfect and, in turn, all the protein, DNA, and RNA molecules must be perfect. For these to be perfect the atoms and monomers that form each molecule must be perfect. Starting, then, with perfection of the monomers, since they are the smallest chemical unit, it should follow that the living cell and all its parts will be normal, and will have its biological ability to duplicate itself.

The pharmacopeia has been made up of naturally occurring substances such as the plants and their alkaloids, the minerals, the venoms of various animal life, etc. They have proved exceptionally valuable in the treatment of many diseased conditions. Still, they have not been sufficient to meet all disease states. For this reason there has been a continuing search for new and more perfect substances that will assist in the correction of the still incurable diseases.

These investigations have turned from naturally-occurring materials to those synthetically produced which have been made possible by a greater knowledge of polymer chemistry. It has been found that these new polymerized chemicals are often able to exert a profound biological effect on the living organism. As a result of these findings, a wealth of new materials has come into biological usage and these are able to play a vital role in physiology. Many of these could be selected for proving and added to the homœopathic materia medica. Besides the synthetic biochemicals used in common practice today, the entire fields of enzymes, phospho-lipoids and others are still to be explored. It is quite possible that practical experience with the activity of these substances will yield clues to other and even more valuable substances.

SUMMARY

Some fundamental principles of protein chemistry have been reviewed, in order to correlate biology with the molecular components of cellular life. An attempt has been made to ascertain the activity of several new substances as therapeutic agents by use in the flocculation test. Preliminary observations suggest that a variety of new synthetic substances heretofore neglected may open a new approach in the management of disease.

—*Jourl. of the Am. Inst. of Homœopathy, Jan.-Feb., '59.*