



## CHAPTER XX

# BIOCHEMISTRY

**B**iochemistry was recognized as a separate discipline in the beginning of the 20th century with the establishment of the first Department at the University of Liverpool. Many scientists would not know that the second Department exclusively devoted to biochemistry was set up in the world at the Indian Institute of Science (IISc) in Bangalore in 1921. Biochemistry is a mother discipline for all biological sciences, as it deals with molecular reactions of biological phenomena in all living cells. Biochemistry has grown and flourished in India with advanced level teaching in nearly 50 universities and research in over 75 departments including those of national laboratories. The growth of this discipline in India has kept pace with the rest of the world by the generation of new ideas and application of advanced technologies. This

article highlights important achievements in the past five decades. The account is divided into two periods: 1950-80 and 1980-2000, recognizing that novel molecular biological tools and technologies became available in the early 1980s which were quickly adopted in the Indian laboratories for seeking answers to a wide spectrum of fundamental biological questions.

No comprehensive account is attempted. Some of the important research findings made during the first three decades are summarized under a few headings:

### SANITATION AND HEALTH

**S**anitation biochemistry was an important subject pursued at IISc in the early years particularly with respect to natural purification of sewage in Bangalore

by using activated sludge process and examining the role of protozoa and *Vorticella* sp. These activities led to the setting up of the National Environmental Engineering Research Institute at Nagpur. The biochemistry of pathogenic bacteria was initiated at the Central Drug Research Institute (CDRI) Lucknow, Vallabhbhai Patel Chest Institute (VPCI) Delhi, and IISc, which dealt with delineating the pathways of degradation and biosynthesis of

amino acids, utilization of sugars and mechanism of action of antitubercular drugs. Biochemistry of Kala Azar was taken up at the All India Institute of Experimental Medicine (AIEM), Kolkata, now called Indian Institute of Chemical Biology (IICB) Kolkata, which was very relevant in the context of the re-emergence of this disease in eastern UP and Bihar. The biochemistry of filarial worms and helminth par-

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asites was investigated at Lucknow and Chandigarh. Respiratory diseases were studied at VPCI. Drug metabolism and pesticide metabolism were other areas of research carried out during this period at Bangalore and Lucknow respectively. Biochemical adaptation to high altitude and low temperature environment prevalent in the Himalayan peaks was studied at Bangalore and Delhi.

### FOOD RESEARCH

The early work at IISc on foods and food processing centered around the development of nutritive food formulations and fortification with vitamins. These early studies paved the way for the establishment of the Central Food Technological Research Institute (CFTRI), in Mysore, which has become one of the premier institutions of CSIR today. Food research was also carried out in the Food Technology Department of the Bombay University and the Hindustan Lever Research Laboratories. The pioneering work at BARC, Mumbai, has resulted in the development of extremely useful methodologies for preserving canned foods and spices using radiation.

### ENZYMOLGY

Enzymes are the central players in biochemistry and have attracted the attention of several researchers over the last five decades. Enzymology which had its beginning at IISc, Bangalore, has become even more important in the post-genomic era since we need to understand the functions of thousands of genes discovered in silico based on the sequence information of several organisms. In the early 1950's emphasis was on the study of enzymes involved in the metabolism of amino acids, lipids, vitamins, plant hor-

mones and nucleic acids. The most important contribution during this period was the study of reactions in trans-glycosylation. With the availability of sophisticated spectroscopic and kinetic techniques, subsequent work in the 1960s was centered round the regulation of enzyme activity by small molecules. Metabolic pathways for the bioutilization of aromatic compounds and characterization of the enzymes involved as well as their mechanism of action attracted serious consideration during this period. The structure-activity relationship of ribonuclease and its intermediates in the *pH* and temperature-induced denaturation process was a major contribution from IISc. Amino acid and nucleic acid metabolism in *Mycobacterium tuberculosis* and *Mycobacterium smegmatis* were studied in great detail during this period at IISc.

An active and vibrant school of enzymology was developed during this period at the University of Calcutta. The group studied the biosynthesis of ascorbic acid and this research has been continued even during 1980-2000. The enzymes regulating the biosynthesis of inositol phosphates, RNA, DNA and protein biosynthesis were studied in Kolkata.

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Enzymes of carbohydrate metabolism and thyroxin were investigated at IICB. Epimerases and their regulation were studied at the Jadavpur University. Enzymology research also flourished during this period at the Madras University where work was carried out on phosphoprotein phosphatase, niacine-tryptophan interrelationship and trace element metabolism. Pioneering work on the structure of collagen and theoretical work on the conformation of proteins were carried out in Chennai during 1960's.

