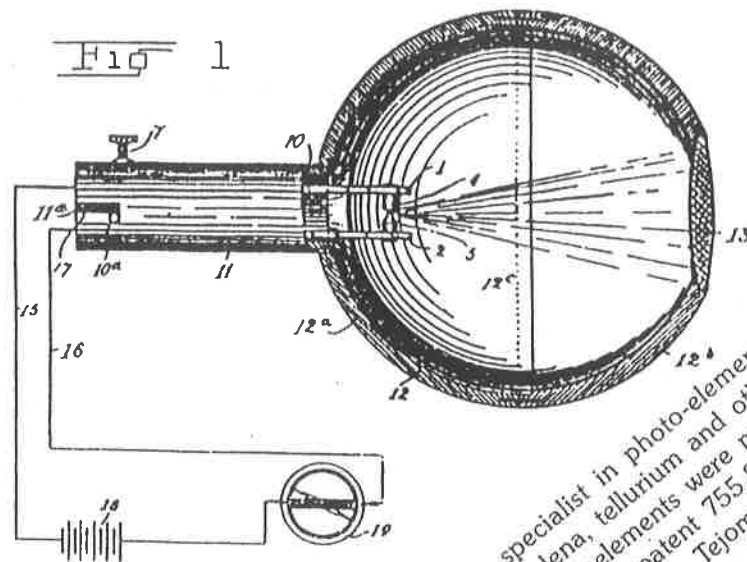


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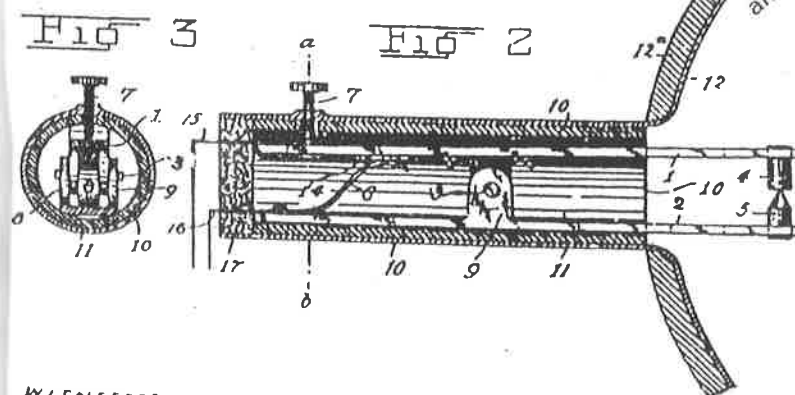
PATENTED MAR. 29, 1904

J.C. BOSE  
DETECTOR FOR ELECTRICAL DISTURBANCES.  
APPLICATION FILED SEPT. 30, 1901.

NO MODEL



According to Bruno Lange, specialist in photo-elements, the first observations on galena, tellurium and other detector minerals as crystal photoelements were made by J.C. Bose and described in U.S. patent 755,840 of 1904. Bose named his light detector as 'Tejometer' derived from the Sanskrit word for radiations, 'tej'. In a review on a lead salt photoconductors, Moss refers to Bose's use of galena crystal as a detector of light waves. J.C. Bose's galena detector is, in fact, considered today to be the most sensitive detector for near and middle infrared waves and laid the foundation of optical communication.



WITNESSES:

Frederick White  
Thomas Mallard

INVENTOR:

Jugadish Chandra Bose,  
By his attorney,  
Nathaniel C. Orin

ANNUAL REPORT  
OF  
THE BOSE INSTITUTE  
FOR  
1997-98



**Bose Institute**

93/1, Acharya Profulla Chandra Road  
Calcutta-700 009

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## From the Director's Desk

Dear Friends,

I take much pleasure to present before you the Annual Report of the Institute for the year 1997-98. During the year, we have witnessed a lot of vibrancy in our activities, exuberant enthusiasm, and several achievements. Major efforts had

been directed to ensure that the aims and objectives of our research are focussed towards some meaningful conclusion, no matter whether it is in the basic science area and/or in the applied field.

Dr. P. Chakrabarti of the Biochemistry Department was awarded the Fellowship of Indian Academy of Sciences.

An International publication by Dr. Dipankar Home on "Conceptual Foundation of Quantum Physics" is a major step forward to bring the name of the Institute into the International arena. This book of about 400 printed pages is a major addition in this complex area of Quantum Physics, which baffled the minds of Physicists, Mathematicians and others over the years.

Dr. Amita Pal has been invited to prepare a Monograph on production of bioactive materials in *in vitro* plant tissue culture, Prof. Arun Lahiri Majumder published a Review on L-myoinositol-1 Phosphate Synthase, Dr. Jayati Basu was invited to participate in the prestigious Gordon Research Conference and IUBMB Congress.

Dr. Samir Sikdar of PMCG Section filed a US Patent on Plastid Transformation of Arabidopsis Thaliana jointly with other inventors of Rutgers University, USA, and received his first royalty payment.

Several other Patent applications had been filed by our Scientists on development of new peptides with unique properties of anti-body binding affinity, identifying bacterium having novel properties for degradation of toxic organohalogenated pesticides from food, a method for selective induction of apoptosis in Cancer cells as a new approach to Cancer therapy, development of instruments such as Hanging Wire Fluid Velocimeter-cum-acoustic Pressure Gauge.

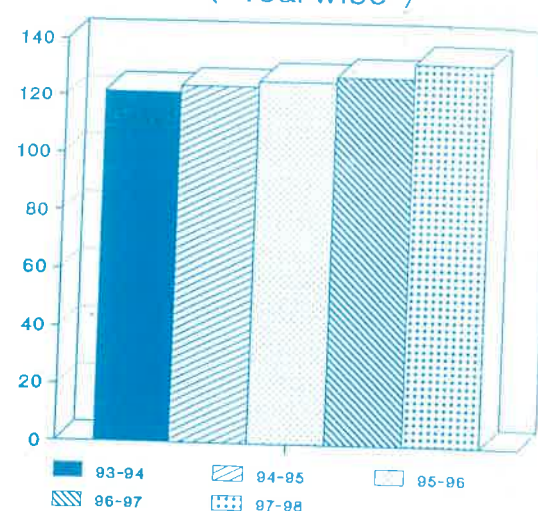
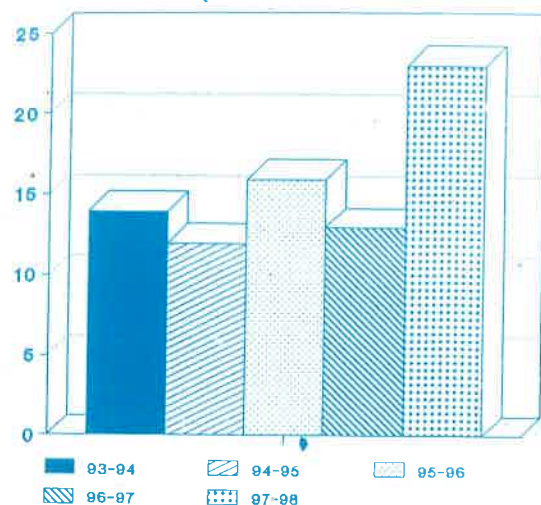
This year our students have a number of achievements to their credits. Ms. Suranjan Guha received INSA Young Scientist Award in the area of Biological Sciences in 1998, and also Nilratan Sarkar Award in 1997 given by the Institute.

Smt. Shampa Khasnobis of Chemistry Department got best Poster Presentation Award, Dr. Sanjoy Kr. Ghosh of the Physics Department was awarded full financial coverage to attend a number of International Meetings, Summer School in Italy, Japan and in various other places in India.

The Institute had also witnessed renewed interests for inter-departmental and inter-institutional collaborative efforts. Many Physicists are collaborating with the

During the year the Institute had published 122 full-length research papers, produced 23 Pd. Ds., and brought 67 funded projects from National and Inter-

### Number of Publications ( Yearwise )



It is also heartening to announce that a large number of sophisticated equipments, such as FACS Vantage and FACS Calibre, Capillary Electrophoresis, Protein Sequencer, Stop Flow Kinetic Measurement System, Phosphorimager, Liquid Scintillation Counter, Jel Documentation System, Atomic Absorption Spectrophotometer, 500 MHz FT-NMR, Spectrofluorimeter, a large number of Ultra-Centrifuges, PCR Instruments, HPLC, Spectrophotometers, Jel Docs, IR etc., had been procured, installed and are being utilized successfully. A Central Facility Management System, with the participation of a large number of scientists, who have personal knowledge about the usage of different instruments, had been formed for proper maintenance and functioning of these equipments. This instrument facility is also being extended to others, whosoever may like to use them. This, I am sure, will help our scientists to produce advanced level data to enable them to publish in high impact factor journals.

One of the major tasks which the Institute has completed is in the field of better communication facilities within the campuses, and in between the campuses. Very soon we will witness INTERNET facility introduced independently in both the campuses, such that not only international communications through INTERNET would become available but also the students, scientists and others in the Main Campus would be able to communicate with the Centenary Building through that connectivity. Local Area Networking has been completed within the Institute, in the Library, DIC, Administration and Accounts, so that two-way communications through the electronic media becomes possible.

Partial implementation of the recommendations of the 5th Pay Commission has been effected, a part of the arrears have been paid already. Enhanced stipend/salary for Scholars/PD Fellows/Research Associates had been introduced. For the Pensioners, enhanced pensionary payments have been made.

The much infamous Howrah Warehouse fire incidences are in the minds of all. We were asked by the "Green Bench" of the Hon'ble High Court of Calcutta to provide expert guidance to deal with the hazardous materials stored there and for its safe disposal. A team of experts were sent there along with Customs Officers, Officers from the West Bengal Pollution Control Board, and Police Authority. Scientists from our Environmental Sciences Section along with others from our Workshop conducted detailed investigations on identification of toxic chemicals, and suggested methods of their safe disposal. Tons of such both solid and liquid chemicals were required to be safely disposed. Scientists are also helping the Santaldih Thermal Power Station, Bandel Thermal Power Station, Kanchrapara Railway Workshop for controlling the levels of air pollution. Waste water monitoring work is going on in various industries. Expert data were produced for gadgets developed by entrepreneurs for containment for air pollutants from car exhausts, air purification gadgets, water filters etc.

An account of the chronological progress in terms of research publications, number of Ph. Ds. produced, number of funded projects and the earnings, patents etc., are shown in various figures for proper appreciation about the overall performance of the Institute during the preceeding year.

Since the Annual Report being handed over to you has given a detailed account about the researches conducted by individual scientists in specific departments, I would only like to highlight the major findings and/or observations.

As mentioned above, Dr. Dipankar Home of the Department of Physics published a book on "Conceptual Foundations of Quantum Physics" in his single authorship, which was published by the Plenum Press, USA. This is a major addition to literature so far as the present concept of Quantum Physics is concerned, along with various future projections. The Department played a major role in holding a highly successful Symposium on "Science at High Altitude" and has since come out with a publication compiling the various papers presented by the scientists looking into the Physical, Chemical, Biological and Environmental issues pertaining to high altitude situations. This Symposium was held at Sir J.C. Bose High Altitude Research Station of the Bose Institute during May 1997. Further work is going on for the improvement of Superheated Drop Detector designed and fabricated by Prof. S.C. Roy with the help of our own Workshop. The Department published 28 full-length papers, 1 Book, and filed 1 Patent application.

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Among the major highlights of the Department of Botany are further progress in the area of molecular analysis of plant response to salinity stress, particularly in inositol metabolism, polyamine biosynthesis and ABRE-binding factor. Major efforts to analyse the genome of rice in relation to submergence tolerance and aroma, Palong and Brassica, regarding their genotypic variation by AFLP and RAPD analysis are continuing. Clonal propagation of *Nyctanthes* has been achieved. A pollen calender having allergy causing potential in Calcutta and Howrah region has been prepared. The Department published a total of 20 full-length papers, 6 Ph. Ds. and brought 13 funded projects.

The Department of Microbiology identified a 20 kDa submit pillus protein from non-01 non-0139 *Vibrio cholerae* strain 10325, and established its role in the intestinal colonization of its diarrheogenic mechanism. Leishmanial infection has been shown to impair the level of drug metabolising enzymes which explains why the patients suffering from *Leishmania* may poorly respond to drug treatment. In collaborative studies, with the Immunotechnology Section it was established that Protein A of *S. aureus* could reduce the parasite burden in *Leishmania* infected animals. The Department published 5 full-length papers, produced 3 Ph. Ds. and brought 10 funded projects.

The highlights of the Department of Biochemistry are – the transgenic tobacco and tomato plants expressing the trypsin inhibitor gene from mustard, which have been found to be resistant to lepidopteran insects. Transcription status on a galactokinase gene of *Mycobacterium smegmatis* have been shown to be positively regulated by cAMP and amino acid glutamate. PCR-based diagnostic methods to differentiate *E. histolytica* from *Dispar* has been developed. DNA fingerprinting technique of AFLP has been used, in collaboration with PMCG Section, to characterize different strains and species of *E. histolytica*. The Department also celebrated the Silver Jubilee of its establishment by organizing a Symposium on "Biochemistry" A Journey Through Three Decades". The Department published a total of 16 full-length papers, produced 4 Ph. Ds. and brought 16 funded projects during the year.

The highlights of the Department of Biophysics are – that the crystallographic structure of Calotropin DII has been solved. Also the Department cocrystallized a series of complexes of amino acids and nucleotides. The difference in cognate/noncognate tRNA-tRNA synthetase binding was attributed to a conformational change. The nature of structural change involved in the phosphorylation of P protein of CHP virus was identified. A theoretical model of protein electron transfer was developed with a successful application to Ru-Cyt-c. The Department published 10 full-length papers and brought 5 funded projects.

The highlights of the Animal Physiology Section are the findings that Estradiol-17Beta, a vertebrate female sex steroid, showed potent stimulatory effect on silkgland activity of silkworm, *Bombyx mori*. Therefore, this steroid could be effectively used in sericulture for enhancement of silk production. Work on basic fibroblast growth factor (bFGF) on newborn rat neuronal cells showed that it can be differentiated through phosphorylation of a 90-93 KD transcription factor. This factor then homodimerizes and translocates to nucleus where it binds to specific nucleotide

sequence to activate specific gene transcription. The Section published 6 full-length papers and brought 2 funded schemes.

In Plant Molecular & Cellular Genetics Section work on Integrated Pest Management Policy is continuing. The Section has identified four stable somatic hybrid lines with more *Brassica juncea* genome component focusing on Cytoplasmic Male Sterility, *Alternaria* blight resistance, drought tolerance and low glucosinolate content. Transgenic *Brassica* plants with GUS + NPT II gene had been produced. The Section produced 1 Ph. D. and brought 3 funded projects during the year.

The newly created Environmental Sciences and Immunotechnology Sections had also made appreciable progress in their areas of activity. The Environmental Sciences Section had isolated two bacteria - BI-100 and BI-102 having the unique ability to degrade organochlorine pesticide (HCH) at a very fast rate. A Patent application for the same has been submitted. The Section has developed an Arsenic selective water filter for decontamination of Arsenic from drinking water. Search for immunomodulatory compounds from exotic Indian plants have been initiated. From desmotricum, anti-microbial, immunomodulatory and anti-tumour compounds have been isolated, which are being tested for confirmation of the preliminary results. Further progress has been made on the immunotherapeutic potential of Protein A to mitigate Aflatoxin intoxicated death in poultry. The Section has published 2 papers and brought 6 funded projects, and also extended its consultancy for testing and monitoring of various environmental pollutants in different industries, which also brought in considerable amount of funds to the Section.

The Immunotechnology Section pursued their on-going work and made a significant observation that it is possible to induce selective killing of Cancer cells without harming the normal cells, which had been a long cherished goal of Cancer researchers. This selective induction of apoptosis is mediated through soluble mediators released by activated immunocytes. Such knowledge might help in the development of suitable strategy for treatment of Cancer. A 20 mer peptide had been identified from the structural segment of Protein A having the unique property of binding the antibody globulin and having anti-tumor properties. The hematopoietic toxicity rendered by anti-AIDS drug, AZT, could be reversed using Protein A. Cloning and expression of Protein A in both bacterial and mammalian cells had been initiated. The Section published 4 papers and brought in 2 funded projects.

The Madhyamgram Experimental Farm, Library, Workshop, RSIC, DIC and Publication Unit had all served the Institute with the best of their ability. The Library published a paper and has been trying to modernise its facilities. The Workshop helped different Departments for maintenance of various electrical and mechanical gadgets and also made some small equipments for different laboratories. It had supplied 2 units of Crescograph to Birla Industrial and Technological Museum, Calcutta, on payment basis.

RSIC activities showed some moderate income through the services rendered using different sophisticated instruments. They have also made 3 publications.

The DIC, apart from serving the Eastern Sector of India, had also helped the Institute to establish a Local Area Network for massive computerisation and to

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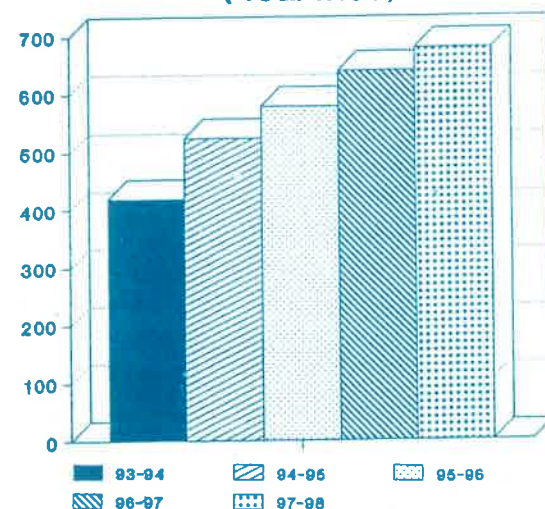
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establish connectivity within the Departments and in-between the Departments, including the arrangement to connect both the campuses through electronic media. Two softwares have been developed by the Unit and deposited to PDB. A software on comprehensive data base has been developed.

The Publication Unit had come out with 3 volumes of Book entitled "Acharya J.C. Bose - A Scientist and Dreamer" edited by Prof. P. Bhattacharyya. These

Books contain J.C. Bose's scientific work published in various journals, and information about many other incidences and comments of historic importance. M/s. Dasgupta & Co. Pvt. Ltd., Calcutta, has been entrusted with the responsibility of Sole Indian Distributorship for selling these publications.

### GRANT (Rs. in Lakhs) (Yearwise)



During the year under report the Institute received Rs. 679.75 lakhs as grant-in-aid from DST and also could fetch about Rs. 2.00 crores from outside funding. The interest income of the Institute was about 40.00 lakhs and miscel-

laneous earnings were about 13.00 lakhs. So, funds available for spending during this financial year was about Rs. 909.874 lakhs.

Thus, it will be quite apparent from the above that the Institute is marching forward with its full vigour and renewed enthusiasm. Now that a major equipment facility have been created in the Institute by incorporation of a large number of sophisticated equipments, the scientists are in a position to publish their papers in high impact factor journals. With the induction of quite a good number of young scientists during the past couple of years, the momentum in our net productivity is yet to be achieved. I am quite sure that in months and years ahead of us, the Institute will be able to show better performance and also will react more and more to fulfill the societal obligations.

Wishing you all a very good year ahead of us.

(P. K. Ray)  
Director



During the year 1997-98, Bose Institute initiated a comprehensive programme in multi-disciplinary research on Science at High Altitude. In collaboration with Saha Institute of Nuclear Physics and the Eastern Centre of Research in Astrophysics, Bose Institute organized a National Symposium on "Science at High Altitude" at Darjeeling during May 8-11, 1997, with external financial support from Council of Scientific & Industrial Research, New Delhi, Department of Science & Technology, Govt. of India, New Delhi, Indian National Science Academy, New Delhi and Bruker (India) Ltd. Various aspects of Physical, Chemical, Biological, Environmental and Medical Science issues related to the high altitude environment were discussed by leading experts. Several programmes, which could be initiated at an early date, were identified and a Task Force, under the Chairmanship of Dr. A. P. Mitra, FRS, was formed to coordinate activities in various areas of high altitude research. The proceedings of the symposium were published by Allied Publisher, New Delhi, in a volume edited by Prof. Sibaji Raha, Prof. P. K. Ray and Prof. Bikash Sinha.

During the Symposium, the Acharya Jagadis Chandra Bose High Altitude Research Station was formally inaugurated at the Darjeeling campus (Mayapuri and Haimavati buildings) of Bose Institute by Hon'ble Shri Subhas Ghising, Chairman of the Darjeeling Gorkha Hill Council. This station would serve as the base station for all high altitude research by the faculty of Bose Institute as well as other interested scientists of the Nation at other neighboring experimental stations at still higher altitudes.

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## Physics

**Faculty :** Prof. S.C. Roy, Prof. M.H. Engineer (Chairman), Dr. A.K. Chatterjee, Dr. S. N. Changdar, Prof. S. Raha, Dr. I. Bose, Dr. D. Home, Dr. B. Roy, Dr. B.K. Chatterjee, Dr. S.K. Saha, Dr. T.P. Sinha.

### HIGHLIGHTS

A book on "Conceptual Foundations of Quantum Physics" by Dr. D. Home has been published by Plenum Press, USA. The department played a major role in holding a highly successful national symposium on Science at High Altitude at Darjeeling during May, 1997. Improvements on the Superheated Drop Detector pioneered by Prof. S.C. Roy have been carried out by a member of the academic staff.

#### A. Acoustics

**A.1. Study of viscous fingering type instability at the interface of vertically driven liquid layers :**

**B. Roy and M.H. Engineer**

A Viscous fingering type instability has been observed in an oil-water system consisting of castor oil+chloroform, kept above a layer of 0.2% surfactant solution in distilled water. The density difference and interfacial tension between the two liquids are kept very small and the system is vertically driven in a steady circulatory flow by acoustical means. For sufficiently strong drive, a long viscous fingering instability develops as the less viscous liquid tunnels into the more viscous one. In this particular oil-water system the thickness of the viscous finger is found to change with change of diameter of the driving acoustic beam, while keeping the dimension of the liquid container fixed. The reason for this behaviour lies in the nature of the steady driving force. The steady circulatory flows generated by it are more or less confined around the tube axis, depending on whether the ratio of driving transducer radius to the radius of liquid container is smaller or larger. Although the finger profile changes, it remains stable as a whole i.e. there is no further instability at its tip. This is in marked contrast to the behaviour of an analogous system where the two liquids were Petroleum ether +  $\text{CCl}_4$  and water. We conjecture that the reasons are to be found in the different viscosity ration present in the two cases.

**A.2. Illusion in human hearing :**

**M. H. Engineer and A. Chatterjee (RSIC)**

Perceptual illusions in human hearing were investigated. Apparatus to generate arbitrary sequences of pure tones was built. When two such different sequences are fed, separately, into each ear an illusion occurs. The sequences heard differ strikingly from those actually impinging on the eardrums.

After refining the apparatus we studied the structure of the illusion further to determine whether there are any similarities to the phenomenon of "backward masking" discovered, in the perception of skin touching, by Libet's group.

#### B. Nuclear Physics

**B.1. Electronic Structure and Chemical Bonding Mechanism in Silver Oxide :**

**A. K. Chatterjee and Aniruddha Deb**

The compound silver oxide has interesting properties because of its role in fast ion conducting glasses of the type  $\text{AgI-Ag O-B}_2\text{O}_3$ . A study in detail of the electronic structure of silver oxide is necessary for the understanding of the chemical bonding between silver and oxygen in glasses. This facilitates in the understanding of the local structure of the glasses and the mechanism of ionic conduction. the electronic structure and chemical bonding mechanism of silver oxide ( $\text{Ag}_2\text{O}$ ), is studied on the basis of band-structure calculations, using the full potential linearized augmented plane wave (FLAPW) method. In accordance

with, experimental observations, silver oxide, is found to be a semiconductor. Total and partial densities of states and electron densities were calculated and are utilized to give an interpretation of chemical bonding. The admixture of the Ag (5s) states to the Ag 4d-O 2p bands resulted to be essential for the covalent bonding effect, since pure 4d-2p band, with bonding and antibonding states fully occupied, do lead to a covalent energy gain. It is found that there is a significant deviations from a simple ionic picture due to the depletion of the valence band of Ag-Ad electrons leading to a non-spherical charge density around silver.

### B.2. Formation and Coupling of Magnetic moments in Heusler alloy $\text{Cu}_2\text{MnAl}$ :

A.K. Chatterjee and Aniruddha Deb

The microscopic mechanism responsible for both the formation and coupling of magnetic moments in Huesler alloy  $\text{Cu}_2\text{MnAl}$  are identified. We found that the Cu atoms serve primarily to determine the lattice constant, while the Al atoms mediate the interaction between the Mn-d states. There is no significant direct interaction between the Mn-d atoms, but the occupied d-states of Mn are delocalized by their strong interaction with the Cu-atom d-states. The localized character of the magnetization results from the exclusion of minority-spin electrons from the Mn-3rd shell. The coupling between the localized magnetic Mn moments can be described with the Heisenberg Hamiltonian and the sign of the exchange constants results from a competition between the intra-atomic magnetic energy and interatomic Al-atom mediated covalent interactions between the Mn-d states. The sensitive dependence of magnetic order on the occupation of the mediating p-d hybrid states accounts well for experiments by Webster in which this occupation is varied by alloying. Our analysis is based on the self-consistent, spin-polarized energy-band calculations using the full potential augmented plane wave (FLAPW) method, for ferromagnetic spin alignments.

### B.3. Positron lifetime in polymer :

A.K. Chatterjee and Aniruddha Deb

In the positron annihilation life time measurement spectrometer we have used at

present BaF<sub>2</sub> scintillation crystal. With this new arrangement the resolution of the spectrometer is found to be 200 psec. We are now measuring life time of positrons in protein samples with this high resolution spectrometer.

### C. Applied Radioactivity :

S. N. Changdar

Diffusion studies in liquids were continued using both sliding cell technique and sheared boundary technique. Hg-203 and P-32 were the two radioactive isotopes which were used for study in multicomponent systems. Isotope effect was tested in different systems. A new semi-empirical formalism is being contemplated for concentrated aqueous solutions.

### D. Radiation Physics and Condensed Matter

### D.1. A new concept of gelation :

Nirmal K. Halder, **B.K. Chatterjee** and  
**S.C. Roy**

Recently carried out experiment to study the change in viscosity in polymeric liquid with concentrations of suspended particles confirms a new concept of gelation in contrary to the conventional model of gelation. Common concept of gelation is the immobilization of fluid and is usually associated with cluster size and which will produce shear elasticity in the liquid. This occurs at a time when the clustering probability exceeds a threshold which is usually known as the "gel point". It had been reported earlier that the viscosity diverges (on irradiation) but without producing any elasticity, in contrary to what is expected from the conventional model of gelation. We present a new concept of gelation on the basis of hydrodynamic interactions between suspended particles which is capable of explaining the observed divergence. According to this concept, gelation is a two step process and we have been able to identify two types of gels; one is the inelastic gel which does not have a shear elasticity but is immobile due to the largeness of viscosity, and other is the conventional elastic gel.

### D.2. Ionic transport and reaction processes in nonpolar liquids :

B. K. Chatterjee

Transport of copper ions in octamethylcyclotetra siloxane are being investigated.

Attempts are being made to cut down the image current and hence get a clean pulse of copper ions by using an extra grid just in front of the ion collector.

**D.3. Study on neutron sensitivity of superheated drop detectors (SDDs) :**

Mala Das, B. Roy, S.C. Roy and B.K. Chatterjee

Using the horizontal tube air displacement system of volumetric method (reported in Bose Inst. Ann. Rep. 1995 - '96) the maximum rate of nucleation, life time of superheated drops and efficiency of neutron detection for SDDs made of different liquids e.g. Freon-12 (b.p. - 29.7°C), Freon-22 (b.p. - 40.8°C), Freon-21 (b.p. 8.92°C), Freon-502 (b.p. - 45.6°C), etc, were determined in presence of same neutron flux from <sup>3</sup>Ci Am-Be source. It has been found that the SDDs made of Freon-22 sample is the most efficient one to detect neutrons among the four liquids investigated so far. With the present system of measurement the minimum measurable dose was found to be 0.231 μ Sv/cc of Freon-22 in the SDD and the sensitivity to neutrons was observed to be  $1.2 \times 10^7$  neutrons / cm<sup>2</sup> / cc of Freon-22.

The actual dose of neutrons at different distances from the centre of the source were measured by our standard Neutron Monitor model NM2 of N.E. Technology Ltd., England procured from the joint grant of Institute and AERB. Variation of our SDD responses as a function of dose was found to be linear.

Investigations have been made to study the effect of temperature on the SDD made of Freon-12. In one set of experiment the effect of heat shock on the performance of SDDs made of Freon-12 were studied. It was observed that within the temperature range 20°C to 60°C the nucleation rate and efficiency of neutron detection was larger than those in the case where the temperature was increased slowly. Investigations on variation of neutron detection efficiency with temperature is being continued.

For determining the nucleation rate of the superheated drops by counting each drop vaporisation acoustically, a compact electronics is under development.

**D.4. Mossbauer quadrupole splitting of Fe(2+) ions in insulators under vibronic coupling :**

T.P. Sinha

The Mössbauer effect has been widely used to study the electronic states of  $\text{Fe}(2+)$  ions in solids. The information about the electronic charge distribution is obtained from the study of nuclear quadrupole splitting and isomer shift, while the study of the magnetic hyperfine spectrum furnishes the information about the spin ordering. The effect of orbit-lattice interaction (vibronic coupling) as a perturbation has been used on the electronic states of  $\text{Fe}(2+)$  ions in  $\text{NH}_4\text{CoCl}_3$  to explain the observed temperature dependence of quadrupole splitting. This interaction, through weak in general, may be quite important in the case of  $\text{Fe}(2+)$  ion which is strongly coupled to lattice vibrations and its effect should be manifested in Mössbauer studies which can measure extremely small energy changes.

#### D.4. Free radicals in *Oryza sativa* and *Hevea brasiliensis* :

T. P. Sinha and S. Sen-Mandi

Electron paramagnetic resonance studies to understand the status and role of free radicals and their scavenging systems in dry embryos of *Oryza sativa* (rice seeds) and in bark samples of *Hevea brasiliensis* (rubber plants) have been carried out. The free radicals recorded here are carbon-based derived from quinones.

### E. Theoretical Physics, Astrophysics and Cosmology

**E.1.1. High Energy Particle and Nuclear Physics (Theory) :**

(a) *Finite Temperature Field Theory :*

Abhijit Bhattacharyya (now at Variable Energy Cyclotron Centre, Calcutta since March 2, 1998), Sanjay Kumar Ghosh and **Sibaji Raha**

Various aspects of the many-body system of hadrons and quarks at high temperature and zero baryon density have been investigated in the framework of different models, like the Zimanyi - Moszkowski model, the Walecka model and the quark-meson coupling model.

The dissociation rate of pions into a q pair in the medium of quarks and gluons at high temperature has been calculated. The variation of the effective masses of diverse mesons with temperature and their decay widths have been studied.

(b) *Microscopic Approaches to Hadronization:*

Sanjay Kumar Ghosh, Abhijit Bhattacharyya (now at VECC) and **Sibaji Raha**, in collaboration with Dr. Janere Alam of VECC, Calcutta and Prof. Bikash Sinha of VECC and Saha Institute of Nuclear Physics, Calcutta.

Stochastic pionization in a non-equilibrium, highly excited system of quarks and gluons produced in ultrarelativistic heavy ion collisions has been investigated in the framework of the Smoluchowski theory of coagulation. It has been found that even if some pionic degrees of freedom start manifesting themselves at temperatures substantially higher than the critical temperature for deconfinement, pions begin to appear in sizable numbers only when the temperature of the system becomes comparable to or slightly lower than the critical temperature. Fluctuations that are typical of a first order phase transition are also seen ; current efforts are aimed at deciphering whether these fluctuations are real and physically meaningful or artifacts of the involved numerical procedure.

(c) *Hadronic Structure Functions and Quantum Chromodynamics :*

Sanjay Kumar Ghosh and **Sibaji Raha.**

Studies on incorporating the dynamical effects of colour confinement on hadronic structure functions have been continued. In the course of these investigations, the method of Jacobi Polynomials, suggested by some authors as a very reliable tool to calculate the  $Q^2$  evolution, has been critically examined and found to be not so accurate as claimed in the literature. Coupled equations for valence and sea quark and gluon distributions are now being solved numerically.

(d) *Properties of Multiquark hadrons :*

Dr. Sanjay Kumar Ghosh, in collaboration  
with Prof. S. C. Phatak of IOP, Bhubaneswar

The formation of multi-quark hadrons in energetic heavy ion collisions is a novel possibility. In this context, the lowest lying six-quark or dibaryon state, i.e. dihyperon, has been studied in the framework of the Chiral Colour Dielectric model. The dihyperons are found to be stable, with masses below the two hyperon threshold, for different parameter sets of the model. A manuscript containing these results has been accepted for publication in Physical Review.

**E.1.2.** *Astrophysics and Cosmology – theoretical and observational :*

(a) Many-body Theory of Strongly Interacting Matter and Equation of State :

Sanjay Kumar Ghosh, Abhijit Bhattacharyya (now at VECC), Shibaji Banerjee and **Sibaji Raha**, in collaboration with S.C. Phatak of Institute of Physics, Bhubaneswar.

The equation of state of hot and dense hadronic matter is a field of high current interest, both for heavy ion collisions as well as for astrophysical processes like Neutron Star structure, supernovae and such. Such studies aimed at understanding the equation of state of hadronic matter and the hadron-quark phase transition have been carried out, using different effective models for the hadronic sectors and the Chiral Colour Dielectric model for the quark sector. It has been found that all the models in the hadronic sector predict a phase transition to quark matter at high temperature. On the other hand, at zero temperature, only the Walecka model and its non-linear version undergo a phase transition to quark matter. A manuscript with these results by Dr. Abhijit Bhattacharyya and Dr. Sanjay K. Ghosh has been accepted for publication in International Journal of Modern Physics.

The Walecka model for superdense hadronic matter, although very popular due to its simple structure, has many drawbacks, the most serious ones being the high incompressibility at nuclear matter density and the dropping of the baryon masses to zero at moderately high densities and temperatures. Efforts have been made to improve on these by including the mesonic corrections self-consistently. The preliminary results show a substantial softening of the

equation of state at higher densities. Also the variation of baryon masses with density is found to be much slower than in the original Walecka model. A full calculation for asymmetric baryonic matter is currently in progress.

Neutrino emissivity from kaon-condensed baryonic matter at neutron star densities is being calculated.

(b) *Study of Strangelets in Cosmic ray fluxes:*

Shibaji Banerjee, Sanjay Kumar Ghosh, M.H. Engineer and **Sibaji Raha**, in collaboration with Bikash Sinha (VECC) and SINP, Calcutta), Debapriyo Syam (Presidency College, Calcutta), Tarun K. De (Bajkul Milani Mahavidyalaya, Midnapore) and **Rabi N. Mukherjee** (IP, Bhubaneswar).

Propagation of strangelets in the upper atmosphere, taking account of the earth's magnetic field at the latitude of Darjeeling and Sandakphu, has been studied. Calibration experiments for the CR-39 solid state nuclear track detector have been designed and beam time applied for at various accelerators in the country. An image and contour analyzing system for automatic scanning and recording of the etched nuclear tracks in SSNTD-s has been installed successfully and used for calibration purposes with previously exposed detectors.

### E.2.1. A Conceptual Analysis of Quantum Zeno Paradox : Measurement, and Experiment :

**Dipankar Home** in collaboration with  
M.A.B. Whitaker, Dept. of Pure and Applied  
Physics, Queen's University, Belfast, Northern  
Ireland.

Arguments on controversial points concerning quantum measurement theory and the quantum Zeno effect are developed. In particular it is argued that (1) the quantum Zeno effect is a genuine result of quantum theory and current quantum measurement theory, independent of the projection postulate; (2) the effect is of very general nature and rests on analogous arguments to those involved in Bell's theories; (3) the term "quantum Zeno effect" may usefully be restricted to experiments where a measuring device exerts a nonlocal

negative-result effect on a microscopic system. (4) since no decay is truly exponential, theoretically all decay phenomena should exhibit the quantum zeno effect under observation, continuous or discrete. A detailed study is made of the experiments claiming to demonstrate the effect; it is found that they do not meet our criterion above.

### E.2.2. Quantum Zeno effect : Relevance for local realism, macroscopic realism, and non-invasive measurability at the macroscopic level :

**Dipankar Home** in collaboration with  
M.A.B. Whitaker of Dept. of pure and Applied  
Physics, The Queen's University of Belfast, UK.

Variants of the quantum Zeno effect are analysed. It is deduced that the predictions of some variants are incompatible with local realism, and that, provided the laws of quantum theory remain valid at the macroscopic level, all variants of quantum theory remain valid at the macroscopic level, all variants of quantum Zeno prediction must be incompatible with non-invasive measurability at the macroscopic level.

**E.2.3.** Comment on "Why quantum mechanics cannot be formulated as a Markov process":

**Dipankar Home** in collaboration with I. Hardy, E. J. Squires of Dept. of mathematical Sciences, University of Durham, England, and M. A. B. Whitaker of Dept. of Pure and Applied Physics, Queen's University, Belfast, Northern Ireland.

As a sequel to the earlier work by D. Home with L. Hardy, E.J. Squires and M.A.B. Whitaker [Phys. A 45, 4267 (1992)], it is demonstrated explicitly by using a quantitatively precise model that the behaviour of a quantum mechanical two-state oscillator may be simulated by a classical jump process.

#### E.2.4. Collapse-Induced Quantum Nonlocal Effect :

**Dipankar Home** in collaboration with  
G.Kar of Physics and Applied Mathematics Unit,  
Indian Statistical Institute, Calcutta.

This work brings out some empirically significant new aspects of what we call

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collapse-induced quantum nonlocal effects resulting from the use of the hypothesis of wave function collapse.

**E.2.5. Conceptual Foundations of Quantum Physics – An Overview from Modern Perspectives :**

**Dipankar Home**

A comprehensive treatise on the foundational problems of quantum mechanics was completed and published (Plenum Press, New York). The book explains in detail how a number of fundamental conceptual problems of quantum mechanics have become amenable to precise quantitative formulations as well as experimental studies. It also indicates new research directions in this field.

**E.2.6. Geometric Phase :**

**M. H. Engineer**

The Bristol Geometric Phase group has suggested that the Exclusion Principle might be related to the geometry of the underlying Hilbert space. I consider their implementation of this obvious possibility to be unsatisfactory. I have attacked the problem using Majorana's representation for arbitrary spins.

**E.3.1. Strongly correlated systems and Magnetism. :**

**Indrani Bose, Saurabh Gayen and Emily Chattopadhyay**

Recently, superconductivity has been discovered in a spin ladder compound doped with holes. This discovery has motivated a large number of studies on doped ladder systems. Earlier, Bose et al constructed a ladder model with two holes and showed through rigorous, analytical results that two holes can form a bound state, the hole Copper pair. In the light of discovery of superconductivity in a ladder compound, exact hole dispersion relations for the scattering, bound and anti-bound states of two holes have been explicitly calculated. The bound state wave function has been shown to be of modified d-wave symmetry. The original electronic model has been mapped onto a model of hard core bosons

and dominance of superconducting pairing correlations has been demonstrated.

Work has been initiated on spin and electronic models which have matrix-product ground states as exact ground states. The models describe a variety of real systems exhibiting novel phenomena like coexistence of long range order and the Haldane gap, new impurity states in the Haldane gap, destruction of the spin liquid state by the presence of non-magnetic impurities and so on.

**E.3.2. Statistical and cross-disciplinary Physics :**

**Indrani Bose, Indranath Chaudhuri and Asimkumar Ghosh**

A new type of phase transition analogous to the percolation transition has been discovered in the distribution of activator concentration in the reaction-diffusion model of activator and inhibitor. The pattern of activator concentration is obtained in the non-equilibrium steady state. The connectivity of the pattern is lost below a critical probability of site occupation in the original square lattice. Study of the scaling behaviour of the clusters near the percolation threshold is in progress.

The site percolation threshold has been determined for a series of depleted square lattices. The depletion has been made in a regular manner. When the depletion is small, one finds that the percolation threshold is the same as that obtained in the case of random depletion. Determination of the critical temperature of the Ising model on the depleted lattices is in progress.

A reaction-diffusion model involving activator, inhibitor and substrate has been constructed and linear stability analysis has been performed to determine the phase diagram. The phase diagram is rich with a variety of phases including a Turing region of stationary patterns. The possibility of chaotic states is being investigated.

A model has been formulated to explain the punctuated equilibrium behaviour that has been observed in a recent experiment on bacterial evolution.

## PUBLICATIONS

1. Interpolation of elastic scattering cross sections of photons B.K. Chatterjee and S.C. Roy, *Ind. J. Phys.* **71B**, 245, 1997.

