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HISTORY OF BIOCHEMISTRY

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ABSTRACT

Biochemistry in broad terms is the study of the chemical composition of the living matter and the biochemical processes that underlie life activities during growth and maintenance. This article is an attempt to explore the metamorphosis of biochemistry from a pupa entwined in its own cocoon to a vibrantly colored phenomenon. Studies pertaining to this discipline of science began with Biochemistry interfaces with biology and chemistry even before nineteenth century with studies concerned with the chemical processes that take place within living cells. Modern biochemistry developed out of and largely came to replace what in the nineteenth and early twentieth centuries was called physiological chemistry, which dealt more with extra cellular chemistry, such as the chemistry of digestion and of body fluids. The name Biochemistry was coined in 1903 by a German chemist named Carl Neuber. However, work in this very living, aspect of chemistry had started much earlier. Claude Bernard is accredited with the Sirehood of Biochemistry. During the later part of the nineteenth century eminent scientists contributed a great deal to the elucidation of the chemistry of fats, proteins and carbohydrates. At this period some very fundamental aspects of enzymology were under close scrutiny. Study of nucleic acid is central to the knowledge of life but its fusion with biochemistry started with works of Fredrick Sanger and Har Gobind Khurana. Their experiments involved a subtle bland of enzymology and chemistry that few would have thought possible to combine. The scientists were busy removing the mist that was mitigating the light of knowledge but they still lacked an insight into the cell. In 1990's research turned to finding the structural details of cell. The field of molecular biochemistry was also progressing at an almost unstoppable speed having expanded its horizons beyond human imagination with the introduction of PCR, creating waves of appreciation from every field of medicine and then coming out of the lab to help establish better therapies for various diseases by introduction of gene therapy. Biochemistry has promises to the world of science in development of new path-breaking research and coming times would surely prove these promises to be fulfilled.

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Biochemistry in broad terms is the study of the chemical composition of the living matter and the biochemical processes that underlie life activities during growth and maintenance. Biochemistry as we know today is relatively new, but to unravel the secrets of life has been the quest of humans that started the dawn of human knowledge, which has never since seen a sunset. It seems very likely that in those primitive times man asked himself questions, but unfortunately fell in a trap of easy reasoning. Supernatural phenomenon intrigued him, and he tried to reason everything accordingly.

It was some 500 years ago, beginning with the renaissance in Europe and the application of the method of science, true answers began to be found to questions, which has fascinated man for thousands of years. However even when laws and theories of physics, chemistry and astronomy had become a commonplace, and had led to the solutions of many mysteries, phenomenon concerning life were still shrouded by the belief of its being beyond science. Basic laws of biology did not emerge till the beginning of this century.

Any attempt to trace the history of biochemistry must go back to the beginning of organic chemistry from which it mainly took root. Theophratus Bombastus Von Hohenheim (1493-1541) better known as Paracelsus was the earliest to refer to some chemical aspects of medicine in his writings. If we consider chemical composition of the living and chemical processes and reactions, which form the basis of the living matter as biochemistry, then studies pertaining to this discipline of science began with the early chemists like Priestley, Lavoisier, Scheel and others. This was even before the 19th century. These studies were confined to the fulfillment of the curiosity about the composition of the animals and plants. Trailing these initial forays into chemistry were studies of chemical processes involved in respiration and fermentation; and chemical quantitative analysis of ‘organic substances’, which necessarily meant substances made by the living organism (James M Orten, 1982).