Hexokinase, enzymes of the citric acid cycle and phosphatases were extensively probed at the University of Poona. Enzymology was also actively pursued in Mumbai at TIFR, BARC and Cancer Research Institute (CRI). At TIFR, major emphasis was laid on alkaline phosphatase and genetics of pyruvate kinase and hexokinase. Lactate dehydrogenase, hexokinase and enzymes of folate metabolism were studied at BARC. Enzymes from snake venom and dihydrofolate reductase were studied at CRI. Enzymes of carbohydrate metabolism were also studied Nagpur and Hyderabad. At Aligarh and Ahmednagar, the emphasis had been on the physico-chemical and evolutionary aspects of proteins like haemoglobin and albumin. Research work at Ahmednagar resulted in deriving a phylogenetic tree based on the sequence of amino acids in proteins.

### VITAMINS AND NUTRITION

The Department of Biochemistry, Calcutta University, gained considerable importance for its work on vitamin C particularly on the biosynthesis of ascorbic acid and the role of vitamin C in diverse biological processes. Significant contributions have been made on vitamin A at the Department of Biochemistry, IISc. The National Institute of Nutrition, Hyderabad, has done excellent work in the field of nutrition research, particularly on vitamin A deficiency, vitamins B and nutritional disorders such as pellagra, marasmus, and kwashiorkor.

### LIPIDS AND BIOMEMBRANES

The Bangalore group has made significant contributions to our understanding of the absorption of phospholipids, glycerides and cholesterol in addition to the biosynthesis of phospholipids in animal tissues, glycolipids in photosynthetic tissues, herbicide effects on plant lipid metabolism and biogenesis of cholesterol. A major finding has been the identification of ubiquinone in non-mitochondrial membranes which is now being shown to be important as an antioxidant in conjunction with vitamin E. The effect of vitamin A deficiency and hyper-vitaminosis on lipid metabolism in *Mycobacterium tuberculosis* were studied at VPCI, Delhi. Biogenesis of mitochondria was actively studied at Bangalore and Madurai. Physical approaches have also been used to study the membrane functions at Bangalore. Liposomes and their importance in drug delivery were extensively studied at IICB, Kolkata.

### ENDOCRINOLOGY

Endocrinology was an active area of research during the period 1960-80 which included a) mechanism of action of gonadotropins and steroid hormones, b) biosynthesis of iodotyrosine and thyroxine in extrathyroidal tissues, c) regulation of biosynthesis of gonadotropins, d) biochemical and immunological approaches to contraception, e) mechanism of action of centchroman, e) comparative endocrinology of birds, fishes and other lower vertebrates, f) gonadotropin inhibitors g) glucagon and insulin secretion in diabetes, h) adrenal steroids and gastric ulcer, i) use of plant products as contraceptives and j) antisteroid drugs as inhibitors of sperm maturation. Several aspects of male and female reproduction were studied at IISc, All India Institute of Medical Sciences (AIIMS), New Delhi, and Institute for Research in Reproduction (IRR), Mumbai. Reproduction-specific vitamin-carrier proteins have been isolated and characterized. One of the major efforts during this period was to explore the feasibility of immunological approaches to contraception.

## PLANT BIOCHEMISTRY

There was sustained research activity in plant biochemistry in several places during this period, which included the study of pathways of aromatic amino acid metabolism, plant hormones, enzymes from plant sources, mechanism of drought tolerance, mechanism of host-parasite interactions, photosynthetic processes in C3 and C4 plants, role of nitrate and nitrite reductases in nitrogen fixation.

## NEUROCHEMISTRY

Christian Medical College (CMC), Vellore, was the first school of neurochemistry and glycobiology in India. The scientists there demonstrated the cause of an inborn error of metabolism, metachromatic leucodystrophy by establishing the deficiency of the enzyme arylsulphatase in the brain tissue.

The M.S. University of Baroda, has developed a research programme in the area of neurochemistry especially related to nutrition and brain function. The group in the University of Calcutta has done a lot of work on mode of action of psychopharmacology of drugs.

The National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, has a strong research group working on motorneuron disease, neuroepidemiology and clinical neurophysiology. The Institute has also been engaged in the basic and applied areas of mental health and psychiatric disease.

Neurochemical research was conducted at AIIMS, New Delhi; CMC Hospital, Vellore; CRI, Mumbai; Post Graduate Institute of Medical Research, Chandigarh; IICB, Kolkata; National Institute of Nutrition, Hyderabad; and IISc, Bangalore. The studies included sensory perception in developing brain, neurotoxicity of snake venom, effects of malnutrition in brain development, myelogenesis and synaptogenesis, nucleic acid metabolism and psychopharmacology.

## MOLECULAR BIOLOGY

In the beginning, the discipline of molecular biology concerned with only the aspects of nucleic acid metabolism, protein synthesis and gene regulation. However, subsequent developments in recombinant DNA technologies gave a new turn to our understanding of the various biochemical aspects of life processes. With the recent advent of biotechnology as a very important component of Life Sciences, molecular biology is now understood as only a tool or means to unravel cellular processes.