2. Photoelectric cross sections derived from measured total attenuation coefficient of photons near absorption edges of heavier atoms, B.Roy, B.K. Chatterjee, S.C. Roy, Nanak Bhattacharya, and N. Choudhury, *Appl. Radiat. Isot.* **48**, 785-788, 1997.

3. Optimisation of Scattering Geometry. Swapan K. Saha, B.K. Chatterjee, and S.C. Roy, *Meas. Sci. Tech.* **8**, 1262-1266, 1997.

4. Change of viscosity with concentration of suspended particles and a new concept of gelation. N. K. Halder, B.K. Chatterjee, and S.C. Roy, *J. Phys. (Condensed Matter)* **9** 8873-8878, 1997.

5. Variation of gamma ray and particle fluxes during the total solar eclipse of 24 October, 1995. Abhijit Bhattacharyya, Sukumar Biswas, Barun K. Chatterjee, Mala Das, Pradipta K. Das, Tapan K. Das, Tarun K. De, M.H. Engineer, Rabi N. Mukherjee, Sibaji Raha, S.C. Roy, Swapan Saha, A.K. Sen, Bikash Sinha, and Debapriyo Syam, *Astrophysics and Space Science* **250**, 313-326, 1997.

6. Determination of the rate of Cold Fusion reaction using a calorimetric method. B.K. Chatterjee, Pradipta Roy and S.C. Roy, *Sci. & Cult.* **64** 29-30, 1998.

7. A new kind of neutron detector using superheated liquid. Mala Das, B. Roy, B.K. Chatterjee and S.C. Roy, *Proc. of DAE Symposium on Nuclear Physics (Bangalore)*, **40B** 452-453, 1997.

8. Kaon mass in dense matter. A. Bhattacharyya, S.K. Ghosh, S. C. Pathak and S. Raha, *Physics Letters*, B401 **213**, 1997.

9. Finite temperature effects on electromagnetic probes of quark - gluon plasma. A. Bhattacharyya, J. Alam, S. Raha and B. Sinha, *International Journal of Modern Physics*, A12 5639, 1997.

10. Dissipative effects in quark-gluon plasma diagnostics. S. Sarkar, P. Roy, J. Alam, S. Raha and B. Sinha, *Journal of Physics*, G23 469, 1997.

11. Thermal masses and equilibration rates in the quark - gluon phase. J. Alam, P. Roy, S. Sarkar, S. Raha and B. Sinha, *International Journal of Modern Physics A2* 5151, 1997.

12. Quark gluon plasma diagnostics in a successive equilibration scenario. P. Roy, J. Alam, S. Sarkar, B. Sinha and S. Raha, *Nuclear Physics*, **A624** 687, 1997

13. Mössbauer and magnetic studies of intermetallic Fe-Mn-Ti alloy. Sinha T.P., Roy R., Mandal P., Bhattacharya M. and Majumdar C.K. *Ind. J. Phys.* **71A**, 267, 1997.

14. Active oxygen and their scavengers in rice seeds (*Oryza sativa* cv. IET 4094) aged under tropical environmental conditions. Nandi S., Sen-mandi S. and Sinha T.P. *Seed Science Research* **7**, 253, 1997.

15. Status of free radical and its scavenging system with stimulation in *Hevea brasiliensis*. Das G., Raj S., Pothan J., Sethuraj M.R., Sinha T.P. and Sen-Mandi S. *Plant. Physiol. & Biochem.* **24**, 1, 1997.

16. Mössbauer quadrupole splitting of Fe(2+) ions in  $\text{NH}_4\text{CoCl}_3$  under vibronic coupling. Sinha T.P., Roy R. and Majumdar C.K. *Solid State Phys. (India)* **40C**, 424, 1997.

17. Structural and dielectric behaviour of  $(\text{Mo}_{1/2}\text{W}_{1/2})\text{O}_3$  ceramic. Choudhary S.N., Sinha S.K., Sinha T.P. and Choudhury R.N.P. *Solid State Phys. (India)* **40C**, 340, 1997.

18. Exact ground and excited states of frustrated antiferromagnets on the  $\text{CaV}_4\text{O}_9$  lattice by Indrani Bose and Asimkumar Ghosh, *Phys. Rev. B* **56**, 3149, 1997.

19. Collinear Néel-type ordering in partially frustrated lattices by Uma Bhaumik and Indrani Bose, *Phys. Rev. B* **58**, 77, 1998.

20. Lattice animals and percolation model under rotational constraint by Indrani Bose, in the Proceedings of the International Workshop on Percolation theory and Particle Systems ed. by Rahul Roy (Indian Statistical Institute, New Delhi 1996), pages 173-194

21. Rugged Energy Landscapes by Indrani Bose, *Physics Teacher* **38**, 60, 1996.

22. New topics in condensed matter physics syllabus by Indrani Bose. *Physics Teacher* **39**, 21, 1997.

23. Quantum zeno effect : Relevance for local realism, macroscopic realism, and non-invasive measurability at the macroscopic level. Home D., and Whitaker M.A.B., *Phys. Lett. A* **239**, 6 1998.

24. Response to Comment on DNA Molecular Cousin of Schroedinger's Cat : A Curious Example of Quantum Measurement. Home D., and Chattopadhyaya R., *Physical Review Letters* **80**, 6, 1998.

25. Comment on Why Quantum Mechanics cannot be formulated as a Markov Process. Home D., Hardy L., Squires E.J., and Whitaker M.A.B., *Physical Review A* **56**, 4, 1997.

26. A Conceptual Analysis of Quantum Zeno: Paradox, Measurement and Experiment. Home D., and Whitaker M.A.B., *Annals of Physics* **258**, 237, 1997.

27. Collapse-induced Quantum Nonlocal Effect. Home D., and Kar G., *Foundations of Physics* **27**, 12, 1997.

28. Science and Superstition in Society. M. H. Engineer, *Science and Culture*, **63**, 229-234, 1997.

#### Book

"Conceptual Foundations of Quantum Physics - An Overview from Modern Perspectives". Home D., Plenum Press, New York, 1997 (ISBN) No. 0-306-45660-5).

#### Participation in Conferences/Symposia/ Training Programmes/Awards and Honours received :

##### Prof. S.C. Roy :

Attended the Symposium on Science at high Altitude held in Darjeeling during May 8-12, 1997. He also attended the Advance Workshop on Internet held at Goa during March 25-27, 1998.

##### Prof. Sibaji Raha :

Acted as the Convener for Physical Sciences at the National Symposium on Science at High Altitude at Darjeeling during May 1997. ; delivered a colloquium at Saha Institute of Nuclear Physics, Calcutta in September 1997.; delivered a colloquium and a seminar at Physical Research Institute, Ahmedabad in September 1997.; gave an invited talk at the National Symposium on Hundred Years of Electron Discovery at Calcutta University in November 1997.; participated at the 15th Annual Meeting of the International Society for General Relativity and Gravitation at IUCAA, Pune during December 1997 and gave a talk. ; participated at the International Conference on Modern Trends in Physics at Utkal University, Bhubaneswar during January 1998 and gave two invited plenary talks. ; acted as the Member Secretary for the National Task Force for Science at High Altitude. ; served in the TPSC Calcutta Centre sub-committee as a member.

##### Dr. Indrani Bose :

Delivered a talk on Protein Folding in the one-day workshop held at the Bose Institute, Calcutta (May 1997) ; delivered a Colloquium talk at the Bose Institute on Turing Patterns, sandpiles and biological evolution (March 1998); invited to give (i) a talk at the workshop titled Condensed Matter Days held at Visva-Bharati University, Santiniketan, West Bengal (August 1997).

(ii) a talk at the Conference on Physics and Chemistry held at I.A.C.S., Calcutta in celebration of 50 years of the Indian independence September 1997) ; a Colloquium at S.I.N.P., Calcutta (October 1997) ; a talk on Growth and Form at the Presidency College, Calcutta (1997) ; a talk at the National Symposium on Superconductivity held in the Central University of Hyderabad (December 1997) ; a talk at the Centre of Theoretical Studies, I.I.Sc., Bangalore (December 1997) ; a talk at the International Conference on Nonlinear Dynamics : integrability and chaos held in the Bharathidasan University, Tiruchirapalli (February 1998) ; five lectures in the Refresher Course on Theoretical Physics for undergraduate Physics teachers, organised by

Calcutta University (January - February 1997); a set of lectures on Magnetism in the Post-M.Sc. class of the Saha Institute of Nuclear Physics, invited to become a member of the organising committee of the Workshop 'Condensed Matter Days' held in Visva-Bharati, Santiniketan (August 1997).

##### Dr. B. Roy :

Participated and presented a poster in the Symposium on "Condensed Matter Days - 1997" held at Dept. of Physics, Visva - Bharati, Santiniketan during August 29 - 31, 1997 ; attended the National symposium on "Contemporary Physics, Visva - Bharati, Santiniketan during August 29 - 31, 1997 ; attended the National Symposium on "Contemporary Physics: Some Aspects" held at Physics Dept, Presidency College, Calcutta from November 6 - 8, 1997, for celebration of the 50 years of Independence.

##### Dr. B.K. Chatterjee :

Participated in the symposium on Science at High Altitude held at Darjeeling in May 1997 and an invited talk on "Studies of reactions of dimethyl methyl phosphonate with atmospheric ions".

##### Dr. T.P. Sinha :

Participated in the Workshop of Neutrons as Probes of Condensed Matter held at Solid State Physics Division, Bhabha Atomic Research Centre, Mumbai from July 7 to 25, 1997 ; presented three papers in the meeting "Condensed Matter Days - 1997" held at Department of Physics, Visva-Bharati, Santiniketan from August 29 to 31, 1997; participated in the School on "Magnetism : Modern Methods and materials" held at Department of Physics, Goa University, Goa from October 13 to 25, 1997 ; presented two papers in the Annual DAE Solid State Physics Symposium held at Department of Physics,

Cochin University of Science & Technology, Cochin from December 27 to 31, 1997.

##### Dr. Sanjay Kumar Ghosh :

Participated in the National Symposium on Science at High Altitude at Darjeeling during May 1997 and gave a talk ; participated in the Summer School on High Energy Physics and Cosmology at the International Centre for Theoretical Physics, Trieste, Italy during June - July, 1997 ; participated in the Yukawa International Seminar on Non-perturbative QCD at the Yukawa Institute of Kyoto University, Japan during December 1997 and gave a talk.

##### Abhijit Bhattacharyya :

Visited Institute of Physics (Bhubaneswar), Tata Institute of Fundamental Research and Bhabha Atomic Research Centre (Mumbai) and Indian Institute of Astrophysics and Indian Institute of Science (Bangalore) under the Theoretical Physics Seminar Circuit (TPSC) and delivered seminars and colloquia at the respective institutes/centres ; participated in the National Symposium on Science at High Altitude at Darjeeling during May 1997 and gave a talk.

##### Mala Das :

Participated and presented paper at "DAE Nuclear Physics Symposium, 26-30 December 1997" at Bangalore University, Bangalore.

##### Shibaji Banerjee :

Participated in the National Symposium on Science at High Altitude at Darjeeling during May 1997.

##### Patent Applied :

Dr. B.K. Chatterjee, Dr. B. Roy and Dr. Swapan K. Saha applied for patent of Govt. of India on "Hanging wire fluid velocimeter cum acoustic pressure gauge" in Oct. 1997.

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Bose Institute

#### GRANTS-IN-AID SCHEMES

| Investigator/<br>Co-investigator | Title                                                                                | Financed by                       |
|----------------------------------|--------------------------------------------------------------------------------------|-----------------------------------|
| Prof. S.C. Roy                   | Fabrication of pressure reactor and electronic circuitry to detect bubble nucleation | Defence Laboratory, Jodhpur       |
| Prof. S. C. Roy                  | Investigations on the use of superheated drop detector in neutron detection          | Automatic Energy Regulatory Board |
| Dr. B. K. Chatterjee             | Ionic transport and reaction processes in nonpolar liquids                           | CSIR                              |

#### RESEARCH SCIENTISTS/RESEARCH ASSOCIATES/POST-DOCTORAL FELLOWS/ SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS

A. Bhattacharya, Aniruddha Deb, Sanjay Ghosh, Sibaji Banerjee, Mala Das, Indranath Chowdhury, Ashim Kumar Ghosh (guest worker w.e.f. July 1997 to June 1998), Emily Chottopadhyay (joined on 2.1.1998), Som Shubhro Bandyopadhyay (joined on 25.11. 1997), Sonali Saha (joined on 26.11.1997)

#### SUPPORTING STAFF MEMBERS

G. R. Paul, N.C. Dey, A. K. Ganguly, K. C. Das, S. P. Singha, S. Basak (worked in the Dept. upto 7.9.1997), Shyam Sundar Mallick, Sbasis Banerjee, Manas Datta, Subrata Das, Tarun Kumar Maji, B. P. Ram, B. Dutta, D. K. Thakur, S. K. Basu, Kissan Das, R. K. Mourya, Prabir Halder.

## Chemistry

**Faculty :** Prof. P. Chakrabarti, Prof. A. Ghosh, Prof. P. Bhattacharyya (Chairman, till 10.08.97), Prof. Parimal C. Sen (Chairman, from 11.08.1997), Dr. Manas Chakrabarty, Dr. Suniti Misra, Dr. Kalipada Das, Dr. Joyoti Basu, Dr. Manikuntala Kundu, Dr. Pradip Das

#### HIGHLIGHTS

Clinical trial of Eicosa Pntaenoic Acid (EPA) on volunteer patients suffering from cardiovascular disorders has been started. A new protein kinase (PKx) has been purified and characterized from goat testis is seemed to be involved in fertility regulation. Anti bacterial and anti diabetic compounds have been isolated from Indian Medicinal plants. Mechanisms of drug resistance in *Shigella dysenteriae* and mycobacteria have been proposed. The mechanism of molecular chaperone activity of  $\alpha$ -crystallin protein at different temperatures has been established.

#### INSTITUTIONAL PROJECT II

**A.1.** Clinical trial of EPA on volunteer patients suffering from cardiovascular disorders at S.S.K.M. Hospital :

**A. Ghosh,** D. Banerjee, D. Pal and J. Dey in collaboration with Dr. M. Panja, Dr. K.N. Ghosh Hazra and Dr. A. Hoque of S.S.K.M. Hospital and Dr. S.K. Tripathi of Nil Ratan Sarkar Medical College Hospital.

A clinical trial of EPA on patients suffering from Angina pectoris and at high risk for cardiac attack has been initiated at S.S.K.M. Hospital. According to the protocol, approved by the Drug Controller General of India, patients were admitted with frequent anginal pain, associated with hypertension and high cholesterol, triglyceride and LDL levels. Each patient was given a dose of 2 g of EPA per day in the form of 500 mg soft gel capsules, orally, for a period of 4 months.

Six patients satisfying the inclusion criteria were able to complete the scheduled trial. Frequency of anginal pain was found to reduce considerably. Blood lipid profile indicated considerable reductions of cholesterol, TG and LDL levels and brought down to specified limits. Values for SGPT and SGOT were found to be normal in all of the patients, indicating that the EPA administered had no toxicity.

The trial is being continued on a sizable number of patients for further confirmation of the initial results.

**A.2.** A comparative study on the lipid profiles of fresh and sun-dried Bombay duck (*Harpadon nehereus*) :

**A. Ghosh,** J. Dey, D. Pal and D. Banerjee.

*Harpadon nehereus*, commonly known as Bombay duck fish, is abundantly available in the eastern and western coastal areas of India. Generally this fish is mostly consumed by the low income group of population because of its very low price. A large portion of the catch is sun-dried for preservation and export to other countries.

Preliminary investigations indicated the occurrence of considerable high levels of polyunsaturated fatty acids (PUFA), particularly of  $\omega$ 3 series which have received considerable attention because of their various biological activities including regulation of cardiovascular disorders. The other group of bioactive compounds detected and estimated was the glycerol ethers, known to have bacteriostatic and fungistatic properties. Besides, these alkyl-glycerol ethers are also known to have anti-inflammatory, hemopoietic and anti-tumor activities. Minor differences in composition of two samples were noted.

**A.3.** Studies on the lipid profiles of Indian sardine, *Sardinella fimbriata* :

**A. Ghosh,** A. Bhattacharya, D. Banerjee and D. Pal.

*Sardinella fimbriata*, commonly known as sardine fish, is abundantly available in the coastal regions of India. There are large number of fish

canning industries for export of this marine fish. From these fish canning industries, sizable quantities of sardine oils are available as byproduct, which is misused as a thinner for paints.

Current awareness of PUFA, particularly of  $\omega 3$  series of marine origin, which have large number of bioactive compounds for medicinal use, prompted us to examine this species for its proper utilization.

Preliminary results indicated the occurrence of high levels of PUFA of  $\omega 3$  series.

### INSTITUTIONAL PROJECT III

#### A.1. Cryopreservation in relation to sperm membrane damage :

**A. Ghosh** and **J. Chakrabarty**, in collaboration with Dr. G.C. Majumder, I.I.C.B., Calcutta.

The objective of this project is to identify novel cryoprotectants to delineate the biochemical basis of sperm membrane damage during cryopreservation and thawing of cells and to develop a novel technology for improving the cryoresistance potential of spermatozoa by manipulating membrane structure of these cells. The semen cryopreservation technology will be highly beneficial for solving some of the problems of cattle breeding and human infertility.

In the first phase of the programme, motility of goat spermatozoa were determined after cryopreservation and thawing in various conventional as well as non-conventional media.

#### B.1. Characterization of an unsaturated fatty acid activated protein kinase (PKx) from goat spermatozoa distinct from PKC and PKN:

**Parimal C. Sen**, Koushik Roy, Atin K. Mandal, Rita Sikdar, Subrata Majumder (Microbiology), Y. Ono (Kobe University, Japan).

The cytosolic fraction of goat cauda epididymides possesses a protein kinase (PKx) activity which is activated by unsaturated fatty acids with arachidonic acid as the best activator in absence cAMP, Ca etc. Phosphati-

dyl choline, phosphatidyl ethanolamine, phosphatidyl serine and diacyl glycerol have no effect either alone or in combination. The membrane fraction does not show any appreciable kinase activity even after detergent treatment. PKx migrates as a single band of apparent molecular mass 120 kDa on 10% SDS-PAGE after sequential chromatographic separation on DEAE-cellulose anion exchange, phenyl sepharose hydrophobic, high Q anion exchange and protamine agarose affinity columns. The cytosolic extract was treated with 20  $\mu$ M cAMP prior to DEAE cellulose chromatography to remove the catalytic subunit of contaminating PKA. PKx phosphorylates histone 1, histone 11s, protamine sulphate but not casein. However, the best activity was obtained with PKC substrate based on C-PKC pseudo substrate sequence. The kinase phosphorylates two endogenous cytosolic proteins of 68 and 60 kDa.  $\text{Ca}^{2+}$  and  $\text{Mn}^{2+}$  inhibit PKx activity in micromolar range. Staurosporine, a well known PKC inhibitor, and heparin are found to inhibit PKx activity at very low concentration. Lyso phosphatidyl choline and certain detergents at very low concentrations ( $<0.05\%$ ) stimulate enzyme activity to some extent. The immuno crossreactivity study with antibody against C4 domain of PKC suggests that protein kinase under study is not related to any known PKC family. Even the antibody against C-terminal end of PKN (a related protein kinase reported in rat testes found to be activated by arachidonic acid) does not cross react with our protein kinase. Hence, we believe that the protein kinase (PKx) reported here is different even from the PKN of rat testes. The phosphorylation of endogenous proteins by PKx may be involved in cell regulation including fertility regulation and signal transduction.

#### B.2. Purification and functional characterization of an 70 kDa inhibitor protein of Na, K-ATPase from goat testis cytosol :

**Parimal C. Sen**, Atin K. Mandal and Koushik Roy.

A protein isolated from goat testis cytosol is found to inhibit Na, K-ATPase from rat brain microsomes. The inhibitor has been purified by

ammonium sulphate precipitation, followed by hydroxyapatite chromatography. The purified fraction appears as single protein band of approximate molecular mass 70 kDa on 10% SDS-PAGE. The concentration at which 50% inhibition ( $I_{50}$ ) occurs is in the nanomolar range. The inhibitor seems to bind to Na, K-ATPase reversibly at or close to ATP binding site, but away from ouabain (a specific inhibitor of Na, K-ATPase) binding site. However, affinity of the inhibitor towards ATPase is less than ouabain as evident from the 3H-ouabain binding study. Tryptophan fluorescence study and CD analysis suggest conformational changes of Na, K-ATPase on binding to the inhibitor.

#### B.3. Enterotoxin (STa) induced activation of guanylate cyclase proceeds through stimulation of protein kinase C (PKC) in rat enterocytes :

**Parimal C. Sen**, Koushik Roy and Arindam Basu in collaboration with Dr. Uma Ganguly, National Institute of Cholera & Enteric Diseases.

Heat stable enterotoxin (STa), produced by enterotoxigenic strain of *E. coli*, belongs to a family of low molecular mass (1,900-2,000) peptide toxins and is a major cause of secretory diarrhoea in human and other animals. STa is known to bind to intestinal receptors leading to activation of guanylate cyclase, elevation of cytosolic cGMP level and enhances  $\text{Cl}^-$  secretion from intestinal cells. We have prepared intact enterocytes of rat and treated with STa. The excess toxin was removed by repeated centrifugation and washing. Whole cells were homogenized and cytosolic and particulate fractions were collected after ultracentrifugation at 100,000 g for 1 hr. The control experiment with enterocytes was run simultaneously without STa.  $\text{Ca}^{2+}$ / phospholipid dependent PKC activity was measured in both the cases. The enzyme activity in the membrane fraction was found to be four fold higher in STa treated one, while the cytosolic PKC activity remain unaltered compared to the control one. The stimulation of PKC activity by STa seems to be interesting and may be related to the guanylate cyclase activation.

### C.1. Chemistry of Plant Products :

#### (a) Search for antibacterial principles from medicinal plants

**Manas Chakrabarty** in collaboration with Dr. M.R. Saha and G.B. Nair of N.I.C.E.D., Calcutta.

The antibacterial property of the aqueous alcoholic extracts, the petrol extracts and the ethyl acetate extracts (of the defatted marc) of leaves, seeds, bark and whole plant of *Vitex negundo*, *Ocimum basilicum*, *Berberis aristata* and *Withania somnifera* were tested against various enteropathogens like *Vibrio cholerae*, *E. coli*, *Salmonella spp.*, *Shigella spp.* and *Klebsiella spp.* The petrol extracts were resistant to all the bacteria, whereas other extracts were weakly sensitive (MIC : 50-400  $\mu$ g/ml) to mainly *V. cholerae* strains.

### C.2. Synthetic Heterocyclic Chemistry

#### (a) Newer Syntheses of Pyrrolocarbazoles.

**Manas Chakrabarty**, Shampa Khasnobis and Subho Ghosh.

In pursuit of our D.B.T. P.D.F. programme on the microbial transformation of pyrrolocarbazoles (PCs), the following two newer approaches towards the synthesis of PCs were taken up :

(i) In the first approach, 2-iodophenylindolylamine was attempted to be prepared by the Goldberg reaction of an appropriate bromindole and 2-iodoacetanilide. Two products were formed, whose characterisation and cyclisation are underway.

(ii) In the second approach, based on Moody's indole synthesis by the cyclisation of azidocinnamates, 9-methyl-3-formylcarbazole was prepared by the Vilsmeier-Haack formylation ( $\text{POCl}_3/\text{DMF}$ ) of 9-methylcarbazole.

#### (b) Hetero Diels-Alder Reactions.

**Manas Chakrabarty** and Shampa Khasnobis.

As a follow-up of the hetero Diels-Alder reactions, stated in last year's A.R., the hetero Diels-Alder reaction of 1-ethoxycarbonyl and 1-benzoylindole-3-carboxaldehyde *N,N*-dim-

ethyl- hydrazones with the dienophile, diethyl azodicarboxylate was studied. A number of products were formed and these are being characterised.

**D.1. Unfolding and refolding studies of  $\beta$ -lactoglobulin and lysine modified  $\beta$ -lactoglobulin :**

**K.P. Das**, Chaitali Bhattacharya and A. Bhattacharya.

Structure of thermally unfolded and refolded form was characterised by FT-IR, CD and Fluorescence Spectroscopy. The refolded  $\beta$ -lactoglobulin regained native secondary structure, but tertiary packing was somewhat loose. This opened up channels to access highly hydrophobic pockets located deep inside the  $\beta$ -barrel structure. Refolding, however, was found to be pH dependent. Access to the interior pockets were facilitated at pH away from isoelectric pH but denied at pH close to i.e.p. Whether unfolding by non-thermal means and subsequent refolding gives rise to similar characteristics of refolded form is under investigation.

**D.2. Molecular chaperone function of  $\alpha$ -crystallin :**

**K.P. Das**, J. Bhattacharya and S. Bhattacharya.

The role of thermal activation in inducing chaperone function of  $\alpha$ -crystallin has been studied. It has been established that the previous belief that  $\alpha$ -crystallin is not active as a chaperone below 30°C is incorrect.  $\alpha$ -Crystallin can function as a molecular chaperone even at 18°C since it can bind aggregation prone substrates. However, increase of temperature enhanced the chaperone activity.

In order to understand the mechanism of chaperone-substrate binding, the kinetics of the binding process by stopped-flow kinetic measurements is in progress. Surfactants have also been found to prevent some protein aggregation during unfolding and refolding.

**E.1. Structure and function of the human erythrocyte membrane :**

**Joyoti Basu**, Raja Bhattacharya, Amit K. Das, Biswajit Pal and Prasun K. Moitra

**Mapping the anion exchanger-binding site on human erythrocyte membrane protein 4.2 :**

Human erythrocyte membrane protein 4.2 is a membrane cytoskeletal protein that associates with the cytoplasmic domain (CDB3) of the anion exchanger, band 3. It is one of the proteins of the human erythrocyte that is linked to hereditary spherocytosis. The protein 4.2-CDB3 association accounts for one of the vertical interactions of the cytoskeleton with the overlying lipid bilayer. The CDB3-interacting site of protein 4.2 was mapped to a 23000  $M_r$  N-terminal domain by partial proteolysis and blot overlay assay using renatured protein 4.2-derived polypeptides and biotinylated CDB3. This domain was then expressed as a series of overlapping recombinant glutathione S-transferase fusion proteins. The CDB3 binding domain mapped to two non-overlapping recombinant polypeptides in the N-terminal half of the protein.

**F.1. Actin filament ordering :**

**Pradip Das**, N. Chakrabarti and N. Mitra.

Well aligned nematic domain of actin filaments have been made at the air-fluid boundary, as is evident from the streaklike fluctuations perpendicular to the director. The threshold concentration in mg/ml for appearance of the nematic phase is given by  $F = 36.25/L$  (Flory) and  $F = 14.94/L$  (Onsager) ( $L$  = filament length in  $\mu m$ ). The persistence length of F-actin is 4  $\mu m$ ,  $F \sim 9$  mg/ml (Flory) and  $F \sim 4$  mg/ml (Onsager). However, since there is attractive interaction between the filaments, the observed threshold concentration is lower than that predicted by theory.

**F.2. Lyotropic polymorphism in F-actin :**

**Pradip Das**

Polymer supermolecular structures bind the entire solvent in the F-actin system (interstructural swelling). This is followed by intrastructural swelling in which solvent molecules penetrate the G-actin moiety causing it to unfold. The solvent itself becomes ordered, oriented and thermally less mobile. The anisotropic interaction between the polypeptide chain segments and solvent molecules leads to the appearance of chiral smectic and smectic B phases.

**INSTITUTIONAL PROJECT V**

**A.1. Drug targets and drug resistance mechanisms in mycobacteria :**

**Parul Chakrabarti, Manikuntala Kundu, Joyoti Basu**, Sanjay Mukhopadhyay, Suresh K. Bhardwaj, Seabrat Mahapatra and Saumita Das.

**(i) Analysis of the high molecular mass penicillin-binding protein (PBP) 1 of *Mycobacterium leprae* :**

Through genetic manipulations it has been established that the region between amino acid residues L147 and R314 is necessary for insertion of this PBP into the membrane. The penicillin-binding module of this PBP could be expressed in *E. coli*, extracted from inclusion bodies and refolded into an independent penicillin-binding entity in the absence of the non-penicillin-binding module. This is the first report of a high molecular mass PBP of class A where the penicillin-binding module has been expressed as a soluble protein which can bind penicillin with an efficiency equal to that of the full-length protein.

**(ii) Characterization of drug resistance involving efflux mechanisms in mycobacteria :**

An ethidium-bromide resistant mutant has been isolated by adaptation of *M. smegmatis* mc<sup>2</sup>155 in the presence of increasing concentrations of ethidium bromide. This mutant was also found to be resistant to the frontline antitubercular drug isoniazid. Accumulation of ethidium bromide was found to be lower in the mutant than in the wild type, an effect which was offset by the proton motive force uncoupler carbonylcyanide *m*-chlorophenyl hydrazone (CCCP), suggesting the involvement of an efflux mechanism in the resistance of this mutant.

**(iii) Cloning and sequencing of the *rmlD* gene of *M. smegmatis* :**

The *rmlD* gene encodes the last enzyme involved in the biosynthesis of rhamnose. Rhamnose is a constituent of the special linkage unit, -L-Rhap-(1 $\rightarrow$ 3)D-GlcNAc-P-linking the reducing end of the galactan to the 6-position of some of the muramic acids of peptidoglycan. This gene has been cloned and

partially sequenced from *M. smegmatis*. Characterizing the genes of the *rml* cluster and their products should help in identifying new drug targets on the mycobacterial cell wall.

**B.1. Fertility regulation : Role of BIM in rat testis :**

**P. Bhattacharyya** and D. Ghoshal in collaboration with A. Pakrashi, I.I.C.B..

The anti-implantation effect of BIM by virtue of its action on macrophage and brain ATPases is now established in female rats. In this programme we have studied the effect of the bone marrow cytokine BIM on testicular function in male rats. BIM was shown to have a reversible effect on seminiferous tubules. It seems that the maturation process of spermatozoa is affected. Withdrawal of BIM has been shown to reverse this effect.

**B.2. Immunomodulatory effect of the rodent bone marrow cytokine-BIM in human carcinoma.**

**P. Bhattacharyya**, D. Ghoshal and J. Chatterjee.

BIM is a nonspecies specific rodent bone marrow secreted immunomodulatory cytokine. Partial sequence of BIM compared in a peptide data sequence library showed considerable homology with human IL 11, 12 and 14. As IL-12 is a potent immunomodulator in stressed situations and as BIM had a close structural homology with it, BIM was used in human volunteers suffering from terminal carcinoma ( $n=7$ ). These patients have all undergone conventional treatment in vain (5 males and 2 females). Injection of BIM (conc. 12  $\mu g/ml/50Kg$  b.wt.) showed upregulation of the suppressed immune system. The pain associated with carcinoma which could not be controlled by painkiller injection as fortwin, voveran, etc., is totally under control without any painkiller after 3 injection of BIM. Vomiting and nausea associated with liver and intestinal carcinoma and which could not be controlled by regular injection, is also under control. These two physiologic events gave the patients a tremendous model booster. Five patients that did not take any immunosuppressive drug including antibiotics and are

on herbal medicines are leading a normal life even after one year of initiation of the BIM therapy. There is no pain, vomiting or nausea no bleeding in a uterine carcinoma female patient. Two patients have expired, one after taking immunosuppressive antibiotic injection. The other discontinued the treatment. It seems, as has also been observed in poultry, that BIM works much better in an ayurvedic environment.

**B.3. Immunomodulatory effect of two herbal preparations in increasing bio-availability of anti-tubercular drugs in tubercular patients.**

**P. Bhattacharyya**, D. Ghoshal, J. Chakraborty, P.K. Debnath and M. Sau.

The bioavailability of antitubercular drug isoniazide, rifampycin and ethambutol is studied in tubercular, non HIV patients with or without two herbal preparations. It is observed that in presence of the herbal preparations the bioavailability of ATD has increased significantly. Moreover, an upregulation of the antibody response and WBC count is also noted. Clinically, the patients on ATD and herbal preparations are improving much more than those only on ATD.

**B.4. Hepatoprotective and immuno-modulatory studies of an Indian Medicinal plant :**

**P. Bhattacharyya**, S. Sinha, D. Ghoshal and S. Datta.

The effect of a herbal protein, CI-1 from *Cajanus indicus* was evaluated as a probable hepatoprotective and immuno-potentiating in control and stressed mice. It was noted that CI-1 could offer protection to the experimental animals from the deleterious effects of different hepatotoxins in both pre and post exposure conditions. CI-1 (conc. 100 µg/ml) improved both humoral and cell-mediated immune response. In mice immunized with SRBC, CI-1 enhanced HIR by 24.6% over control ( $P < 0.01$ ). Further, CI-1 facilitated DTH response to SRBC in sensitized mice and also enhanced leucocyte and macrophage migrations in immunized mice. Both bone marrow and thymus secretory protein levels were found to be significantly increased on CI-1 treatment. The results thus indicate that

CI-1 modulates both humoral and cell-mediated immune response.

**B.5. Anti-diabetic activity of an Indian medicinal plant :**

**P. Bhattacharyya**, D. Ghoshal and S. Sinha.

Streptozotocin (STZ), induced diabetic rats were treated both by a herbal formulation (leaf extract, genus-Lagerstromia) and insulin from few hours to one day whereas administration of this leaf extract showed marked reduction in blood glucose level after 24 hours and persisted for 15 days and reached the near normal values. Further studies are going on to isolate, purify and characterise the active principle from the above plant, responsible for hypoglycemia.

**C.1. Adrenal catecholamines and phospholipids during water-deprivation stress in mice :**

**Manas Chakrabarty** and Subho Ghosh in collaboration with Prof. Asok Ghosh and Dr. (Mrs.) Santasri Sengupta of Histophysiology Laboratory, Department of Zoology, University of Calcutta.

The profile of adrenomedullary catecholamines (CAM) and phospholipids (PL) was studied in chronic water-deprived mice (*Mus musculus*). The contents of both epinephrine (E) and norepinephrine (NE) were found to increase significantly, which reflect that the accumulation of CAM in mice adrenal medulla may be dependent on the degree of osmotic and/or hypovolemic cues working in a specific manner. In the case of PL, while phosphatidyl ethanolamine (PE) and phosphatidyl inositol (PI) increased significantly, there was a sharp decrease in the phosphatidyl glycerol (PG) content. The increase in PL reflects its positive role in water-deprivation stress. It is evident that PE, PI and PG play adaptive roles in the environmental stress studied.

**C.2. Effects of lithium chloride, an antidepressant, on the histophysiology of adrenal medulla of pigeons :**

**Manas Chakrabarty** and Subho Ghosh in collaboration with Prof. Asok Ghosh and Dr. (Mrs.) Santasri Sengupta of the Department of Zoology, University of Calcutta.

In order to explore whether lithium chloride acts also (in addition to through hypothalamus) in the adrenomedullary pathway, histological and biochemical investigations were conducted in domestic pigeons (*Columba livia*). Atrophy and overall degeneration of the medullary portions along with an appreciable depletion of adrenomedullary CAM, both E and NE, were observed. It has been demonstrated that lithium salts also act through the adrenal medulla.

**D.1. Mechanisms of antibiotic resistance in *Shigella dysenteriae* :**

**Manikuntala Kundu**, Anindya Sundar Ghosh, Jasimuddin Ahamed, Kamlesh K. Chauhan and Jayanti Gangopadhyay

(i) *Involvement of an efflux system in high-level fluoroquinolone resistance of *Shigella dysenteriae* :*

We have isolated fluoroquinolone-resistant mutants of *S. dysenteriae* by adaptation of a clinical isolate C152 to increasing concentrations of norfloxacin (NFX). The results obtained with these mutants indicate that high-level quinolone resistance arising from exposure of *S. dysenteriae* to NFX may involve mechanisms other than a mutation in the *gyrA* gene. The decreased accumulation of quinolones in the mutant NRM16 compared to the parent strain and the effect of CCCP in bringing accumulation to similar levels in C152 and NRM16 suggested a role of a pmf-dependent efflux pump in high-level quinolone resistance of NRM16. Since efflux pumps confer resistance to a structurally diverse array of antibacterials simultaneously, this aspect was also investigated. Among other drugs tested, there was a 2-fold increase in MIC of tetracycline and a small but consistent increase in MIC of chloramphenicol in NRM16.

(ii) *Quinolone resistance in clinical isolates of *Shigella dysenteriae* :*

Several quinolone resistant clinical isolates of *S. dysenteriae* were analyzed for mutations in the *gyrA* gene. The following mutations were detected : Ser 83 → Leu and Asp 87 → Gly.

In addition, strains having no mutations in the *gyrA* gene but resistant to fluoroquinolones, were also obtained. These strains showed lower

levels of accumulation of norfloxacin, indicative of an active efflux mechanism in fluoroquinolone resistance in these cases.

(iii) *Beta-lactam resistance due to an oxacillin-hydrolyzing expanded spectrum beta-lactamase in a clinical isolate of *S. dysenteriae* :*

An oxacillin-hydrolysing beta-lactamase from a clinical isolate of *Shigella dysenteriae* has been purified and characterized earlier. The gene of this beta-lactamase, present in a large plasmid of *S. dysenteriae* has been cloned in *Escherichia coli*. A 3.5 kb DNA fragment obtained by Sall digestion has been found to hybridize with a known OXA-I probe and this fragment contained the full length gene of the beta-lactamase, since *E. coli* expressing this gene was found to be resistant to cefotaxime, ceftizoxime and other third generation cephalosporins.

## PUBLICATIONS

1. Characterization of a beta-lactamase from *Mycobacterium smegmatis* SN2. D. Basu, D.V. Narayankumar, J. Van Beeumen and J. Basu. *Biochem. Mol. Biol. International*, **43**, 557-562, 1997.
2. Characterization of a porin from *Mycobacterium smegmatis*. S. Mukhopadhyay, D. Basu and P. Chakrabarti. *J. Bacteriol.*, **179**, 6205-6207, 1997.
3. Altered permeability and beta-lactam resistance in a mutant of *Mycobacterium smegmatis*. S. Mukhopadhyay and P. Chakrabarti. *Antimicrob. Agents Chemother.*, **41**, 1721-1724, 1997.
4. Involvement of an efflux system in high-level fluoroquinolone resistance of *Shigella dysenteriae*. A.S. Ghosh, J. Ahamed, K.K. Chauhan and M. Kundu. *Biochem. Biophys. Res. Commun.*, **242**, 54-56, 1997.
5. Involvement of a 43-kilodalton outer membrane protein in beta-lactam resistance of *Shigella dysenteriae*. A.K. Kar, A.S. Ghosh, K.K. Chauhan, J. Ahamed, J. Basu, P. Chakrabarti and M. Kundu. *Antimicrob. Agents Chemother.*, **41**, 2302-2304, 1997.

6. Dual multimodular class A penicillin-binding proteins in *Mycobacterium leprae*. S. Lepage, P. Dubois, T.K. Ghosh, B. Joris, S. Mahapatra, M. Kundu, J. Basu, P. Chakrabarti, S.T. Cole, M. Nguyen-Disteche and J.M. Ghuyssen. *J. Bacteriol.*, **179**, 4627-4630, 1997.

7. Drug targets and drug resistance mechanisms in mycobacteria. P. Chakrabarti. *Proc. Acad. Sci., India, LXVII, Sect. B. Part III*, 169-179, 1997.

8. Montmorillonite clay catalysed synthesis of Bis(indol-3-yl)methanes and 1,2-Bis(indol-3-yl)ethanes. A.K. Maiti and P. Bhattacharyya. *J. Chem. Research (S)*, 425, 1997.

9. Hepatoprotective effect of a protein isolated from *Cajanus indicus* (Spreng.) on carbon tetrachloride induced hepatotoxicity in mice. S. Datta, K. Basu, S. Sinha and P. Bhattacharyya. *Ind. J. Exp. Biol.* **36**, 175-181, 1998.

10. Anti implantation effect of a bone marrow cytokine-BIM. D. Ghoshal, P. Bhattacharyya and A. Pakrashi. *Ind. J. Physiol. Pharmacol.*, **42**, 352-358, 1998.

11. Salmonella antigen induces differential bone marrow cytokine secretion in control and malnourished rats. D. Ghoshal, S. Datta and P. Bhattacharyya. *Asia Pacific. J. Clin. Nutrition*, **7**, 177-181, 1998.

12. Immunomodulatory effect of a rodent bone marrow cytokine in improving antibody response in New castle disease vaccinated chicks. D. Ghoshal, D. Chakraborty, G. Chakraborty and A. Chatterjee. *Ind. J. Animal Science*, **68**, 1998.

13. Lipids and fatty acids of air breathing fish *Boleophthalmus boddarti*. D. Banerjee, D. Pal, T.K. Patra, S. Misra and A. Ghosh, *Food Chemistry*, **60**, 303-309, 1997.

14. Lipid composition of parshey roe (*Mugil parsia*). P.C. Sen, J. Dutta and A. Ghosh. *J. Indian Chem. Soc.*, **74**, 470-473, 1997.

