



BOSE INSTITUTE
ANNUAL REPORT
1999-2000

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FOUNDER DIRECTOR

ANNUAL REPORT
OF
THE BOSE INSTITUTE
FOR
1999-2000



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

Compiled and edited by the Annual Report
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Third Cover : Illustrates the instruments fabricated and used by Sir J. C. Bose

Our Institute

Bose Institute, now a leading R & D organisation of the nation, was founded by Acharya Jagadis Chandra Bose in 1917 with an intention for the advancement of science and diffusion of knowledge. The Institution is in the service of the nation for the last 83 years through its pursuit of advancement of knowledge in science and technology and by producing efficient and skilled scientific man-power that the country needs for its development. The Institute caters to this need through six departments (Physics, Chemistry, Botany, Microbiology, Biochemistry and Biophysics) four sections (Plant Molecular Cellular Genetics, Animal Physiology, Immunotechnology and Environmental Science) and through other service centres like RSIC, DIC, Library, Workshop and Publication, etc. There are also experimental field stations at four different locations in West Bengal (Darjeeling, Falta, Madhyamgram and Shyamnagar) mainly concentrating in applied research.

The Institute has two campuses, one located at 93/1 Acharya P. C. Road near Raja Bazar where the Bose Institute was originally founded (commonly known as Main Campus) and the other at Bagmari Road near Kankurgachi (commonly referred to as Centenary Campus, as it was built to commemorate the birth centenary of the founder). The departments of Physics, Chemistry, Botany, Library, Workshop, J. C. Bose Museum, Publication and RSIC are located in the Main Campus, while the departments of Biochemistry, Microbiology, Biophysics, PMCG, Animal Physiology, Immunotechnology and Environmental Science Sections, DIC, a part of the Library, Administration and Accounts are located in the Centenary Campus.

The Institute was started by a gift of Rupees four lakhs by the founder (invested in land, building and G. P. notes) and funds raised from public donations. The Institute is at present funded by the Department of Science and Technology, Govt. of India.

- "It is for advancement of world's science."
- "Its object is the foundation of a branch of knowledge which owes its birth of India."
- "It obtains its strength and inspiration from the realisation, that it is through such work that India will make her great contributions to the empire of knowledge, and win her true place in the Federation of Nations."

At present Bose Institute is conducting research in different major basic science areas and Biotechnology.

The major Institutional research programmes are :

- (1) Improvement of plant Productivity, Nitrogen Fixation and Photosynthesis using modern Biotechnology and Plant Breeding.

MANAGEMENT COMMITTEE

Members of the Governing Body

1. Shri Sanjoy Sen
2. Shri Debabrata Bose - Secretary
3. Prof. R. N. Chakraborty
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7. Prof. S. N. Chatterjee
8. Shri Somnath Sanyal
9. Dr. A Chatterjee
10. Director, Bose Institute

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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
18. Prof. Asis Dutta
19. Member of Parliament—Vacant (Lok Sabha)

Nominees of the
Governing Body

Two Scientists from outside
West Bengal nominated by
DST., Govt. of India

Registrar, Bose Institute - Non Member Secretary

Invitees : 1. Representative of the Academic Council
2. Representative of Bose Institute Employees Association.

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
6. Registrar - Secretary

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 records its thanks to the British Council, Federal Republic of Germany, United States of America, the Rockefeller Foundation, the ECU and others 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 Committee for their help and interest.

**SCIENTIFIC AND
TECHNICAL REPORTS**

Physics

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

HIGHLIGHTS

Considerable progress has been achieved in the areas of neutron dosimetry, quantum disentanglement, teleportation, propagation of strangelets through the terrestrial atmosphere, Cosmological dark matter and quantum antiferromagnets, among other areas.