In the early years before 1980s, the major areas of research were 1) bacteriophage genetics and control of gene expression in phage-infected bacteria, 2) bacterial genetics: chromosome mapping, study of genes specifying DNA recombination and replication, 3) regulation of DNA repair function, 4) ribosome structure and function, 5) molecular basis of host-virus interaction, 6) structural studies on DNA, tRNA, 5S RNA and protein-nucleic acid interaction, 7) chromatin structure and function, 8) molecular mechanisms underlying differentiation in plants and lower eukaryotes and sporulation in bacteria, and 9) regulation of X-chromosome inactivation. The Molecular Biology Unit at Banaras Hindu University (BHU), Varanasi, has contributed significantly to the molecular basis of host-virus interaction and structure and function of the *E.coli* ribosome. This group has shown that the structure of ribosomal RNA plays a significant role in protein synthesis. Salient contributions were made at IISc, Bangalore, on alternate structures in DNA, modified bases in tRNA, immunological aspects of nucleotides, regulation of heme biosynthesis, chromatin structure and function, animal viruses, hormonal regulation of gene expression and so on.

The School of Biological Sciences at Madurai, has been actively engaged in teaching and research in Molecular Biology over the years. The important research interests of this school had been 1) gene regulation during bacterial sporulation and development of *Artemia salina*, 2) transcription mutants of *E.coli*, and 3) organelle gene expression

plants. Significant contributions were made on transcriptional regulation of gene expression using coconut endosperm nuclei as the experimental system at Bose Institute, Calcutta. DNA repair using the phage systems were actively pursued at the Biophysics Department, Calcutta University. The Molecular Biology Unit at TIFR Bombay, has been a major centre of Molecular Biology research since its inception in 1962. The areas of research included genetic transfer mechanisms in *E.coli*, theoretical and experimental work on ribosome biogenesis and protein structure, neurobiology, molecular aspects of olfaction in *Drosophila*, yeast genetics, pattern formation and molecular biology of tumor viruses. The Centre for Cellular and Molecular Biology (CCMB) at Hyderabad was established during this period by CSIR and the initial work was on molecular mechanism of action of seminalplasmin and reverse transcriptases. The School of Life Sciences at JNU also started during this period and the initial efforts were on translational control mechanisms in eukaryotes. The group at JNU has worked extensively on protozoa parasite, *Entamoeba histolytica*, particularly on genome structure, and also on the molecular genetics of nitrogen fixation and have also shown the presence of multiple chromosomes in *Azotobacter vinelandii*. More recently another group at JNU has reported a novel gene encoding hyaluronic acid binding protein located on human chromosome 17 related to signal transduction pathway.

### BIOCHEMISTRY SINCE 1980

As mentioned above, developments of the molecular biological tools including the recombinant DNA technologies have given a new direction to our approach to understand life processes. Also the bor-

derline between the different disciplines is no longer water tight. We can broadly classify the recent progress into the following areas namely, Biochemistry and Biotechnology, Cell biology, Immunology and Structural Biology. The contributions in these areas at various centres has been summarized. It is to be mentioned that the establishment of the Department of Biotechnology by the Government of India as a separate Scientific Department has given a great stimulus to research and training in this field in India over the last two decades. The Department of Biotechnology in addition to supporting the development of infrastructure

at the major research centres, has also contributed to the development of manpower by opening M.Sc. courses in Biotechnology at several universities. It has also started postdoctoral training at a few select institutions.

Protein structure and molecular enzymology continues to be one of the major areas being pursued at IISc, using site-directed mutagenesis approach. The enzymes studied include serine hydroxy methyl

transferase, restriction-modification enzymes, proteins involved in genetic recombination, histones and other basic proteins, enzymes involved in DNA repair, enzymes involved in fatty acid biosynthesis and others. There has been sustained research on molecular aspects of genetic recombination in *E.coli*, yeast and mammals. Over the last decade molecular aspects of infectious diseases particularly tuberculosis, malaria and Japanese encephalitis virus have received serious attention. The molecular virology of animal and human viruses is being extensively studied with the objective of developing vaccines in the near future. Gene regulation in prokaryotic and eukaryotic systems using model

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genes has been extensively studied which includes cytochrome P450 in rat liver, tRNA genes in silk worm, *mom* gene from bacteriophage, *TGF beta*, cryptic genes of *E.coli*. Some of these studies have led to recent attempts to develop DNA vaccines. Research groups at IISc and JN Centre, Bangalore have recently obtained valuable information on certain important biosynthetic pathways in the malarial parasite. Protein folding is receiving critical attention as one of the important areas in which several important contributions have been made by researchers at IISc and National Centre for Biological Sciences (NCBS), Bangalore.