15. Purification and functional characterization of a low molecular mass Ca, Mg- and Ca-ATPase regulator protein from rat brain cytosol. D. Bhattacharyya and P.C. Sen. *Biochem. J.*, **130**, 95-101, 1998.

16. Carbazole Alkaloids from *Murraya koenigii*. M. Chakrabarty, A.C. Nath, S. Khasnobis, M. Chakrabarty, Y. Konda, Y. Harigaya and K. Komiyama. *Phytochemistry*, **46**, 751-755, 1997.

17. Two novel carbazole alkaloids from *Murraya Koenigii* and a useful spin-off. M. Chakrabarty, A.C. Nath and Y. Harigayn. *Chimia*, **51**, 530, 1997.

18. A brief survey of the synthesis and bioactivity of pyrrolocarbazoles, an emerging class of condensed heterocycles. M. Chakrabarty and S. Khasnobis. *J. Indian Chem. Soc.*, **74**, 917-925, 1997.

19. Ethanolic or aqueous formic acid (1:1) - a new efficient reagent for the regeneration of ketones from phenylhydrazones. M. Chakrabarty and S. Khasnobis. *Synth. Commun.*, **28**, 1361-1368, 1998.

20. Effect of incorporation of hydrophobic, hydrophilic and ionic groups through lysine modification of  $\beta$ -lactoglobulin on its adsorption at alumina/water interface. A. Bhadhuri and K.P. Das. *J. Disp. Sci. Technol.* **19**: 435-449, 1998.

21. Covalent grafting of tryptophan to solid surfaces inhibit protein adsorption. A. Bhadhuri and K.P. Das. *J. Disp. Sci. Technol.* **19**, 452-464, 1998.

## GRANTS-IN-AID SCHEMES

| Investigator/<br>Co-investigator                 | Title                                                                                                           | Financed by |
|--------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|-------------|
| Prof. Parul Chakrabarti<br>Dr. Manikuntala Kundu | Molecular Basis of Non-specific resistance in Mycobacteria : Role of Efflux Pumps.                              | DBT         |
| Prof. Parul Chakrabarti<br>Dr. Joyoti Basu       | Drug Permeation through Mycobacterial Cell Wall.                                                                | DST         |
| Dr. Joyoti Basu                                  | Studies on the role of palmitoylation of human erythrocyte proteins.                                            | DST         |
| Dr. Joyoti Basu<br>Dr. Manikuntala Kundu         | Protein palmitoyl transferase : a potential regulator of dynamic protein palmitoylation.                        | DBT         |
| Dr. Manikuntala Kundu                            | Role of outer membrane proteins in drug resistance in <i>Shigella dysenteriae</i> .                             | CSIR        |
| Dr. Manikuntala Kundu                            | Biochemical approaches towards the therapy of shigellosis.                                                      | DST         |
| Dr. G.C. Majumder<br>Prof. A. Ghosh              | Cryopreservation in relation to sperm membrane damage.                                                          | DST         |
| Prof. P. Bhattacharyya                           | Antihepatotoxic agents from some Indian Medicinal Plants.                                                       | CSIR        |
| Prof. Parimal C. Sen                             | Regulation of ion transporting enzymes by protein kinase, stimulator or inhibitor proteins in goat spermatozoa. | CSIR        |
| Dr. P. Nandy<br>Prof. Parimal C. Sen             | Ion channel in artificial membranes.                                                                            | DST         |
| Dr. K.P. Das                                     | Conformational specificity of $\alpha$ -crystallin for its function as molecular chaperone.                     | CSIR        |
| Dr. Pradip Das                                   | Quantitation of orientational ordering in F-actin smectic B phase.                                              | CSIR        |

## Ph. D. Awarded

| Name                               | University | Year | Title                                                                            | Supervisor                           |
|------------------------------------|------------|------|----------------------------------------------------------------------------------|--------------------------------------|
| Amit Das                           | Calcutta   | 1997 | Studies on structure and function of the human erythrocyte membrane              | Dr. J. Basu                          |
| Sebabrata Mahapatra                | Calcutta   | 1997 | Biochemical studies on <i>Shigella</i>                                           | Dr. M. Kundu                         |
| Padur Thiruvengadachari Srinivasan | Jadavpur   | 1997 | Studies on various structural and functional aspects of thalassemic erythrocytes | Prof. P. Chakrabarti and Dr. J. Basu |

| Name                                 | University | Year | Title                                                                                                                                           | Supervisor         |
|--------------------------------------|------------|------|-------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| Tapan Kr. Mukhopadhyay               | Calcutta   | 1998 | Penicillin-binding proteins of mycobacteria                                                                                                     | Dr. J. Basu        |
| Debashis Pal                         | Jadavpur   | 1997 | Studies on the Liver Lipid Profiles of Sting ray, <i>Dasyatis bleekeri</i> (Blyth) from Hooghly-Matlah Estuarine Complex of West Bengal, India. | Dr. S. Misra       |
| Dipankar Bhattacharyya               | Jadavpur   | 1997 | Regulation of Ion Transporting Adenosine Triphosphatase Activities by Endogenous Proteins/Peptides.                                             | Prof. P.C. Sen     |
| Amar C. Nath                         | Jadavpur   | 1998 | Studies on Secondary Metabolites and Synthetic Approaches to Some Heterocycles                                                                  | Dr. M. Chakrabarty |
| Anuradha Bhaduri (nee' Bandopadhyay) | Jadavpur   | 1997 | Studies on the Interaction of Chemically Modified Globular Proteins on Chemically Modified and Unmodified Solid Surfaces.                       | Dr. K.P. Das       |

#### Participation in Conferences/Symposia, Training Programmes/Awards and Honours received

##### Prof. Parul Chakrabarti :

Delivered an invited talk and chaired a session in the 17th International Lectin Meeting in Wurzburg, Germany, September 24-27, 1997; Delivered an invited talk on "Drug targets and Drug Resistance Mechanisms in Mycobacteria" at the Institut fur Physiologische Chemie, Ludwig-Maximilians-Universitat, Munich, Germany, September 20, 1997; Gave a series of lectures to the final year students of the Biochemistry Department, Calcutta University on microbial cell wall and mode of action of antibiotics.; Acted as an expert for the assessment of the Faculty Members of the Indian Institute of Chemical Biology, Calcutta and the Indian Institute of Science, Bangalore.

Was the Convenor of the Fourth Seminar on Biochemistry and Biophysics of the West Bengal Academy of Science and Technology held on March 30, 1998 in Bose Institute, Calcutta.

##### Prof. Amitabha Ghosh :

Participated in the symposium "Science at High Altitude" held at Darjeeling, during May 7-10, 1997 and delivered a lecture entitled,

"EPADHA an indigenous marine derived medicine for prevention and cure of Coronary Artery Diseases (CAD)".

##### Prof. Parimal C. Sen :

Attended and presented a paper in the International Conference on Stress, Adaptation, Prophylaxis and Treatment at Calcutta and delivered an invited lecture at West Bengal Academy of Sciences; received Professor R.C. Shah Memorial Lecture Award, 1997-98, from Indian Science Congress Association; delivered an invited lecture at Indian Science Congress Association, Hyderabad, in connection with the receiving of Prof. R.C. Memorial Award.

Koushik Roy and Atin K. Mandal attended the International Conference on Stress, adaptation, Prophylaxis and Treatment at Calcutta.

##### Dr. Manas Chakrabarty :

Along with Shampa Khasnobis, presented a Poster in the 17th NASOC held at Science City, Calcutta on September 23-25, 1997; participated, along with Shampa Khasnobis, in the "Symposium on Recent Trends in Bio-organic and Synthetic Organic Chemistry" held at IACS, Calcutta during December 3-5, 1997; participated in the "International Conference on Stress Adaptation, Prophylaxis and Treatment",

held on January 10-12, 1998 at Oberoi Grand Hotel, Calcutta; as a Resource Person, delivered an Invited Lecture entitled, "Sugar-shaped Alkaloids" on February 5, 1998 at the 9th Refresher Course in Chemistry, arranged by the Academic Staff College, University of Calcutta, Calcutta; participated, along with Dr. Subho Ghosh and Ms. Shampa Khasnobis, in the "4th Seminar on Biochemistry and Biophysics" held on March 30, 1998 at Bose Institute, Calcutta; taught as a Guest Lecturer in the B.Tech., Pt. I, First Year class in Polymer Science and Technology Department, University of Calcutta, Calcutta for 1997-98; elected a Council Member of the Indian Chemical Society, Calcutta for 01.01.98 - 31.12.2000.

Shampa Khasnobis received the "Best Poster Presentation Award" at the "17th NASOC on Natural Products and Drug Development" held in honour of the 80th Birth Anniversary of Prof. (Mrs.) Asima Chatterjee at Science City, Calcutta during September 23-25, 1997.

##### Dr. K.P. Das

Delivered an invited lecture at Delhi University on "Molecular chaperones in protein folding" during the Platinum Jubilee Celebration of Delhi University and Indian Chemists Convention held in December, 1997.

##### Dr. Joyoti Basu

Participated in the Gordon Conference on Red Cells in New Hampshire, USA during July 13-18, 1997; delivered an invited lecture at the 17th IUBMB Conference in San Francisco, USA during August 24-29, 1997; participated in the symposium, "From peptidoglycan biosynthesis to antibiotic resistance: present and future" at the University of Liege, Belgium.

##### Dr. D. Ghosal

Attended APPPT meeting Lucknow on December 1997 and International Conference on Stress adaptation, Prophylaxis and Treatment at Calcutta.

#### RESEARCH SCIENTISTS/RESEARCH ASSOCIATES/POST-DOCTORAL FELLOWS/ SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS

Dipankar Bhattacharyya, Sanjoy Mukherjee, Shampa Khasnobis, Sarmistha Dutta, Anindya Sunder Ghosh, Dr. Debasish Ghosal, Dr. Dipankar Banerjee, Debasish Pal, Dr. Swati Sinha, Dr. Amit K. Das, Dr. Anupa Bhattacharyya, K.K. Chauhan, Dr. Amlan K. Das, Dr. Jaya Bhattacharyya, Atin K. Mandal, Koushik Roy, Alok Kanti Kar, Jasimuddin Ahamed, Dhiman Basu, Raja Bhattacharyya, Chaitali Bhattacharyya, Mukunda M. Sarkar, Pinakiranjan Jana, Joydeep De, S.K. Bhardwaj, Nita Mitra, Soumita Das, Jitamanu Chakrabarty, Jayanti Gangopadhyay, Seabrata Mahapatra, Sandipta Bhattacharya, Biswajit Pal, Sandip Pal, Dr. Subho Ghosh, Prasun Kanta Moitra.

#### SUPPORTING STAFF MEMBERS

Nirmal Kumar Das, Prasanta Pal, Anirudha Aich, Debaprasad Roy, Sukumar Maitra, Gopinath Mukherjee, Mrityunjay Gayen, Paresb Bhattacharya, Alok Kundu, Pranab K. Dey, Bhabani Prasad Banerjee, Chittaranjan Ghosh, Ekhas Ali (till 15.09.97), Debdas Nandy, Rama Mukherjee, Tapas Ghosh, Arindam Basu, Dipak Chandra Konar, Gopal K. Biswas, Amarnath Bhatta, Subhas Chandra Pal, Kodan Das, Asoke Kumar Maity, Sachidanand Ram, Kalyan Das.

## Botany

**Faculty :** Prof. S. Gupta (Chairman from 01.12.97), Prof. S. N. Ganguly, Prof. B. Ghosh, Prof. A. N. Lahiri Majumder (Chairman—upto 30.11.97), Prof. K. K. Mukherjee, Prof. Swati Sen Mandi, Dr. B. B. Mukherjee, Dr. P. K. Saha, Dr. D. N. Sen Gupta, Dr. S. Bhattacharya, Dr. S. Gupta Bhattacharya, Dr. T. K. Ghose.

### HIGHLIGHTS

Significant progress has been made in the molecular analysis of plant response to salinity stress particularly in inositol metabolism, polyamine biosynthesis and ABRE—binding factor. Massive effort has been started to analyse the genome of rice in relation to submergence tolerance & aroma, Palong and *Brassica* regarding their genotypic variation by RAPD. Clonal propagation of *Nyctanthes* has been achieved. Pollen calendar of big cities like Calcutta & Howrah has been made.

### INSTITUTIONAL PROJECT I

#### A.1. Regulation of Polyamine Metabolism During Salinity Stress :

M.K. Chattopadhyay, A. Bose, A. Banik, **D.N. SenGupta, Bharati Ghosh**

During the last few years we are engaged in establishing a correlation between salinity stress and polyamine metabolism in rice and *Brassica* (Physiol Plantarum, Plant Cell Physiol, Phytochemistry, Review in Current Science etc.). We have already studied the expression of the key enzyme arginine decarboxylase (ADC) during stress both at protein and RNA level (Plant Mol. Biol). Exogenous polyamines were also used to find out the protective role of higher polyamines during stress (Communicated). As the salt tolerant cultivars accumulate more amount of higher PAs (Spd & Spm) than the salt sensitive cultivars during NaCl stress, studies on the regulation of higher polyamine biosynthesis have been undertaken.

We already measured the SAMDC activity during stress and control conditions in rice (Ph.D. thesis, Manas Kr. Chattopadhyay) and designed the oligonucleotide primers for *E. coli* SAMDC and Spd-Synthase, the two major enzymes involved in higher PA biosynthesis. In northern blot analysis with samples containing total RNA from control and stressed shoots, these two probes (prepared from *E. coli* DNA by PCR) could not detect any hybridizable band either for SAMDC or Spd-Synthase. The com-

puter analysis in the gene bank revealed no plant cDNA/genomic DNA clone for Spd-Synthase. But SAMDC has been cloned from several plant systems including rice. Recently, we have amplified the cDNA for SAMDC by RT-PCR from rice poly(A)+RNA and the characterization is under progress. We are also in a position to clone the SAMDC(rice)\Spd-Synthase(*E.coli*) genes in pBI121 (expression vector) for transformation through *Agrobacterium*.

#### A.2. Delay of Fruit Ripening by Antisense RNA approach :

**Bharati Ghosh, D.N.SenGupta, Mousumi SenGupta**

To delay fruit ripening in tomato our goal is to inhibit endogenous ethylene biosynthesis in fruit by antisense RNA approach. Therefore we have generated the cDNA for ACC synthase 2 (entire ORF) by RT-PCR using PolyA+ RNA prepared from ripened tomato fruit. To get partial cDNA from 5' end two oligo nucleotides were synthesised and by fill in reaction ds DNA of 100bp length was developed. Both partial and the full length cDNA for ACS2 will be subcloned in pBI121 (Ti based plasmid) for transformation of *Agrobacterium* and then tomato. Tissue culture of Tomato and Banana has been practiced to standardise the condition for regeneration of plantlets from leafdiscs and shoot apex respectively. Next, we want to start leaf disc transformation with Agro to develop transgenic tomato plants.

### Annual Report

#### A.3. Molecular Mechanism of Polyamine Action in Protection against Abiotic Stress in *Indica rice* :

**Bharati Ghosh, Papia Roy, Saswati Laha**

Polyamines are important modulators of biological processes including tolerance of plants to stress, but how they are involved in such diverse phenomenon is not clear. PA could bind to the negatively charged phospholipids head groups on membrane thereby influence stability and permeability. Stress triggers a signal transduction pathway which results in adaptation or tolerance. As the higher PAs are elevated during stress in tolerant cvs, the identification of molecular mechanism of all these phenomenon by PAs may provide the ideas for biotechnological manipulation of PA level to improve tolerance to stress. There is a lack of specific molecular probe as well as sufficient knowledge about cellular and subcellular localisation of PA biosynthetic enzymes. On this background we have started our work i. Role of PAs in signal transduction cascades ii. Regulatory mechanism affecting the activity and expression of H+ ATPase in relation to PAs.

#### A.4. Salinity stress and gene expression in rice :

**D.N.Sengupta, Gouri Majumdar, Kakoli Banerjee**

Previously we have reported our findings on post-translational activation of ABRE-binding factor from rice nuclear extract by gel mobility assay (Fig. 1). Identification of the factor(s) binds

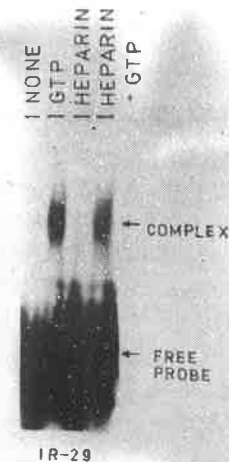


Fig. 1 Gel mobility shift assay showing activation of <sup>32</sup>P-ABRE (Emla) binding factor (S) in nuclear extract of rice (IR-29) leaves. Addition of GTP to equal amount of control nuclear extract showed enhancement of complex formation. Heparin inhibits the enhancement partially.

to ABRE is our goal. Two groups from USA and Japan have already cloned cDNAs from rice which are called as RITA1 and OSBZ8. Using their sequence specific and poly A+ RNA from salt treated roots of IR-29 plants we have amplified the entire ORF of OSBZ-8 (Fig.2), so that they

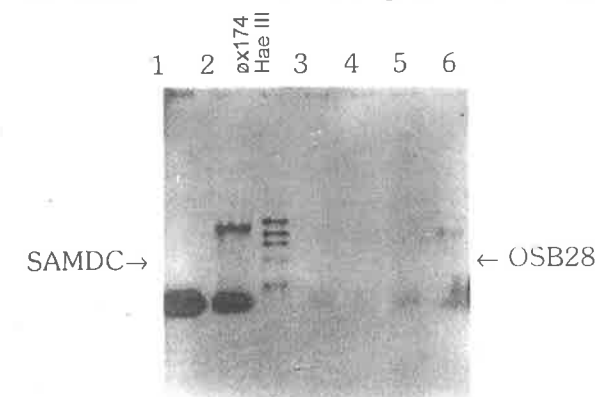


Fig. 2 RT-PCR showing amplification of CDNA using poly A+ RNA from roots of control and salt treated (200mM, 24 hr) rice (Pokkali) plants. Lanes 1, 3 & 4 were from control RNA and lanes 2, 5 & 6 were from RNA of NaCl treated roots. Lanes 1 & 2 with primers for Rice Sam deCarboxylase, lanes 3 & 5 with primers for RITA-1 (BZIP) Class factor and lanes 4 & 6 with primers for OSBZ8 (BZIP Class factor)

could be expressed in *E. coli* for their protein and then antibodies. To compare the transcript level of salinity stress inducible genes in tolerant and sensitive rice cultivars, the entire gene for rab21(=rab16A) has been PCR amplified, which will be used as probe for northern analysis. Differential display or DD-PCR using total RNA from roots of salt tolerant rice cultivar has been standardized and will be repeated to clone salinity stress inducible genes from rice.

#### A.5. Studies on DNA polymerase beta from Rice :

**Dibyendu N. Sengupta, M. Sanath Kumar, Sankar Bakshi**

Our preliminary observation on the enhancement of ddNTP-sensitive DNA polymerase activity in rice shoot tips after treatment with EMS, a mutagenic agent, was confirmed by partial purification of the enzyme followed by activity gel assay. Although hydrogen peroxide, a potent oxidative damage agent, inhibits germination of rice seeds at 5% for 12 hrs. no enhancement of DNA pol beta was detected. Using yeast Pol IV oligonucleotides amplification of YDPIV gene has been done. Subcloning in plasmid is going on. Financial support from CSIR is gratefully acknowledged.

**A.6. Inositol metabolism in relation to salinity stress : Implications in the chloroplast functions :**

**A.N. Lahiri Majumder**, N.C. Hait, S. Guha Roy, A. Roy Choudhuri and Sriparna Bagchi.

The salt-mediated enhancement of the chloroplast inositol synthase in rice has been further studied. A precursor 80 KD synthase protein seems to be processed to a 60 KD active synthase in presence of light/salt. The activation may be related to phosphorylation state of the protein.

Full length coding sequences for inositol synthase from *Oryza* and *Porteresia* have been obtained by RT-PCR. Nucleotide sequence comparison reveal 95% identity between the two synthases. Whether such differences contribute to the salt tolerance of the enzyme protein constitutes the ongoing programme.

### B. Plant Breeding and Tissue Culture

**B.1. Cytogenetical investigations in Beta spp. and studies on wild gene pool of Chenopodium and Ipomoea :**

**K. K. Mukherjee**, S. K. Mitra and S. Das

Plant regeneration was obtained by direct bud formation from petiole and from thin cell layer explants taken from *Beta palonga* grown *in vitro*. The buds were mainly induced in the blade petiole transition zone ( BPT ) of the explants. High frequency bud regeneration was observed the petiole and thin layer explants of few selected lines of *B. palnoga*.

Studies on seed storage proteins of two diploids (  $2n=18$  ), one hexaploid (  $2n=54$  ) *Chenopodium album* and *C. murale* (  $2n=18$  ) showed both similarities and dissimilarities amongst the species of *Chenopodium* suggesting that the diploids *C. album* and *C. murale* contribute towards the evolution of semi-cultivated hexaploid *C. album*.

Relationship of 12 *Ipomoea* species was determined analyzing 64 morphological traits in the form of dendrogram showing three clear cluster combination of species. The grouping reflected the separation of evolved members of

the genus studied, in a single cluster. Studies on allelopathic behavior of *Ipomoea* seed extracts revealed significant inhibitory effect of some *Ipomoea* seed extracts over the others. Inhibitory and inhibited species were included in separate groups as revealed through morphological studies. Intergeneric allelopathy was also observed over rice, jute and mustard seed germination.

**B.2. Phenotypic and genotypic studies with Tephrosia purpurea, Albizia procera and Brassica juncea :**

**P.K. Saha**, B. Das and A. Roy

Pure line populations of four seed coat colour varieties (Yellow, Brown, Black and Variegated) of *Tephrosia purpurea* were isolated from mixed population. Phenotypic variabilities of four seed coat colour varieties were studied in detail. Water uptake patterns and testa ultrastructure (through SEM studies) showed variation according to the category of seed. Seed protein profile and esterase banding pattern showed no marked differences among the four phenotypically distinct seeds.

Another leguminous seed *Albizia procera* was studied for their germinability and dormancy status. It was found that seeds of same population showed variations in their germinability. Only 20% of the seed population are non-dormant but majority (80%) of the population are dormant. Attempt has been made to investigate the testa ultrastructural differences among these non-dormant and dormant seeds.

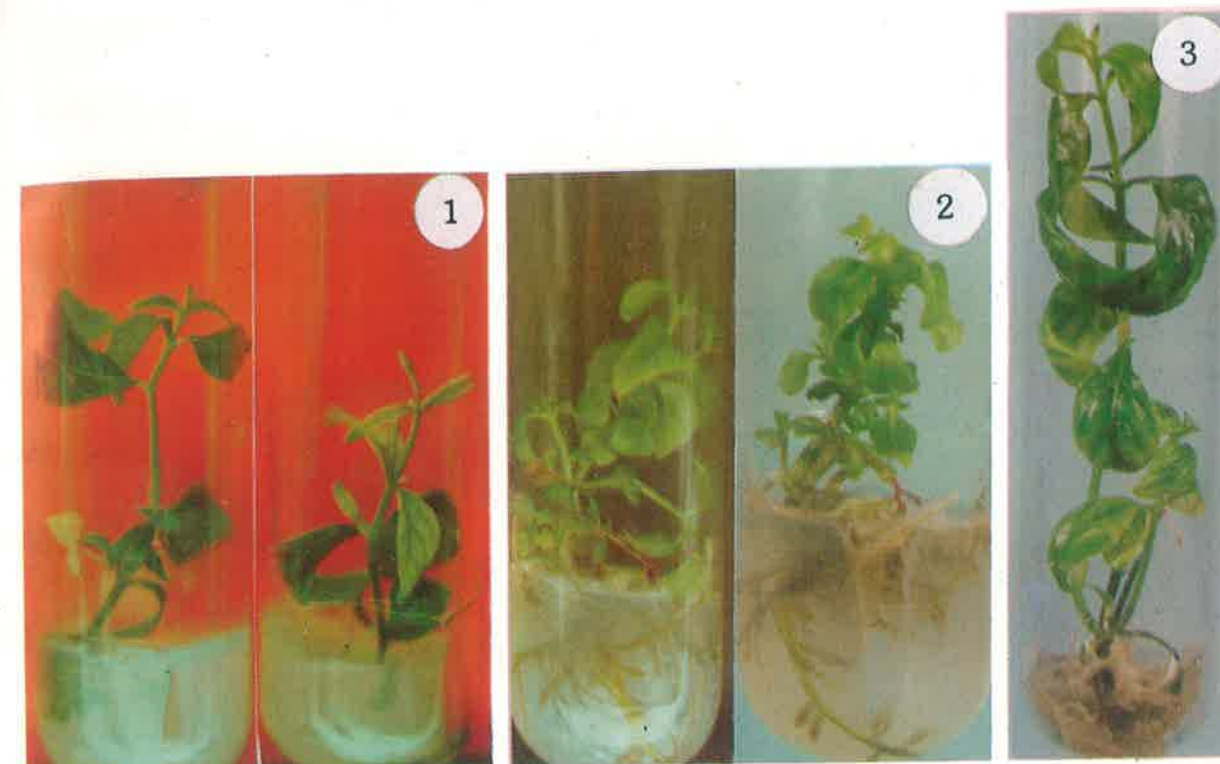
Intravarietal hybridization between two cultivars of *Brassica juncea* (cv. RLM-198 and cv. RJ-15) was carried out for isolation of recombinants having higher potential and comparatively tolerant against aphids. A number of segregated population were isolated.

**B.3. Generation and Demonstration of tissue culture raised planting materials from Andrographis paniculata :**

**S. Gupta** and Debjani Sinha Ray

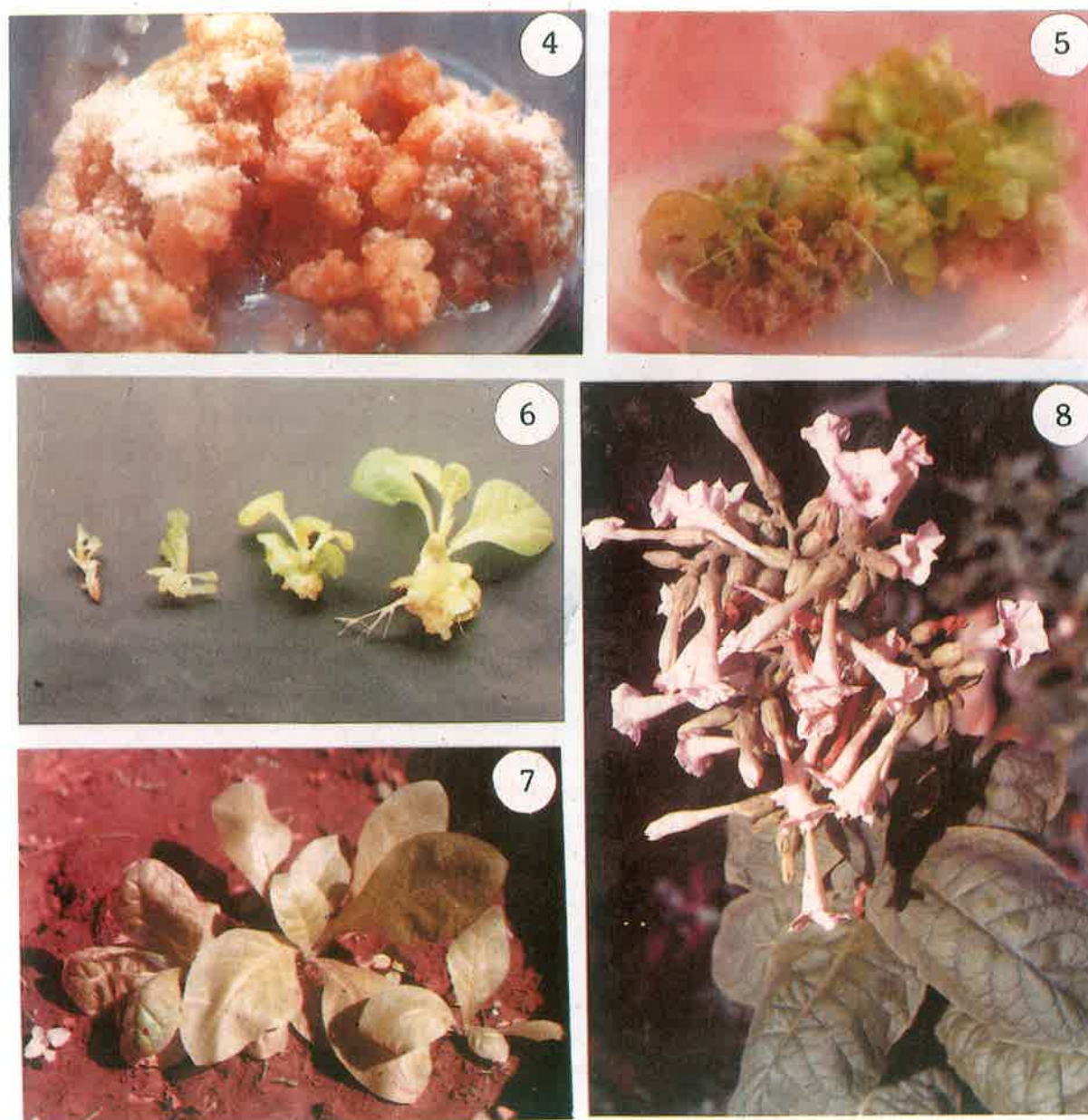
Disinfected (0.1%  $HgCl_2$ , 10 min treatment) nodal explants of *Andrographis paniculata* were inoculated on different nutrient media (MS; 1/

## MICROPROPAGATION OF *Andrographis paniculata* (KALMEGH)



1. Establishment of shoot bud and its growth in medium containing NAA.
2. Multiple shoot formation and root initiation with normal growth in medium containing lower concentration of NAA.
3. Growth of plantlets in hormone free medium.

## *in vitro* PROLIFERATION AND REGENERATION OF DISEASE FREE PLANTS OF *Nicotiana tabacum* VAR. JAYASRI (Tobacco)



4. Growth of callus initiated from leaf disc.
5. Regenerating Callus.
6. Plantlets at different stages of growth.
7. Regenerated plants transferred to soil.
8. Flowering plants in field condition.

2 MS, B5, White's) containing different growth regulators. Best growth and development of the explants were obtained on 1/2 MSNCM medium (MS + 0.1 mg/l NAA + 10% (v/v) coconut milk). Usually 2 shoots (1-1.25 cm long) with two green leaves grew from the axillary buds from an explant within 30 days growth period. The same medium is suitable for subculture of the materials for successive passages. To increase the number of shoots per explant subculturing on 1/2 MS medium with higher concentration (0.5 mg/l) of NAA and 10% (v/v) coconut milk was found suitable (within 30 days growth period 3-4 branches developed). Shoots were further proliferated and elongated after subculturing on the same medium. The process was repeated several times to increase the number of plants in culture. If the *in vitro* grown shoots (2.5-3 cm long) were transferred to rooting medium (1/2 MS + .05 mg/l-1 NAA) profuse rooting from the cut ends were obtained. The sequential treatment and the number of plantlets obtained within a stipulated time is given below:

|                                                                              |                                                          |                                                                       |                            |                                                                             |                                                |
|------------------------------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------------------------|----------------------------|-----------------------------------------------------------------------------|------------------------------------------------|
| Nodal explant<br>(MS+NAA+<br>15% CM)<br>shoots with one<br>node<br>0.8-1 cm) | →                                                        | Establishment<br>medium<br>(1/2 MS+NAA+<br>10% CM)<br>1-2 shoots/node | →                          | Multiplication<br>(1/2MS+N AA<br>.5 mg/l<br>+10% CM)<br>4-6 shoots/<br>node |                                                |
| Plantlets                                                                    | Rooting<br>Medium<br>(MS+NAA<br>(rooting<br>at the base) | ←                                                                     | Shoot<br>pcs.<br>(1-1/2cm) | ←                                                                           | Elongation and<br>proliferation<br>same medium |

Sequential treatments to obtain 4-6 plantlets from a single nodal explant within 4 passages (each passage = 30 days).

### B.4. Genetic approaches for the improvement of submergence tolerance in Rice :

S. Gupta and Sarat Kr. Raj

The androgenic double- haploid (DH) submergence tolerant lines (17 in number) developed from our laboratory were maintained at the Madhyamgram Farm. Agromorphological evaluation of these lines revealed their significant differences in plant height, tillering ability, 50% flowering, spikelet fertility and grain quality. Among these, four lines are shown to be promising. To analyse the genotypic differ-

ences between these DH lines, protocol for DNA amplification using PCR has been standardized. Polymorphism has been obtained between the five lines (developed from the cross between Pankaj/FR-13A) by PCR amplification with isolated genomic DNA against the primer P265.560 (Amplicon length polymorphism marker linked to Blast resistance of rice - Lu et al. Chin. Sci. Bull. (1994) 39: 2103-2105). The banding pattern has been shown in Fig. 3. Generation of polymorphism among other lines and with other sets of primer are in progress.

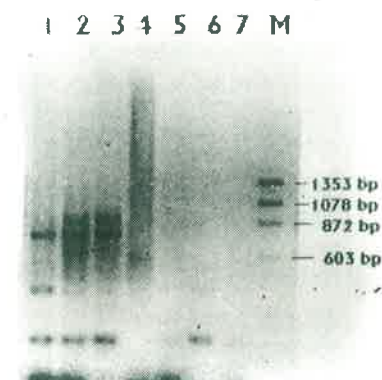


Fig. 3 Polymorphism generated by PCR amplification with the primer p 265.560 and separated by Agrose (2.0% w/v) gel electrophoresis. Lane 1, Pankaj ; lanes 2 to 6, five DH lines ; lane 7, FR-13A ; M=molecular weight marker ( $\phi$  x 174/Hae II digest)

To an aim to generate PCR based DNA marker for submergence tolerance quantitative trait loci (QTL) from F2 segregating population, a cross has been made between IR42 (submergence sensitive variety) and FR-13A (submergence tolerant variety) to raise a suitable F2 population. Genomic DNA isolated from these two parents has been successfully amplified with three different primer sets specific to Rice chromosomes to see the difference in banding pattern between them. Amplification with other primers are in progress.

### B.5. Physiological characterization of NaCl- and mannitol-adapted callus lines in *Brassica juncea* :

B.B. Mukherjee and Sangita Basu

NaCl (salt) and mannitol (drought) tolerant callus lines of *Brassica juncea* L. Czern var.

RW-85-59 were isolated by plated cell suspension culture technique against 43mM mannitol respectively. Callus lines, adapted to high concentration of NaCl (171mM) and mannitol (329mM) were then established by direct adaptation process. Adapted callus lines showed considerable accumulation of free proline in NaCl/mannitol containing media compared to the controlled callus grown on stress free medium. A significant increase of intensity of one particular acid phosphatase isoenzyme band in the adapted calluses irrespective of NaCl or mannitol stress, indicated that it may be used as an osmotic stress-marker in this system. Short-term salt/osmotic -shock treatment with high concentrations of osmotica revealed that only the adapted line retained the maximum amount of free proline within the cells for osmoregulation.

**B.6. Regeneration of salt adapted callus of indica rice (var. Basmati 370) in saline condition :**

**B.B. Mukherjee** and Sangita Basu

NaCl adapted callus of *Oryza sativa* var. Basmati 370 showed 55% regeneration in R2B5 Basal medium supplemented with IAA and Kinetin. Regeneration was low in 85mM NaCl but a concentration of 128mM was inhibitory to regeneration. SEM study showed that organogenesis and somatic embryogenesis occurred from the same callus. The rate of survival and endogenous free proline content of the plants regenerated from the NaCl adapted callus was significantly higher than for those obtained from unadapted callus.

**B.7. Micropropagation of economically important and ornamental plants :**

Sanghamitra Bhattacharya & **Sabita Bhattacharya**

True-to-type clonal propagation of *Nyctanthes arbortristis* (L) and *Jasminum officinale* var. *grandiflorum* (Vahl.) was confirmed through biochemical analysis. Similarity observed in the leaf protein profile, analysed through SDS-PAGE in case of both mother plants and the micropropagated shootlets reveal the chances of a similar genetic make up in both. The isozymic pattern (Peroxidase, esterase, acid phosphatase and alkaline phosphatase) were also seen to be identical when comparison were

made between leaf extracts of the parent plant and the *in vitro* regenerated ones.

The histochemical analysis of the nodal sections of either of the plant material suggested a common axillary origin of the regenerated shootmass. SEM studies also revealed such a type of emergence pattern of the juvenile shoot buds. The anatomical investigation was further extended through TEM studies.

**B.8. Genetic Approaches for the Improvement of Aromatic Rice Suitable for the Agroclimatic Conditions of West Bengal : Mapping of the Rice Aroma Gene(s):**

**T.K. Ghose**, S. Bhattacharya, S. Gupta Bhattacharya, B.B. Mukherjee and S.N. Ganguly, T. Ray Chaudhuri, M. De (In collaboration with S. Das, PMCG)

The main objectives of the project are identification and improvement of aromatic rice suitable for the agroclimatic conditions of West Bengal, a non-traditional zone for Basmati quality rice production, through appropriate breeding techniques including anther culture and DNA-marker assisted selection.

The highlights of achievements of this project up to March 1998 are as follows:

After screening 300 aromatic cultivars, a world collection, 88 rice lines, including Basmati, were found to maintain their characteristic scent at Madhyamgram (North 24 Parganas, West Bengal) for the last eight generations. Partial agromorphological characterization of these lines has been made. Genetic variance,  $D^2$  and principle component analyses on the agromorphological trait data showed that there is ample genetic variation amongst the varieties for improvement.

Segregation analysis of F2 leaf samples showed monogenic recessive control of aroma in 15 Aromatic/Non-aromatic cross combinations, while digenic recessive control operated in one such combination.

Large scale anther culture from three heterobeltiotic hybrids was initiated in November 1996, and very high callusing response, more than 100%, has been obtained.

GLC analysis against the standard chemical confirmed the presence of 2 acetyl-1-pyrroline

in the rice cultivars maintaining aroma at Madhyamgram (W.B.).

Protocols for DNA amplification using PCR, and AFLP has been standardized.

A promising F2 mapping population has been characterized for aroma, agronomic traits, and other quality parameters.

**B.9. Germplasm Screening And Development OF Molecular Markers For Important Agronomic Traits through Use of RAPD/AFLP Techniques :**

**Swati Sen-Mandi**, Somen Nandi, Subhra Talai, Arup Ratan Das

Seed vigour is an important agronomic quality that determines crop stand establishment in the field. Benefits of using elite varieties and biotechnological inputs( including biofertilizers, pesticides and improved irrigation system) can only be realized when high vigour seed is used in cultivation. With the increasing emphasis on direct seeding cultivation in rice and in view of growing scarcity of land, labour and water suitable to agriculture, it is becoming imperative to use high vigour seed in rice cultivation.

This calls for use of molecular marker assisted selection( MAS) of high vigour varieties (i) in breeding programmes (ii) for use under stressful ecological conditions (iii) for identifying parents to be used in conventional breeding as well as in genetic engineering programme.

Programme on development of molecular marker for vigour/tolerance to abiotic stress at seedling stage has been initiated with varieties that were short listed on the basis of ( a) germination performance in the laboratory (b) germination performance under field (stress) conditions and (c) tolerance to ageing (seed deterioration) due to spontaneous radiation (stress) under hot and humid condition.

Variation in isozyme pattern at different developmental stages of germination of esterase (respiration associated enzyme) and superoxide dismutase (SOD), peroxidase (free radical scavenging enzymes) was shown to correlate with germination performance viz. vigour of seed. Such studies were used to screen germplasm for seed vigour quality. The results

obtained corroborated with the short list(of varieties) prepared on the basis of laboratory germination studies. Isozyme pattern of SOD in different vigour varieties was similar to that observed in age-related different vigour seed stocks (obtained as a function of storage period) within a single variety. Results indicate that in addition to germination performance, variation in seed storability is also a manifestation of seed vigour.

DNA fingerprints of selected varieties were developed through RAPD analysis of genomes using random( 8-10 mer) primers (Fig. 4)to identify unique band(s) correlating with the trait of interest. This will help towards establishing molecular markers through RAPD/AFLP analysis of DNA followed by DNA sequencing of appropriate bands and identification of specific primers. Using a cluster analysis computerised programme NTSYS-pc, DNA fingerprints are also being used to develop dendograms for providing an understanding of the interrelationships between varieties.

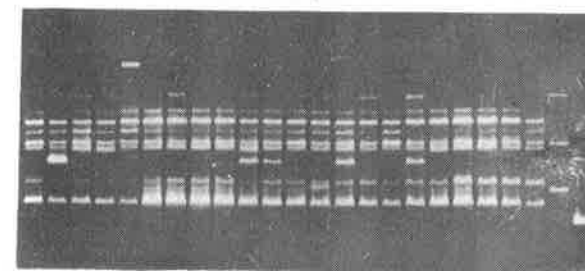


Fig 4 : An example of ethidium bromide stained RAPD products with primar A-5.