Biochemistry interfaces with biology and chemistry and is concerned with the chemical processes that take place within living cells. Modern biochemistry developed out of and largely came to replace what in the nineteenth and early twentieth centuries was called physiological chemistry, which dealt more with extra cellular chemistry, such as the chemistry of digestion and of body fluids. Scheele (1742-1786) isolated and studied number of substances like citric acid and lactic acid. Lavoisier (1743-1794) was the earliest to notice that the burning inside the body is similar to the combustion and oxidation of organic materials. This may be said as the beginning of animal calorimetry. Leibig (1803-1873) showed that plants depended on the simple organic substances (P M Bhargava, 1982). The theory of vitalism – that animal materials fundamentally differed from the lifeless substances in that only living matter can synthesize them, proved to be a myth when Wohler (1800-1882) synthesized urea in the laboratory. The earliest book relating to biochemistry was "Animal chemistry" published by Swedish chemist Berzelius in 1806. Berzelius(1779-1848) embarked on a systematic program to try to make accurate and precise quantitative measurements and insure the purity of chemicals. After Lavoisier, Berzelius is known as the father of chemistry. He determined the exact elementary constituents of large numbers of compounds. The results strongly confirmed Proust's Law of Definite Proportions. He published a table of atomic weights in 1826 that was in good agreement with modern values (AVSS Rama Rao, 1980).

The first major biological theory of universal importance was the cellular theory, which was postulated by Schleiden in 1838 with respect to plants and, by Schwann in 1839 with respect to plants and animals. Essentially this theory purports that the cells of an organism are discreet relatively autonomous entities. Two events helped to lay the early foundations of biochemistry. First was the synthesis of urea by Wohler in 1828, which showed that organic substances produced by the biological systems follow same laws of chemical combination as inorganic substances and thus seriously challenged Vitalism theory popularly accepted those days. Second were the experiments of Pasteur that showed that organisms couldn't be created in test tube from non-living materials. These discoveries provided a strong motivation for furthering the scientific investigation into phenomenon concerning life. The two subsequent major steps were the enunciation of the theory of evolution by Darwin and the laws of inheritance by Mendel in the last half of the last century (Deshmukh UD, Phatarphekar GV, Dandekar SP, 2001).

From these fragmentary beginnings biochemistry started to evolve into a distinct entity and efforts started to be made in this direction. It was then called 'Physiological

Chemistry'; first volume of 'Zeitschrift für Phisiologiche Chemie' came out in 1877. The 'Journal of Biological Chemistry' (JBC) came in 1906, representing the 'American Society of Biological Chemists'.

The name Biochemistry was coined in 1903 by a German chemist named Carl Neuber. However, work in this very living, aspect of chemistry had started much earlier. Claude Bernard is accredited with the Sire hood of Biochemistry. He was the first to conceive and present to the scientific community, the idea of a 'fixed internal milieu'. This was as early as – or, perhaps, as late as, when seen on the backdrop of chronology of science as a whole – the later half of the 19th century. The idea of Biochemistry as a distinct, well-defined part of bioscience took shape without difficulty for, where it takes place- the cell, was already well delineated and defined. The cell, through mechanisms not wholly understood, was known to withstand the changes, and keeps the milieu constant. This is the best approximation of homeostasis and what biochemistry is all about.

During the later part of the nineteenth centaury Chevreul, Kossel and Emil Fischer contributed a great deal to the elucidation of the chemistry of fats, proteins and carbohydrates. At this period some very fundamental aspects of enzymology were under close scrutiny at the same time. This was also the time when Embden and Meyerhof were creating history studying the formation of pyruvate. Elsewhere Bodansky and Somogyi as enzymologists were carving out their names to be remembered for generations to come. Banting and Best discovered insulin by tying a string around the pancreatic duct of several dogs. When they examined the pancreases of these dogs several weeks later, all of the pancreatic digestive cells were gone and the only thing left was thousands of pancreatic islets. They then isolated the protein from these islets and this was found to be insulin. Insulin was the first hormone identified (late 1920's). Note that there are other hormones produced by different types of cells within pancreatic islets (glucagon, somatostatin, etc) but insulin is produced in far greater amounts under normal conditions making the simple approach used by Banting and Best quite successful (www.endocrineweb.com).

In the later half of 1930's the pioneers of the anaerobic glycolysis continued to work and further consolidated the foundation they had laid. Embden published more work on his own about anaerobic pathway. A little later Cori CF continued the work that they had started, by isolating the enzymes to catalyze the conversion of glucose to glucose-1- phosphate and then the one that helps the next step the conversion of glucose-1- phosphate to glucose- 6- phosphate (Deshmukh UD, Phatarphekar GV, Dandekar SP, 2001).