INSTITUTIONAL PROJECT VI

A. Acoustic

A.1. Auditory Illusions and Time Perception :

M. H. Engineer

Time is perceived in a strange way in human consciousness. An example is the Auditory Illusion discussed in last year's Annual Report. It was thought that the time delay of around half a second between the physical and perceived time of events might be explained by advanced action and backward causation. Fundamental physics recognises both possibilities, the only exception being the decay of the K_0 particle. The physics literature on the subject is vast. It starts with Boltzmann and Maxwell and is still of interest in, for example, the detection of spatially separated Entangled states in Quantum mechanics. A selection of the literature has been studied in detail. The study continues, as do the experiments on illusions.

B. Applied Radioactivity and Liquid Dynamics :

S. N. Changdar

Studies were continued to observe the effect of coupled flow on diffusive motion of ions and molecules in complex liquid systems, mainly ternary systems. The radioactive isotopes used as tracers were Hg-203 and Tl-204. Both the diffusion arrangements, namely the sheared cell and the sliding cell, were used for these measurements. Direct evidence on isotope effect and convection effect was sought.

C. Radiation Physics and Condensed Matter :

C.1. Neutron spectrometry using superheated drops : S. C. Roy, B. K. Chatterjee, B. Roy and Mala Das

The neutron energy spectrum of Am-Be neutron source was constructed from neutron detection efficiencies at different temperatures of R-114 superheated drop detector utilising the spectrometry principle developed last year. The present results agree well with the other available results, obtained by using monochromatic neutron sources and with more than one superheated liquid. This clearly demonstrates the validity of our spectrometry principle.

C.2. Gamma-ray sensitivity of R-12 superheated drop detector : B. Roy, S. C. Roy, B. K. Chatterjee and Mala Das

Gamma-ray detection threshold temperatures of R-12 superheated drop detector have been found out for three photon energies (59.54keV, 662keV, 1250keV). The nucleation was detected using an active device developed in our lab, by counting the number of drops vapourised per min with varying the temperature of the detector over a wide range. The results show that the threshold temperature for detection increases with the energy of the incident photons.

Sensitivity of R-12 superheated drop detector at room temperature towards low energy photon has also been observed for the first time in our laboratory. This discovery is important to the users of R-12 detector in neutron dosimetry to take note of this photon sensitivity, in assessing the neutron dose correctly.

C.3. Limit of superheat of liquids : **S. C. Roy, B. Roy, B. K. Chatterjee** and Mala Das

The limit of superheat of three refrigerant liquids (R-12, R-22, R-114) have been found out utilising the new improved method developed last year. From the experimental observation, reduced limit of superheat has been found out. The limit of superheat measured by the present method supercedes all other measurements and theoretical predictions in reaching closest to the critical temperature and warrants to improve the theoretical calculations.

C.4. Structural and ferroelectric studies of Na-modified PLZT : **T. P. Sinha** in collaboration with S. R. Shannigrahi, Prof. R. N. P. Choudhary and Prof. H. N. Acharya of Indian Institute of Technology, Kharagpur

Polycrystalline samples of $\text{Pb}_{0.92}(\text{La}_{1-z}\text{Na}_z)_{0.08}(\text{Zr}_{0.60}\text{Ti}_{0.40})_{0.98+0.04z}\text{O}_3$ ($z = 0.0, 0.1, 0.3, 0.5, 0.7$) have been prepared using the metal-alkoxide sol-gel technique. Differential thermal analysis and x-ray diffraction studies show that the above materials can be formed in a single-phase perovskite structure at 550 °C. The formation of grain structure, particle size and their distribution have been studied by scanning electron microscopy and transmission electron microscopy. Systematic studies of compositional dependence of ferroelectric properties of the compounds have been done. High value of dielectric constant $\sim 10^4$, remanent polarization ($14\text{--}24 \mu\text{C cm}^{-2}$), pyroelectric coefficient ($46\text{--}57 \text{ nC cm}^{-2} \text{ K}^{-1}$) and small piezoelectric parameters have been observed for all the compounds. The density of the sample increases with increasing Na^+ concentration. A combination of donor and acceptor doping in lead-zirconate-titanate in the studied materials lead to stable ferroelectric states over a wide usable range.