**B.10. Identification and study of Biochemical and Ecophysiological components of seed vigour for direct seeding cultivation of rice :**

**Swati Sen-Mandi**. (In collaboration with Directorate of Rice Research, Hyderabad)

Possibility of a laboratory-to -land transfer of an "invigoration" seed pretreatment of rice seed for cultivation under stressful environmental condition viz. drought at seeding stage is being assessed under the National Network Programme of Directorate of Rice Research (ICAR). The cell-molecular mechanism for enhancement in germination process due to this pretreatment has been standardised in this laboratory.

**B.11. Physiological and Biomolecular Investigations on Tapping Panel Dryness syndrome in rubber :**

**Swati Sen-Mandi, Gitali Das, T.P. Sinha.**

Tapping panel dryness syndrome in rubber tree refers to drying up of rubber latex due to pre-coagulation of the latex in the tapping panel. This results in non-secretion of rubber latex at the tapping cut on rubber trunk. Unambiguous report on clonal variation in occurrence of TPD is still lacking. Electron Paramagnetic Resonance spectrometer study of free radical and simultaneous assessment of superoxide dismutase enzyme in the laticiferous cells/latex of five different clones of Rubber showed that the bark of rubber trees when over-exploited with ethephon, accumulates more free radical in comparison to that of the untreated trees (Fig. 5). In a similar comparative study the level of SOD appeared less in the treated plants. Results suggest that an increase of free radical

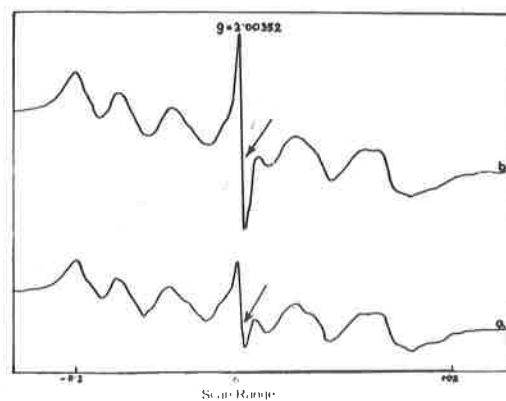


Fig 5: Electron Paramagnetic Resonance (EPR) spectra of (a) normal and (b) stimulated (1/2 S d2 with 10 % ethephon) rubber trees.

and associated decrease of the scavenging system i.e. imbalance in the free radical and their scavenging enzyme system may be one of the factors responsible for bringing about TPD syndrome in rubber.

## INSTITUTIONAL PROJECT II

### A. Chemistry of Natural Products

**A.1. Isolation and characterisation of a new cucurbitacin from the fruits of *Luffa cylindrica***

(*L.*), a new phenolic growth inhibitor from the leaves of mangrove plant *Exocorea agalacha* and a new triterpene from *Rubus ellipticus* :

**S. N. Ganguly,**

From the petroleum ether (60-80°) extract of *L. cylindrica* fruits a white solid, crystallised from petroleum ether-chloroform, m.p. 192° was isolated. On the basis of physical evidences coupled with chemical conversion the compound was identified as a new cucurbitacin and the structure was established.

A new phenolic plant growth inhibitor, m.p. 110°,  $C_{10}H_{12}O_4$  was isolated from the methanolic extract of the leaves of the plant *Exocorea agalacha*. On the basis of the physico-chemical evidences the structure of the compound was given.

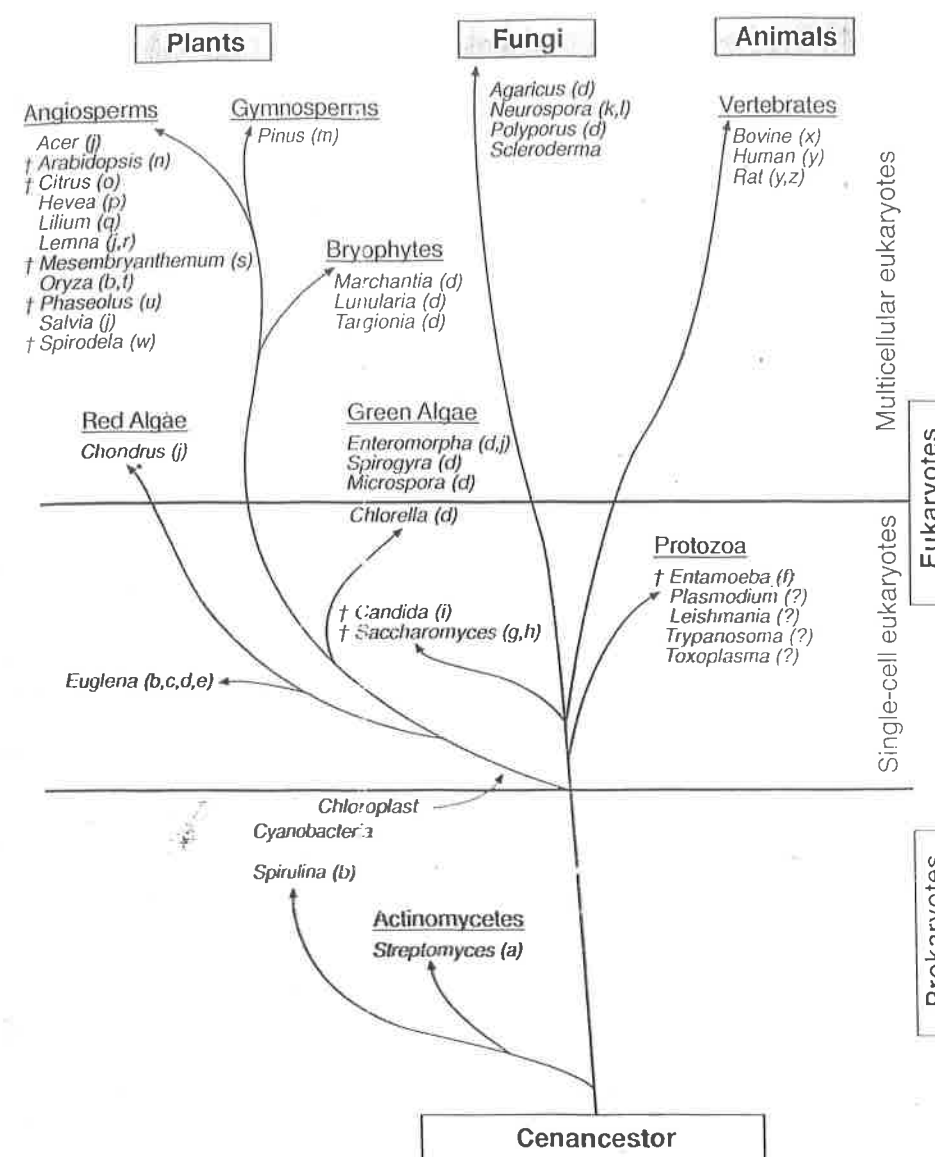
From the methanolic extract of the leaves of *Rubus ellipticus*, a new triterpene, m.p. 251° - 53°,  $C_{30}H_{50}O_5$  ( $M^+$  490) was isolated. On the basis of physical and chemical evidences the structure of the compound was given.

During our investigations on "Microwave-induced organic Reaction Enrichment" (MORE) chemistry techniques, we are able to synthesise mahanimbine, a pyrano carbozole with a very good yield.

**A.2. Plant inositol synthase : Phylogeny and evolution :**

**A. N. Lahiri Majumder.**

Based on the distribution of inositol synthase in the biological kingdom, a model for phylogenetic relationship among the protein/gene has been proposed. (Fig. 6) While the soluble inositol synthase might have originated from an eubacterial precursor, the chloroplast inositol synthase may have its origin in a cyanobacterium like organism. Analysis of the inositol synthase gene sequences reveal high conservation among parasites, fungi and higher plants, suggesting that the protein/gene has been conserved throughout evolution. The chloroplast protein may, however, have a very different gene structure and may, in fact, constitute a gene having an origin in the *Synechocystis* like prokaryote.



Fig—Distribution of 1-P synthase along phylogenetic lines suggesting the antiquity of the enzyme. The branches of the tree illustrate paths of common descent and relative branching order (not drawn to scale of organisms in which 11-P synthase has been demonstrated. Lower case letters in parentheses on the figure indicate reference to the cited work documenting the presence of 11-P Synthase: a. Pittner et al. b. Raychaudhuri et al. c. Adhikari et al. d. DasGupta et al.; e. Loewus et al. [Loewus, 1986#66]; f. Majumder and Lohia [83]; g. Donahue and Henry [34]; h. Dean-Johnson and Henry [71]; i. King et al. [88]; j. Loewus and Loewus [26]; k. Ezcamilla et al. [36]; l. Zsindley et al. [31]; m. Gumber et al. [38]; n. Johnson and Sussex [69]; o. Abu-Abied and Holland [89]; p. Loewus et al. [90]; q. Loewus et al. [39]; r. Ogunyemi et al. [91]; s. Ishitani et al. [68]; t. Funkhouser and Loewus [30]; u. Wang and Johnson [92]; v. Majumder and Biswas [27]; w. Smart and Fleming [67]; x. Mauck et al. [33]; y. Adhikari and Majumder [41]; z. Meada and Eisenberg [20]. The ancestral organism (Cenancestor) at the base of the chart is defined as in Dollittle [75], as the last common ancestor of all living things. The lines of descent leading to modern eukaryotes and eubacteria diverged early from this ancestor. Not depicted are representatives of the Archaea, believed to have branched from the common stem leading to the Eukarya after the divergence of the eubacteria [75]. No *INOI* homolog has been detected in the one completed genome sequence from the Archaea [77,78]. Indicates genera from which an *INOI* gene homolog has been cloned and sequenced. (BB acta 1348, 245-256, 1997)

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## INSTITUTIONAL PROJECT IV

### A.1. Environmental biopollution in relation to human health :

**Swati Gupta Bhattacharya**, Pampa Chakroborty, Indrani Chowdhury, Moon Moon Sen, Atin Adhikari and Debajyoti Ghosh (In collaboration with Prof. Sunirmal Chanda)

An aeroallergen immunoblotting technique using chemiluminescent kit was used for the detection and quantification of allergens and antigens of *Borassus flabellifer* pollen (Palmyra palm) directly on the adhesive tapes during pollen season.

Two allergenically active components of 33 and 66 kD molecular weight of *Phoenix sylvestris* Roxb. pollen were identified by immunoblotting after separation through  $(\text{NH}_4)_2\text{SO}_4$  fractionation and gel filtration. This was further supported by CIE.

The presence of two common allergenically active components were identified by ELISA inhibition and immunoblotting technique between the fruit and pollen of *Carica papaya*.

A comprehensive study has been done to investigate airborne culturable and non-culturable spores in a rural area of Howrah District. The results reveal that spores originated from the genera of *Cladosporium*, *Aspergillus*/*Penicillium* group, nonculturable ascospores and basidiospores, etc. and culturable moulds originated from *Aspergillus flavus*, *Rhizopus nigricans*, *Aspergillus niger*, *Cladosporium cladosporioides*, etc. are the major contributors. Higher statistical correlation has been observed between change in meteorological parameters and change in airspora.

Airborne pollen grains have been recorded with three different types of sophisticated air sampler from five outdoor air sampling stations at Domjur, Howrah. From the statistical correlation between meteorological parameters and airborne pollen load it has been found that temperature play an important role on anther dehiscence and pollen dispersal. Several pollen types were recorded round the year including pollen grains of Poaceae, Chenopodiaceae, etc. Pollen grains from four

varieties of rice (*Oryza sativa*) and two wild grasses showed presence of a common allergenic protein as evidenced by Western blot and Dot blot studies.

### Participation in Conference/Symposia/ Training Programme/Awards and Honours received.

#### Dr. Swati Gupta Bhattacharya :

Attended workshop on 'Information Superhighway' on November, 21 & 22nd at Bose Institute organized by Indian Physics Association, Calcutta Chapter and D.I.C., Bose Institute. ; Attended annual symposium on "Recent Trends in Genetic Engineering and Molecular Genetics" on December 18-19, 1997 at Meghnath Saha Auditorium organized by Dr. B.C. Guha Centre for Genetic Engineering and Biotechnology, University College of Science, 35 Ballygunge Circular Road, Calcutta - 700 019. ; Attended and participated in the workshop on "Individual Man and his Role in Combating Pollution" on 30 August, 1997 organized by National Environmental Science Academy. ; Attended AICP Monitoring meeting held at Delhi on 7-8 August 1997. ; Delivered an invited talk on 27-28 March in a National Seminar on the "Contribution of woman scientists in Botanical Research since Independence" to celebrate the 50th year of Independence at Botany Department, Visva Bharati, Santiniketan. ; Acted as a member of Board of Examiners, North Bengal University.

Mr. Atin Adhikari and Miss Moon Moon Sen

Attended and participated in the first workshop on "Individual Man and his Role in Combating Pollution" on 30 August, 1997 at Sisir Mancha, Calcutta organized by National Environmental Science Academy.

**Prof. Bharati Ghosh** presented invited talks/ chaired session in the following meetings/ symposia :

National conference on "Impact of Biotechnology and Modern Horticulture on rural development", Jadavpur University, on Nov 20-22, 1997 and chaired a session. ; National conference on 'Recent trends of Microbiology

and Plant Physiology in India' Burdwan University, 26-27th Sept. 1997. ; National Symposium on "Current trends in Plant Physiology and Biochemistry", School of Life Sciences, University of Hyderabad, Jan. 29-31, 1998. ; International Symposium on "Stress adaptation, Prophylaxis and treatment", Dec. 19-22, 1997, Hotel Oberoi Grand, Calcutta. ; Delivered Prof. S.M.Sircar Memorial Lecture on 26th Nov., 1997 at Dept. of Botany, Calcutta University. ; Delivered an invited lecture under the aegis of DSA Programme in Burdwan University on Feb. 5th, 1998. ; National Conference on "Eminent women scientist in India, Botany Dept. Visva Bharati University, Bolepur, on March, 1998. and chaired a session.

She was also invited to deliver a lecture and chair a session in DST-JSP bilateral India-Japan workshop in Feb. 5-7th, 1998 in INSA.

**Dr. D.N.SenGupta** presented invited talks in

National conference on "Current trends in Plant Physiology and Biochemistry", School of Life Sciences, Univ. of Hyderabad, Jan.29-31, 1998. ; International conference on Stress adaptation, Prophylaxis and treatment, Dec. 19-22, 1997, Hotel Oberoi Grand, Calcutta. ; Delivered an invited lecture in focal theme on Indian Science Congress at Hyderabad.

Dr. Manas Kr. Chattopadhyay received the JSPS, Govt. of Japan, fellowship to work in Tokyo University, Japan.

A. Roy and **P. K. Saha** presented a paper in the meeting of XX1 PTCA, held in Delhi University in January 1998

#### Prof. A.N. Lahiri Majumder

Delivered invited talk in "Recent trends in Plant Science Research" at the Hyderabad University, January, 1998.

Delivered invited talk in "Biotechnology of Extremophiles" and presented concept proposal at TERJ, New Delhi in January, 1998.

Delivered invited talk in International conference on "Stress adaptation, prophylaxis and treatment" at Calcutta in January, 1998.

**Dr. Sabita Bhattacharya** and Sanghamitra Bhattacharya attended the National Conference on Impact of Biotechnology and Modern Horticulture held in Jadavpur University, Calcutta on Nov. 20-22, 1997 and presented a poster "En Mass Micropropagation of *Thunbergia erecta* : use of nodal explant as the source. "attended workshop on 'Information Superhighway' on november, 21 and 22 at Bose Institute organized by Indian Physics Association, Calcutta Chapter and D.I.C. Bose Institute. ; annual Symposium on 'Recent Trends in Genetic Engineering and Molecular Genetics, on December 18-19, 1997 at Meghnad Sha Auditorium organized by Dr. B.C. Guha Centre for Genetic Engineering and Biotechnology, University College of Science, 35, Ballygunge Circular Road, Calcutta-700 019.

**Dr. T.K. Ghose** attended the National Symposium on Current Trends in Plant Physiology and Plant Biochemistry, School of Life Sciences Univ. of Hyderabad, January 29-31, 1998.

**Prof. Swati Sen-Mandi** visited Cambridge, U.K. on invitation for attending 25th anniversary celebrations to commemorate the admission of women to Churchill College, Cambridge University, U.K.

Technology Transfer / Training programme organized in the laboratory of Prof. Swati Sen-Mandi.

Imparted training on DNA fingerprinting of rubber clones to scientist (Dr. Gitali Das) of Rubber Research Institute of India, Rubber Board. (Sep-Oct. 1997) for transferring technology to Rubber Research Station, Agartala.

Summer Training on Germplasm Screening through DNA fingerprinting in rice imparted to Mr. Anindya Sen, M.Sc (P) student at the School of Biotechnology, Faculty of Science, Banaras Hindu University.

## GRANTS-IN-AID SCHEMES

| Investigator/<br>Co-investigator                                                                         | Title                                                                                                                                                                      | Financed by                              |
|----------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------|
| Prof. B. Ghosh/<br>Dr. D.N. Sengupta                                                                     | Use of antisense RNA to delay fruit ripening                                                                                                                               | DBT                                      |
| Prof. B. Ghosh/<br>Dr.D.N. Sengupta                                                                      | Molecular mechanism of Polyamine action in protection against abiotic stress in Indica rice.                                                                               | DST                                      |
| Prof. B. Ghosh/<br>Dr.D.N. Sengupta                                                                      | Cloning of Molecular markers involved in salt tolerance and their over expression to enhance salt tolerance in rice plants                                                 | DBT<br>(CPMB<br>Phase II)                |
| Dr. D.N. Sengupta                                                                                        | Characterization and Cloning of Plant DNA Polymerase I                                                                                                                     | C.S.I.R.                                 |
| Dr. D.N.Sengupta                                                                                         | Molecular Analysis of Regulation of Salinity stress and Absciscic Acid Inducible Genes in rice                                                                             | DST                                      |
| Prof. S. Gupta                                                                                           | Generation and Demonstration of Tissue Culture raised planting materials                                                                                                   | W.B. SC & ST                             |
| Prof. S. Sen-Mandi                                                                                       | Identification and study of Biochemical and Ecophysiological components of seed vigour for direct seeding cultivation of rice                                              | ICAR                                     |
| Prof.S. Sen-Mandi                                                                                        | Physiological and Biochemical Rubber investigation on Tapping Panel Research Dryness Syndrome in Rubber. (In collaboration with Rubber Research Institute of India) Kerala | Rubber Research<br>Institute of<br>India |
| Prof. S. Sen-Mandi/<br>Prof. P. Bhattacharyya                                                            | Potential use of AFLP Techniques in DNA fingerprinting and Cataloguing of Tea Germplasm : developing molecular markers for drought tolerance in elite Tea clones.          | DBT and<br>Tea Board                     |
| Prof. A.N. Lahiri Majumder<br>Prof. Bharati Ghosh                                                        | Inositol and polyamine metabolism in relation to environmental Stress in rice                                                                                              | Indo-US                                  |
| Prof. A.N. Lahiri Majumder                                                                               | Chloroplastic myo-inositol synthase from <i>Englena gracilis</i> : Molecular studies and photoregulation.                                                                  | DST                                      |
| Prof. A.N. Lahiri Majumder<br>Dr. S. R. Sikdar                                                           | Metabolic Engineering of inositol pathway in rice : implications in the chloroplast functions                                                                              | DBT<br>CPMB (Phase II)                   |
| T.K. Ghose, S. Das,<br>S. Bhattacharya, S. Gupta<br>Bhattacharya,<br>B.B. Mukherjee, and<br>S.N. Ganguly | Genetic Approaches for the Improvement of Aromatic Rice Suitable for the Agroclimatic conditous of West Bengal : Mapping of the Aroma gene (s).                            | DBT<br>CPMB (II)                         |

## Ph.D. Awarded

| Name                       | University | Year | Title                                                                                                                                                      | Supervisor                      |
|----------------------------|------------|------|------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| Gourab<br>Gangopadhyay     | C. U.      | 1997 | <i>In-vitro</i> studies on salt and water stress in <i>Nicotiana tabacum</i> L. var. Jayasri and <i>Brassica juncea</i> L. Czern. 85-59                    | Prof.S.Gupta                    |
| Srabani<br>Biswas          | C. U.      | 1997 | Studies on germination of some leguminous seeds with special reference to their ultrastructure                                                             | Dr.P.K.Saha                     |
| Manas K.<br>Chattopadhyay  | J. U.      | 1998 | Polyamins metabolism during salinity stress in rice ( <i>Oryza sativa</i> L.)                                                                              | Prof.B.Ghosh<br>Dr.D.N.Sengupta |
| M. Sanat<br>Kumur          | J. U.      | 1998 | Purification and Characterisation of DNA polymerase from shoot tips of germinated seedlings of rice                                                        | Dr.D.N.Sengupta                 |
| Jayashree<br>Banerjee      | C.U.       | 1998 | Isolation characterisation bioassay of naturally occurring growth regulators from plants of economic importance including mangroves of the Gangetic Delta. | Prof.S.N.Ganguly                |
| Aniruddha<br>Roy Choudhury | C.U.       | 1998 | Myo-inositol synthase from plant sources Biochemical and regulatory properties                                                                             | Prof. A.N. Lahiri<br>Majumder   |

## PUBLICATIONS

1. Ghosh, Bharati (1997) Polyamines and linity stress. In recent Advances in oil seed *Brassica* p 223 - 232 eds. Kalia H.R. and Gupta, S.K. *Kalyani Publishers*, N.Delhi.

2. Chattopadhyay M.K. and Ghosh, Bharati Molecular analysis of Polyamine biosynthesis in higher plants. *Current Science*, **74** (6), 517 - 22, 1998.

3. Roy, Malabika and Ganguly, S. N. Isolation and Characterisation of Indole-3-methylethanoate form *Camellia sinensis* (L.) O. Kuntz and its biological acitivity. *Nat. Prod. Sci.* **3**, 106 1997.

4. Dutta, S., Ghatak, K. L. and Ganguly, S. N. Isolation and structure elucidation of a new pentacyclic triterpene from the leaves of *Ru-*

*bus ellipticus*. *Nat. Prod. Sci.* **3**, 108 1997.

5. Mukherjee, K. K., Mitra, S. K. and Ganguly, S. N. A new coumarin form the seeds of jute (*Corchorus olitorius* L.). *Nat. Prod. Sci.* **4**, 51 1998.

6. Bhattacharya, A. and Saha, P. K. Germination behaviour of seed of *Cassia tora* at different temperatures. *Weed Res.* **37** : 87-92 1997

7. Roy, A. and Saha, P. K. Induction and plant regeneration form microspore callus in two Indian cultivars of mustard (*B. juncea* Czern. & Coss.). *Proc. Ind. Natn. Sci. Acad.* **B 63** : 89-98 1997.

8. Mandal, N. and Gupta, S. Studies on histomorphological course of events during androgenesis of rice (*Oryza sativa* L.). *Phytomorphology* **46**(4). 357-364, 1996.



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9. Basu, S., Gangopadhyay, G., Gupta, S. and Mukherjee, B.B. Screening for cross tolerance against related osmotic stress in adapted calli of salt sensitive and tolerant varieties of rice. *Phytomorphology* **46**(4). 357-364, 1996.

10. Gangopadhyay, G., Basu, S., Mukherjee, B.B., and Gupta, S. Effect of salt and osmotic shocks on unadapted and adapted callus lines of tobacco. *Plant Cell Tissue and Organ Culture*. **49**, 45-52, 1997.

11. Mandal, N. and Gupta, S. Anther culture of an interspecific rice hybrid and selection of fine grain type with submergence tolerance. *Plant Cell Tissue and Organ Culture*. **51**, 99-82, 1997.

12. Das, S. and Mukherjee, K.K. Studies on morphology and allelopathy for understanding species relationship in *Ipomoea* spp. *Journal of The National Botanical Society*. **50**, 1-8, 1997.

13. Bhattacharya, S. and Bhattacharya, S. Rapid multiplication of *Jasminum officinale* L. by *in vitro* culture of nodal explants. *Plant Cell Tissue and Organ Culture* **51**, 57-60, 1997.

14. Chakroborty, P., Chowdhury, I., Gupta-Bhattacharya, S. Roy, I. Chatterjee, S. and Chanda, S. Aerobiologic Clinical and Immunological studies on *Borassus flabellifer* pollen : an evidence for a 90 kD allergen. *Annals of Allergy Asthma and Immunology*. **80**(4) : 311-317, 1998.

15. Chakroborty, P., Gupta-Bhattacharya, S. and Chanda, S. Clinical tests and chemical

analysis of some common aeroallergen from Calcutta. in "Environment and Aerobiology" (Ed. by Ashok Jain). *Res. Periodicals & Book Publishing House*, Houston, USA. p. 239-243, 1997.

16. Das, G., Raj, S., Pothen, J., Sethuraj, M.R., Sinha, T.P. and Sen-Mandi, S. Status of free radical and its scavenging system with stimulation in *Hevea brasiliensis*. *Plant. Physiol. Biochem.* **24**(2): 1-4, 1997

17. Gupta, S., Chattopadhyay M.K., Chatterjee P., Ghosh B., and Sengupta D. N., Expression of abscisic acid-responsive elementbinding protein in salt-tolerant indica rice (*Oryza sativa* L. cv. Pokkali). *Plant Molecular Biology*. **37** (4) : 629-637, 1998

18. Raychoudhuri, A., Hait N.C., Dasgupta S., Bhaduri, T. J., Dev, R., and Lahiri Majumder, A., L-Myo-inositol-1-Phosphate synthase from plant sources : Characteristics of the chloroplastic and cytosolic enzymes. *Plant Physiol.* **115**, 727-736, 1997

19. Lahiri Majumder, A., Johnson, M.D., and Susan, A. Henry, L-myo-inositol-1-Phosphate synthase. *Biochim Biophys. Acta*. **1348**, 245-256, 1997.

20. Bohnert, H. J., Adames, P. Chmara, W., Ishitani, M., Kamasani, U.R., Katsuhara, M. Majumder, A. L. Marquez, S., Michalowski, C. B., Nelson, D. and Jensen R. D. *Plant Molecular Biology and Biotechnology*, Eds. Tewari, K. K. and Singhal G. S. (Narosa Publishing House) 153-167, 1997.

#### RESEARCH SCIENTISTS/RESEARCH ASSOCIATES/POST-DOCTORAL FELLOWS/ SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS

Sangita Basu, Sanghamitra Bhattacharya, Pampa Chakraborty, Biswajit Das, Sreejit Kr. Mitra, Sarat Kr. Raj, Tathagata Roychoudhury, Sankar Bakshi, Somen Nandi, M. Sanat Kumar, Sudhi Ranjan Gupta, Gourab Gangopadhyay, Dr. Malabika Roy, Dr. Runu Das, Netai Chand Hait, Kakali Banerjee, Piyali Chatterjee, Aniruddha Roychoudhury, Atin Adhikary, Moon Moon Sen, Madan Dr. Indrani Chowdhury, Amitava Roy, Sriparna Bagchi, Anindita Bose, Manas Kr. Chattopadhyay, Devasree Dutta, Gouri Majumder, Subhra Talai, Arup Ratan Das.

#### SUPPORTING STAFF MEMBERS

Narendra N. Choudhury, Dilip Kr. Sanyal, Sankar Basu, Sukumar Majumder, Dr. Ramit Poddar, Chanchal Chakraborty, Jadab Kr. Ghosh, Binoy Krishna Modak, Chaitali Roy, Kaberi Ghosh, Madan Mohan Nath, Shyamal Kr. Ghosh, Subikash Bayen, Sankar Prasad Banerjee, Dilip Kr. Chakraborty, Subarna Ghosh, Nar Bahadur Rai, Shyamapada Halder, Kanailal Chatterjee, Sukadeb Das, Sudhir Jana, Sasanka Sekher Bera, Sambhunath Debnath, Dhiren Kumar Das, Rabindra N. Roychoudhuri, Bipul Kr. Nag, Kanan Singh, Jagannath Das, Subhas Mandal, Munna Turi.

**Faculty :** Prof. P. K. Chakrabartty (Chairman), Prof. A. C. Ghose, Prof. Timir B. Samanta, Dr. Roma Chakraverty, Dr. Pradosh Roy, Dr. S. Majumdar, Dr. S. K. Dasgupta

Role of a 20 kDa subunit pilus protein in intestinal colonization of a diarrheogenic non-01/non-0139 *Vibrio cholerae* strain 10325 has been established.

Leishmanial infection in animals was shown to impair the level of cytochrome P<sub>450</sub> which may cause poor residual drug management. However, protein A treatment reduced the parasite burden in *Leishmania* infected animals.

#### A. Interaction of aluminium with iron transport system in rhizobia :

**P. K. Chakrabartty**, Anup Halder (in collaboration with Dr. Nupur Roy)

Under iron depleted condition the growth of the wild type *Rhizobium* sp. (*Cicer arietinum*) BICC 651 and its siderophore overproducing mutant N15 decreased with increasing concentrations of aluminium upto 100  $\mu\text{M}$  beyond which no further effect of aluminium on the growth of the organism was evident. This effect appears to have a relationship with siderophore production the level of which was maximal in presence of 100  $\mu\text{M}$  aluminium. The siderophore non-producing mutant A35 practically failed to grow in iron depleted culture but in culture supplemented with 5  $\mu\text{M}$   $\text{FeCl}_3$ , a slight but increased growth was evident in presence of high concentration of aluminium. The reason for this apparent anomaly is under investigation.

**Molecular and cellular basis of bacterial and parasitic diseases ;**

### A.1. Cholera and *Vibrio cholerae* :

**A.C. Ghose**, R. K. Nandy, S. Mukhopadhyay  
and B. Nandi

A diarrheogenic strain of non-O1/non-O139 *Vibrio cholerae* (10325) of serogroup O34 was

earlier shown to express a new type of pilus composed of 20-kDa subunit protein. Amino-terminal sequence data (determined up to 20 amino acid residues) of this protein showed complete homology with the corresponding sequence of a 22-kDa outer membrane protein (OMPw) of *V. cholerae* O1.

Southern hybridization experiments demonstrated the involvement of the same genetic loci in coding for both the proteins. Serological data suggested that the OMPw protein is poorly expressed in O1 strains. Subsequent studies, however, established the role of this pilus protein in the intestinal colonization of the *V. cholerae* strain 10325.

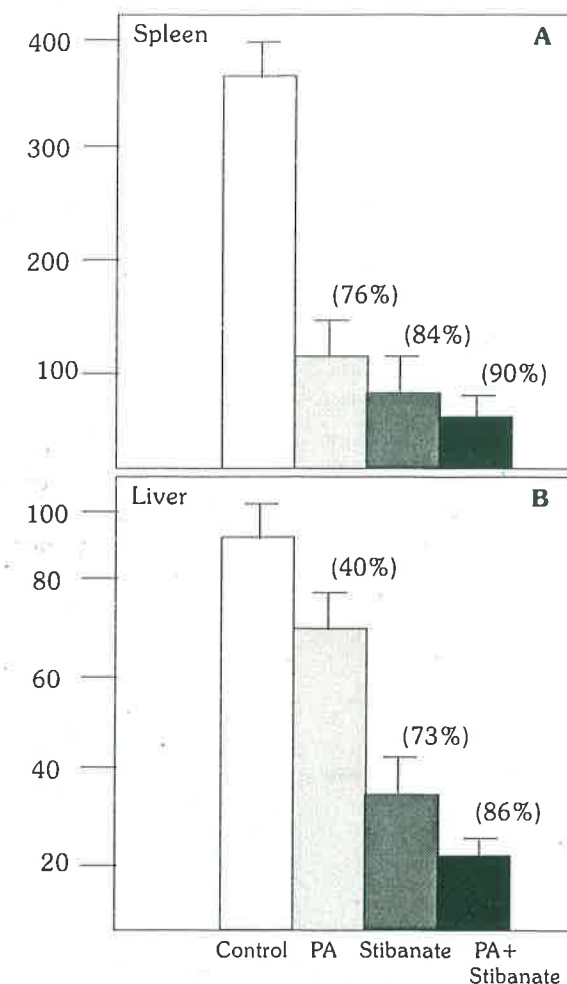
### A.2.1. *Leishmaniasis* :

**A.C. Ghose** and A. Mookherjee (in collaboration with Prof. P. K. Ray)

Leishmanial infection in experimental animals was shown to induce enhanced synthesis of reactive nitrogen intermediates e.g. nitric oxide or NO by their mononuclear phagocytic cells. Peritoneal macrophages could also be induced to produce NO by *in vivo* or *in vitro* treatment with the immunomodulator protein A (PA). Interestingly, PA treatment (alone or in combination with the drug "stibanate") significantly reduced the parasite burden in *L. donovani* infected animals and the therapeutic effect of PA was shown to be mediated primarily through the killing of intracellular parasites by NO. Involvement of NO in immunosuppression associated with visceral leishmaniasis was also established as

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its persistent release down regulated the T-lymphocyte function in progressively ill animals.



Therapeutic effect of the treatment of *L. donovani* infected hamsters with Protein A (PA), stibanate and a combination of PA + stibanate. Mean parasite load in the organs of experimental & control group of animals (5 in each group) is shown. Percent reduction of parasite load in experimental groups is shown within the parenthesis.

### A.2.2. *Leishmaniasis* :

**Timir B. Samanta**, Nilanjana Das and Himan Mukerjee

The kinetics of infection in golden hamsters with *L. donovani* showed impairment of cytochrome P<sub>450</sub> which was concomitant with the induction of nitric oxide synthase (NOS). Both these phenomena attained their maximum height when the parasite load in liver was maximum. The precise role of nitric oxide in impairment of cytochrome P<sub>450</sub> during infection and possible protection of impairment during

therapy with anit-leishmanial agent are being investigated.

### A.3. Tumor necrosis factor- $\alpha$ (TNF- $\alpha$ ) induced altered cell function :

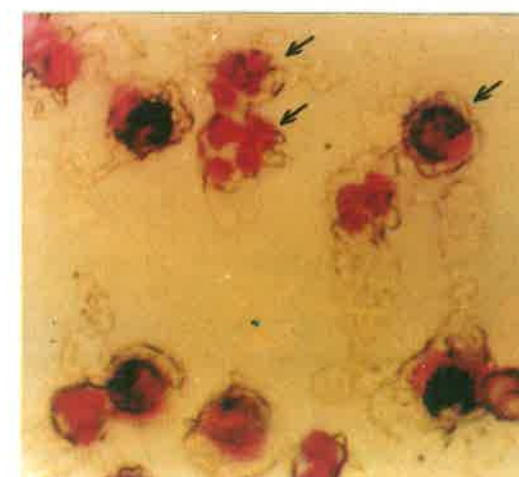
**Subrata Majumdar**, Sonali Das, Sandip Bhattacharyya

We have extended our programme on apoptosis by staining nuclei with DAPI and Wright Giemsa Stain. Fragmented nuclei were

## CONTROL



**TNF 1000 U/ml**



Nuclear fragmentation induced by tumour necrosis factor- $\alpha$  in human neutrophil :

Human neutrophils were treated with tumour necrosis factor- $\alpha$ . Cells were stained with Wright Giemsa. Nuclear fragmentation is denoted in cells indicated by arrows. Control plate shows the untreated neutrophil.

observed under fluorescence microscope. To probe the hypothesis that ceramide plays a key role in apoptosis, we measured ceramide production in TNF treated neutrophil and we observed a substantial amount of ceramide production in these cells.

**A.4. Signal transduction mechanism in *Leishmania* infected macrophage :**

**Subrata Majumdar, Sanjukta Ghosh and Sandip Bhattacharyya**

Protein Kinase C (PKC) mediated signaling cascade was observed in *Leishmania* infected intra-peritoneal mouse macrophage. Activity of Ca-dependent PKC was found to be inhibited depending on the infection ratio and time of infection whereas Ca-independent PKC was unaffected in infected macrophage. Endogenous protein phosphorylation was also found to be inhibited.

**A.5. Molecular genetics of mycobacteria :**

**Sujoy Kr. Das Gupta, Mamta Chawla and Shreyasi Chatterjee**

The mycobacterial disease, tuberculosis, is a major health problem, particularly in India. In order to understand the molecular genetics of mycobacteria in more details and to develop efficient tools for genetic engineering of mycobacteria we have been investigating the replication of *E. coli*-mycobacteria shuttle vectors derived from pAL5000, a mycobacterial plasmid. During the course of the study we observed a unique phenomenon of structural instability of the shuttle vectors induced by frequent transposition that IS1096, a *M. smegmatis* transposon, at a cluster of sites located within a 60bp domain in the vector, in between two oppositely oriented genes, coding for aminoglycoside phosphotransferase (the kanamycin resistance gene) and  $\beta$ -lactamase (the ampicillin resistance gene) respectively. Our results demonstrate that IS1096 can transpose at a high frequency at certain topologically preferred sites. Also transposition of IS1096 induces frequent deletions in its neighborhood possibly by an imprecise excision mechanism. Based on the information thus obtained we have constructed several simplified vectors that were found to be stable in mycobacterial hosts.

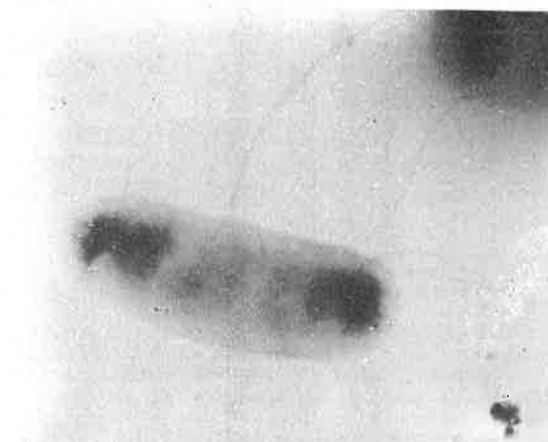
We have also been continuing our attempt to construct a convenient phage based transformation system for mycobacteria. We have already constructed a cosmid shuttle vector for mycobacteria utilizing the cohesive end (cos) site of a mycobacteriophage L1. Various aspects of the packaging of the cosmid vector are currently being investigated.

**B. Industrially important microbial enzymes.**

**B.1. Biotransformation of organic compounds by microbes : Studies on penicillin acylase :**

**Timir B. Samanta and Anjan Pal**

A bacterial strain, A-13, isolated in our laboratory was partially characterized by microbial and biochemical techniques. It was found to belong to the genus *Alcaligenes*. A semi-synthetic medium comprising of peptone - beef extract and phenyl-acetic acid was found to be most suitable for growth and penicillin



Transmission electron micrograph of penicillin acylase producing *Alcaligenes* sp.

acylase production by the strain. Penicillin acylase produced by A-13 is an inducible enzyme and is best induced by phenyl acetic acid. The enzyme was found to be located in periplasmic space of the bacterium. The acylase produced by A-13 has got wide substrate specificity ranging from semi-synthetic penicillin to cephalosporins.

**B.2. Molecular recognition of chiral compounds by microbes :**

**Timir B. Samanta and S. Das**

Chirality plays an important role on efficacy of  $\beta$ -blockers, anaesthetics and pesticides. Most of these molecules currently used as racemates are handicapped by the adverse effect of one of its enantiomers. In fact, the pharmacokinetic and pharmacodynamic studies of these molecules as racemates do not represent the precise picture. Attempts were made to isolate microbes capable of separation of the enantiomers of metoprolol tartarate ( $\beta$ -blocker), ibuprofen (analgesics) and thalidomide (sedative) separately used as substrate under liquid culture conditions. Characterisation of the bioconverted product is underway.

**B.3. Microbial starch degrading enzymes :**

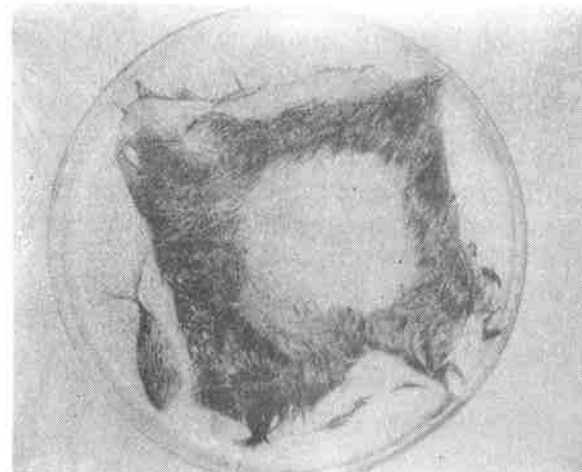
**P. K. Chakrabartty, Sanjeev Pandey and Mithu De**

Enrichment culture of soil suspension at high temperature followed by dilution plating on starch containing medium and staining with iodine have afforded isolation of several thermostable starch degrading bacteria. The enzyme activity of the selected isolates are being studied.