In this era of booming science, laureates in chemistry made contributions, which might equally well have been awarded a prize in Physiology or Medicine. Butenandt received the 1939 prize in Chemistry for his work on sex hormones; de Hevesy was awarded the 1943 Chemistry prize for introducing isotopes as tracers in the study of chemical processes, a technique of great value in biomedical research, while Sanger was awarded two Chemistry prizes for discoveries of great importance in modern biotechnology (Liljestrand G, 1972). Edward Tatum and Gorge Beadle proposed one-gene, one- enzyme hypothesis which earned for their book, in 1958, the Nobel Prize in Physiology or Medicine. During this fruitful collaboration with George Wells Beadle, Tatum took charge of the chemical aspects of their joint work on the genetics of eye-colour in *Drosophila* and, when he and Beadle decided to give up their work on *Drosophila* and to work instead with the fungus *Neurospora crassa*, it was Tatum who discovered that biotin was necessary for the successful cultivation of this fungus on simple inorganic media and thus provided these two workers with the genetic material that they needed for the work (G M Blackburn and M J Gait, 1990). The above achievement of interlinking genetics with biochemistry of the cell opened up immense possibilities for the molecular biology to be intricately linked with biochemistry. In many respects biochemistry and molecular biology represent the realization of the dream of early twentieth-century mechanistic biologists, who were convinced that the most fundamental biological processes could ultimately be understood in terms of the laws of physics and chemistry.

For over 60 years, men and women were fascinated and stimulated by their awareness that study of nucleic acid is central to the knowledge of life. It was the ingenious experiments of Fred Griffith that started it all, in 1923, he was able to differentiate the difference between ‘S’ a virulent, and ‘R’ non-virulent forms of the pneumonia bacterium. Griffith went on to show that this bacterium could be made to undergo a permanent, heritable change from non-virulent to virulent type. This discovery was bombshell in bacterial genetics. Oswald Avery and his group at the Rockefellers Institute in New York setout to identify the molecular mechanism responsible for the changes Griffith had discovered, now technically called bacterial transformation. They achieved a breakthrough in 1940 when they found out that non-virulent R pneumococci could be transformed irreversibly into a virulent species by treatment with a pure sample of high molecular weight DNA. Avery concluded that 'DNA is responsible for the transforming activity' and published that analysis in 1944.

In 1951, Francis Crick and Jim Watson joined forces in the Cavendish laboratory in Cambridge to tackle the problem of DNA structure. Almost incredibly, they attempted no other line of direct experimentation but drew on the published and unpublished results of other research teams in order to construct a verity of models, each to be discarded in favor of the next until they created one that satisfied all the facts. It is common to ascribe the publication of Watson and Crick's paper in Nature in April 1953 as the end of 'classical' period in the study of nucleic acid, upto which time basic discoveries were made by a few gifted academicians in an otherwise relatively unexplored field (science world.wolfram.com).

In the 1940s and 1950s the disciplines of chemistry and biology were so separate that it was a rare occurrence for an individual to embrace both. Two young scientists who were just setting out on their careers at that time were exceptional in recognizing the potential of chemistry in solving the problems of biology and both in their different ways, were to have a substantial and lasting effect in the field of nucleic acids. One was Fredrick Sanger, a product of the Cambridge Biochemistry School, who in the early 1950's set out to determine the sequence of protein insulin. This feat had been thought unattainable, since it was widely supposed that proteins were not discrete species with defined primary sequence. Even more remarkably, he went on to develop methods of sequence determination first of RNA then DNA. These methods involved a subtle bland of enzymology and chemistry that few would have thought possible to combine. The award of two noble prizes to Sanger (1958 and 1980) hardly seems recognition enough.