C.5. First-principles study of the optical properties of perovskite : Sonali Saha and **T. P. Sinha** in collaboration with Prof. A. Mookerjee of S. N. Bose National Centre for Basic Sciences, Calcutta.

The first-principles tight-binding linear muffin-tin orbitals method with atomic sphere

approximation using density functional theory in its local density approximation has been used to calculate the electronic band-structure, density of states (DOS) and charge density of perovskites ABO_3 ($A = \text{Ba, Sr, Ca}$ and $B = \text{Ti}$) in cubic phase. From the DOS analysis as well as charge density studies, it has been concluded that the bonding between A and BO_3 is mainly ionic and the BO_3 entities bond covalently. Using the band structure and site projected DOS, the interband contributions to the optical properties of ABO_3 have been analyzed. The real and imaginary parts of the dielectric function and hence the optical constants (such as reflectivity, refractive index and absorption coefficient) are calculated. The calculated spectra are compared with the available experimental results.

D. High Energy Physics, Astrophysics and Cosmology :

D.1.1. High Energy Particle and Nuclear Physics : Dr. Sanjay Kumar Ghosh and **Prof. Sibaji Raha**, in collaboration with Dr. Abhijit Bhattacharyya (VECC, Calcutta)

Modification of the NJL model to include explicit gluonic degrees of freedom is being investigated.

D.1.2. Astrophysics and Cosmology - theoretical and observational :

(a) Mass limit of compact quark stars :

Mr. Shibaji Banerjee, Dr. Sanjay Kumar Ghosh and **Prof. Sibaji Raha**

The mass limit of compact cold stars made up of quark matter has been investigated. It has been found that there exists an upper limit on the mass of such stars, which is determined by universal constants like the Planck Mass and the difference between the perturbative and the nonperturbative vacua of Quantum Chromodynamics (QCD), the fundamental theory of strong interactions. It has been shown that this limit is very close to the Chandrasekhar limit, for the canonical value of the Bag constant which mimics the energy difference between the perturbative and the nonperturbative vacua. A paper with this finding has been published.

(b) Study of Strangelets in Cosmic ray fluxes : Mr. Shibaji Banerjee, Dr. Sanjay Kumar Ghosh and **Prof. Sibaji Raha**, in collaboration with Dr. Debapriyo Syam (Presidency College, Calcutta) and Dr. Amal Mazumdar (Marburg University, Germany)

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. Ionisation loss as well as charge and mass capture reactions have been included in a fully relativistic formulation. A manuscript has been accepted for publication and another one submitted for publication.

(c) QCD Phase transition in the early universe and baryonic dark matter :

Mr. Shibaji Banerjee, Dr. Sanjay Kumar Ghosh and **Prof. Sibaji Raha**, in collaboration with Dr. Abhijit Bhattacharyya (VECC, Calcutta) and Prof. Bikash Sinha (VECC and SINP, Calcutta)

The gravitational clumping of stable strange quark nuggets, formed in the cosmic first order quark-hadron transition, has been investigated. It has been found that they remain in thermal kinetic equilibrium with the rest of the universe till the neutrino decoupling temperature ($\sim 1 \text{ MeV}$). Below this temperature, they clump under gravitational attraction, the collapse time being comparable to the lifetime of the universe at that epoch. It has been shown that the total mass of nuggets within the Jeans length at that time amounts to $0.5 M_{\text{Sun}}$, which is the most probable mass of the **MA**ssive **C**ompact **H**alo **O**bjects (MACHO) detected through gravitational microlensing. A manuscript with this result is currently under preparation.

(d) Calibration of Passive Solid State Nuclear Track Detectors for Cosmic Ray Studies :

Mr. Shibaji Banerjee and **Prof. Sibaji Raha**, in collaboration with Dr. Debapriyo Syam (Presidency College, Calcutta), Dr. Amal Mazumdar (Marburg University, Germany) and Prof. Bikash Sinha (VECC and SINP, Calcutta).

Various polymers available in the market are being tested for their suitability as detectors of charged nuclear fragments as well as their

ruggedness against exposure to very cold temperature.