**B.4. Microbial proteases :**

**Roma Chakraverty and Papia Mitra**

The extracellular protease obtained by growing *Streptomyces nogalater* (AC 80) on



Dehairing of goatskin by an enzyme produced in the culture filtrate by a soil isolate *Streptomyces nogalater* AC 80. Loss of hair in the skin after treatment with the enzyme is evident as a bald patch.

wheat bran substrate, moistened with basal salt medium, showed dehairing capacity when applied to goatskin pieces.

An attempt was made to isolate caseinase over-producing as well as non-producing mutants of the strain. This could provide a means for overproduction of proteolytic activity on one hand, and also to identify the type of proteolytic activity responsible for dehairing of goat skin on the other.

The wild type strain was subjected to UV radiation. The resultant colonies were grown separately on caesins—as well as gelatin medium and screened for proteolytic activity by the nutrient gelatin agar mercuric chloride flooding method. The diameter of the clearance zones formed by hydrolysis of caesin and gelatin gave the measure of proteolytic activity in each case. A mutant showing higher activity and another showing reduced activity, as compared to the wild type, were isolated. These are being studied for enzyme production as well as dehairing of goatskin.

**B.5. Microbial  $\beta$ -amylase :**

**Roma Chakraverty and Rina Rani Roy**

*Syncephalastrum racemosum* producing significant amount of  $\beta$ -amylase was found to utilize strachy wastes of maize, tapioca, arrow root, wheat, rice and pulse, collected from market dumps. Thus its ability to biodegrade waste starches could be effectively exploited for maltose production at a nominal cost.

**C.1. Distribution of arsenic resistance genetic determinant in diverse species of soil bacteria :**

**Pradosh Roy and Anupama Saha**

The relationship between soil arsenic content and arsenic resistance pattern of viable bacteria indigenous to soil samples was investigated. A linear relationship was observed between percentage of arsenic resistant bacteria and arsenic content of soil samples. A simple bioassay based on monitoring the arsenic resistant bacterial count can simply be used to predict the arsenic content of the respective soil samples. To understand the genetic diversity of arsenic resistance in different species of bacteria, DNA of arsenic resistant soil isolates were hybridized with two distinct arsenic resistance operons (*ars* operon) of gram negative and gram positive bacteria. Most of the isolates (90%) showed the presence of *ars* operon homologue of either of the two distinct genetic

systems. The isolates which did not hybridize with any of the two known *ars* operons, could have a third yet unknown arsenic resistance genetic determinant.

## PUBLICATIONS

1. TK Sengupta, R.K. Nandy, S. Mukhopadhyay, R.H. Hall, V. Sathyamoorthy and A.C. Ghose : Characterization of a 20-kDa pilus protein expressed by diarrhoeagenic strain of non-O1/non-O139 *Vibrio cholerae*. *FEMS Microbiology Letts.*, **160**, 183-189, 1998.

2. P. K. Chakrabartty : Ecology of Rhizobia. In : Recent Advances in Ecobiological Research, Vol. 1, (Ed. Sinha, M.P.) : A.P.H. Publishing Corporation, New Delhi, pp. 233-245, 1997.

3. Ranadhir Chakraborty, Chirojyoti Deb, Anuradha Lohia and Pradosh Roy : Cloning and characterisation of a high copy number novel insertion sequence from chemolithotrophic *Thiobacillus ferrooxidans*. *Plasmid*, **38**, 129-134, 1997.

4. Timir B. Samanta and Rittika Chanda : Impairment of cytochrome P<sub>450</sub> during leishmaniasis : Role of nitric oxide synthase. *Indian J. Clin. Biochem.*, **12**, 55-59, 1998

5. S. K. Gupta, A. Mandal, S. Majumdar and A. Dutta : Local cyclophosphamide in the treatment of 7 patients with foot and ankle infection. *J. Foot Surgery*, **12**, 54-48, 1997.

### Participation in Conferences/Symposia/ Training programme/Awards and Honours received

**Prof. A.C. Ghose** acted as a member of the Scientific Panel of PRC of the Indian Council of Medical Research ; delivered an invited talk and chaired a session in the XXIV Annual Conference of Indian Immunology Society held at Calcutta on December 22-24, 1997 ; attended and delivered an invited lecture in the "Symposium on Biochemistry : A journey through three decades" held at Bose Institute on January 22-24, 1998 ; delivered a series of lectures to the M.Sc. (Biochemistry) students of Calcutta University.

**Prof. Timir B. Samanta** delivered an invited talk at the 6th National Bio-organic Symposium held at Indian Institute of Science, Bangalore, June 5-7, 1997 ; delivered an invited talk at the Symposium on Recent Trends in Tropical Disease Research held at Mahatma Gandhi Institute of Medical Sciences, Sevagram, September 5-6, 1997 ; delivered an invited talk at the national seminar on Bioprocessing and Recombinant-DNA Technology held at Central Drug Research Institute, Lucknow, March 27-28, 1998 : was elected a member of the National Academy of Sciences, India, 1998.

**Prof. P. K. Chakrabartty** delivered an invited lecture on "Role of siderophore in iron aquisition by *Rhizobium* and its interaction with aluminium" at the National Symposium organised by Department of Botany, Burdwan University during Sept. 26-27, 1997 ; chaired a Session in the National Symposium on Frontiers in Microbial Technology held on February 14, 1998 at the Department of Botany, Calcutta University ; acted as a Joint Secretary in the International Conference on "Stress Adaptation, Prophylaxis and Treatment" organised by Bose Institute during January 10-12, 1998.

**Dr. Roma Chakraverty** attended and presented a paper at the 9th National Conference on Aerobiology, held at Hyderabad from 27th to 30th November, 1997.

**Dr. S. Majumdar** delivered a plenary lecture on the topic "Differential substrate specificities between the PKC isotypes" on Dec. 27, 1997 at Nagpur University under the auspices of Golden Jubilee celebration and International Symposium on "Biochem '97", Nagpur, Maharashtra ; delivered an invited lecture in the XXIVth Indian Immunology Society at Science City, Calcutta. on 23<sup>rd</sup> December, 1997.

**Dr. S. Dasgupta** delivered the Dr. R.N. Chakraborty Memorial Oration lecture on the "Role of genetic engineering in MDR tuberculosis" at the XXIII Annual Conference and CME Programme '98 organized by the Doctors Club, Central Hospital, S.E. Rly. GRC.

Ranjan K. Nandy attended and presented a paper in the XXIV Annual Conference of Indian Immunology Society held at Calcutta on December 22-24, 1997.

Sm. Papia Mitra participated and presented a paper in the National Symposium on Frontiers in Microbial Technology on February 14, 1998 held at the Department of Botany, University of Calcutta.

## GRANT-IN-AID SCHEME

| Investigator/<br>Co-investigator | Title                                                                                                                                                                                 | Financed by |
|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| Prof. A.C. Ghose                 | Characterization of a new type of pilus expressed by pathogenic strains of non-O1 <i>Vibrio cholerae</i> and evaluation of its role in intestinal colonization as well as protection. | CSIR        |
| Prof. A.C. Ghose                 | Use of an immunomodulator protein A in the treatment of visceral leishmaniasis.                                                                                                       | ICMR        |
| Prof. A.C. Ghose                 | Development of bioconjugates for diagnosis and treatment of microbial infections. (Indo-Hungarian Collaborative Project).                                                             | DST         |
| Prof. Timir B Samanta            | Studies on protection of impairment of cytochrome P450 during experimental leishmaniasis in golden hamster : Role of nitric oxide synthase.                                           | CSIR        |
| Dr. P. Roy                       | Development and evaluation of a microbial based arsenic assay kit.                                                                                                                    | DBT         |
| Dr. S. Majumdar                  | Alteration of the human neutrophil function by TNF- $\alpha$ ; Influence of apoptosis on signal transduction pathway.                                                                 | CSIR        |
| Dr. S Majumdar                   | Signal transduction mechanism in visceral leishmaniasis in mice : Role of TNF in leishmaniasis.                                                                                       | DAE         |
| Dr. S Dasgupta                   | Investigation into the mechanism of extrachromosomal replication of the mycobacterial plasmid pAL5000.                                                                                | CSIR        |
| Dr. S. Dasgupta                  | Understanding the mechanism of replication of the mycobacterial plasmid pAL5000 using an <i>in vitro</i> approach.                                                                    | CSIR        |
| Dr. S. Dasgupta                  | A cosmid vector for cloning genes linked to viability of virulent mycobacteria in macrophages.                                                                                        | DBT         |

## Ph.D. Awarded

| Name                    | University          | Year | Title                                                                                                                                                            | Supervisor         |
|-------------------------|---------------------|------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------|
| Asish Ghosh             | Calcutta University | 1998 | Studies on the antigens of <i>Leishmania donovani</i> promastigotes stimulating humoral and cell-mediated immune responses in visceral leishmaniasis (kala-azar) | Prof. A.C. Ghose   |
| Sumana Ghosh (Majumder) | Calcutta University | 1997 | Studies on keratinolytic soil actinomycetes                                                                                                                      | Prof. A.L. Chandra |
| Mousumi Ghosh           | Calcutta University | 1997 | Studies on major xylanolytic enzyme system of <i>Aspergillus sydowii</i> MG49 and immobilisation of the organism for extracellular xylanase production           | Prof. Geeta Nanda  |

RESEARCH SCIENTISTS/RESEARCH ASSOCIATES/POST DOCTORAL FELLOWS/  
SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS

Dr. Anup Kr. Halder, Dr. Sarmistha Chakraborty, Dr. Soumitra Pal, Mr. Santanu Chakrabarti, Dr. Eliza Chakrabarty, Dr. Indrani Chakrabarty, Sm. Nilanjana Das, Sm. Papia Mitra, Sm. Sonali Das, Sm. Mousumi Basu, Sm. Saona Sengupta, Sri Pratap Mukherjee, Sri Subhajit Dasgupta, Sri Ananda Mukherjee, Sri Ranjan Nandy, Sm. Anupama Saha, Sri Anjan Pal, Sm. Mamta Chawla, Sm. Shreyoshi Chatterjee, Sm. Sarmistha Mukherjee, Sm. Rina Roy, Sri Sandip Bhattacharya, Sm. Sanjukta Ghosh, Sri Asish Ghose. Sri Bisweswar Nandi, Sri Shuvendu Das, Sri Sanjeev Pandey,

## SUPPORTING STAFF MEMBERS

Sri R.R. Sanyal, Sri S.K. Das (I), Sri S.K. Das (II), Dr. Bandana Banik, Sm. Gita Dey, Sri N.K. Malakar, Sri B.N. DeSarkar, Sri B.N. Mandal, Sri P.K. Chattopadhyay, Sri P. Gupta, Mr. S. Gazi, Sri D. Sarkar, Sm. Babli Marik, Sri Prabir Halder, Sri Debashis Majumdar, Sri B. Bahali, Sri N. Patra, Sri J. Prasad, Sri B. Bhattacharyay, Sri R. Prasad, Sri B. Ram, Sri B. Das, Sri S. Das. Sri Rabin Pal, Sri Sankar P. Bari, Sri Purnendu Manna, Sri Narayan Patali.



A "Microbial Culture Repository" Created in the Department of Microbiology, was formally inaugurated on February 23, 1998 by Prof. P.K. Ray, the Director of the Institute in presence of Prof. P.N. Nandy, the Chief-guest. Both Prof. P.K. Ray and Prof. P.N. Nandy discussed the importance of such a facility, particular in the Eastern part of India. The repository is located in the Department of Microbiology and has the necessary facilities. The facility is open to scientists for deposition of well characterised microbial strains.

The silver jubilee year of the Department of Biochemistry was celebrated by holding a meeting **Symposium on Biochemistry : A Journey Through Three Decades** on January 22-24, 1998. It was a grand event, marked by the presence of several past associates of the department, as well as a number of well known biochemists who enlivened the three days discussing science and remembering the past. Various areas of Gene Regulation, Plant Biochemistry, Structural Biology and Parasitology were discussed. The symposium was inaugurated by the eminent biochemist Prof. A.N. Bhaduri. The founder of the department, Prof. B.B. Biswas was felicitated on this occasion, which also marked his seventieth birthday.



National Science day was observed on March 2nd 1998. The theme of this year was "Fifty year of Independence" and this was reflected through a week long programmes on lecture, quiz and scientific model competitions among school students. The photograph shows Prof. D.P. Burma, former Head of Centre for molecular Biology, Banaras Hindu University distributing prizes to the school students.

## Biochemistry

**Faculty :** Prof. N. C. Mandal (Chairman), Prof. R. K. Mandal, Prof. S. Biswas, Prof. B. Bhattacharyya, Dr. P. Sinha, Dr. Prabhakar Gupta (upto 09.7.97), Dr. P. Chakrabarti, Dr. A. Lohia, Dr. R. Chattopadhyaya, Dr. P. Parrack.

### HIGHLIGHTS

Transgenic tobacco and tomato plants, expressing the trypsin inhibitor gene from mustard, have been found to be resistant to lepidopteran insects.

Transcription of a galactokinase gene of *Mycobacterium Smegmatis* has been shown to be positively regulated by cAMP and the amino acid glutamate.

Diagnostic methods using PCR on stool samples have successfully differentiated *E. histolytica* from *E. dispar*. DNA fingerprinting technique of Amplification Fragment Length Polymorphism (AFLP) has been used to characterize different strains and species of *E. histolytica*.

To mark the occasions of its silver jubilee and the seventieth birthday of its founder member, Prof. B. B. Biswas, the Department of Biochemistry organised the "**Symposium on Biochemistry : A Journey Through Three Decades**" at Bose Institute, Jan. 22-24, 1998.

### INSTITUTIONAL PROJECT - I

#### A.1. Seed Protein Genes and Transgenic Plants :

**R.K. Mandal**, Surekha Mandal and Pallab Kundu

Stable transformation of tobacco and tomato plants with a trypsin inhibitor gene from *Brassica juncea* and its expression in transgenic plants were reported last year. Some of T<sub>1</sub> and T<sub>2</sub> generation lines were bioassayed for insect resistance by feeding the plant leaves to larvae of laboratory reared tobacco cutworm *Spodoptera litura*. The lines expressing the mustard trypsin inhibitor gene retarded the growth of larvae and were lethal if fed early after hatching. The results indicate that the inhibitor gene can be potentially used for producing insect resistant plants. Tomato plants transformed with and expressing TVMV-coat protein gene have been tested (in collaboration with Dept. of Agronomy, University of Kentucky, USA) and have been found to be resistant to tobacco etch virus, a member of the same potyvirus group.

#### A.2. Sequencing and characterization of Cis-acting upstream regulatory sequences and transacting factors of At2S<sub>3</sub> promoter :

**Susweta Biswas**, Samarpan Mazumder and Asim Poddar (in collaboration with B.B. Biswas, Calcutta Univ., Science College)

This is in continuation of the work on expression of the reporter gene  $\beta$ -galactosidase (GUS) under specific *Arabidopsis thaliana* promoter (2S<sub>3</sub>) in *Agrobacterium*, yeast and plant. Since relative level of expression of GUS gene under full length 1.5 kb

2S3 promoter vis-a-vis 0.94 kb At2S3 promoter was found to be more or less same in all these systems, it was reasoned that 0.94 kb At2S3 contains necessary and sufficient informations typical of a fully active promoter. The 940 bp<sup>5</sup> upstream regulatory region of At2S3 gene of *A. thaliana* was sequenced. A putative TATA box lies within the sequence CCTATAAATAC (located at -74) which shows 9 out of 11 box identity with the plant storage protein gene consensus sequence CCTATAATTA. A putative CAAT box lies with -94 to -88 from the translation start point A. There is also enhancer like sequences GCCA in many regions of the promoter. Comparison

of the 5' upstream regions of the At2S3 promoter in BLASTIN computer programmes give the best possible score of the query sequence (At2S3 promoter) against various promoters. It is seen that At2S1, At2S3 and At2S4 (i.e. other three isomers of At2S gene family) gene promoter showed significant homologies with At2S3 promoter.

### INSTITUTIONAL PROJECT III.

**A.1. Studies on the Lethal Interaction of Bacteriophage Lambda Replication Gene P with its host *E. coli* :**

**N. C. Mandal**, Lopa Adhikary, Indrani Datta, and Niranjan Das

Earlier attempt to clone the mutant *rpl* gene of *E. coli* which confers resistance to lambda P gene lethality, was not succeeded, possibly, for the fact that such clone in high copy number vector is lethal to *E. coli*. So a low copy number *E. coli* cloning vector was constructed. This contained the *E. coli* p15A origin of replication so that it will be compatible with both lambda *ori* and *ColE1 ori* plasmids. This carries the chloramphenicol resistance gene. This new vector was named pIN and has a size of ~5 kb. An *E. coli* genomic library taking DNA from the *rpl* mutant bacteria was prepared in pIN and this was screened for putative clones bearing *rpl* property. Two such clones have been identified bearing inserts of ~ 2 Kb and 4 Kb. Further characterization of the *rpl* clone is in progress.

**A.2. Studies on Gene Regulation in *Mycobacteria* :**

**N. C. Mandal**, Amitabha Chakraborty and Madhumita Basu

In continuation of the study of the regulation of galactokinase gene expression in *Mycobacterium smegmatis*, the above gene in *Bam*HI- *Acc*I fragment from pGK6 plasmid was subcloned in pUC19 vector and about 70% of the DNA segment was sequenced in an automatic DNA sequencer. The sequencing data suggest that the putative promoter and the upstream regulatory region are located in the *Acc*I site proximal region. The *galK* gene from

*ginI* mutant (glutamate independent galactose utilizing mutant) was cloned in pTAC3734 plasmid. The expression of galactokinase from the *galK* clone (pGK6) derived from wild-type bacteria and that from *ginI* mutant in wild-type *E. coli* suggest that the *ginI* mutation has not affected neither *galK* nor its upstream positive regulatory sites. This indicate that the *ginI* mutation has possibly affected a trans-acting positive regulatory element in *M. smegmatis*. The expression of *galK* in *E. coli* has been shown to be cAMP/CRP dependent. Preliminary experiments with cell-free extracts from *M. smegmatis* indicate the presence of cAMP-binding protein in this bacterium. Work is being done to confirm the location of the *galK* promoter and to determine its transcription start site.

**A.3. Studies on Developmental Regulation in *Mycobacteriophage L1* :**

**N. C. Mandal**, Prajna Mandal, Chandrani Chattopadhyay and Rajat Bhattacharyya

To characterize the L1 promoter in pCM8 clone, the 700 bp DNA fragment containing the promoter was subcloned in pUC19 and sequenced. Using *E. coli* and mycobacterial RNA polymerases and the above 700 bp DNA template, *In vitro* transcription is being standardised. The 800 bp DNA fragment of L1 containing the early regulatory gene *G27* was subcloned in pUC19 and partially sequenced. The size of the insert is being reduced by nested dilution to get a minimum sized DNA fragment containing the whole of the *G27* gene.

To understand the role of *G23* gene of L1 in post transcriptional regulation of late regulatory gene of the phage, the *ts* mutant which did not complement *G23ts23* mutation at 42°C was characterized. This mutant was defective in late transcription but not in exonuclease synthase. This mutation was mapped in between *G24* and *G25* genes of L1. The wild-type counter part of the mutant gene has been cloned and is being characterized. By analysing the revertants of L1 *G23ts23* mutant isolated at 42°C, certain *G23* by-pass (extragenic suppressor) mutants have been isolated which have overcome the *G23ts* defect for phage growth at 42°C but still retained the *ts*

defect in exonuclease synthesis. This mutant is being characterized to identify the late regulatory gene which is the target of post-transcriptional regulation by *G23*.

**A.4. Studies on a Complex and Novel NTP-NDPase (apyrase) from *Mimosa pudica* leaflets :**

**Susweta Biswas** and Rinku Ghosh

In order to characterize the NTP-NDPase (apyrase) enzyme further, a cDNA library of *Mimosa pudica* leaves was constructed and by screening with antibody raised against the protein apyrase, the gene of interest has been isolated and subsequently cloned and over-expressed in *E. coli*. The full length cDNA insert was subcloned in Bluescript and the nucleotide sequence of 1.99 Kb *Eco*RI fragment of apyrase gene was determined by automated sequencer. To locate the open reading frame on the DNA sequence, partial N-terminal amino acid sequence of the protein apyrase (Isoform I) was determined. The open reading that ends with a TGA stop codon encodes a polypeptide of 331 amino acid with a calculated molecular mass of 36,410 which is in good agreement with the estimated molecular mass of the 36 KD apyrase isolated from *Mimosa pudica*.

A consensus ATP binding domain consisting of G(x) 4GK (S/T) (x) 6 (I/V) has been found to be present in this enzyme. Ten Ca<sup>2+</sup> binding loops as found in calmodulin are present in the sequence of apyrase gene. Computer data bank search revealed a striking percent similarity of this enzyme with calmodulin of different plant species and also with touch genes (TCH3 and TCH4) from *Arabidopsis thaliana*.

**A.5. Tubulin Substructure and Identification of its Structural, Functional and Regulatory Domains :**

**B. Bhattacharyya**, Suranjana Guha, Sankar Bhattacharyya, Taradas Sarkar and Tapas Kumar Manna

Tubulin unfolding in urea proceeds via the formation of a partially unfolded intermediate state, stable in 2 M urea, that unfolds further in higher urea concentrations. The intermediate state had spectroscopic properties reminiscent of a molten globule and negligible *colchi-*

*cine binding* activity. Refolding of totally unfolded tubulin in 8 M urea yielded an intermediate like state characterized by partial burial of tryptophans and partial recovery of secondary and tertiary structures, although *colchicine-binding* activity of the protein was not regained. Further folding of this intermediate-like state, toward the native conformation, with respect to both structural and functional parameters did not occur. However, a significant percentage of *colchicine binding* activity and native like tertiary structure was recovered when refolding was initiated from partially denatured protein samples, viz., from <1.2 M urea. Thus, although high concentration of urea induced loss of structure and activity was irreversible, the conformational changes induced in restricted regions of tubulin by lower concentrations of urea, which are probably crucial for its various functional properties, could be reversed.

**A.6. Structure-Function Relationship of Colchicine-Tubulin Interactions :**

**B. Bhattacharyya**, Suroopa Banerjee, Shalmali Chakraborty, Pradip Mahapatra and Pampi Basu

Interaction of antimetabolic drugs with tubulin being continued in this laboratory. Colchicine binding activity of tubulin containing different proportions of dimers and monomers are analysed using Scatchard plots. There are two slopes in the Scatchard plot correspond to higher and lower affinity for the dimer and the monomer respectively. Thermodynamic parameters show that the binding with dimeric tubulin is entropy driven and the binding of monomer with colchicine is enthalpy driven. We are trying to find out how the C-termini of tubulin regulate colchicine binding to tubulin while the C-termini are not the binding sites for the drug.

**A.7. Chromosome Transmission in Yeast :**

**P. Sinha**, Kaustav Sanyal, Atasi Poddar and Santanu Ghosh

Chromosome replication and a proper segregation of the duplicated copies to daughter cells are essential features of a normal cell cycle.

We are continuing our studies on the identification and characterization of genes required for hi-fidelity chromosome transmission in the budding yeast *S. cerevisiae*. Earlier we had cloned several genes (*MCM16* - *MCM19*; *MCM21*, *MCM22*) which are needed for chromosome segregation. These gene products

were shown to be involved in the assembly of a competent kinetochore establishing kinetochore-microtubule interactions during mitosis. In a bid to understand the molecular mechanisms by which the corresponding proteins act, we decided to study the subcellular localization of these proteins by fusing each of them to a

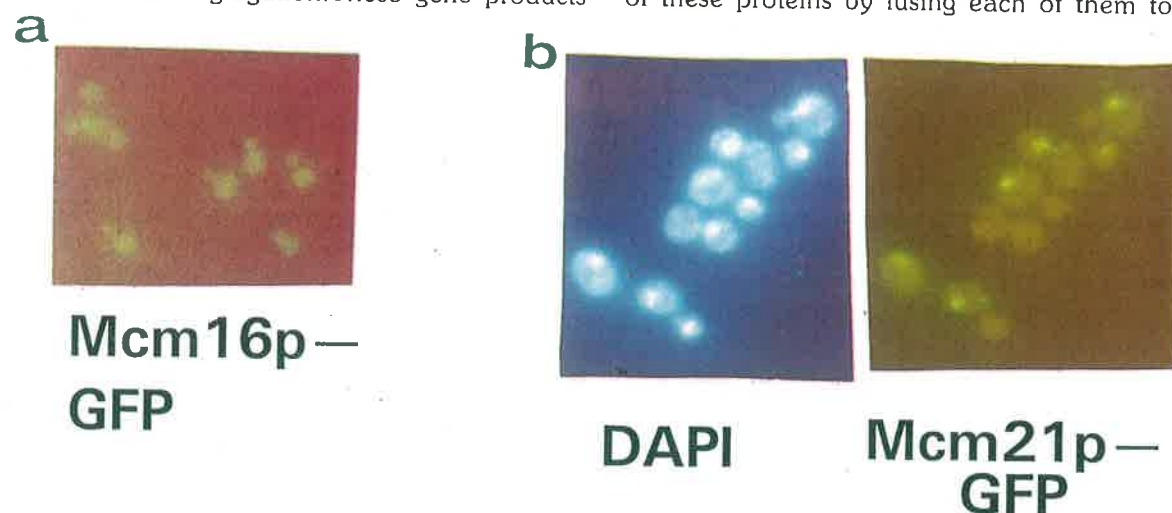


Fig.1 Subcellular localization of Mcm16 and Mcm21 proteins using the fluorescent tag, the green fluorescent protein (GFP). (a) Mcm16 is localized to the entire cell as the whole cell glows green. (b) Nuclear localization of the Mcm21 protein tagged to GFP. Staining with DAPI identifies the positions of the nuclei in whole cells while the green fluorescence is indicative of the presence of Mcm21 protein.

fluorescent tag GFP, the green fluorescent protein. ORFs corresponding to *MCM16* and *MCM21* genes were fused to GFP and the localization of the Mcm proteins was monitored by the fluorescent tag. Fig. 1 shows that the Mcm16p was localized to the entire cell while the Mcm21p, in some cells was localized to the nucleus. This suggests that Mcm16p does not bind directly to the kinetochore but could be regulating the activity of another protein which is either a constituent of the kinetochore or regulates its assembly. Mcm21p appears to present in the nucleus in some cells. Since Mcm21p does not have a known nuclear localization signal, it could be complexing with some other nuclear protein to take a ride to the nucleus. It would be rewarding to identify such interacting proteins. For this purpose, we have initiated work on the isolation of suppressors of the mcm mutations and other genes which interact with the *MCM* genes.

We have also purified Mcm16, Mcm19, Mcm21 and Mcm22 proteins from *E. coli* and raised antibodies to them. These antibodies will be used for tracking the corresponding proteins in various studies, such as subcellular localizations, *in vitro* centromere or microtubule-binding studies etc.

**A.8. Interrelationship between the main-chain and side-chain conformations in protein structures, and its implications for sequence comparison, entropy of folding and protein engineering experiments :**

**P. Chakrabarti** and Debnath Pal

The main-chain conformation of protein residues are adequately given by the Ramachandran plot on which the side-chain is assumed to have a very negligible influence. Our studies using the Protein Data Bank have shown that the backbone torsion angles can be affected by the side-chain conformation, and this effect is different

for different amino acid residues, which can thus be classified into 7 groups. Instead of having one Ramachandran plot, each residue (except Gly, Ala and Pro) is now represented by three plots (at three different side-chain torsion angles,  $\chi_1$ ). Correlations between these sets of plots provide similarity indices between various residues which are being used to generate a new algorithm for structure-based sequence comparison (as opposed to the one using Dayhoff's mutational table). Using these plots we have calculated the loss in the main-chain conformational entropies for various residues on folding which are found to be correlated with hydrophobic values. These observations have implications for protein engineering experiments.

**A.9. New interactions, residue environments and solvent accessibilities in protein structures :**

**P. Chakrabarti**, Uttamkumar Samanta and Debnath Pal

Geometrical features of various types of novel interactions like  $S...C=O$ ,  $S...aromatic$ ,  $C-H...aromatic$ , are being enumerated; these interactions not only provide stability, but are also useful for protein function. The environments of Trp residues, which are very important for studying protein structures and dynamics using fluorescence spectroscopy, have been found out from an analysis of known protein structures. Trp residues are also found at the binding sites of many enzymes, and important lessons on molecular recognition and binding affinity can be obtained by analysing the Trp environment of these proteins. We are now working on how the solvent accessibility of a Trp residue change depending on the number of residues in its environment and on substrate binding, and the difference, if any, in the environment of Trp residues in globular and membrane proteins.

**A.10. Aromatic-aromatic interactions in and around helices and their use in the design of helical peptides :**

**P. Chakrabarti** and Ajoy Koomer

Stacked and edge-to-face interactions between two aromatic residues are known to be stabilizing. We are analysing if such motifs are found when a pair of aromatic residues belonging to a helix or a helix and its immediate neighbourhood are spatially close, and if

so, if there is any pattern in the sequence in the intervening region. The results will be used to design sequences with high helicity brought about by aromatic-aromatic contacts.

**A.11. Crystallographic studies on C-reactive protein :**

**P. Chakrabarti** and Ajoy Koomer

C-reactive protein (CRP) is an acute-phase protein. CRP from a fish source is a pentameric protein which has been isolated and characterised at IICB. We have initiated a project to crystallize the protein for structure determination.

**A.12. Crystallographic studies of modified DNA oligomers :**

**Rajagopal Chattopadhyaya**, Bhaswati Goswami

Syntheses of ten oligomers were completed previously for studying properties of T-T photodimers. They are being exposed to UV and changes in the DNA structures will be probed by various biophysical and crystallographic methods.

**A.13. Crystallographic studies of lipase from *Aspergillus niger* :**

**Rajagopal Chattopadhyaya** and V.M.H. Nambodiri

The lipase purification was completed and the enzyme has since been characterized as a 35.5 kDa protein containing 2.8% by mass carbohydrate. The enzyme retains activity at temperatures up to 70°C and could thus be commercially important in the detergent industry. N-terminal sequencing of 36 residues have been completed, as well as amino acid composition. We are now trying to determine the complete protein sequence. Crystallographic trials are also under way.

**A.14. Crystallographic studies of lambda repressor and mutants :**

**Rajagopal Chattopadhyaya** and Kausik Ghosh

High quality X-ray diffracting crystals of the carboxy terminal domain of the wild type lambda repressor and a mutant were successfully grown. One of them have been found to diffract to high resolution in the newly installed X-ray Image Plate at Saha Institute.

**A.15. Model building studies of the intact lambda, P22, 434 and Lex A repressors :**

**Rajagopal Chattopadhyaya** and Kausik Ghosh

Models for all these four repressors have been built so as to reflect assemblies important in biological systems, using biochemical and biophysical data previously generated in dozens of laboratories around the world. The modeling problems are basically solved and they are at various stages of completion.

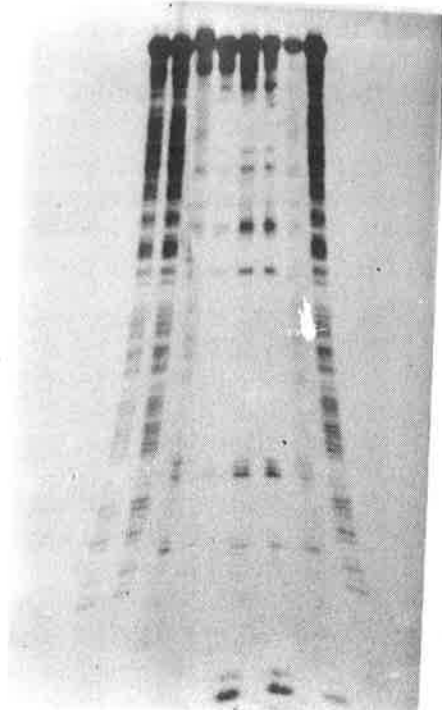
**A.16. Structural Basis of Control of Transcription Initiation in *E. coli* :**

**P. K. Parrack** and Jayanta Mukhopadhyay

In *E. coli*, the transcription activator protein CRP (Cyclic AMP Receptor Protein) adopts an

active conformation when it is complexed with cyclic AMP (cAMP). Recent studies indicate that there are two types of binding sites for cAMP in CRP. Each type can bind two cAMP molecules per CRP molecule which is a homodimer. The two modes of binding lead to different conformational states of the protein. At low cAMP concentrations the complex can activate transcription. However, with cAMP molecules bound to the lower affinity site (at high concentration of cAMP), the conformation of the complex resembles that of unliganded CRP. *In vitro* transcription experiments carried out by us indicates that the level of activation goes down at high cAMP concentrations, in the *galactose* operon of *E. coli*.

**Fig.1A**



**Fig.1A :** DNase I footprint of a 300 bp DNA fragment containing gal promoter and upstream region, labeled at the 3'-end of the template strand. Lanes (from left) 1, 2 and 8 : Maxam-Gilbert A+G sequence markers; lanes 3 and 7 : Control lanes without protein; lanes 4,5,6 contain increasing concentrations of RNA polymerase. The protected region at the top represents gal promoter footprint and that at the bottom, the TB-site footprint.

**Fig.1B :** DNase I footprint of the same fragment, labeled on the same strand at the 5'-end. Lanes 1 and 7 : A+G sequence markers; lanes 2 and 6 : Control lanes; lanes 3-5 : increasing concentrations of RNA polymerase. Here the TB footprint is visible at the top and the gal promoter footprint at the bottom.

**Fig.2. :** *In vitro* transcription from the same DNA fragment. The first two lanes are results from identical experiments, the third lane is an RNA marker with 100, 200, 300, 400 and 500 nt bands. Transcripts P1 (97 nt) and P2 (102 nt) can be seen near the 100b marker while the transcript from TB site is visible near the 200b marker.

**Fig.1B**



**Fig.2**



**A.17. Role of upstream sequences in promoter recognition by RNA Polymerase :**

**P. K. Parrack** and Runa Sur

Binding of *E. coli* RNA polymerase to certain nonpromoter sequences in the *E. coli gal* promoter region has been studied in detail. One such sequence, known as "tight binding" or TB site has been located in the promoter-upstream region of the *gal* operon, by DNase I footprinting. It covers 44 base pairs, centered around -100, contains a classical Pribnow box TATAAT, and can give rise to run-off transcripts *in vitro*. *In vivo* experiments wherein the promoter activity of the wild type promoter (containing TB site) has been compared with a 'TB-less' or 'TB-only' situation using the promoterless vector pTAC, show that TB site modulates promoter activity in this system, although it does not give rise to translational products on its own.

**A.18. Structural Studies on the cII protein of bacteriophage lambda :**

**P. Parrack** and Ajit Bikram Datta

The cII protein of  $\lambda$  phage is a transcription activator which activates the  $P_{RE}$  and  $P_{int}$  promoters in  $\lambda$  phage and promotes lysogeny. cII is a small, tetrameric protein with M.W. 4 x 11 kDa. Not much is known about its structure.

To obtain the protein in milligram quantities required for structural studies, the cII gene has been cloned into a high expression vector (pET3a) under T7 promoter control. The clone (pAB119) is found to express the protein upon induction with IPTG, in high yield (about 30% of the total cellular protein). Purification of cII is in progress.

**INSTITUTIONAL PROJECT NO. IV**

**A.1. Fish Genetic Stock Identification and Conservation :**

**R.K. Mandal** and B. K. Padhi

Nuclear ribosomal RNA gene, mitochondrial DNA and *Mbol* satellite DNA from mrigal (*Cirrhinus mrigala*) and magur (*Clarius batrachus*) have been used as probes to identify

species, genetic stocks and interspecific hybrids. The extent of hybridization in the hatchery as practised by fish breeders has been found to be 8-10%, a fact indicative of the danger to fish breeding programme in the future. Gamet viability and long term preservation of catfish sperm have been studied also. Species specific RFLP patterns in snakehead fishes (murrels) and their taxonomic implication has been investigated.

**INSTITUTIONAL PROJECT NO. V.**

**A.1. Caffeine induced  $Ca^{2+}$  efflux from Trophozoites of *E. histolytica* :**

**Susweta Biswas** and Banabehari Giri

Caffeine induced  $Ca^{2+}$  efflux from trophozoites of *Entamoeba histolytica* caused the change of morphology of the cells within minutes of application. Pseudopod formation stopped and cells became rounded. Trophozoites treated with caffeine (< 5 mM) for 6 h were able to grow after washing of caffeine and putting back to the fresh culture medium. Whereas trophozoites treated with caffeine above 20 mM for the same period failed to grow in the fresh culture medium indicating induction of encystation and excystation by caffeine *in vitro*. With the higher concentration of caffeine, excystation could not be obtained instead some necrotic effect on cell nucleus and DNA leading ultimately to the death of the cells has been observed. The fragmentation of chromosomal DNA was also monitored with staining the caffeine-treated cell *in vivo* by DAPI and by FACS scanning. Strikingly caffeine could inhibit the growth of *E. histolytica* *in vitro* similarly as that caused by the commonly used drug, metronidazole. That caffeine could potentiate the action of metronidazole has also been noted.

Since total  $Ca^{2+}$  pool in *E. histolytica* could be released by  $InsP_3$ ,  $InsP_4$  and caffeine, it is possible to check the growth of *E. histolytica* by regulating any one of the modules of  $Ca^{2+}$  mobilization. Caffeine exhibited dramatic effects leading to death of the cells *in vitro*. If it could be mimicked, *in vivo* containment of the amebiasis might be possible by adjusting the

concentration of caffeine and metronidazole not lethal to host cells.

#### A.2. Molecular Genetics of *Entamoeba histolytica* :

**Anuradha Lohia**, Anasuya Ganguly, Ardhendu Maaji, Suchismita Das, Sulagna Ghatak and Kamala Neogi

**Cell Cycle of amoeba** : Dynamic changes in the DNA content of encysting *Entamoeba invadens* was studied. Along with changes in the DNA content we also studied the changes in intracellular calcium during encystation. Other studies reported earlier are being continued.

**MCM gene family of amoeba** : The cDNA for *Eh MCM2* and *Eh MCM5* are being sequenced. Deletion analysis to identify the location of putative promoter elements for *Eh MCM3* is continued.

**DNA fingerprinting** : Diagnostic methods using the polymerase chain reaction on stool samples have successfully differentiated *E. histolytica* from *E. dispar* in our laboratory. We have extended this study to characterise different strains and species of *Entamoeba* using the DNA fingerprinting technique of Amplification Fragment Length Polymorphism (AFLP). We have shown that *E. dispar* and *E. histolytica* are totally dissimilar. We have also characterised a pathogenic strain of *E. histolytica* isolated at Kothari Centre of Gastroenterology and shown that it is different from the classical HM1:IMSS strain and similar to the attenuated pathogenic strain 200:NIH.

**L-myo-inositol 1-phosphate synthase from amoeba**: L-myo-inositol 1-phosphate synthase catalyses the primary reaction for the synthesis of inositol, which is a precursor of essential macromolecules like membrane phospholipids, GPI anchor proteins and lipophosphoglycans. These play a determinant role in the pathogenesis of protozoan parasites such as *Leishmania* and *Entamoeba*. Using molecular cloning techniques we have isolated and characterised the *INO1* gene coding for the enzyme L-myo-inositol 1-phosphate synthase from *Entamoeba histolytica*. Simultaneously, we have purified the native enzyme from *E. histolytica* trophozoites.

The gene product and the purified enzyme were both shown to be recognised by a heterologous anti-I-1-P synthase antibody from the phytoflagellate *Euglena gracilis*. Phylogenetic analysis of I-1-P synthase sequences from different eukaryotes suggest that it is highly conserved across species and the origin of this enzyme precedes the evolutionary divergence of modern eukaryotes.

**Note**: DNA fingerprinting was done in collaboration with *Dr. Tapas K. Ghosh* and L-myo-inositol-1-phosphate synthase study in collaboration with *Prof. A.N. Lahiri Majumder* and *Nitai C. Hait* of the Department of Botany.

#### PUBLICATIONS

1. Chatterjee, M., Chakraborty, S.L., Chattopadhyay, B.D. and Mandal, R.K. Production of lactic acid by direct fermentation of starchy wastes by an amylase-producing *Lactobacillus*. *Biotechnol. Lett.* **19**: 873-874, 1997.
2. Padhi, B.K. and Mandal, R.K. Species identification of snake-head fishes by nuclear DNA RFLP : its taxonomic implications. *Current Science*, **73**:907-908, 1997.
3. Padhi, B.K., Ghosh, S. and Mandal, R.K. Cloning and characterization of *Mbol* satellite in *Cirrhina mrigala* and *Clarias batrachus* (Pisces). *Genome* **41**:34-39, 1998.
4. Padhi, B.K. and Mandal, R.K. Gamete viability of *Heteropneustes fossilis* (Bloch) *in vitro*. *J.Aqua.Trop.* **13**:37-41, 1998.
5. Hirock J. Datta and Nitai C. Mandal, Identification of an early positive regulatory gene of mycobacteriophage L1, *Journal of General Virology* **79**: 205-210 1998.
6. Suranjana Guha & B. Bhattacharyya; Refolding of Urea-Denatured Tubulin : Recovery of Nativelike Structure and Colchicine Binding Activity from partly unfolded States. *Biochemistry* **36**, 13208-13213, 1997 .
7. Suranjana Guha & B. Bhattacharyya; The Colchicine-Tubulin Interaction : A Review. *Current Science* **73**, 351-358, 1997.
8. A.K. Bothra, S. Roy, B. Bhattacharyya, C. Mukhopadhyay; Molecular dynamics Simulation of Colchicinoids and its implication for

the colchicine-tubulin Interactions. *J. Biomolec. Struct. Dynamics*, **16**, 240-248, 1998.