The other pioneer in this field was Har Gobind Khurana; who was convinced that chemical synthesis of polynucleotides could make an important contribution to the study of the fundamental process of flow of information from DNA to RNA to protein. He ingeniously devised a combination of nucleic acid chemistry and enzymology to form a general strategy of gene synthesis, which in principle remains unaltered to this day. It is ironic that even upto the early 1970s, Khurana's gene syntheses were regarded, by many biologists, unlikely to have practical value. Today, synthetic genes are used routinely in the production of proteins [8]. Looking back now, this unique achievement marked the beginning of an exceptional era for science and its success permitted the then contentious technology of recombinant DNA to emerge and flourish.

The emergence of a new paradigm in any field of science generates, along with the excitement of a new frontier and perspective, an uncertainty about its full implications. This was especially true for the geneticists that fueled the emergence of the recombinant DNA technology during the 1970s. The concerns about recombinant DNA had their antecedent in the creation of a DNA molecule containing the entire Simian Virus 40 genome joined to a segment of DNA containing three genes responsible for galactose metabolism in *Escherichia coli.* But improvements in the technology, most notably the ability to clone DNA segments from virtually any organism on our planet, triggered a new yield of important drugs, industrial products and improved agricultural varieties (Jackson, D.A., Symons, R.H. and Berg P, 1972.).

Unraveling of secrets of our own metabolism also continued and gave rise to a tremendous increase in human understanding of phenomenon still considered to be beyond the reach of human knowledge. Bergström, Samuelsson & Vane carried out structural work on prostaglandins in 1959-1962.They were mainly interested in transformation products of arachidonic acid. This led to the discovery of endoperoxides, thromboxanes and the leukotrienes, and this group has mainly been involved in studying the chemistry, biochemistry and biology of these compounds and their role in biological control system. The research had implications in several clinical areas, particularly in thrombosis, inflammation and allergy (http://nobelprize.org).

Similarly cholesterol was the object in many studies. In his Nobel Lecture of 1964, Konrad Bloch summarized his brilliant studies on the biological synthesis of cholesterol. At the end of his talk, Bloch predicted that the next era in cholesterol research would involve the elucidation of homeostatic control mechanisms. A decade later, in 1973, the LDL receptor concept was advanced to explain the homeostasis of plasma cholesterol and to account for regulatory abnormalities in cholesterol metabolism that were observed in patients with familial hypercholesterolemia. During the next 12 years, the LDL receptor was transformed from a genetic abstraction to a well-characterized protein whose structural domains have been well studied. Brown & Goldstein received Nobel Prize in 1985for their studies regarding regulation of cholesterol metabolism.

But it was not only cholesterol metabolism that was being studied; the control of cell metabolism was also given its due importance. There was a lot of work being carried out during this time for discovery of various mechanisms by which metabolism was regulated in a cell. Earl Sutherland had discovered cyclic AMP as a "second messenger" of epinephrine - and glucagon-mediated effects on glycogenolysis in liver preparations. Sutherland's work made an impression on his colleague Murad. Later Murad working along with Furchgott & Ignarro discovered that nitric oxide was a signaling molecule in the cardiovascular system.

A landmark discovery was made relating to antibodies with the devoted efforts of Niels K. Jerne, Georges J.F. Köhler and César Milstein. They established monoclonal antibodies and received the Nobel Prize in Physiology or Medicine 1984. Susumu Tonegawa a molecular biologist from Japan worked for years investigating the role of somatic rearrangement in the activation of the rearranged antibody gene, and the antigen receptor of T cells. He discovered a tissue-specific transcriptional enhancer in the immunoglobulin heavy chain gene and by identifying, cloning, and sequencing genes coding for the polypeptide subunits of the T cell receptor. For his tremendous efforts he was awarded the Nobel Prize in Physiology or Medicine 1987.Equally interesting work was been done in field of cancer, as scientists were trying to find answers to what intrigued them for centuries J. Michael Bishop in 1989 explored the contributions of proto-oncogenes to the genesis of human cancer, added to the repertoire of proto-oncogenes by several experimental strategies. He pursued the physiological functions of proto-oncogenes in normal organisms, and shared in the discovery of the protein kinase encoded by src.