Structural Biology is a discipline in which India has made a mark in international science. This work began with the initial efforts of G.N.Ramachandran on conformations in polypeptides. More recently IISc has shown leadership in protein crystallography, a subject which is also being pursued in at least half a dozen relatively new research groups across the country. The results of research in the area of lectins from the Bangalore group are now well recognized particularly in relation to structure and specificity of interaction with sugars.

Over the past decade CCMB has become a premier research centre in biology. The Institute has developed a salt-inducible expression system in *E.coli* which has now been commercialized. This is a good example in which basic research has been transformed into a commercial product. CCMB has also developed a Bkm-derived probe for DNA fingerprinting, again an outcome of an important study in basic science. The development of this technology was the determining factor in the establishment of the Centre for DNA Fingerprinting and Diagnostics (CDFD) at Hyderabad by the Department of Biotechnology. Characterization of microbial biodiversity of the Antarctica and molecular basis of adaptation to low temperature, and the demonstration that crystallins may have a role in protecting the eye lens, and that one of the crystallins has a chaperone activity may function in modulating protein folding are other important

findings of CCMB.

The National Institute of Immunology (NII), New Delhi, which was established by the Department of Biotechnology has been concentrating on various aspects of immune system, drug delivery, regulation of gene expression in the Baculovirus system, development of vaccines, immunomodulators from plant sources and intracellular protein trafficking in pathogen-infected macrophages. NII has also contributed significantly in the area of reproductive biology and various approaches to contraception. Extensive work was carried out at NII and CRI on the feasibility of developing a vaccine against leprosy bacterium (*Mycobacterium leprae*).

The Department of Biochemistry at Bose Institute, Kolkata, has been a major centre of research for several decades. Presently this Department is actively pursuing the biochemical and biophysical aspects of tubulin and antimetabolic drug interactions. More recently structural biology of aminoacyl tRNA synthetases is being pursued using several biophysical techniques. Bacterial genetics continues to be one of the important areas of research. Yeast chromosomes and parasite biology are other major areas of research in this department. Theoretical aspects of protein structure and folding and experimental determination of protein structure by X-ray, and NMR are some of the new activities initiated recently.

The IICB at Kolkata is addressing several issues related to health and disease and more particularly parasitic diseases like leishmaniasis and cholera. Protein Engineering is one of the important areas that is being pursued here. The Institute is also known for its work on neurobiology and molecular endocrinology. More recently, work on human genetics has also been started particularly in the areas of gene flow across ethnic populations in India, animal models of inherited eye disorders and genomic diversity in Indian population. Significant contributions have been made on Indian population genetics from groups at the Indian Statistical

Institute (ISI), Kolkata.

The Institute of Microbial Technology (IMTECH), Chandigarh, is one of the relatively recent institutions in the country, which has concentrated on molecular biology of cholera, yeast genetics, and production of recombinant proteins of therapeutic interest. The Centre of Biochemical technology, New Delhi, has been known for studies on allergy caused by pollen and fungi and applied immunology. However more recently, it has diversified its interests into functional genomics and is engaged in active research using the information of human genome sequence on neurological disorders. The Centre is also engaged in using genome information to address the basis of respiratory disorders, tuberculosis and haemoglobinopathies. CDRI, Lucknow, has recently embarked on using the recent developments in the area of structural biology and genomic information in its efforts towards future drug research. TIFR, Bombay, continues to add to our knowledge of plant developmental biology, neurobiology and mechanisms of genetic recombination. The School of Life Sciences, University of Hyderabad and the Osmania University have been the major centres of masters degree training and research. The scientists of these institutions are investigating DNA repair and ageing, neurochemistry, plant biochemistry, biochemistry of eicosanoids such as prostaglandins and leukotrienes.

The Department of Zoology of the BHU is known for studies on the molecular aspects of

ageing. The Department of Biochemistry at the University of Delhi South campus is one of the younger departments in the country but has already made significant work on molecular biology of tuberculosis, use of phage display for whole genome epitope mapping to identify immunodominant B-cell epitopes and development of a HIV detection kit. The researchers of this Department have received world wide attention for their work on intracellular protein trafficking and the development of a novel delivery system using Sendai virus model. Liposome-mediated drug delivery is also being actively pursued by them.

The International Centre for Genetic Engineering and Biotechnology, New Delhi, has developed strategies for developing vaccines against malaria, HIV and chloroplast transformation.

In conclusion, it may be stated that rapid strides and achievements in the areas of Biochemistry and other related disciplines have been made in India over the last decade. The number of publications in journals of international repute (high impact factor) have been steadily increasing. These have been possible because of the changing trends in leading laboratories in the world, development of novel techniques and tools and importantly generous support provided by the scientific departments, especially Department of Biotechnology, Government of India, in terms of humanpower development and upgradation of infrastructure in terms of high-technology equipment over the last decade.