9. SS Gangopadhyay, SS Ray, P Sinha and A Lohia. Unusual genome organisation in *Entamoeba histolytica* leads to two overlapping transcripts. *Mol Biol Parasitol* **89**: 73-83, 1997.

10. Gangopadhyay, S. S., Ray, S. S., Sinha, P. and Lohia, A. Unusual Genome organisation in *Entamoeba histolytica* leads to two overlapping transcripts. *Mol. Bioch. Parasitol.* **89**, 73-83, 1997.

11. Ray, N., Poddar, A., Lohia, A. and Sinha, P. The *mcm 17* mutation of yeast causes size dependent segregational defect of a minichromosome. *Current Genetics* **32**, 182-189, 1997.

12. Chakraborty, R., Deb, C., Lohia, A. and Roy, P. Cloning and characterisation of a high copy number novel insertion sequence from

chemolithotropic *Thiobacillus ferrooxidans*. *Plasmid* **38**, 129-134, 1997.

13. Gangopadhyay, S. S., Ray, S.S., Kennady, K., Pande, G. and Lohia, A. Heterogeneity of DNA content in axenically growing *Entamoeba histolytica* HM1:IMSS clone A. *Mol. Biochem. Parasitol.* **90**, 9-20, 1997.

14. Ray, S.S., Gangopadhyay, S.S., Pande, G., Samuelson, J. and Lohia, A. Primary structure of *Entamoeba histolytica* g-tubulin and localisation of microtubule organising centres. *Mol. Biochem. Parasitol.* **90**, 331-336, 1997.

15. Mukhopadhyay, C. and Lohia, A. Homology modeling of *Entamoeba histolytica* ferredoxin. *J. Biomol. Str. Dynam.* **15**, 663-672.

16. Home, D. and Chattopadhyaya, R., Reply to Comment on "DNA Molecular Cousin of Schrodinger's Cat : A Curious Example of Quantum Measurement", *Physical Review Letters*, **80**, 1349 1998.

#### Ph.D. Awarded

| Name of the        | University          | Year | Title                                                                                           | Supervisor       |
|--------------------|---------------------|------|-------------------------------------------------------------------------------------------------|------------------|
| Gopal Chakrabarti  | Jadavpur University | 1996 | Studies on the interaction of B-ring analogs of colchicine with tubulin                         | B. Bhattacharyya |
| Nabanita Sarkar    | Jadavpur University | 1996 | Studies on structure-function relationship of tubulin using aminonaphthalenes and related drugs | B. Bhattacharyya |
| Suranjana Guha     | Calcutta University | 1997 | Spectroscopic studies on conformations of goat brain tubulin in different solution conditions   | B. Bhattacharyya |
| Hirock Jyoti Datta | Calcutta University | 1997 | Genetic studies of the temperate Mycobacteriophage L1.                                          | N. C. Mandal     |

## GRANTS - IN - AID SCHEMES

| Investigator/<br>Co-investigator | Title                                                                                                                                                           | Financed by   |
|----------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| Prof. N. C. Mandal               | Studies on the Lethal interaction of the Bacteriophage lambda replication gene <i>P</i> with its <i>E. coli</i> . (Terminated on 31.1.98)                       | DBT           |
| Prof. N.C. Mandal                | Studies on the mechanism of action of gene <i>G23</i> in post-transcriptional regulation of bacteriophage L1 gene expression                                    | CSIR          |
| Prof. S. Biswas                  | Study of Signal Transduction in plants : Inositol trisphosphate receptor from <i>Vigna radiata</i> , its gene cloning and characterization of different domains | CSIR          |
| Prof. S. Biswas                  | Signal Transduction pathways operative in <i>Entamoeba histolytica</i> mediated by Inositol phosphates                                                          | DAE           |
| Prof. S. Biswas                  | Biochemical and Biophysical studies on the novel NTP-NDPase (apyrase) linked with the sensitivity of <i>Mimosa pudica</i> leaflets                              | DST           |
| Prof. B. Bhattacharyya           | Studies on carboxy termini of tubulin: Its structural and functional implications                                                                               | CSIR          |
| Dr. Pratima Sinha                | The role of Mcm family of proteins in initiating DNA replication of eukaryotic replication origins                                                              | DAE           |
| Dr. Pratima Sinha                | Characterization of <i>MCM</i> genes required for DNA replication and segregation in the yeast <i>S. cerevisiae</i> .                                           | CSIR          |
| Dr. P. Chakrabarti               | Protein structure and function through data base analysis and crystallography                                                                                   | DST           |
| Dr. A. Lohia :                   | Studies on the expression of <i>ras</i> superfamily proteins in <i>Entamoeba histolytica</i> .                                                                  | DBT           |
| Dr. A. Lohia                     | Identification of pathogenic strains of <i>Entamoeba</i> by a PCR based diagnostic assay.                                                                       | CSIR          |
| Dr. A. Lohia                     | Transformation of <i>Entamoeba histolytica</i> to study the role of proteins involved in the growth, virulence and drug sensitivity of the parasite.            | US-India Fund |
| Dr. A. Lohia                     | Role of tocotrienols as anti-proliferative agents against <i>Entamoeba histolytica</i> .                                                                        | UNESCO        |
| Dr. R. Chattopadhyaya            | Crystallographic studies of lipase from <i>A. niger</i> (terminated on 31.12.1997)                                                                              | CSIR.         |
| Dr. R. Chattopadhyay             | Biophysical characterization of DNA oligomers containing UV induced thymine dimers                                                                              | DAE.          |
| Dr. P. K. Parrack                | Structural Basis of control of Transcription initiation in Bacteria by the cyclic AMP receptor protein                                                          | CSIR          |

Participation in Conferences/Symposia/  
Training programme/Awards and Honours  
received.

**Prof. R. K. Mandal** presented talks / Chaired sessions in the following seminars/ symposia:

Talk on 'Cloning : Technological and Bio-ethical ; Implications' at Bharatiya Samskriti Samsad, SINP Physical Colloquium, Burdwan Raj College, Ballygunge Science College, C.U., Federation Hall Society and workshop on 'Sci. Commun. & Media Practices' organised by Indian Science News Association, Calcutta, and AIR, Murshidabad Station ; Talk on 'Fish biotechnological research in Bose Institute at the workshop on 'Genetic Improvement of Carps in Asia' organized by Intl. Center for Living Aquatic Resource Management (ICLARM) at CIFA, Bhubaneswar ; S.M. Sircar Memorial Conference by Plant Physiology Forum, Calcutta. ; Symposium on Individual man and his role in combating pollution, organised by Natl. Env. Sci. Academy. Calcutta Chapter. ; Dipta Chakraborty Memorial Symposium on "Science in Post- independence Era" at Bose Institute. ; Symposium on 'Environmental pollution and its control strategies' by Centre for Applied Sci. & Tech., Calcutta. ; Talk on Molecular Anthropology in the Centenary Symposium on Anthropology in the twentyfirst century organised by Bangabasi College ; Talk on 'Combinatorial chemistry and drug research in the twentyfirst century' at the Centenary Symp organised by Chemistry Dept., Bengal Engineering College, Sibpur ; Symp. on Recent Trends of Research in Microbiology and Plant Physiology, at Burdwan University ; Natl. Conference on Biotechnology and Modern Horticulture organised by Eastern India Research Centre in Horticulture and Biotechnology at Jadavpur University ; Acted as Chairman, Organising Committee of the Symposium on Plant Physiology for national Development, Organised by PPF, Calcutta and presented paper ; Recent Trends in Genetic Engg. & Mol. Biol. organised by B.C. Guha Centre for Genetic Engg. and Biotechnology ; Bose Inst. Biochemistry Dept. Silver Jubilee Symposium on "Biochemistry : A Journey Through Three

Decades" ; Participated in Bose Inst. DIC Symp. on 'Bioinformatics'.

**Prof. Mandal** also served as a member of Technical Advisory Committee, CRIJAF, Barrackpore and ISI, Calcutta. He also served as Hony. Lecturer/Examiner/Member Board of Studies of Calcutta, Jadavpur, Kalyani and Vidyasagar Universities.

**Prof. S. Biswas** attended the "Symposium on Biochemistry : A Journey Through Three Decades" organised by the Dept. of Biochemistry, Bose Institute, on January 22-24, 1998, ; Gave an invited talk at Visva-Bharati University, Shantiniketan in "National Seminar on Contribution of Women Scientists in Botanical Research", on 27-28th March, 1998, ; Acted as Vice-President and gave an invited talk in the Conference of "International Conference on Stress Adaptation, Prophylaxis and Treatment" at Hotel Oberoi Grand, Calcutta on Jan. 10-12, 1998 ; Gave an invited talk in S.M. Sircar Memorial Conference by Plant Physiology Forum, Calcutta.

**Prof. N. C. Mandal** Gave a course of lectures on Molecular Biology to the M.Sc. final year students of Biochemistry Department of the Calcutta University ; Acted as a member of the Scientific Advisory Committee of National Institute of Cholera and Enteric Diseases, Beliaghata, Calcutta ; Served as a member of the Sectional Committee of Indian National Science Academy ; Gave an invited talk at IICB, Calcutta in the Workshop on "TRENDS IN BIO-CHEMISTRY" ; Acted on several occasions as member of the Assessment Committee of Indian Institute of Chemical Biology, Jadavpur, Calcutta ; Acted as a member of the Ph.D. committee in Biochemistry of the Calcutta University ; Organized the symposium on "Biochemistry : A Journey Through Three Decades", as Joint Convenor on the occasion of the Silver Jubilee of the Department of Biochemistry, Bose Institute, Calcutta ; Chaired a scientific session in the symposium organized by Dr. B.C. Guha Centre for Genetic Engineering and Biotechnology ; Delivered a course of lectures on "Gene Regulation in Bacteriophage lambda" in the

teaching seminar held at Khandala organized by TIFR in December, 1997 ; Attended Guha Research Conference, Palampur, October 13-17, 1998.

**Prof. B. Bhattacharyya** Visited the laboratory of Dr. C. Briand, Department of Pharmacy, University of Marseille, France in a exchange visitors programme of INSA ; Dr. B. Bhattacharyya delivered invited talk in a one day symposium arranged by West Bengal Academy of Sciences; Elected member of Sectional Committee IX, Biochemistry & Biophysics, Indian National Science Academy, 1998-2000 ; Elected member of the National Committee for IUBMB, Indian National Science Academy; 1998-2000; Organized the symposium on "Biochemistry : A Journey Through Three Decades", as Joint Convenor on the occasion of the Silver Jubilee of the Department of Biochemistry, Bose Institute, Calcutta.

**Dr. P. Sinha** Participated in 'Diversity in Modern Biology' An Interdisciplinary Symposium held at Dept. of Biochemistry, Univ. of Delhi South Campus. New Delhi Sep 21-22. Meeting attended: GRC '97 at Palampur, HP. Courses taught ; Attended Mahabaleswar symposium on Gene Regulation. Lonavala, Dec. 7-14, 1997 ; Delivered courses at University of Calcutta, for M.Sc. (i) Biochemistry and (ii) Biophysics, Molecular Biology students in Yeast Molecular Genetics and at Winter School on 'Human Genetics' held in IICB, Calcutta Jan 10-30, 1998

**Dr. P. Chakrabarti** Elected Fellow of the Indian Academy of Sciences ; Bangalore and Allahabad ; attended the Guha Research Conference, Palampur, October 13-17, 1997. ; Delivered Invited talk at IBY2K, CCMB, Hyderabad, November 24-27, 1997.

**Dr. A. Lohia** Visited the Bernard Nocht Institute, Hamburg, Germany in 1997; Delivered Invited talk at Global Meet on Parasitic Diseases, Hyderabad ; Delivered Invited talk at "Diversity in Modern Biology"

UDSC New Delhi; Delivered Invited talk at the IBY2K symposium at CCMB, Hyderabad. 1997 ; Delivered Invited talk at the Indian society of Cell Biology meeting, Bangalore 1998 ; Delivered Invited talk at the symposium on GI and parasitic diseases, SGPGMS, Lucknow 1998 ; Delivered Talk at Winter school on Humam Genetics at IICB, Calcutta, 1998.

Acting as Member of Governing Body of Manovikas Kendra Rehabilitation and Research Institute, Calcutta ; Life Member of Association for the Promotion of DNA Fingerprinting, and other DNA technologies (ADNAT) ; Member of Editorial Board of Journal of Biosciences, Indian Academy of Sciences.

**Dr. R. Chattopadhyaya** Attended Project Monitoring Workshop organized by CSIR New Delhi in July, 1997 ; Edited a book titled "Biochemistry at Bose Institute" published on the occasion of the Silver Jubilee celebrations of the Department.

**Dr. P. Parrack** Taught X-ray crystallography to M.Sc. part II students of the Dept. of Biophysics, Kalyani University, Kalyani, W.B. and acted as a paper setter and examiner for the same ; Attended the meeting "Transcription Assembly" organised at the Saha Institute of Nuclear Physics, Calcutta on December 27-28, 1997 and gave an invited talk entitled "Identification and Characterization of a Tight Binding site for RNA Polymerase in E.coli gal operon" ; Dr. P. Parrack, Mr. J. Mukhopadhyay, Ms. R. Sur and Mr. A.B. Datta attended the "International Symposium on Genes, Genomics & Development" at the Indian Institute of Science, Bangalore on March 19-20, 1998.

Suranjana Guha a Ph.D. student of Prof. B. Bhattacharyya received following awards :

- 1) *Sir Nilratan Sarkar Award in 1997 from the Bose Institute.*
- 2) *Indian National Sciences Academy Young Scientist Award on Biological Science in 1998.*
- 3) *Humboldt Fellowship.*

## RESEARCH SCIENTISTS/RESEARCH ASSOCIATES/POST-DOCTORAL FELLOWS/ SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS

Dr. B. K. Padhi, Dr. Sk. Abdus Samad, Ms. Kajari Ghosh, Dr. Ruma Das, Dr. Brajadulal Chattopadhyay, Dr. Chaiti Ganguli, Dr. Dipanwita Debnath, Ms. Rinku Ghosh, Ms. Madhumita Basu, Ms. Suranjana Guha, Ms. Lopa Adhikari, Mr. Samarpan Majumdar, Mr. Samudra S. Gangopadhyay, Ms. Pampi Basu, Ms. Pallab Kundu, Ms. Surekha Mandal, Mr. V.M.H. Namboodri, Ms. Runa Sur, Ms. Anasuya Ganguly, Mr. Ajoy Koomer Mr. Tapas Manna, Ms. Sujata Hajra, Ms. Kamala Neogie, Ms. Sulagna Ghatak, Dr. Mita Sen, Mr. Niladri S. Kar, Dr. Amitabha Chakrabarti, Dr. Jayashri Basak, Dr. Nirajan Das, Mr. Banabehari Giri, Dr. Bhaswati Goswami, Dr. Pradip K. Mahapatra, Ms. Suroopa Banerjee, Ms. Shalmali Chakraborty, Ms. Prajna Mandal, Mr. Kausik Ghosh, Mr. Kaustav Sanyal, Mr. Jayanta Mukherjee, Ms. Chandrani Chattopadhyay, Ms. Indrani Datta, Ms. Atasi Poddar, Mr. Santanu Kr. Ghosh, Mr. Shankar Bhattacharyya, Mr. Taradas Sarkar, Mr. Uttamkumar Samanta, Mr. Debnath Pal, Mr. Ajit Bikram Datta, Mr. Rajat Bhattacharyya, Ms. Suchismita Das,

## SUPPORTING STAFF MEMBERS

Mr. Sukumar Saha, Mr. Subhash Debroy, Mr. Durga Charan Das, Mr. Swapan Kr. Das, Mr. Asim Kr. Banerjee, Mr. Narayan Ch. Datta, Mr. Susanta Kr. Das, Mr. Subhash Chakraborty, Mr. Asim Poddar, Ms. Debarati Kanjilal, Sri Durjyodhan Roy, Sri Biswanath Bez, Md. Asraf Ali Molla, Sri Sudhangsu S. Maiti, Sri Basudeb Ghorui, Sri Dulal Ch. Mandal, Ms. Ranubala Das, Sri Ranjit Das,

## Biophysics

**Faculty :** Prof. A.K. Banerjee (Chairman), Dr. S. Roy, Dr. G.Basu

### HIGHLIGHTS

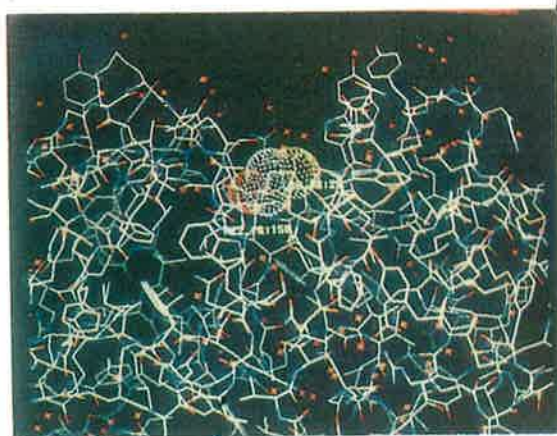
The crystallographic structure of Calotropin DII has been solved by molecular replacement method using Papain as phasing model. We have also cocrystallized a series of small model complexes of amino acids and nucleotides. Biophysical studies have led to the elucidation of protein-ANS/bis-ANS interaction. Difference in cognate/noncognate tRNA-tRNA Synthetase binding was attributed to a conformational change. Studies on inducer-repressor (Gel)-operator complex revealed subtle allosteric control of antirepression by inducer. Nature of Structural change involved in the phosphorylation of P protein of CHP virus was identified. A theoretical model of protein electron transfer was developed with a successful application to Ru-Cyt-c.

### INSTITUTIONAL PROJECT III

**A.1. Structural elucidation of the catalytic mechanism and interaction at active site of two enzymes :** a) *Studies on Nature's another swiss army knife, Calotropin DII, a cysteine Protease* b) *studies on  $\beta$ -lactam- $\beta$ -lactamase interaction :*

Suparna Bhattacharya, Sibani Chakravarty, Asok K. Pal, Sreya Ghosh, Bishnu Mukhopadhyay, **Asok Banerjee**

The resurgence of excitement about proteolytic enzymes has resulted in information explosion and these have importance in medicine and industry. Recently they are implicated in anti cancer and DNA binding activity. We are working for structural A view



of the structure's (Calotropin DII) active site education of a novel cysteine protease. The

crystallographic structure of Calotropin DII has been solved by molecular replacement method using Papain as phasing model. The refinement is in progress with an objective to visualize its substrate specificity, and compare it with papain and other cysteine proteases to rationalize a conclusion. The electron density map is revealing the sequences, and bound waters are being characterized. A series of inhibitor complexes are being attempted.

Another  $\beta$ -lactam analog structure has been solved and complex with the  $\beta$  lactamase macromolecule is being attempted. The characterization of the mode of interaction of this series of  $\beta$ -lactams to  $\beta$ -lactamase is being studied by molecular modelling and docking procedure.

The results are encouraging with prospect of a successful package for receptor based drug design of the antithiol/agents/blockers/thiol-protease inhibitor & potent  $\beta$ -lactam analogues which are important in Medical Science.

**A.2. Visualization of the role of water molecules in the protein-nucleic acid interaction at atomic resolution :**

Asim K. Bera, Suparna Bhattacharya, Sreya Ghosh, B.P. Mukhopadhyay, **Asok Banerjee**

Molecular recognition is a subject of wide spread interest, and protein nucleic acid interactions are of fundamental importance. Their complexation, binding and recognition embrace many aspects of cellular DNA function & genetic expression. The molecular description

of their nature of interaction are emerging recently in several X-ray structural works of trp-repressor/  $\lambda$ -repressor/lac-rep/pec/cro-protein nucleotide complexes. Our group has succeeded to cocrystallize a series of small model complexes of amino acids and nucleotides & has solved the structures of i) a complex of L-serine with two Inosine 5'-monophosphate in highly hydrated form ( $12H_2O$ ) ii) a complex of L-glutamic acid with two Inosine 5'-monophosphate with hydration, iii) a complex of L-glutamine with two Inosine 5'-monophosphate with hydration, iv) a highly hydrated complex of Inosine 5'-monophosphate. A potential model, first of its kind reported, throws some interesting findings which are still to be characterized in macromolecular complexes. Water mediated specific & nonspecific interactions of the partners are found to play a key role in recognition. Other complexes look very similar with inherent disciplines of interaction model. It is unlikely that these structural signatures will not be exploited in biological function by Nature.

**A.3. Modelling & Structural studies of** i) *peptidoglycan - a chemoreceptive constituent of Mycobacterium cell wall and ii) small molecules of biological importance :*

Suparna Bhattacharya, Sibani Chakravarty, Asim Bera, Sreya Ghosh, Bishnu P. Mukhopadhyay, **Asok Banerjee**

i) The cell wall constituents of Mycobacterium are very important since they are prime targets for chemotherapeutic drugs. One major constituent, peptidoglycan has been studied and an interesting pseudo two fold symmetry along glycan chain has been found out. This fits very well with the x-ray diffraction data and our study excludes many proposed hypothetical models and endorses the stereochemically consistent and acceptable model of "Knox and murthy". The kinetic & binding affinity studies of the specific chemical agents/ligands with model peptidoglycan system has clarified source mode of interaction at molecular level. ii) Structural studies of a series of small molecules of biological interest are continuing with a hope to correlate these structures to their functions.

**A.4. Modeling of protein-ANS/bis-ANS interaction**

**Siddhartha Ray**, Anusree Bhattacharya, Durba bandyopadhyay, Asim Bothra, Amit Kumar Mandal, Rajat banerjee, A. Shyamala, Sumita Bandyopadhyay, Sumana Chatterjee, Sunanda Dev

We have shown that fluorescence polarization of both ANS and bis-ANS is excitation wavelength dependent. Bis-ANS excitation maximum is always more red shifted than the corresponding ANS complex.

Calculation of energy migration rate between the two rings suggests that energy migration rate should be high at all values of the naphthyl-naphthyl dihedral angle. Salt and temperature dependence of bis-ANS/protein interaction suggests little ionic interaction and predominant interaction through hydrophobic aromatic rings. We conclude that bis-ANS binds to proteins through interaction with the aromatic rings and with two rings nearly parallel to each other.

Conformational analysis of colchicine and its several important analogs were done. Molecular mechanics studies show that conformational space of these conformation has one low energy conformation. Molecular dynamics of compounds colchicine, is a colchicine and A-C compound show that the value of the dihedral angle C9-C7-C1-C14 (phi), (C7-C1 bond connects the A and C ring), is within 40 degrees to 50 degrees for all the compounds with fluctuations less than 15 degrees. These calculations indicate that there is an overall similarity in the dynamically averaged structure of all the drugs. The A ring and B ring of the compounds are more or less rigid. The C ring is somewhat flexible. The potential difference in binding property of colchicine and isocolchicine may originate from the positional difference of carbonyl oxygen and methoxy group of C ring, which is the only difference in the structures of the two compounds and this has no effect on the motional property and average conformations of these two compounds. From our study it is proposed that the movements occurring at various positions of the drug molecules are significantly correlated and their motion may

play an important role in the biological property of these compounds.

**A.5. Molecular Basis of regulation of gene expression : Aminoacyl-tRNA Synthetases :**

**Siddhartha Ray**

We have shown that the conformational change that occurs upon tRNA(Gln) binding to GlnRS/ATP complex is absent in a noncognate complex. From Van't Hoff plot and CD experiments it was concluded that the cognate tRNA induces a local conformational change in the synthetase that may be one of the critical elements that causes enhanced aminoacylation of the cognate tRNA over the noncognate ones.

A model of the N-terminal half of glutamyl-tRNA synthetase from E. coli was constructed on the basis of similarity in sequence and function of Glutamyl- and Glutaminyl-tRNA synthetases. The glutaminyl-tRNA synthetase does not contain any zinc atom, but glutamyl-tRNA synthetase from E. coli contains one atom of zinc. Molecular modeling is employed to show that the zinc atom is well outside the contact region of the acceptor stem of tRNA. The placement of a zinc atom at a significant distance from the tRNA acceptor stem indicates that the role of zinc is likely to be indirect and structural.

**A.6. Inducer mediated changes in Gal repressor-cognate operator complex :**

**Siddhartha Ray**

Gal repressor inhibits transcription from the gal promoter (PI) when it binds to the cognate operator (O-E). The repression is relieved by the presence of the inducer D-galactose. Compared with its interaction with free repressor, D-galactose, binds to the repressor-operator complex with 11-fold reduced affinity as determined by fluorescence enhancement measurements. Thermodynamic analysis and fluorescence anisotropy showed that the stability of the repressor-operator complex is reduced by only 7-fold by the presence of the inducer in the complex. The formation of the inducer-repressor-operator ternary complex has been confirmed by CD spectral analysis. Fluorescence spectroscopy and energy transfer

experiments suggest that individual allosteric effects of the two ligands, inducer and operator, on Gal repressor are responsible for the slightly weakened stability of the ternary complex compared with the stability of the inducer-repressor and repressor-operator complexes. In vitro transcription results demonstrated full derepression of transcription of the P1 promoter under conditions in which the concentrations of the inducer-repressor binary complex are several fold higher than the dissociation constant of the inducer-repressor-operator ternary complex into inducer-repressor and free DNA.

These results strongly suggest that the inducer binding to the repressor-operator complex does not lead to dissociation of the repressor from the operator during transcription induction. Because Gal repressor inhibits transcription by modulating the alpha subunit of the P1-bound RNA polymerase, we conclude that the inducer binding to the operator-bound repressor only allosterically relieves the inhibitory effect of repressor on RNA polymerase without dissociating the repressor from DNA.

**A.7. Conformational Changes in the P Protein of Chandipura Virus :**

**Siddhartha Ray**

Phosphorylation of P protein of Vesicular Stomatitis Virus as well as Chandipura (CHP) virus is required for transcription activation and replication switch. The structural nature of this crucial conformational change, however, is largely unknown. We have studied the phosphorylation associated conformational change in the P protein of Chandipura (CHP) virus using chemical modification, fluorescence and circular dichroism spectroscopy. The results suggest a global conformational change in the N-terminal domain of the P-protein is induced by phosphorylation and yet the phosphorylated N-terminal domain shows significant flexibility.

**A.8. Studies of Peptide and Protein Structure and Dynamics :**

**Gautam Basu, Manasi Ghose, Lipika Pal and Raja Banerjee**

Protein reorganization energy during electron transfer. In collaboration with Prof. Nobuhiro Go of Kyoto University, we successfully developed a model for the coupling of collective protein motion to electron transfer and subsequently applied this model to the protein cytochrome c. Two papers were communicated and published in J. Phys. Chem.

Studies of peptides and proteins exhibiting the  $3_{10}$ -helical secondary structure. We have completed the first phase of work on documenting all the  $3_{10}$ -helices in known protein structures. Several interesting features characteristic of the  $3_{10}$ -helices have emerged from our study. A comprehensive report is being prepared which will be communicated shortly. Synthesis and spectroscopic studies of a series of peptides containing the amino acid Aib is continuing for establishing a thermodynamic scale for the relative preferences of amino acids between the  $3_{10}$ - and the  $\alpha$ -helical states. Simultaneously, peptide fragments from the protein Lamprey Hemoglobin have been synthesized and NMR / CD studies are ongoing.

**PUBLICATIONS**

1. Gomes, A.C., Pal, A.K., Bera, A.K., Ghatak, K.K., Haldar, U.C., Banerjee, A. Crystal Structure of Trans-9,10-dichlorotricyclo [3.3.2.0<sup>1,5</sup>] decan-2-one, C<sub>10</sub>H<sub>12</sub>Cl<sub>2</sub> O. *Z. Kristallogr. NCS* **212**: 265-267, 1997.
2. Pal, A.K., Bera, A.K., Banerjee, A. Crystal Structure of N-Phenyldichloroacetamide C<sub>8</sub>H<sub>7</sub>NOCl<sub>2</sub>. *Z. Kristallogr. NCS* **213**: 249-251, 1998.
3. Dey, R., Bera, A.K., Pal, A. K., Mukherjee, B.P., Banerjee, A., Crystal Structure of 1,2,3,7-tetramethyl-5, 6-phenanthraquinone, C<sub>18</sub>H<sub>16</sub>O<sub>6</sub>. *Z. Kristallogr. NCS* **213**: 491-493, 1998.
4. Bothra A.K., Roy S, Mandal C, et al. Role of zinc in tRNA-acceptor stem binding by glutamyl-tRNA synthetase from E-coli : A

molecular modeling study *J Biomol. Struct. Dyn.* **15**: 19-25, 1997.

5. Chatterjee S., Zhou Y.N., Roy S., Adhya, S. Interaction of gal repressor with inducer and operator : Induction of gal transcription from repressor-bound DNA. *Proc. Natl. Acad. Sci.* **94**: 2957-2962, 1997.

6. Bothra, A., Bhattacharya, A., Mukhopadhyay, C., Bhattacharyya, A., Roy, S. A fluorescence spectroscopic and molecular dynamics study of bis-ANS/protein interaction *J Biomol. Struct. Dyn.* **15**: 959-966, 1998.

7. Bothra, A.K., Roy, S., Bhattacharyya, B., Mukhopadhyay, C. Molecular dynamics simulation of colchicins *J Biomol. Struct. Dyn.* **15**: 999-1008, 1998.

8. Mandal, A.K. Bhattacharyya, A. Bhattacharyya, S. Bhattacharyya, T., Roy, S.A. cognate tRNA specific conformational change in glutaminyl-tRNA synthetase and its implication for specificity *Protein Sci.* **7**: 1046-1051, 1998.

9. Basu, G., Kitao, A., Kuki, A., & Go, N. Protein Electron Transfer Reorganization Energy Spectrum from Normal Mode Analysis. I. Theory. *J. Phys. Chem. B.* **102** : 2076-2084, 1998.

10. Basu, G., Kitao, A., Kuki, A., & Go, N. Protein Electron Transfer Reorganization Energy Spectrum from Normal Mode Analysis. II. Application to Ru-modified Cytochrome c. *J. Phys. Chem. B.* **102**: 2085-2094, 1998.

**Participation in conferences/symposia/training/programme/Award and honours received.**

**Prof. A. Banerjee** delivered an invited talk on "The Structural view of Nature's another Swiss Army Knife, Calotropin D II, a thiol protease at 2.1 Å resolution" at the Discussion meeting on Macromolecular Crystallography, MBU, Indian Institute of Science, Bangalore, February, 1998.

**Dr. S. Roy** at NIH, USA, in the laboratory of Prof. S. Adhya.

## GRANT-IN-AID SCHEME

| Investigator/<br>Co-investigator | Title                                                                                                                               | Financed by |
|----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|-------------|
| Prof. A. Banerjee                | Studies on model nucleotide-peptide/<br>peptide ligand complex at atomic level<br>by x-ray Crystallography.                         | CSIR        |
| Prof. A. Banerjee                | Structural basis of differences in catalytic<br>mechanism of different Cysteine Proteases,<br>Calotropin DII by X-ray and modelling | DBT         |
| Dr. S. Roy                       | A study of structural and molecular<br>basis of co-operative binding of operator<br>sites of repressor.                             | CSIR        |
| Dr. S. Roy<br>Dr. G. Basu        | Molecular basis of recognition of tRNAs<br>by Aminoacyl-tRNA synthetases                                                            | DST         |
| Dr. G. Basu                      | Theoretical studies of peptide and<br>protein dynamics from a collective<br>motion viewpoint                                        | CSIR        |

RESEARCH SCIENTIST/RESEARCH ASSOCIATES/POST DOCTORAL FELLOWS/  
POOL OFFICERS/SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS

Dr. B.P. Mukhopadhyay, Dr. A. Seal, Dr. A. Shamla, Dr. A. Bhattacharyya, Dr. D. Mukherjee,  
Dr. S. Bandopadhyay, Dr. U. Haldar, S. Dev, A.K. Pal, A.K. Mondal, S. Chatterjee, R. Banerjee,  
M. Ghosh, L. Pal, R. Chattopadhyay, S. Bhattacharya, S. Chakrabarty.

## SUPPORTING STAFF MEMBERS

B. Goswami (upto 12.1.98), B. Marick (from 1.7.97), A. Bhattacharyya, B. Majumder (from  
12.8.97), B. Munshi, S. Jogsharma (from 3.11.97)

## Animal Physiology

Faculty : Prof. A.K.Ray (In-Charge), Dr. R. Ghosh, Dr. G. Sa

## HIGHLIGHTS

Estradiol-17Beta ( $E_2$ ) a vertebrate female sex steroid appears to have potent stimulatory effect on silk gland activity of silkworm, *Bombyx mori* L.  $E_2$  could be effectively used in sericulture for enhancement of qualitative and quantitative aspects of silk production.

Newborn rat neuronal cells have been found to be differentiated by basic fibroblast growth factor (bFGF) through phosphorylation of a 90-93 KD transcription factor (STAT). This phosphorylated transcription factor then homodimerizes and translocates to nucleus where it binds to specific nucleotide sequence (SIE) and activates specific gene transcription.

## INSTITUTIONAL PROJECT I

## A. Environmental Physiology and Hormonal Regulation

A. 1. Improvement of primary culture of fish liver cells :

Rama Ghosh and E.A. Khan

In course of development of the catfish (*Heteropneustes fossilis*) hepatocyte primary culture use of EDTA has been found to be very much potent to isolate substantial number of cells from the fish liver. This finding establishes dispensability of enzyme (collagenase/trypsin) for cell isolation in catfish. We also used matrix prepared from carp skin and catfish skin. Carp skin matrix showed better efficiency for cell attachment than the catfish skin. The fact indicates that no species/ generic barrier exists in matrix between catfish and carp.

A.2. Carbofuran induces the cytochrome P450 1A1 gene in primary culture of fish hepatocytes :

Rama Ghosh, Manik Ghosh and Swades De

Carbofuran is a member of carbamate pesticide used extensively in agriculture. Using catfish (*H. fossilis*) hepatocyte primary culture we have demonstrated an induction of cytochrome P450 mono-oxygenase enzyme activity by carbofuran. Superinduction is a splendid phenomenon which is found in several genes like CYP1A1, c-myc, c-fos, etc. In our recent experiment the CYP1A1 gene has been found to be super induced after administration of the cycloheximide to the pre-induced hepatocytes. In this condition a

super induction as high as 45 % over the control value was found. Such observations will help in development of a bio-monitoring system for aquatic environmental contaminants.

A.3. Production of triploid and unisex fish: Gonadal changes of triploid Indian catfish (*H. fossilis*) during spawning season:

Arun K. Ray and Basant K. Tiwary

Triploidy apparently inhibits gonadal development more in females than in males. Failure of meiosis may prevent oocyte development and spermatogenesis in triploid catfish. Testis of triploid male fish showed regressed lobules with lumen having few developing stages of spermatogonia and surrounding areas filled with connective tissue in spawning season. The female triploid showed, at this season, oocytes significantly smaller in size in comparison to the diploid varieties. In triploid ovary, majority of oocytes failed to reach maturation and eventually turned into atretic follicles. No ripe and vitellogenic stage of oocyte development were observed in triploid ovary.

The study suggests that arrested gonadal development in triploid fish is a key factor for enhanced growth rate. This supports the commercial viability of the triploid catfish in aquaculture.

A. 4. Role of estrogen in the ovarian development in freshwater prawn: Purification of egg yolk precursor protein :

Arun K. Ray and Anuradha Chaudhury

Egg yolk protein precursor vitellogenin, is necessary for oocyte maturation. This protein is

secreted from the hepatopancreatic cells. A primary culture of prawn hepatocytes having four major cell types has already been made. In order to identify which cell type is responsible for vitellogenin secretion efforts have been made to develop immunologic probe.

Vitelin protein obtained from mature prawn ovary was purified and antibody was raised against this protein. Immunologic tests like immunodiffusion, ELISA and Western blot indicated that the hepatocyte cytosol contain a protein having strong cross reactivity with the antibody raised against vitellin. Further efforts are being made to use this probe for identification of the specific type of cells in culture that secretes vitellogenin.

**A. 5. Role of estrogen in the ovarian development in freshwater prawn: Separation of different types of hepatopancreocytes :**

**Arun K. Ray** and Sharmila Chatterjee

The prawn hepatocyte primary culture, that has been developed in our laboratory contains several types of cell. In order to identify the particular cell type responsive to exogenous estrogen attempts have been made to prepare enriched cell fractions from the prawn hepatopancreas for culture. A percoll density gradient cell fractionation gave rise to isolation of four fractions containing one large round shaped cell type and three other medium to small cell enriched fractions. The four different cell type-enriched fractions have been subjected to monoculture and their ability to produce vitellogenin in response to estrogen are being examined.

**A. 6. Thyroid hormone regulation of mammalian brain function :**

**Arun K. Ray** and Nilkanta Chakraborty

In course of our study on the non-genomic action of thyroid hormone we have already demonstrated thyroid hormone-linked inhibition of the  $\text{Na}^+ - \text{K}^+ - \text{ATPase}$  and excitation of the acetylcholinesterase and  $\text{Ca}^{2+} - \text{ATPase}$  activities in synaptosomes of adult rat cerebrocortical preparation.

In successive experiments using depolarised synaptosomal preparation it has been revealed that L-triiodothyronine ( $\text{T}_3$ ) induces dose-dependent entry of  $\text{Ca}^{2+}$  inside the synaptosomal complex.

Such elevated intrasynaptosomal  $\text{Ca}^{2+}$  concentration could be related to localized activation of synaptosomal membrane  $\text{Ca}^{2+}$ -dependent ATPase activity.

**A. 7. Phenylhydrazine-hydrochloride (PHH)-thyroid hormone interaction in rat :**

**Arun K. Ray** and Mitali Pramanik

During the course of our study on the phenylhydrazine hydrochloride (PHH)-induced oxidative stress in juvenile rats we have demonstrated thyroid hormone counteraction of the haematological changes in stressed condition. In our subsequent investigation administration of different doses of PHH showed a dose-dependent fall in serum  $\text{T}_4$  and  $\text{T}_3$  levels by 40 to 70%, except the lowest dose which showed an initial rise in the hormone levels in serum. Histological preparation of thyroid gland of PHH-treated animals showed typical appearance of goitrous structure in comparison to the control animals. Such a dramatic change in the serum levels of thyroid hormone and histological structure of thyroid gland occurred within three days of single injections of different doses of PHH. The results indicate a potential role of PHH as an anti-thyroid agent which remained unknown as yet.

**B. Control of growth and development of silkworm and silk production**

**B. 1. Anabolic effect of estradiol- $17\beta$  on silk gland :**

**Arun K. Ray** and Bela Keshan

Administration of estradiol- $17\beta$  ( $\text{E}_2$ ) to 5th instar larvae of silkworm *Bombyx mori* L., effected an increase in nucleic acid and fibroin contents of silk gland in comparison to the untreated control insects. In our earlier studies indigenous appearance of  $\text{E}_2$  in serum and many metabolic effects of this hormone in the silk worm larvae have been demonstrated. Usually the question arises whether  $\text{E}_2$  plays an indigenous hormonal role in these insects. To get an answer we made an attempt to identify specific  $\text{E}_2$  receptors in silk gland of fifth instar larvae. It has been found that the cytosol obtained from posterior silk gland of different days of fifth instar larvae possess different binding capacity for estradiol. The pattern of alteration of  $\text{E}_2$  binding sites in posterior silk gland had also a

correlation with the  $\text{E}_2$  concentration of cytosol of the posterior silk gland. The binding of  $\text{E}_2$  with its specific receptors could not be displaced with ecdysone. The study favours much for a hormonal role of  $\text{E}_2$  in the silkworm, *Bombyx mori*.

**C. Cell signalling**

**C. 1. Endothelin stimulates sis-inducing factor-DNA binding activity :**

**Gaurisankar Sa** and Tanya Das (Immunotechnology Section)

In contrast to our knowledge of signal transduction pathway mediating cell proliferation, little is known about the pathways of nuclear signalling responsible for ET-induced cellular differentiation. We have demonstrated that ET-1 elicits a distinct signalling mechanism by which it mediates neonatal rat astrocyte differentiation. The initial event is ligand-induced ET receptor aggregation which may involve single or multiple receptor subtypes. Receptor aggregation concomitantly activates JAK-1 (Janus Kinase-1, a nonreceptor tyrosine kinase) through tyrosine phosphorylation. Phosphorylated and activated JAK-1 then tyrosine phosphorylates  $\text{p}^{91}\text{Stat}$  (signal transducer and activator of transcription) protein. The tyrosine phosphorylated  $\text{p}^{91}\text{Stat}$  is then homodimerizes forming a sis-inducible factor (SIF) and translocates to nucleus. The SIF is then additionally phosphorylated at the serine residue by p42 MAP kinase activated by ET through other signalling mechanism and binds to sis-inducible element (SIF), which alone is sufficient, under certain circumstances to mediate ET-induced transcriptional activation of differentiation specific genes.