The scientists were busy removing the mist that was mitigating the light of knowledge but they still lacked an insight into the cell. In 1990's research turned to finding the structural details of cell, discovering that proteins have intrinsic signals that govern their transport and localization in the cell. Erwin Neher discovered Processes initiated inside cells, eventually leading to a cellular response-like secretion of hormones and neurotransmitters. Jens C. Skou discovered an ion-transporting enzyme and Roderick MacKinnon identified the K^+ channels signature sequence.

The field of molecular biochemistry was also progressing at an almost unstoppable speed having expanded its horizons beyond human imagination with the introduction of PCR, creating waves of appreciation from every field of medicine and then coming out of the lab to help establish better therapies for various diseases by introduction of gene therapy. The earliest references that specifically address a scientific approach to carry out human gene therapy are in 1966; the original thinkers were Edward Tatum and Joshua Lederberg. Human gene therapy progressed from speculation to reality in a short time. The first clinical gene transfer (albeit only a marker gene) in an approved protocol took place on 22 May 1989.An unsuccessful attempt was made in 1980 to carry out gene therapy for B-thalassemia with the use of calcium phosphate-mediated DNA transfer. Retroviral-mediated gene transfer was developed in the early 1980s in animal models. This technology is the principal procedure used today. Now there are 11 active clinical protocols on three continents with nine more approved protocols about to begin and over a dozen additional protocols in various stages of development (http://www.asgt.org).

Biochemistry is still unraveling the mysteries of life, as shown by the Nobel prize for 2004 awarded to Aaron Ciechanover, Avram Hershko and Irwin Rose for the discovery of "ubiquitin-mediated protein degradation" (http://nobelprize.org). Predicting the future is a pastime, more wisely left to fortunetellers than to scientists. However, there lies many new fascinating areas of emerging development and the indulgence in the brief extrapolation into the future, is what makes the impact of new, path-breaking research more tangible.

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सारांश

जीव रसायन विज्ञान का इतिहास

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जीवरसायन विज्ञान एक ऐसा विषय है जो कि जीवन व जीव की रसायनिक प्रकिया से और जीवन के विकास से संबान्धित है। यह लेख एक ऐसा प्रयास है जो जीवरसायन विज्ञान के नवजात होने से बड़ा होने तक की सारी गतिविधियों को जानने का प्रयास करता है। १९ वी सदी से पहले जीवरसायन विज्ञान, रसायनशाश्त्र एवं जीव विज्ञाण से संबन्धित था। आज का यह विषय असल मे जन्मा शारीरिक रसायन शास्त्र से जिसका कि १९ वी एवं २० वी सदी के शुरुवाती दौर में विकास हुआ। जीवरसायन विज्ञान को उसका जन्म दिया गया १९०३ में कार्ल न्यूबर नाम के एक जर्मन वैज्ञानिक द्वारा जब कि क्लाड ब्रनाड नामक एक वैज्ञानिक को इसका जन्मदाता माना गया है।

१९ वीं सदी के समाप्त होने तक वैज्ञानिकों ने मेदस, प्रोटिन व कार्बो हायड्रेट की रासायनिक प्रकिया को समझने के लिए बहुत से यत्न किए। न्युक्लिक आसिड के बारें मे जानकारी को जीवन के लिए बहुत एहम माना जाता है। पर जीव रसायन के साथ इसका समावेश हुआ जब फेड्रिक साँगर और हर गोबिन्द खुराना ने इस विषय मे आगे काम किया। १९ वी सदी में वैज्ञानिक इस बात पर ज्यादा ध्यान देने लगे की कोशिकाएँ कैसी बनती हैं और किस प्रकार करती हैं। इन सब कोशिशों के फलस्वरूप था पी. सी. आर. का जन्म। जीव विज्ञान की बहुत सी अधूरी प्रतिज्ञाएँ है जो अभी पूरी नही हुई है पर आने वाला समय जरुर सिद्व करेगा कि जीव विज्ञान बहुत ही महत्वपूर्ण विज्ञान है।