E. FUNDAMENTALS OF QUANTUM MECHANICS

E.1.1. A New Quantum Violation of Noncontextuality invoking Bell's inequality and Entangled State of a Spin-1/2 Particle : **Dipankar Home** in collaboration with Sayande Basu, Dept. of Physics, University of Cincinnati, Ohio, Somshubhro Bandyopadhyay, Dept. of Physics, Bose Institute and Guruprasad Kar, Physics and Applied Mathematics Unit, ISI, Calcutta

Deriving Bell's inequality as a general consequence of the hypothesis of noncontextuality, we demonstrate a testable example of its incompatibility with quantum mechanics. For this purpose, an appropriately formulated entanglement between disjoint Hilbert space of a single spin-1/2 particle is invoked.

E.1.2. Determination of When an Outcome is Actualised in a Quantum Measurement using DNA-Photolyase System : **Dipankar Home** in collaboration with Rajagopal Chattopadhyaya, Dept. of Biochemistry, Bose Institute, Calcutta

The biochemical attachment of photolyase to ultraviolet (uv) absorbed DNA molecules provides a method for registering whether a source has emitted photons. Here, using laws of chemical kinetics and related experimental methods, we argue that the instant, after which this information becomes discernible, can be empirically determined by retrodicting from relevant data when the photolyase binding to uv-absorbed DNA molecules has started occurring. Thus an empirically investigable twist is provided to the quantum measurement problem.

E.1.3. Barrier Perturbation induced "Superarrivals" and "Nonlocality" in a Time-Evolving Wave Packet : **Dipankar Home** in collaboration with Somshubhro Bandyopadhyay, Dept. of Physics, Bose Institute, Calcutta

We compute the time evolving probability of a Gaussian wave packet to be reflected from a rectangular potential barrier which is perturbed by reducing its height. A time interval is found

during which this probability of reflection is enhanced ("superarrivals") compared to the unperturbed case. Such a time evolving reflection probability implies that the effect of perturbation propagates across the wave packet faster than its group velocity - a curious form of "nonlocality".

E.1.4. Optimal Universal Disentangling Machine for two qubit quantum states : Somshubhro Bandyopadhyay in collaboration with S. Ghosh, G. Kar, A. Roy, of Indian Statistical Institute, Calcutta and D. Sarkar of Burdwan University

In this work we obtained the optimality curve starting from most general transformations imposing the isotropy requirement. We studied both the symmetric and asymmetric cases and obtained an upper bound on the reduction factor of the Bloch vectors involved. We also discussed why a nonlocal operation if applied cannot disentangle an arbitrary state better than ours.

E.1.5. Teleportation and Secret Sharing with Pure Entangled States : Somshubhro Bandyopadhyay

It is well known that an arbitrary qubit cannot be teleported with perfect fidelity if the quantum channel is a non-maximal entangled state. But there is of course a possibility to achieve perfect teleportation with a certain finite probability via any pure entangled state. We present a qubit assisted teleportation protocol with any pure entangled state which is optimal in the sense that it provides the highest probability with which perfect teleportation is possible with any pure entangled state.

E.1.6. Qubit assisted Optimal Entanglement Concentration: Somshubhro Bandyopadhyay

We now understand that for perfect quantum communication purposes, for example, quantum teleportation or secure key distribution, it is absolutely necessary to work with maximally entangled states. Thus the situation calls for methods which can concentrate entanglement from a finite ensemble of pure entangled states or can distill entanglement from a finite ensemble of mixed states. In the present work we propose a state dependent protocol for concen-

trating entanglement from a finite ensemble of pure states. We have shown that our method concentrates entanglement optimally, in the sense maximum fraction of singlets can be obtained.