**C. 2. Basic fibroblast growth factor-stimulates cytosolic phospholipase  $A_2$  phospholipase C- $\gamma 1$  and phospholipase D through distinguishable signalling mechanisms :**

**Gaurisankar Sa** and Tanya Das (Immunotechnology Section)

Fibroblast growth factors (FGFs) stimulates proliferation, differentiation and motility of different cell types. The cellular effects of FGF are transduced by its interaction with any one of four members of a family of high affinity, cell surface FGF receptors (FGFRs) that have autophosphorylating tyrosine kinase activity.

Activation of FGFR causes release of various low molecular weight signalling molecules which are required for the pleiotropic effects of FGFs. We report here that basic FGF plays critical role in membrane phospholipid hydrolysis in NIH 3T3 cells that are stably transfected with FGFR1. Basic FGF, upon binding to FGFR1, stimulates cytosolic form of phospholipase A2 (cPLA2), phospholipase C- $\gamma 1$  (PLC- $\gamma 1$ ) and phospholipase D (PLD), the key enzyme of various lipid second messengers, in a tyrosine kinase-dependent manner. In addition to tyrosine phosphorylation cPLA2 catalytic activation requires serine phosphorylation by p42 mitogen-activated protein (MAP) kinase and possibly pertussis toxin-sensitive G-protein coupling. On the other hand, phosphatidyl inositol 4,5 bisphosphate ( $\text{PIP}_2$ ) hydrolysis requires direct phosphorylation at tyrosine residue of the PLC- $\gamma 1$  isozyme. The activation of PLD needs direct or indirect receptor tyrosine kinase and protein kinase C (PKC) activities. Additionally it also requires botulinum toxin C-sensitive Rho-like G-protein activation. All these results suggest that the pleiotropic effects of FGF are exerted through its tyrosine kinase receptors and individual effectors are activated via distinguishable signalling mechanisms according to the cells, need.

**C. 3. Intracellular signalling mechanisms in fibroblast growth factor-mediated differentiation-specific gene expression in rat neuronal cells :**

**Gaurisankar Sa**, Suman Pal and Tanya Das (Immunotechnology Section)

Although the basic fibroblast growth factor (bFGF) has been found to stimulate proliferation and differentiation of mammalian cells we do not know the molecular mechanisms by which bFGF induced transcriptional activation of differentiation-specific gene expression in newborn rat neuronal cells. It has been shown that unlike hepatic cells rat newborn neuronal cells differentiates as a result of basic FGF stimulation. Therefore, these cells were used to deduce the bFGF-mediated signalling mechanisms for cellular differentiation. It has been shown that upon receptor activation bFGF stimulates tyrosine phosphorylation (through receptor or non-receptor tyrosine kinase) of 90-93 KDs transcription factor (STAT). This phosphorylated transcription factor then

homodimerizes and translocates to nucleus where it binds to specific nucleotide sequence (SIE) and activates specific gene transcription.

### PUBLICATIONS

1. E.A. Khan, A.K. Dasmahapatra and Rama Ghosh: Evaluation of EDTA and fish skin extract in primary culture of fish liver cells. *Methods in Cell Science* **19**, 153-159, 1997.
2. M. Sen, S. Chatterjee, A.K. Dasmahapatra and Rama Ghosh: Effect of toxic stress by carbofuran in a carp fish. *Proc. Zool. Soc. Calcutta* **50**, 40-43, 1997.
3. B.K. Tiwary, R. Kirubakaran and A.K. Ray: Introduction of triploidy by cold shock in Indian Catfish, *Heteropneustes fossilis* (Bloch). *Asian Fisheries Science* **10**, 123-129, 1997.
4. S. Das and A.K. Ray: Silkworm ovary- A target organ for estrogen. *Proc. Zool. Soc. Calcutta* **50**, 34-39, 1997.
5. A.K. Ray and P.K. Sarkar: The action of thyroid hormone in adult mammalian neuron: Synaptosome as a novel site. In "Frontiers in Environment and Metabolic Endocrinology", Ed. S.K. Maitra, pp. 229-239, The University of Burdwan, India, 1997.
6. Prasanta K. Ray, Shradha Goenka, Tanya Das, Gaurisankar Sa, Pratima Sinha and Manda Srivastava: Role of nitric oxide in immune function and amelioration of toxicity and carcinogenicity of drugs and

chemicals. In "Biological oxidants and anti-oxidants: Molecular mechanisms and health effects", Ed. T. Gitler, pp. 42-53, AOCS Press, USA, 1998.

### Participation in Conferences/Symposia/ Training Programmes/Awards and Honours received.

**Prof. Arun K. Ray** delivered Course of Lecture in Reproductive physiology in Tripura University, Agartala, in November, 1997, delivered invited lecture in the last session of Indian Science Congress held in January, 1998, at Hyderabad.

**Dr. Gaurisankar Sa** made several deliberations in Society of Biological Chemists Symposium on Biochemistry and Molecular Biology of Health and Diseases, India, 1997; 24th Annual Conference of Indian Immunology Society, India, 1997; International Conference on stress Adaptation, Prophylaxis and Treatment, India, 1998, Dr. Sa also acted as Instructor in the workshop on Flow-cytometry at Bose Institute, 1997, and attended another workshop on flow-cytometry at Bose Institute, in 1998. Dr. Sa acted as Instructor in the Refreshers Course in Zoology, Academic Staff College, University of Calcutta in 1998. Mr. Indrajit Ray, Mrs. Anuradha Chaudhury, Ms. Sharmila Chatterjee, Ms. Bela Keshan and Mr. Manik Ghosh attended Workshop on Information Superhighway held at DIC, Bose Institute, in November, 1997. Mr. Indrajit Ray also participated in Computer Programming Course held at DIC, Bose Institute, in May-June, 1997.

### GRANT-IN-AID-SCHEMES

| Investigator/<br>Co-Investigator | Title                                                                                                         | Financed by |
|----------------------------------|---------------------------------------------------------------------------------------------------------------|-------------|
| Dr. G. Sa                        | Intracellular signalling mechanisms that mediate endotheleing-induced transcription of immediate early genes. | DST         |
| Dr. G. Sa                        | Intracellular singalling mechanisms of basic fibroblast growth factor-stimulated early gene transcription.    | CSIR        |

### RESEARCH SCIENTISTS/RESEARCH ASSOCITES/POOL OFFICERS/POST DOCTORAL FELLOWS/SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS.

Dr. Anuradha Chaudhury, Dr. Swades De (upto 29.8.97), Sharmila Chatterjee (from 1.4.97) Mr. Basant K. Tiwary (upto 29.8.97), Ms. Bela Keshan (upto 07.10.97), Mr. Nilkanta Chakraborty (upto 23.4.97), Mr. Manik Ch. Ghosh, Mr. Suman Pal (from 23.10.97), Mr. Tathagata Chaudhury (from 02.2.98).

### SUPPORTING STAFF MEMBERS

Dr. A.K. Dasmahapatra, I. Ray, S.K. Ghosh, A.K. Santra and N.C. Jana.

# Plant Molecular & Cellular Genetics

**Faculty :** Prof. A. N. Lahiri Majumder (Scientist-in-Charge), Dr. A. Pal, Dr. S. Das, Dr. S. R. Sikdar.

## HIGHLIGHTS

*Allium* lectin ASA II as insecticidal agent has been analysed with an aim to exploit it as a component of Integrated Pest Management policy. Four stable somatic hybrid lines have been developed with more *Brassica juncea* genome component focusing on Cytoplasmic Male Sterility (CMS), *Alternaria* blight resistance, drought tolerance and low glucosinolate content trait from other fusion partners. Stable *Brassica* transgenics (R<sub>2</sub> progeny) with GUS + NPT II gene.

## INSTITUTIONAL PROJECT I

### A.1. Genetics of Mungbean Yellow Mosaic Virus (MYMV) resistance :

Amitabha Roy and **Amita Pal**

Hybrids (F1) were obtained from the crosses between *Vigna mungo*, cv.T9, the susceptible line, with three resistant lines VM-1, VM-4 and VM-7. The genetic characterization of these hybrids are underway. The seeds of the F1 progeny are sown in the field for selfing.

### A.2. Genetic polymorphism between MYMV susceptible and resistant lines :

Amitabha Roy and **Amita Pal**

Genetic polymorphism was identified between MYMV susceptible and resistant lines using PCR technique. There is a negative co-relation with the number of bands appeared with a primer (5'GTG-GAT-CCA-GCC-GAT-GT-3') with the seed yield (level of tolerance). These polymorphic marker /s would be very useful in screening the progeny lines for MYMV susceptible/ resistant traits.

### B . Generation of insect resistant plant with potential lectin gene :

Jayasree Banerjee, Anita Roy and **Sampa Das**,

Different lectin proteins have been isolated & characterised from plant sources of Alliaceae, Amaryllidaceae and Leguminosae.

After proper identification, lectins have been purified through affinity column. The aim of this project is :

i) To monitor the efficacy of lectins against target insects

ii) To isolate, clone and express the effective lectin gene into target crop to protect against targeted insect.

The purified lectin have been characterised through agglutination assay with rabbit erythrocytes. The molecular weight have been determined through SDS-PAGE analyses.

The effectivity of the lectins have been determined through insect assay with a sucking type of homopteran insect, Red Cotton bug, both in artificial diet and antifeedant diet (leaf immersed in toxin solution). In both the cases 10 µM concentration of lectin was found to be optimal for insect mortality.

Additionally, insect assay have been carried out with mustard aphid, our target insect in the month of January and February, 1998 in laboratory condition feeding artificial diet supplemented with different doses of *Allium* lectin.

In a parallel approach lectin gene has been isolated through PCR from the *Allium* genome and cloned for its further use in plant transformation.

### C. Understanding of male sterility and transgenic approach for generating nuclear male sterile lines :

Sanchita Roychowdhury, S. Rajlakshmi and **Sampa Das**

Generation of male sterile lines in *Brassica juncea* through genetic engineering have been taken as challenge to produce hybrid seeds which otherwise entertain self pollination. To generate male sterile *Brassica juncea* lines, transgenic plants have been developed with a chimeric constructs of barnase, a killar gene under the control of TA

## Annual Report

29, a tapetum specific promoter. The southern analyses proved the stable integration of barnase gene in *Brassica* species, although it was unable to generate male sterile line may be due to the fact that the rearrangement of the transgene failed to express temporally or spatially to interfere the pollen development.

In a parallel approach, transgenic *Brassica juncea* lines have been generated with barstar (an inhibitor of barnase) driven by TA 29 promoter to restore male fertility. Stability of the transgenes have been analysed through checking the segregating population.

### D. Genetic manipulation of Oil-yielding *Brassica* species

Soma Paul and **S. R. Sikdar**

Backcrossing was carried out between *Brassica juncea* CMS line (raised through somatic hybridisation between *B. juncea* cv. B-85 and *B. oleracea* ssp. *botrytis* Ogura CMS line) and different cultivars of *B. juncea* in search for maintainer and restorer line with an aim to utilise the CMS lines in future hybrid seed programme.

Selfing was carried out for the two consecutive years of the backcrossed progeny line (BC1) of the somatic hybrid between *Trachystoma aphanoneurum* and *Brassica juncea*. The progenies were stable and the seed set was normal. The next progeny will be tested for the *Alternaria* blight tolerant property.

The selfed progeny derived from backcrossed progeny (BC1) of somatic hybrid raised between *B. juncea* and *Diplotaxis harra* showed an excellent property i.e., unusually long root (up to 80-100 cm in length) which indicates strongly a drought tolerant property. The progeny showed vigorous vegetative growth and the seed set was normal.

Somatic hybrid lines developed through protoplast fusion between *B. juncea* cv. B-85 and a low glucosinolate containing wild Canadian variety of the same species was maintaining through selfing for the further utilisation of those lines in developing stable mustard cultivar, containing low glucosinolate.

Laboratory protocol has been established for transgenic plant production in *Brassica juncea* cv. B-85, a common cultivar grown in West Bengal

through *Agrobacterium*-mediated gene transfer using nptII as marker gene and GUS as reporter gene (both are driven by CaMV 35S promoter). On selfing, in R1 progeny both the genes showed Mendelian inheritance pattern and cosegregation of both the genes. The R2 progeny analysis confirmed that the homozygous and heterozygous condition of the transgene (nptII) in R1 progeny.

### Other than Institutional Projects

#### A. Biochemical analysis of two cotyledons of *Vigna radiata* :

Shamik Das and **Amita Pal** (in collaboration with **Dibeyendu Sengupta**)

Differential *in vitro* behaviour of the two cotyledons of seeds of 9 different cultivars of *Vigna radiata* were earlier established. In order to understand the biochemical and molecular basis for such differential behaviour the present investigation was undertaken. Quantitative and qualitative analyses of the cot and cot(e) explants revealed that the latter has significantly higher amount of soluble protein and more number of polypeptide bands in comparison to the former.

During shoot regeneration the protein content declines steadily in cot(e) explants, while in cot explants there is an initial increase and then decreases drastically, when both the explants were grown together. Further experiments are underway to obtain complete information.

In another series of experiments it was shown that when both the explants were grown together in concentric rings (central and peripheral) in a petriplate, cultural response of one mimics that of the other depending on the number of explants. This finding suggests that migration of some 'metabolite' or 'factor' governed by the nutrient pool towards the explant type, which mimics the response of other type of explant. The direction of the nutrient pool which in turn depends on the larger number of explants. These findings were confirmed by further experiments.

#### B. Micropropagation of Bamboo :

Pinaki Chowdhury, **Samir Sikdar** and **Amita Pal**

Methods are established for *in vitro* shoot development from the mature explants of field grown *Dendrocalamus strictus* and *Bambusa tulda*.

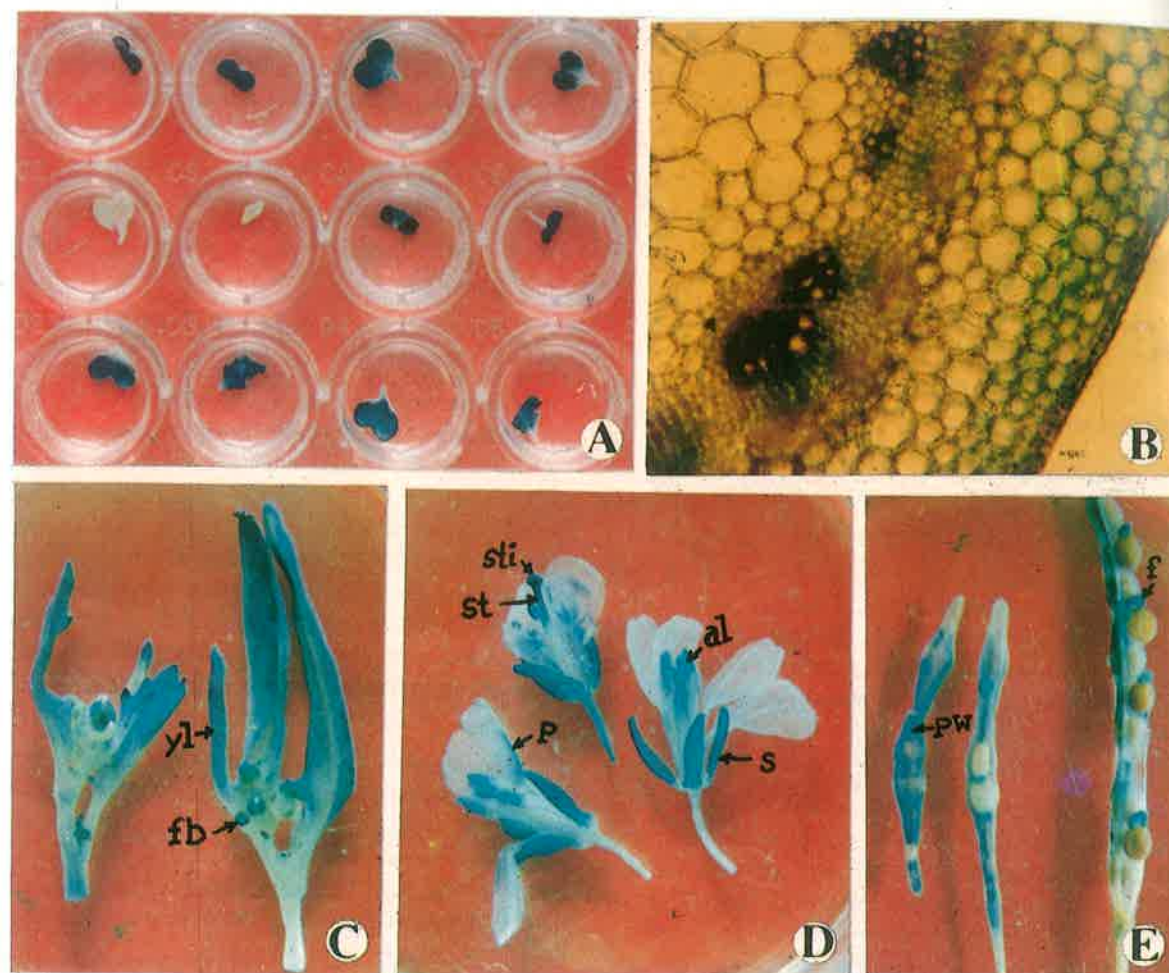


Fig. Histochemical assay for presence of GUS gene in R1 Progeny of transgenic *Brassica juncea* cv. B-85 transformed with GUS and NPT II gene (driven by CMV 35S promoter) through *Agrobacterium* mediated gene transfer : A) Segregation of GUS gene evidenced by blue colouration of cotyledonary leaves in a small population of R<sub>1</sub> progeny when stained with x-gluc ; B) Transverse section of stem showing GUS gene expression in the epidermal, hypodermal and vascular tissues ; C) Flower buds (fb) and young leaves (yl) of the inflorescence axis showing GUS expression ; D) Flowers showing GUS gene expression in sepals (s), petals (p), anther lobes (al), style (st) and stigma (sti); E) An open pod showing presence of GUS gene expression on the pod wall (pw) and funicles (f).

### Participation in conferences/symposia/training programmes/honours and award received.

Dr. Amita Pal delivered an invited talk on "Production of variable diosgenin yielding embryocloned clones of *Costus speciosus*", in the National Seminar on "Contribution of Women- Scientists in Botanical Research", held at the Visva Bharati University, Santiniketan, on 27-28 March, 1998. She also chaired a session in the said seminar.

Dr. Amita Pal served as Paper setter / Examiner for the M.Sc. Special Paper Examination of Botany, Agartala University.

Dr. Amita Pal has participated in an Hands-on training Workshop on "Use of Flow Cytometry in Biology" held at Bose Institute from 17th -21st December, 1997.

Dr. Sampa Das has attended and presented a poster on "Identification of a new plant lectin and monitoring of its insecticidal potential for its ultimate use in crop protection" at the National Symposium on "Current trends in Plant Physiology and Plant Biochemistry" on 29th - 31st January, 1998 held at University of Hyderabad.

Dr. S.R. Sikdar obtained a US patent on "Plastid transformation in *Arabidopsis thaliana*" along with other inventors of Rutgers University.

### Ph. D. AWARDED

| Name             | University | Year | Title                                                                                                                  | Guide     |
|------------------|------------|------|------------------------------------------------------------------------------------------------------------------------|-----------|
| Monalisa Chandra | Calcutta   | 1997 | Regeneration of shoots and transfer of foreign genes in the cotyledonary tissues of <i>Vigna radiata</i> (L.) Wilczek. | Amita Pal |

### GRANT-IN-AID SCHEMES

| Investigator/<br>Co-Investigator | Title                                                                                                                               | Financed by |
|----------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|-------------|
| Dr. Amita Pal<br>Dr. S.R. Sikdar | Generation and Demonstration of Tissue Culture Raised Planting Material of <i>Dendrocalamus strictus</i> and <i>Bambusa tulda</i> . | DBT/WBSCST  |

### RESEARCH SCIENTISTS/ RESEARCH ASSOCIATES/ POST DOCTORAL FELLOWS / SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS :

Dr. Kritika Bhattacharyya, Dr. Pinaki Choudhury, Sanchita Roy Choudhury, Shamik Das, Palash Mandal, Anita Roy, Jayashree Banerjee, Aniruddha Roychowdhury, Sanjoy Guha Roy,

### SUPPORTING STAFF MEMBERS :

Puspita Mukherjee, Ashim Kumar Nath, Bijoy Kumar Mondal, Motilal Singh, Pravash Basu Roy, Bishnu Pada Pradhan, Ganesh Chandra Dey, Arup Kumar Dey, Sk. Eklas Ali and Subal Basak.

## Immunotechnology Section

Faculty : Prof. P. K. Ray, Dr. Nripendranath Mandal

### HIGHLIGHTS

Recent results from our laboratory have demonstrated that Protein A (PA) can induce selective apoptosis in tumor cells while stimulating immunocyte proliferation. Such knowledge might help in the development of suitable strategy for immunotherapy of cancer.

### INSTITUTIONAL PROJECT III

**A1. Molecular Stimulation Study on Proteolytic Fragments of Protein-A and Protein-G with Respect to Their IgG Binding Capacity :**

**Prasanta K. Ray**, Jayati Sengupta, and Chaitali Mukhopadhyay

Many infectious bacteria, notably *Streptococci* and *Staphylococci* have surface proteins which are able to bind tightly Immunoglobulin-G (IgG) and other host proteins. The best known is Protein-A from *Staphylococcus aureus*. This contains five homologous regions, each contains 58 residues. Two  $\alpha$  helices of the Protein-A domain make extensive contacts with residues at the interface between the CH2 and CH3 domains of Fc portion of IgG. Protein-G is an IgG binding protein associated with the cell walls of *Streptococcus* (different strains). The IgG binding domains of Protein-G are 55 residues long and unlike the functionally equivalent domains of Protein-A, are separated from one another by 15 residues 'linker' sequences. There is no significant sequence homology between the IgG binding domains of Protein-G and those of Protein-A. The secondary structure of Protein-G consists of four strands of  $\beta$  sheets and a single  $\alpha$  helix. Being foreign proteins, it can be assumed that these proteins would be subjected to enzymatic cleavage *in vivo* and small peptide fragments derived would be responsible for inducing their various properties *in vivo*. Wisconsin Sequence Analysis Package was used to get different tryptic fragments of both the IgG binding proteins and Molecular Modeling in conjunction with Molecular Mechanics calculations

have been carried out to search the smallest possible tryptic fragments of these proteins retaining Fc binding activity.

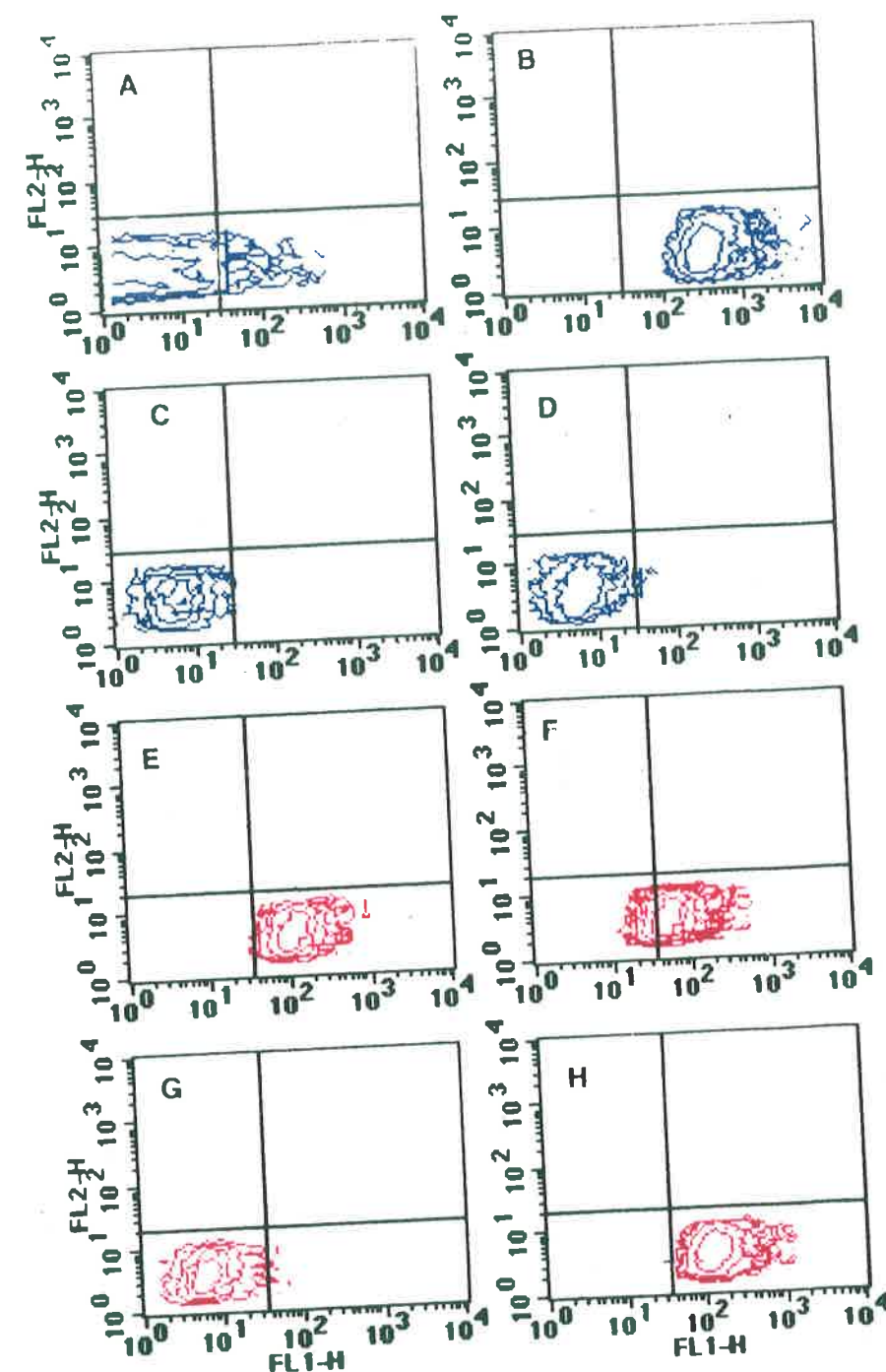
A 16 amino acid (chymotryptic) and 20 amino acid (tryptic) fragments of Protein-A have been already established by us as retaining IgG binding capacity by immunoprecipitation along with capillary electrophoresis.

### INSTITUTIONAL PROJECT V

**A1. Induction of Selective Apoptosis in Ehrlich's Ascites Tumor by Protein A:**

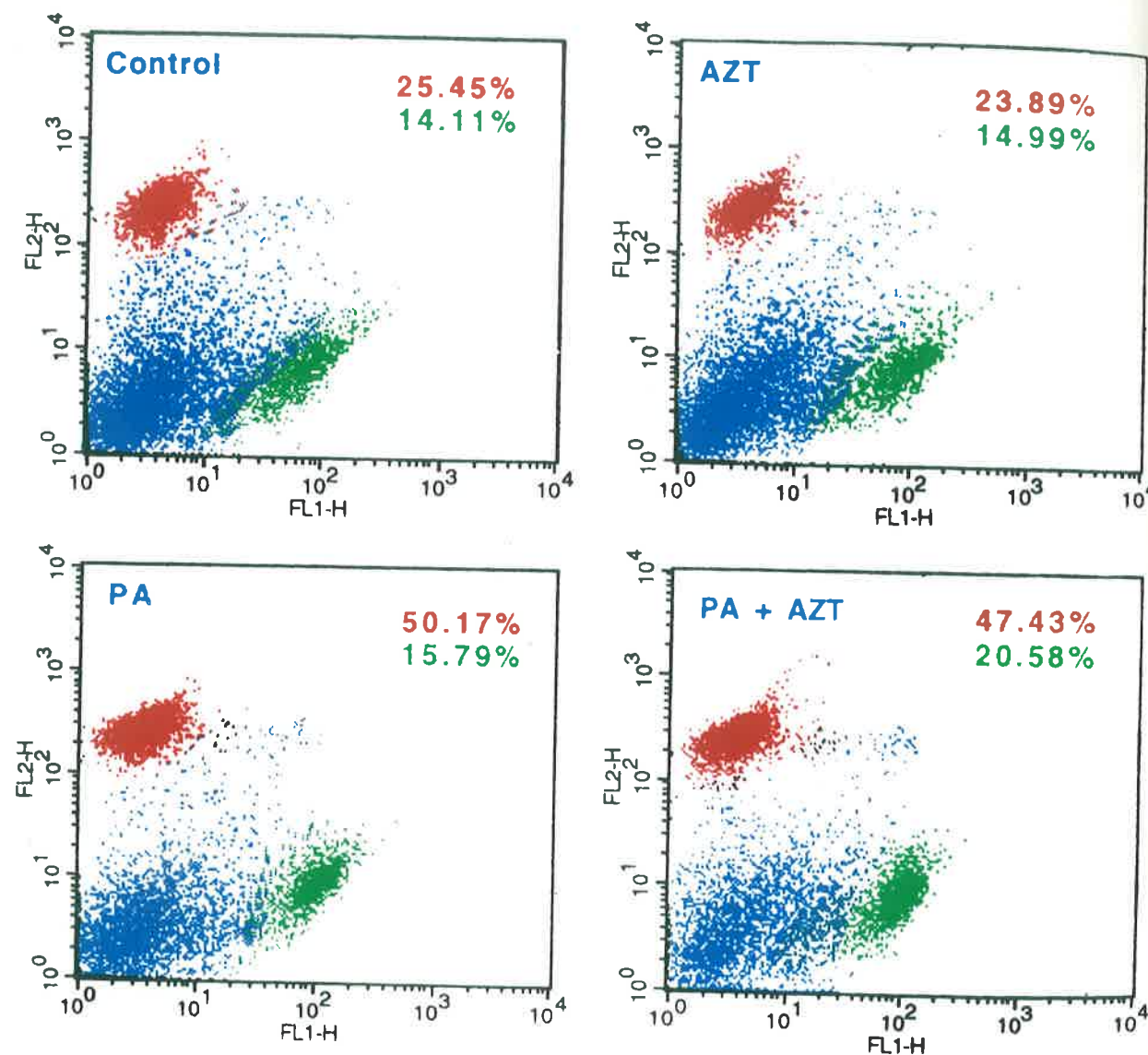
**Prasanta K. Ray**, Tanya Das, and Gaurisankar Sa.

Protein A (PA) of *Staphylococcus aureus*, which has antitumor and immunostimulatory properties, and potentiates the elicitation of interferon- $\gamma$  (IFN- $\gamma$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) by the splenic mononuclear cells, was used successfully to induce apoptosis in Ehrlich's ascites tumor cells (EAC) in Swiss mice, without affecting the splenic mononuclear cells (MNC) of the same tumor bearers. Protein A (1  $\mu$ g/animal, twice a week for 2 weeks) inhibited EAC growth and decreased the mortality of the tumor bearing mice. It appears that there is a cross talk between bcl-2 and p53 gene expressions, if one increases the other decreases or shows no change (Fig.1). Such cross-talks may be a factor determining apoptosis or no apoptosis in any cell. The present observation indicates some novelty in that: a) no bacterial protein so far showed such a property, b) it points out to a causal reverse relationship between bcl-2 and p53 gene



**Fig. 1 Cross-talk between p53 and Bcl-2 expression in determining apoptosis and no apoptosis:** Expressions of p53 (A-D) and Bcl-2 (E-H) were determined Flowcytometrically in Ehrlich's Ascites Tumor Cells (EAC; A, B, E & F) and mice splenic lymphocytes (C, D, G & H). p53 expression was increased in EAC of Protein A-treated mice (A vs. B) but remained unaltered in the splenic cells (C vs. D) of the same host. In contrast, Bcl-2 expression did not change in EAC after Protein A treatment whereas it increased in splenic lymphocytes.

**Fig. 2 Effect of Protein A on AZT-Treated Mice Splenic CD4<sup>+</sup> and CD8<sup>+</sup> Cells**



expressions so far as the induction of apoptosis is concerned, and c) it raises the possibility of developing such an effective therapy of cancer as would not harm the normal cells of the host.

**A2. Involvement of Nitric Oxide in Protein A induced apoptosis:**

**Prasanta K. Ray**, Shradha Goenka, Tanya Das and Gaurisankar Sa

A small non-toxic dose of Protein A, a cell wall protein of *S. aureus*, exhibits a wide array of immunopotentiating properties. Since the role of Nitric oxide (NO) in immunoregulation has been well established, we studied the effect of Protein A on NO production. Our data indicate that PA increases the NO levels in the serum of Swiss albino mice by about 12 fold from its basal level, 12 hours after i.p. inoculation of PA. However, its concentration drops back to the basal value 15 hours post treatment. Splenic lymphocytes and peritoneal macrophages show a significant increase in NO production when cultured with PA, as measured in their culture supernatants. Inhibitors of tyrosine kinase, phospholipase C $\delta$  $\delta$ , and protein kinase C were demonstrated to inhibit NO production in splenic lymphocytes. Thus, it appears that these enzymes participate in the signaling cascade induced by PA, which culminates in the production of NO.

**A3. The Protective Role of Protein-A in Modulating Zidovudine Induced Hematopoietic Toxicity :**

**Prasanta K. Ray**, Amiya K. Ghosh, Tanya Das, and Gaurisankar Sa

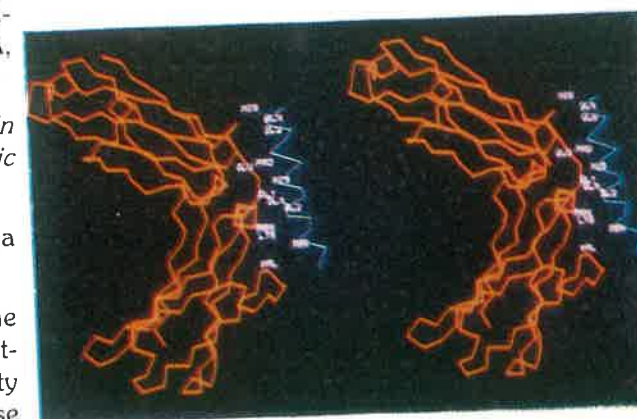
Zidovudine (3'-azido-3'-deoxythymidine) is the FDA approved drug widely used in the treatment of HIV infection because of its ability to act as pseudo substrate for the viral reverse transcriptase (RT). But significant dose-related hematopoietic toxicity has jeopardized the clinical management of AIDS. To ameliorate the toxic effect of AZT, we wanted to examine if Protein A (PA) of *Staphylococcus aureus* could ameliorate the toxicity of AZT. Earlier reports from our laboratory confirmed the antitoxic effect of PA, against toxicity due to different drugs and chemicals. Our results indicated that when

pretreated with PA, AZT could not cause bone marrow malfunction during hematopoiesis in mice. Hematological parameters also showed AZT-induced reduction in RBC, WBC and splenocytes as well as in Hb-content. However when AZT was treated in combination with PA, all hematological parameters showed near normal values. Our flow cytometric data of bone marrow cells showed that a particular population of cell was knocked out severely upon AZT treatment which was replenished by the treatment of PA. From FACS-Scan analysis of splenocytes it has been found that AZT causes slight reduction in the number of CD4<sup>+</sup> cells whereas PA doubles the number of CD4<sup>+</sup> cells (Fig 2). On the other hand PA has significant effect on cytotoxic CD8<sup>+</sup> cells. Further studies are in progress to understand the exact mechanism of PA in alleviating the toxic insults of AZT.

**A4. Functional Studies on Small Peptides of Protein A:**

**Prasanta K. Ray**, Jayati Sengupta and Pratima Sinha.

Since PA is a foreign protein, in the injected animal it may be cleaved into smaller peptide



Stereo view of Ca atom back bones of minimized energy conformation of IgG (Fc:red) complexing with 20 amino acid tryptic fragment (blue). Residues involved in hydrogen bond formation are marked

fragments by the serum proteases. Thus, the whole molecule of PA may not be required for all of its properties. To dissect out the structural entities responsible for one and or the other biological properties, theoretical modeling studies

were done and two peptides were selected (20aa & 16 aa). These peptides were synthesized and their biological properties were checked.

We found that both the peptides have IgG binding capacity (Fig 3) but only 20aa peptide was found biologically active. It increases the serum cytokine level *in vivo* and it showed antitumor effect as was shown by PA. Further investigations are in progress to check the other biological activities with these two peptides.

**A5. Cloning and expression of protein A gene into mammalian system:**

**Nripendranath Mandal**, Srikanta Jana and Prasanta K. Ray.

Protein A is a cell wall protein of *Staphylococcus aureus* Cowan I. It has multifunctional properties such as antitumor, antitoxic, anticarcinogenic, immunomodulatory and mitogenic properties. Many laboratories previously used protein A to remove immune complexes from serum of cancer patients in an attempt to control the disease, it has also been used to ameliorate toxic effects of many drugs or chemicals. Nobody ever tried to use protein A gene as a therapeutic gene to express in mammalian system. Using suitable eukaryotic promoter, specially using an inducible promoter, protein A gene expression study will be conducted. In future this study is being extended to gene therapy in animal models. Apart from obtaining basic information about the gene expression of protein A into mammalian cell line, this study will focuss attention to construct a suitable vector in the field of gene therapy.

At present we have cloned protein A gene into prokaryotic and eukaryotic expression vectors. More experiments are in progress to study the expression of protein A gene.

### PUBLICATIONS

1. Prasanta K. Ray. Protective Role of Stress Genes. *Environment. Health Persp.* **106**, 217-218, 1998.

2. Prasanta K. Ray, Shradha Goenka, Tanya Das, Gaurisankar Sa, Pratima Sinha & Manda Srivastava. Role of nitric oxide in immune function and amelioration of toxicity and carcinogenicity of drugs and chemicals. In *Biological oxidants and anti-oxidants: Molecular mechanisms and health effects*. (Ed. L. Packer) AOCS Press, USA, pp 42-53, 1998.

3. Srivastava, A.K. ; Singh, K.P. and Ray, P.K. Protein A induced protection against experimental candidiasis in mice. *Mycopathologia*, **138** (1), 21-28, 1997.

4. Prasanta K. Ray and Tanya Das. Molecular Adaptation to Toxic Chemicals and Drugs. In *Advances in Organ Biology. Vol. VI* (Ed-Bittar and Das) Jai Inc. Press, USA, vol 6, pp. 251-265, 1998.

### Participation in Conferences/Symposia/ Training Programme/Awards and Honours received

Dr. Tanya Das gave the oral presentations in *24th Annual Conference of Indian Immunology Society*, India, 1997 and *International Conference on Stress Adaptation, Prophylaxis and Treatment*, India, 1998. Dr. Das instructed the workshop on *Flowcytometry* held at Bose Institute 1997 and attended the workshop on *Flowcytometry* held at Bose Institute 1998.

Dr. Pratima Sinha gave the oral presentations in *24th Annual Conference of Indian Immunology Society*, India, 1997 and *International Conference on Stress Adaptation, Prophylaxis and Treatment*, India, 1998. Dr. Sinha participated in the workshop on *Flowcytometry* held at Bose Institute 1997.

Amiya Kr. Ghosh presented a poster in the 24th Annual Conference of Indian Immunology Society and symposium on Immunomodulation in Health and Disease in 1997. He participated in forth seminar on *Biochemistry and Biophysics* organized by West Bengal Academy of Science and Technology.

### GRANTS-IN-AID SCHEMES

| Investigator/<br>Co-investigator | Title                                                                               | Financed by |
|----------------------------------|-------------------------------------------------------------------------------------|-------------|
| Prof. P.K. Ray                   | Development of an Enzyme Linked Immunosorbent Assay for organo-chlorine pesticides. | DBT         |

### RESEARCH SCIENTISTS/RESEARCH ASSOCIATES/POST-DOCTORAL FELLOWS/SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS

Dr. Tanya Das, Dr. Pratima Sinha, Dr. Jayati Sengupta, Shradha Goenka, Amiya Ghosh, Srikanta Jana

### SUPPORTING STAFF MEMBERS

Rina Das, Uttam Kr. Ghosh, Tapan Mandal, Amartya Sen, Ranjit Das

## Environmental Sciences Section

Faculty : Prof. P.K. Ray

### HIGHLIGHTS

Isolated organochlorine pesticide degradable customized bacterium BI-100 and BI-102 and patented. Given expert consultancy 60 multi-nationals and Govt. organisations in National level with respect to control of Air and Water pollution.

### INSTITUTIONAL PROJECT IV

**A.1 Development of arsenic selective synthetic/natural inorganic components for the decontamination of arsenic from drinking water:**

**P.K. Ray, D.P. Modak, Ashis Dalal and Amiya K. Maiti**

Since last decade, West Bengal has been suffering from arsenic pollution in drinking water, using oxides of various metals attempt have been made to absorb the As (III) from water. We have seen that the mixed oxides are more efficient to act as a scavenger than the individual oxides with kd values for arsenic  $5.0 \times 10^4$ . The IR, TGA, DTGA and other structural analysis are initiated. A pilot scale study showed that the filter can efficiently remove arsenic in a continuous dosing of 0.3ppm of arsenic.

**A.2 Microbial degradation of organochlorine pesticides from food products:**

**P.K. Ray, N.C. Mandal, P.K. Chakraborty, P. Bhattacharyya, D.P. Modak, J. Datta, Amiya K. Maiti, and Sujit Ghosh**

The organochlorine pesticides are known to be very toxic and appreciable amount is found in food material. We have undertaken a study to reduce the level of contaminated HCH in various food and agricultural crops by using encapsulated microbes. Highly efficient bacterial strains have been isolated by enrichment culture techniques. The test organism grows efficiently on different isomers of HCH as a sole source of carbon. The bacteria is capable of degrading  $\gamma$ -HCH up to a level of 1500 mg/L in mineral salt medium. The complete degradation of  $\gamma$ -HCH was observed at 72 hrs.

Metabolites have been characterized by GC-MS, HPLC and NMR. Isolation and purification of various enzymes involved in the degradative pathways are being worked out. Metabolites formed during degradational  $\gamma$ -1,3,4,5,6-penta chlorocyclohexene, 1,4,5,6-tetrachlorocyclohexene, 1,5,6-trichlorocyclohexa-1,3-diene, 2-chlorophenol, phenol and catechol. Based on the nature of the metabolites identified, a probable pathway has been proposed for the degradation of  $\gamma$ -HCH.

On the other hand we have also engaged in isolation of specific gene responsible for degradation and it has been found that in one bacterial strain (Sg 10) the gene(s) are located in chromosomal portion, instead of plasmid. As a result, subsequent primer will be synthesized and study would be designed for isolation of specific genes from studied bacteria. Chromosomal insertional mutagenesis study also are in progress. Conjugation with S.17.1 (pSvP 5011) *E. coli* strain having Tn 5; Neo<sup>r</sup> found to be unsuccessful as Sg 10 have neomycin resistance. So we are trying it with Tn 10; Tet<sup>r</sup>.

**A.3. Search of immunomodulatory compounds from exotic Indian plants:**

**P.K. Ray, M. Chakraborty, P. Bhattacharyya, P.K. Debnath, D.P. Modak, Goutam K. Datta and J. Datta**

The herbal product has been known for an effective immuno modulator in Ayurved. But the details scientific investigation is still lacking. Most important Indian Plants used to be as potent drugs such as Amlaki (*Emblica officinalis*), Haritaki (*Terminalia chebula*), Mandukaparni

(*Hydrocotyle asiatica*), Nagbala (*Sida vernicaefolia*), Pipul (*Piper nigrum*), Vaca (*Acarus calamus*), etc. Recently some small peptide of microbial origin has also been known as a potent immunomodulator. Some synthetic Tetra, Octa, and Nano Peptides are also been investigated from our laboratory for its tendency towards the immunomodulatory effect. Besides IL-1, IL-2, IL-6, IL-8, Gammaglobulin, Phospholipid etc. have an immunostimulatory or immuno suppressing effect.

Most of these products are isolated either from organism or cell culture and for the therapeutic uses in large quantities for extraction and isolation is very limited.

Nature created many exotic plants which synthesise varieties of unknown bio-active compounds. And we can use the nature gift for isolation and characterization of these compounds as an immunomodulator. This will work in one hand as a prophylaxis for many diseases and on the other hand the plant materials will be economically viable for the large rural masses.

In this study, we have isolated various chemical constituents from *Desmotrichum fimbriatum* to study (i) anti microbial activities (ii) immuno potentiary effect and (iii) tumor necrosis. We have observed antibacterial activity of the alconor extract part against *E. coli*, *B. subtilis*, *S. aureus*, *S. lutea*, *Myobacterium smegmatis*, *Vibrio cholerae* and antifungal activity against *Aspergillus niger*.

Besides, at a dose of 25 mg/kg body weight through oral route improve the immunological status of the tested animals and splenic cell count increases from  $12 \times 10^8$  count in normal murine to  $20 \times 10^8$  in drug treated murine. The experiment on Ehrlich's ascites tumor bearing mice, the alcoholic extract of drugs at a dose of 25 mg/kg in alternate day, treatment showed remarkable decrease in tumor cell count than the normal. This observation is further supported by DNA cell cycle phase distribution against control. The other studies are in progress.

**A.4. Development of an immunotherapeutic approach to control aflatoxin mediated death in poultry :**

**P.K. Ray, P. Bhattacharyya, D.P. Modak, D. Ghosal and S. Bid**

In India, rapid development of poultry industry has given rise to a huge demand of poultry feed. Improper storage facility, use of low grade oil seeds and warm/humid climate facilitate growth of several toxin producing fungi and other microbes in poultry feed. Birds are dying in poultry farms in thousands because of a feed contaminant called 'Aflatoxin' (AF). This is resulting in huge economic losses in poultry industry in our country which is also considered to be a cottage industry. Aflatoxin is a potent hepatocarcinogenic mycotoxin produced by *Aspergillus flavus* group of fungi on food and feed. Besides, it is reported that AF exposure causes decrease in production of eggs in birds. It seems that AF is posing a multipronged threat to poultry industry.

We have investigated immunomodulatory properties of protein A in chick (normal and aflatoxin intoxicated) for three batches of chick at Ashok Nagar, Howrah and 3 batches in lab condition.

We have observed that the growth of aflatoxin B treated chick, decreases remarkably than the normal birds and after protein A treatment  $1 \mu\text{g} / 45 \mu\text{l}$  showed an excellent recovery of growth rate which is comparable to the normal (control) birds.

**A.5. Development of a database on pesticides :**

**P.K. Ray, D.P. Modak and Jayati Sengupta**

With the advent of 'green revolution', an elevated level of different pesticides are entering constantly into different environmental compartment in many ways. We have developed a database covering all aspects related pesticides especially focusing attention on food contamination and toxicity.

Data were collected (for 145 Pesticides) mainly through Database (Agricola, Toxline Plus etc.) search. Data base is made using FOXBASE package. Tentative name - DATAPEST. It is menu-driven and user's familiar. It will be made available in CD drive, and if possible through internet also.

**A.6. Societal Programme :**

Besides, Environmental Science Section has been carried out consultancy work with respect to Quality control, Monitoring and control of Environmental Pollution in local and national level. Our section has also given Scientific and Technical Expertise to twenty nine Iron Foundries at Howrah for opening their industry which were carried out as per order of the Hon'ble Supreme Court. We have also characterised and evaluated the possible hazard analysis at Das Nagar, Howrah Gas Tragedy Site as per order of the Hon'ble High Court.

Through this consultancy jobs, the section

has earned Rs 8.0 lakhs (approx) in this year, and also Rs 17.00 lakhs through different funded Projects.

**PUBLICATION**

1. D.P. Modak, Study on up take of different heavy metals at sites in Ganga river water. *Indian Biologist*, **30**, No. 2, 1998.

2. Datta, J., S. Ghosh, A.K. Mati, S.K. Das, D.P. Modak, P. Bhattacharyya, A. Ghosh, N.C. Mandal, P.K. Chakrabarty and P.K. Ray Biodegradation of organochlorine Pesticides by soil bacteria. *J. Sci. Ind. Res.* p-380, 1998

**GRANTS-IN-AID SCHEME**

| Investigator/<br>Co-Investigator | Title                                                                                                                                            | Financed by                   |
|----------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------|
| Prof. P.K. Ray                   | Impact of semi intensive prawn culture at Sandeshkhali on Ground water.                                                                          | M/s Hindusthan Lever          |
| Prof. P.K. Ray                   | An approach to develop microbial pesticide degradation technology to reduce burden of long persistent pesticide residues in various food stuffs. | DBT, Govt. of India           |
| Prof. P.K. Ray                   | Development of data base on Pesticide residue in food materials.                                                                                 | DBT, Govt. of India           |
| Prof. P.K. Ray                   | Development of an Immunotherapeutic approach to control aflatoxin mediated death in Poultry.                                                     | DST (Govt. of West Bengal)    |
| Prof. P.K. Ray                   | Environmental Monitoring control of air pollution of Santaldih Thermal Power Station                                                             | WBSEB, (Govt. of West Bengal) |
| Prof. P. K. Ray                  | Environmental Monitoring and control of air pollution of Bandel Thermal Power Station                                                            | WBSEB, (Govt. of West Bengal) |

**RESEARCH SCIENTISTS/RESEARCH ASSOCIATES/POST-DOCTORAL FELLOWS/SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS**

Dr. D.P. Modak, Dr. Jharna Datta, Mr. Amiya K. Maiti, Mr. S. Sengupta, Dr. G.K. Datta, Ms. S. Bid, Ms. S. Chatterjee

**SUPPORTING STAFF MEMBERS**

Mr. A. Adak, Mr. A. Dalal, Mr. S.C. Jana, Mr. S. Roy, Mr. S. Mandal

**Madhyamgram Experimental Farm**

Prof. S.Sen-Mandi (Scientist-in-charge)

**HIGHLIGHTS**

Several research programmes like, micropropagation of economically important plants, germplasm conservation of endangered medicinal plant species, development of sustainable agricultural system, animal and environmental biotechnology are focussed at Madhyamgram experimental farm. Revenue earning programmes through cultivation of short duration crops as well as lease of orchards are undertaken.

Bose Institute has an Experimental Farm at Madhyamgram, situated about 15 km. towards the North from the Centenary campus. Apart from providing experimental plant material and field testing facilities, this farm provides Scientists the opportunity to extend their experimental

irrigation are available with diesel and electrically operated pumps.

Experimental plant materials that have been grown through the years include rice, wheat, millet, jute, cotton, mustard, beet, palang, tomato, *Vigna*, *Impomea*, *Costus* and *Mimosa*. "Part of the land area that remains after fulfilling the experimental requirements of scientists of the Institute, is utilized for growing crops for the purpose of revenue earning. For this purpose crops of lentil, mung, mustard/rape seed, tumeric, palang, beet, tomato etc. are grown. Flowers are grown in the garden as well as in pots. As and when necessary flower pots are brought to the laboratory campuses for decoration and also for sale.

Within the farm there are orchards that contain mango, jackfruit, coconut and safeda trees. The fruit trees are given out on lease each year.

To educate farmers on modern technologies and to establish interaction between scientist and farmers, open day are sometimes arranged at the farm.

In view of the need for field testing of biotechnologically produced plants that in recent times constitute a major thrust area of plant Scientists at Bose Institute, a laboratory building has been set up at Madhyamgram farm. A green house constructed under an FAO/UNDP project



Inauguration of the Green House facility at Madhyamgram Experimental Farm

findings from the laboratory to agricultural land. At present 15 scientists of the Institute conduct their field experiments in the farm. The land area within the boundary wall is about 18 acres that includes land for growing experimental plant materials and revenue earning crops, orchards, a laboratory building, a green house, land for developing contained area and green house for transgenic plants and a pond. Sources of

is being made functional for hardening of micropropagated plants of economic importance. Being on site in a farm complete with a laboratory equipped for biotechnological research and having facilities for planned field experiments and space for developing a 'Contained Area' necessary for testing transgenic plants would serve for undertaking research in diverse areas of plant

biotechnology, including germplasm screening, DNA fingerprinting, lab to land transfer of clonally propagated plants, testing transgenic plants. In addition propagation of endangered medicinal plants, development of modern technologies for improvement of culture of fish and silkworm, mass scale production and utilization of biofertilizer and studies on residual effect of pesticides are also being planned.

Bose Institute

#### SUPPORTING STAFF MEMBERS

Sri Pulak Kr. Roy, Sri Kachiram Kole, Sri Bimal Ch. Modak, Sri Basudev Jana, Sri Amal Krishna Das, Sri Rakhal Ch. Jana, Sri Dilip Pramanick, Sri Nalini Ranjan Malik, Sri Chittaranjan Mallick, Sri Tarapada Roy, Sri Subhas Senapati, Smt. Sabita Basiya, Sri Sukumar Das, Sri Rajkishor Lenka, Sri Rabin Talukdar, Sri Jagobandhu Nayak, Sri Laxmikanta Pradhan, Sri Nodiram Kayal, Sri Tapas Chakraborty, Sk. Inal Ali, Sri Sukanta Chakraborty, Sri Bhanu Kisku, Sri Mahesh Das-Gupta, Sri Subrata Banerjee.

## Library

Ms. R. Mitra (**Librarian, Acting**)

The Library primarily serves the scholars, faculty members and staff of the institute. It has two wings with 15 staff members. Researchers and scientists of other institute can be a member of the library with an annual membership fee. The collection consists of both biological and physical sciences. Library subscribes quite a number of useful journals. Computerisation of library activities will be started soon using the library management software LIBSYS.

#### Acquisition

Library contains 31,641 volumes of books, journals and theses upto 31.3.98. Library purchases 157 books in 1997-98. 27 theses have been deposited in the library in the same year.

#### Circulation

Institute readers can make home issue of the journals and books for 15 days only. Current journals can be issued for overnight use.

#### Photocopying services

Library provides photocopying facilities to institute as well as outside users on payment basis. 1,23,968 (RPG/RICOH) copies and 36,412 (Modi) copies in the Centenary Building Library and 68,516 copies in the Main Campus Library were given during the period. Under Inter-Library loan system, photocopies were also supplied to INSDOC.

#### PUBLICATION

1. Chatterjee, Chinmoy and Sarkar, Pradip. Internet service provider in India. Proc. XXI National Conference of IASLIC. 87-90, 1997.

#### Participation in Conferences/Symposia/ Training Programme/Awards and Honours received.

Samir Pal and Gopa Biswas attended the course "Computer Fundamentals DOS, Wstar", held at DIC, Bose Institute on 5-16 May, 1997.

Pradip Kumar Sarkar attended the Workshop on "Customer Satisfaction management in Library and Information Setting", organised by IASLIC and British Council, Calcutta from 15-20 September, 1997.

Rukmini Mitra, P. G. Das, Pradip Sarkar, Banhisikha Chaudhury participated in the 2nd IPA Workshop on Information Superhighway, organised by Indian Physics Association, Calcutta Chapter and DIC, Bose Institute on 21-22 November, 1997.

Pradip Kumar Sarkar participated in the XXI IASLIC Conference held at Tamil Nadu Agricultural University, Coimbatore from 26-29 December, 1997.

#### SUPPORTING STAFF MEMBERS

R. Bhattacharya, P. G. Das, K.P. Hait, S. K. Pal, P. K. Sarkar, B. S. Chaudhuri, G. Biswas, A. Kolay, G. Mukherjee, S. Bhuiyan, M. Jogsharma, A. K. Nandi, D. Das, D. Turi.

## Workshop

Mr. A. Mukherjee (Superintendent)

The workshop consisting the following units e.g. (i) Mechanical (ii) Electrical (both campuses) (iii) Refrigeration (both campuses) (iv) Glass blowing (v) Carpentry (vi) Drafting (vii) Electronic & (viii) store. During the year under report Workshop received about 1800 requisitions from various departments of the Institute.

Mechanical section received about 200 jobs during the year 1997-98. Most of them are completed and some are on process. The Mechanical unit designed and fabricated as per requirement of the scientists. The jobs undertaken by the workshop are (1) Radio Active Preserver (2) Remodelling of a Magnetic Stirrer of Glass Vessel Fermentation (3) Gel Electrophoresis Apparatus (4) Camera stand for Photography (5) Repairing of Gasometer, pumps and Air compressor/Vacuum pump, etc.

Electrical section for both campuses received about 1200 nos of jobs during the year 1997-98. Electrical section undertook repairing jobs like Voltage Stabiliser, Water Bath, Shakers, Autoclaves, Magnetic Stirrer, motor for pump and other motors including rewinding and heat varnishing, repairing of fans have also been done. This section undertook the responsibility of audio and lighting system during the symposium held in the Institute.

Refrigeration unit having 1 (one) mechanic in each campus maintain some 0° Cold Room and A.C. Machines of both campuses. Some welding

works have also been done by the Refrigeration Mechanic. Received about 100 jobs.

Glass Blowing Section having 1 (one) Glass Blower received 95 nos of requisitions and completed all the jobs. Glass Blowing section has done some special type of glass works as per requirement of the Scientists.

Carpentry Section having one carpenter and one helper completed the partition of Culture Room including making false ceiling, Crescograph Box, Glass case, Xerox Machine table, Projector stand etc. Carpentry section received 82 nos. of job during the 1997-98 most of them are completed.

Drafting section received more than 100 requisitions from various departments for scientific drawing all of them have been completed. Drafting section have done the drawing of electrical distribution system for both campuses.

Internal telephone system are looked after by the Electronic unit with satisfactory and also maintained the EPABX system of both campuses. This unit received 90 nos of job during this period. Most of them are completed.

Store : To Purchase and issue of various types of materials for maintenance of Electrical, mechanical, building and refrigeration. Store also purchase tools and instruments, EPABX Machine and Telephones.

### SUPPORTING STAFF MEMBERS

#### Main Campus :

Mrinal Chandra Roy, Ashutosh Sarkar, Samar Kr. Dutta, Sisir Kr. Ghosal, Tarapada Das, Dipen Sen, Bankim Dutta, Subhas Chakraborty, Jogendra Nath Das, Basanta Kr. Shit, Bholanath Saren, Sourendra Nath Chakraborty, Abdul Rahaman Molla, Panchanan Santra, Panchugopal Karak, Walter D' Rozario, Sk. Kd. Raffick, Rajkumar Das, Pranab Banerjee, Subrata Basak, Lochan Shee, Smt. Sairoon Begum, Gopal Chandra Das, Sk. Md. Farruck, Kishori Mohan Saha, Brahmdeo Prasad, Kalachand Sen, Thakur Gond, Nageswar Das, Kalipada Das.

#### Centenary Campus :

Dipak Dutta, Sithil Mukherjee, Anil Sengupta, Pitambar Patra, Sk. Md. Ehia Hossain, Tapan Kumar Mondal, Ashit Banerjee, Murari Mohan Shee, Baidyanath Murmu.

## Regional Sophisticated Instrumentation Centre

Dr. A. Chatterjee (Scientist-in-Charge)

The RSIC continued to provide data to users in the Institute as well as in the region, maintaining the instruments in the best possible manner. The resume of activities are shown in the table. Attempts to acquire a new Scanning Electron Microscope continue but still remain unfulfilled.

Consultancy services to public and industrial organizations continued to be provided as in the past.

RSIC personnel also conducted lecture courses in Calcutta and Jadavpur Universities.

### RESUME OF ACTIVITIES — FROM APRIL 1997 TO MARCH 1998

| Internal Users       |                |                | External Users       |                |
|----------------------|----------------|----------------|----------------------|----------------|
| Name of Instrument   | No. of Samples | Revenue Earned | No. of Samples       | Revenue Earned |
| X-RAY DIFF           | 2              | Rs. 3,180/-    | 12                   | Rs. 33,040/-   |
| EPR                  | 222            | Rs. 11,100/-   | 22                   | Rs. 3,050/-    |
| AAS                  | 314            | Rs. 10,550/-   | 311                  | Rs. 18,625/-   |
| TIME OF USE (IN HRS) |                |                | TIME OF USE (IN HRS) |                |
| SEM                  | 32             | Rs. 4028/-     | 151                  | Rs. 20,286/-   |
| GLC                  | 39             | Rs. 4800/-     | 128                  | Rs. 6450/-     |
| FLS                  | 136            | Rs. 6,840/-    | 70                   | Rs. 3,340/-    |
| TEM                  | 328            | Rs. 29,040/-   | 242                  | Rs. 24,860/-   |
| HPLC                 | 231            | Rs. 42,966/-   | 7                    | Rs. 1550/-     |
| Total                |                | Rs. 1,12,504/- |                      | Rs. 1,11,201/- |

Grand Total — Rs. 2,23,705

### PUBLICATIONS

1. Synthesis and Spectroscopic Characterisation of Cobalt(III) Complexes with S-Benzyl Dithiocarbamate of 5-Methyl-3-Formyl Pyrazole (HMPzSB) : X-ray crystal structure of [Co(MPzSB)<sub>2</sub>]Cl. A. Mitra, T. Banerjee, P. Roychowdhury, S. Chaudhuri, P. Bera and N. Saha (1997) *Polyhedron*, **16**, 3735-3742.

2. Isoalangidiol Monoacetate, a Triterpene Alcohol. K. Bhattacharyya, S. Chaudhuri, B. Achari, B. Mazumdar and S. K. Mazumdar, *Acta Crystallogr.*, **C53**, 1299-1301 1997.

3. Unusual Substitution of -SCH<sub>3</sub> by -OH in 9,10-Dihydrophenanthrene. S. Sarkhel, A. Goel, V. J. Ram, S. Chaudhuri and P. R. Maulik, *Acta Crystallogr.*, **C53**, 1713-1714, 1997.

## SUPPORTING STAFF MEMBERS

Dr. Dilip Kumar Mukherjee, Dr. Siddhartha Chaudhuri, Mr. Arabinda Biswas, Mr. Tapan Datta, Mr. Jaideb Chattopadhyay, Mr. Arup Kumar Sen, Mr. Samir Kumar Mukherjee, Mr. Pradip Kumar Karmakar, Mr. Jaganmoy Guin, Mr. Subhas Chandra Maikap, Mr. Pathik Ranjan Ray, Mr. alakananda Sarkar, Ms. Tanima Modak Dhar, Mrs. Manisha Banerjee, Mr. Debabrata Polley, Mr. Sankar Santra, Mr. Sanjoy Santra, Ms. Saraswati Sen, Mr. Shyam Sundar Kundu, Mr. Gobinda Das, Mr. Amit Kumar Mahinder.

## J. C. Bose Unit and Museum

Mr. Dibakar Sen (In-Charge)

J. C. Bose Unit & Museum took the responsibility of duplication of another set of J. C. Bose's portable Crescograph instrument jointly with Bose Institute Workshop.

Shri Sen collected some photocopies of rare newspaper cuttings on J. C. Bose from U.S.A. during his visit to U.S.A. in June 1997.

A good number of visitors both from India and abroad including the students from different Universities visited the Museum. Besides, the J.C. Bose Unit & Museum furnished information regarding Acharya J. C. Bose and his works to various organisations, eminent persons on request.

Special display in the Museum were arranged to mark the Foundation Day of the Institute on November 30, 1997.

**Participation in Conferences/Symposium/Training programme/Award and Honour received :**

Dibakar Sen deliverde lectures on :

"J.C. Bose : Scientist, Poet and Philosopher-all in one" in Ramakrishna Mission (Belur) on December 13, 1997 ; Ramakrishna Mission (Purulia) on December 29, 1997; Ramakrishna Mission (Narendrapur) in December 1997 ; "Life and Works of Acharya J.C. Bose" in Academic Staff College Orientation Programmes, Burdwan University on November 06, 1997, University of Calcutta on January 31, 1998 ; "J.C. Bose's instruments" at Max Mueller Bhavan organised by the Institute of Electrical & Electronics Engineers, INC, Calcutta Section on August 22, 1997. "J.C. Bose" in Manmatha Nath High School, Barrackpore on November 22, 1997; "Contribution of J.C. Bose in the societalisation of Science" at Bangiya Bijnan Parishad on the topic on January 25, 1998.

Sri Dibakar Sen was invited in the International Microwave Symposium organised by I.E.E.E. and M.T.T.-S held in Denver, Colorado, U.S.A. during June 09-13, 1997, jointly with Prof. A.K. Sen, Director, E.C.R.A. There Shri Sen and Prof. Sen Jointly demonstrated few

experiments of Acharya J.C. Bose together with an Exhibition which had created both admiration and awe in the minds of the participating scientists coming from all over the world.



In order to pay their homage and respect to Acharya J.C. Bose, on the concluding day drinks were served in bottles labelled with Acharya J.C. Bose's photograph

of his Royal Institution demonstration on January 29, 1897 with marking "Bose Centennial". Shri Sen collected one bottle for display at J.C. Bose Museum.

In the J.C. Bose Memorial Technical Session, Shri Sen delivered lecture about the history of the gradual development of Bose's Millimeterwave Instruments & Components. A part of Shri Sen's lecture has been introduced in a joint paper with Prof. D.L. Sengupta, Life Fellow, I.E.E.E. & Prof. T.K. Sarkar of Syracuse University published in the Proceedings of I.E.E.E. special issue January 1998.

## SUPPORTING STAFF MEMBER

N. Das.

## Distributed Information Centre

Dr. T. C. Ghosh (In-Charge)

Bioinformatics Centre, Bose Institute was incepted in 1988 with Genetic Engineering and Biomolecular modeling as the major thrust area. Since then the Centre is functioning as a repertoire of information related to Bioinformatics. The Centre works as the backbone of the research and development activities in various fields like, Genetic Engineering, Biocrystallography, Biocomputing, Molecular Modeling, etc. The Centre has expanded its userbase significantly over the last few years. Apart from the host and neighbouring institutes, scientists from farflanked places like, Benaras Hindu University, IIT Kanpur, NEHU, RRL Jorhat, Tripura University, Andaman & Nicobar islands, etc. are utilising the facilities of the Centre.

**Major achievements during the year 1997-98.**

1) Establishment of Local Area Network (LAN) in both the campuses of Bose Institute.

2) Two programs "PDBVIEW" and "LOOP SEARCH" have been developed and deposited to PDB.

3) "IBASE -A comprehensive database on Intron has been developed.

4) A National symposium cum workshop was organised on "Trends in Bioinformatics during March 24-27, 1998.

### Bioinformatics Resource Created:

1. **PDBVIEW:** PDBVIEW is a PC based software program that can search PDB entries by compound, source and header. The Boolean operators "and, or, not" can be used for searching. The program then asks for the display option. The display of an individual entry contains the header, compound, and source fields. It also asks whether the sequence is to be displayed or not. The sequence display appears at the bottom of the screen as a line with horizontal boxes of different types for helix and sheet components. The length of the boxes

is proportional to the secondary structural elements.

A repeated display of a homologous family of proteins helps one to identify the common secondary structural elements in them. This program is also helpful for browsing the PDB files and for a two dimensional representation of the secondary elements of a protein.

The program uses several databases (generated from the PDB files) containing various fields and secondary structural information. These databases have to be generated prior to the searching. The program that can read the CD-ROMs containing PDB entries and generates the databases are available on request from dicdbt@boseinst.ernet.in or root@boseinst.ernet.in. This package we had deposited to Protein Data Bank and appeared in **Quarterly Newsletter Published by Brookhaven National Laboratory Protein Data Bank in release # 82, October 1997.**

**2. LOOP SEARCH:** The program LOOP SEARCH has been developed in C and can be compiled on unix workstations having a CD-ROM drive. The program will read the PDB entries from the CD-ROM drive. The program takes the secondary structural information from the file. The structural elements are then arranged sequentially and the lengths of the loop residues connecting two such elements are calculated. The loops are categorised based on their connectivity, e.g., a loop connecting two helical segments are assigned as category 1, connecting one helix and one strand-2, connecting two beta strands-3, and connecting beta strand and helix-4. The output file contains the entry name, loop length, loop category and the sequence in one letter code. The output file is in the FASTA format.

The output file is very useful for homology modeling purposes, where finding a loop of similar length and same category might aid in the modeling process. Also, creation of a

National Symposium-cum-Workshop on Trends in Bioinformatics was organized by Bioinformatics Centre, Bose Institute during March 24-27, 1998. The Symposium-cum-Workshop was intended to provide the participants with an overview of the recent advances and developments in Bioinformatics. Invited lectures by experts in the field of (i) Database Construction management and mining; (ii) Genome analysis and structural Bioinformatics were scheduled. Dr. J. R. Arora, Advisor, Dept of Biotechnology, Govt. of India, inaugurated the Symposium-cum-Workshop. 45 participants from all over the country attended the Symposium-cum-Workshop. The Symposium was sponsored by D.B.T., Govt. of India.



Scientists attending the National Symposium-cum-Workshop on Trends in Bioinformatics.



Dr. T. Madhan Mohans' talk on "Current Trends in BTIS Program" in National symposium-cum-Workshop on Trends in Bioinformatics.

general database of loops will be possible. This package was deposited to Protein Data Bank and it appeared in **Quarterly Newsletter Published by Brookhaven Laboratory Protein Data Bank in release #82, October 1997.**

**3. IBASE:** A comprehensive database on intron sequences has been developed in DOS environment. It is accomplished by an utility program "EXTRACT". EXTRACT is a menu oriented keyword-based retrieval program which can read any EMBL entry and can search through them using keywords that occurs in any entry in the annotation part. IBASE originates from EMBL nucleic acid sequence database.

#### Workshops/Seminars/symposia Organized by the Centre :

1. A Symposium cum Workshop on "Information Super Highway" was organised during November 21-22, 1997. Number of registered participants was 40.

2. A National Symposium cum Workshop was organised during March 24-27, 1998. The Symposium was inaugurated by Dr.J.R.Arora, Advisor, DBT, Govt. of India. There were 45 participants from all over the Country. The Symposium was highly appreciated.

#### Networking and Communications :

During the year 1997-98, BIC has established a Local Area Network (LAN) in both the campuses of Bose Institute.

#### PUBLICATIONS

1. Role of Zinc in tRNA acceptor stem binding by Glutamyl-tRNA Synthetase from E.coli: A molecular modeling study. A.Bothra, S.Roy, B.Bhattacharya, C.N.Mandal and C.Mukhopadhyay. *Journal of Biomolecular Structure and Dynamics*, **15**, 19-25, 1997.

2. Similarities inferred from the studies of long range correlation among mitochondrial DNA sequences S.K.Gupta & T.C.Ghosh. *Ind. J. Biophys. & Biochem.* **34**, 259-265, 1997.

3. Modeling of Entamoeba histolytica Ferredoxin. C. Mukhopadhyay & A. Lohia, *Journal of Biomolecular Structure & Dynamics*, **15**, 663-672, 1997.

4. Molecular Dynamics Simulation of Glycoprotein Glycans of Immunoglobulin G and Immunoglobulin M. C. Mukhopadhyay, *Biopolymers*, **45**, 177-190, 1998.

5. Molecular dynamics Simulation of Colchicinoids. A. Bothra, S. Roy, B. Bhattacharyya, C. Mukhopadhyay, *Journal of Biomolecular Structure & Dynamics*, **15**, 999-1008, 1998.

6. A Fluorescence Spectroscopic and Molecular Dynamics Study of bis-ANS/Protein Interaction. A. Bothra, A. Bhattacharyya, C. Mukhopadhyay, B. Bhattacharyya and S. Roy, *Journal of Biomolecular Structure & Dynamics*, **15**, 959-966, 1998.

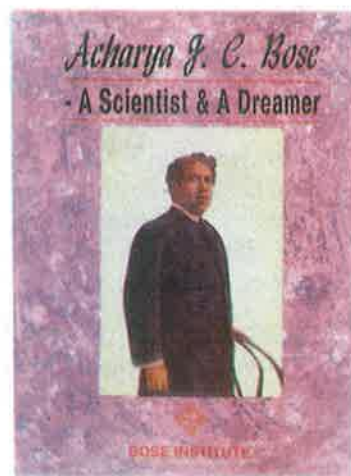
#### SUPPORTING STAFF MEMBERS

Dr. T.C.Ghosh, Mr. V.S.Sundararajan, Mr. T.K.Bhattacharyya, Ms. S.Majumdar, Mr.S.K.Gupta, Mr.J. Mandal, Dr. I. Dey ( RA)

## Publication

Mr. A.K. Das Gupta (**Superintendent**)

Since its inception in 1980, the Publication Section has been doing all sorts of publications from this institute, i.e. Transactions of the Bose Research Institute; Annual Report (both English and Hindi versions)/ Memorial Lectures; Director's Report; Folders; Posters and Leaflets of different Symposium and Training programs.



On 30th November, 1997, last three volumes of the book entitled "Acharya J.C. Bose - A Scientist and a Dreamer" edited by Prof. Prantosh Bhattacharyya were released. This book contains Bose's scientific works published in "The Electrician", "Journal of the Asiatic Society of Bengal", "Philosophical Magazine", "Proceedings of the Royal Society", "Modern Review" as well as lectures delivered before the Royal Institution of London, British Association of Glasgow, and many other items of historic importance.

M/s, Das Gupta & Co. Private Limited, 54/3, College Street, Calcutta - 700 073 has been appointed as sole Indian distributor of our publications. They will sale all of our Institutional publications within India and the Union territories only.

### SUPPORTING STAFF MEMBER

C.K. Sasmal

## Administration

**Prof. P.K. Ray (Director)**

Prof. Prantosh Bhattacharyya (**Registrar, Officiating**)

Mr. K. Chaudhuri (**Astt. Registrar I**), Mr. R.K. Kundu (**Astt. Registrar II**)

Mr. S. Pal (**Accounts Officer**)

### SUPPORTING STAFF MEMBERS

#### Centenary Campus

R.N. Bhattacharyya, S.P. Sit, A. Bera, P. Das, Ankur Dutta, S.R. Dechoudhury, A.K. Sengupta, T. Mukherjee, E. Pal, T. Chakraborty, N. Roy Ghatak, S. Kar, Subir Sen, A. Chakraborty, Debidas Saha, Amit Kumar Ghosh, R.R. Pandey, M.S. Mitra, Dhruvajyoti Sen, Sujut Kr. Ghosh, Dhruvajyoti Sen, Sujit Kr. Ghosh, B.K. Mandal, M.C. Das, Rita Nath, Sisir Chakraborty, Swapan Kr. Singha, Rubi Sarkar, Sudam Jana, P. Mullick, R. Chakraborty, Rina Das, Sanjay Kr. Chaki, Debashis Koley, Somnath Das, A Bhowmik, Sujata Pandit, Sukumar Das, S. Chatterjee, R.C. Sarkar, Dipak Ranjit Chosh, K. Sur, S. Devi, C. Rangwa, T.K. Banerjee, K. Turi, S. Turi, K. Basar, N. Yadav, Md. K. Ansari, S. Guchait, Kanai Hazra, P. Bhunya, Nemai Das, Kanak B. Hazra, Duryodhan Nayak, Sanjib Das, Bablu Mondal, Kashinath Pul, Kamala Turi.

#### Main Campus

Arun Kumar Das Gupta (**Superintendent**), Samar Chakraborty (**Overseer**), Sankar Nath Sinha, Rina Roy, Sanat Kr. Dey, Mahendra Nath Shee, Santosh Patra, Ramani Kanta Jana, Tarun Chakraborty, C. K. Sasmol, R. Ram.

### Acharya Jagadis Chandra Bose Memorial Lecture

The 59th Acharya Jagadis Chandra Bose Memorial Lecture was delivered by **Prof. V. Ramalingaswami**, National Research Professor, AIIMS, New Delhi on "The Doly Affair"—A Dilemma for Humankind.

### Lectures

**Dr. Gourisankar Ghosh**, University of California, San Diego, USA, "DNA Recognition by the REL/NFK  $\beta$  family of transcription factors".

**Dr. Cichik Ghosh**, Cornell University Medical College, New York, USA, "Endocytic trafficking to the trans golgi network".

**Dr. Ananda L. Roy**, Tufts University School of Medicine, Boston, USA, "TF-II-I : a mediator between signal transduction and transcription.

**Dr. Anuradha Lohia**, of Biochemistry, Bose Institute, "Division and multiplication of Entamoeba Histolytica—your intestinal guest !"

**Dr. Satya Priya Sarkar**, Boston Biomedical Research Centre, Boston, USA, "Genetic Engineering approaches to study calcium transduction in muscle functions."

**Dr. G. P. Das**, Solid State Physics Division, BARC, Mumbai, "Torce Concepts in Quantum Mechanics : Applications to Diamond Polytypes".

**Dr. Asmita Sen Gupta**, Dept. of Physics, Visva-Bharati University, Santiniketan, "Coupling of Positron Experiments with Semiconductors—An Achievement Review".

### Acknowledgement

The Council takes the opportunity to express, its grateful thanks to the Department of Science and Technology, Government of India, for providing generous funds in the form of Grants-in-aid, and for the support of research projects, and also to other external agencies such as the Government of West Bengal, Department of Atomic Energy, Council of Scientific and Industrial Research, Indian Council of Agricultural Research, Indian Council of Medical Research, etc., for their support in several forms. The Council also record its thanks to the British Council, Federal Republic of Germany, United States of America, the Rockefeller Foundation, the ECU and other for their support in several forms, such as research grants, gift of equipments, books, travel grants, collaborative project, etc. The Council further records its thanks to the Governing Body, Bose Institute, and to the members of the various Selection Committees for their help and interest.

### Members of the Council

- |                                                                                                                |   |                                            |
|----------------------------------------------------------------------------------------------------------------|---|--------------------------------------------|
| 1. Shri Sanjoy Sen                                                                                             | } | Nominees of the<br>Governing Body          |
| 2. Prof. S. Bose                                                                                               |   |                                            |
| 3. Shri Debabrata Bose                                                                                         |   |                                            |
| 4. Prof. R.N. Chakraborty                                                                                      |   |                                            |
| 5. Prof. (Mrs) Debi Chakraborty                                                                                |   |                                            |
| 6. Mr. Somnath Sanyal                                                                                          |   |                                            |
| 7. Prof. S. N. Chatterjee                                                                                      |   |                                            |
| 8. Secretary, Department of Science & Technology, Government of India, or his nominee                          |   |                                            |
| 9. Director General, C.S.I.R. or his nominee                                                                   |   |                                            |
| 10. Joint Secretary and Financial Adviser, Department of Science and Technology, Govt. of India or his nominee |   |                                            |
| 11. Representative of the Lok Sabha                                                                            |   |                                            |
| 12. Principal Accountant General (A & E), West Bengal                                                          |   |                                            |
| 13. Secretary, Higher Education (Nominee of the Govt. of West Bengal)                                          |   |                                            |
| 14. Municipal Commissioner, Representative of the Calcutta Municipal Corporation                               |   |                                            |
| 15. Director, Bose Institute - Ex-officio                                                                      |   |                                            |
| 16. Deputy Director, Bose Institute - Ex-officio                                                               |   |                                            |
| 17. Prof. Sandip K. Basu                                                                                       | } | Two scientists from<br>outside West Bengal |
| 18. Prof. G. Padmanaban                                                                                        |   |                                            |

Registrar, Bose Institute - Secretary

- Invitees :* 1. Representative of the Academic Council  
2. Representative of Bose Institute Employees Association.

**Members of the Governing Body**

1. Shri Sanjoy Sen
2. Prof. Sunando Bose
3. Shri Debabrata Bose - Secretary
4. Prof. S.D. Chatterjee
5. Prof. R.N. Chakraborty
6. Dr. (Mrs.) Debi Chakraborty
7. Prof. D. Banerjee
8. Prof. B.B. Baliga
9. Prof. S.N. Chatterjee
10. Shri Somnath Sanyal
11. Director, Bose Institute

**Members of the Finance Committee**

1. Shri Sanjoy Sen - (Representative of the Council) - Chairman
2. Shri Debabrata Bose - (Representative of the Governing Body)
3. Joint Secretary and Financial Adviser to the Department of Science and Technology, Govt. of India, or his nominee
4. Principal Accountant General (A & E), West Bengal
5. Director, Bose Institute - Ex-officio

**Members of the Centre Management Committee, RSIC, Bose Institute, Calcutta**

1. Director, Bose Institute, Calcutta—Chairman
2. Head—RSIC, Bose Institute, Calcutta—Member-Secretary
3. Representative of DST, Govt. of India
4. Prof S. C. Roy, Deptt. of Physics, Bose Institute, Calcutta
5. Prof. B. Bhattacharyya, Deptt. of Bio-chemistry, Bose Institute, Calcutta
6. Prof. N. Chatterjee, Calcutta
7. Dr. R.B. Singh, Director, Forensic Sc. Lab., Lucknow
8. Prof. S.K. De, Rubber Technology Centre, Kharagpur
9. Prof. K.S. Mukherjee, Department of Chemistry, Viswa Bharati University, Santinketan.



An international conference on "Stress adaptation, prophylaxis and treatment" held at **The Oberoi Grand, Calcutta** was organised by **Bose Institute** during January 10-12, 1998. Scientists all over the world attended the conference to interact among themselves and exchange their ideas in management of stress. In fact, they talked about various stress induced by physico-chemical, biological and environmental factors and their control mechanisms at cellular and molecular level. The deliberations in the scientific sessions during the conference was rich in content and thought provoking. The conference was first of its kind in this country. We got a bird-eye view on the subject, which will be discussed in greater details in years ahead.