F. CONDENSED MATTER (THEORY) AND STATISTICAL PHYSICAL

F.1.1. Quantum antiferromagnets and strongly correlated systems : **Indrani Bose** and Emily Chattopadhyay

Haldane gap antiferromagnets are integer spin systems which have a gap in the excitation spectrum. Y_2BaNiO_5 is a linear chain $S = 1$ Haldane-gap antiferromagnet which can be doped with holes by replacing some of the Y ions by Ca ions. The holes are mobile in the chain. Experiments provide evidence for new states appearing in the gap on Ca doping. The dynamic structure factor has two peaks. We have used the matrix product formalism to study this problem. The hole excitation spectrum has been determined analytically. Work on the dynamic structure factor is in progress. The possibility of doping induced magnetization plateaux is under investigation. Y_2BaNiO_5 provides the first example of a doped quantum spin liquid in one dimension.

Quantum phase transitions occur at $T = 0$ as a function of some parameter. At a critical value of the parameter, the nature of the ground state changes. In the critical region, various ground state properties exhibit power-law behaviour. We have studied two antiferromagnetic spin models in two dimensions and studied quantum phase transition in these systems. In one phase, the excitation spectrum is gapped and the ground state is disordered. In the other phase, long range magnetic order exists and the spectrum is gapless. We have calculated the critical exponent associated with the vanishing of the gap.

F.1.2. Cross-disciplinary physics: **Indrani Bose** and Indranath Chaudhuri

The earlier work on bacterial evolution has been extended. A recent experiment on bacterial evolution has quantified the roles of chance (mutations), history and adaptation in bacterial

evolution. We have constructed a model which can reproduce the experimental results in a qualitative manner. Work is in progress on establishing connections between our model and the usual population genetics approaches.

PUBLICATIONS

- Roy S C 1999 Elastic scattering of photons: Perspectives and present status, *Proceedings of the European Conference on Energy Dispersive X-ray Spectrometry 1998 (EDXRS-98)* (Edited by J.E. Fernandez and A. Tartari) pp. 31-36.
- Roy S C, Kissel Lynn and Pratt R.H. 1999 Elastic scattering of photons; *Radiation Physics and Chemistry* **56**, 3.
- Bradley David, Roy S C, Kissel Lynn and Pratt R H 1999 Anomalous scattering effects in elastic photon-atom scattering from biomedically important elements; *Radiation Physics and Chemistry* **56**, 76.
- Roy S C 1999 Elastic scattering of photons: Perspectives and present status; *X-ray Spectrometry* **28**, 376.
- Das Mala, Roy B, Chatterjee BK and Roy S C, 1999 Superheated drop neutron spectrometer; *IRPS Bulletin*, **13**, pp. 3-4 (September issue).
- Engineer M H and Roy B 2000 Three dimensional viscous fingering instability; in *Nonlinear Dynamics Integrability and chaos* (Eds. M. Daniel, K. M. Tamizhmani and R. Sahadevan) Narosa Publishing House, 421.
- Banerjee S, Ghosh SK, Raha S and Syam D, 1999 Strangelets in terrestrial atmosphere; *Journal of Physics* **G25** L15
- Bhattacharyya A, Ghosh SK and Raha S, 1999 ρ mass modification in He^3 - a signal for restoration of chiral symmetry or test for nuclear matter models?, *Physical Review* **C60** 081202
- Raha S and Syam D, 1999 Finite Size Effects in multiparticle production processes; in: *The State of Physics at the end of the 20th Century*, (Eds. F. Cooper, I. Sarcevic, C.-I. Tan and G. West), p. 125, *World Scientific*, Singapore.
- Bhattacharyya A, Alam J, Sarkar S, Roy P, Sinha B, Raha S and Bhattacharjee P, 1999

Cosmological QCD phase transition and dark matter ; Nuclear Physics **A661** 629

11. Banerjee S, Ghosh SK and Raha S, 2000 The Chandrasekhar limit for quark stars; *Journal of Physics* **G26** L1

12. Bhattacharyya A, Alam J, Sarkar S, Roy P, Sinha B, Raha S and Bhattacharjee P, 2000 Relics of the Cosmological QCD Phase Transition; *Physical Review* **D61** 083509.

13. Bose I and Gayen S 1999 Exact ground and excited states of a t-J ladder doped with two holes; *J. Phys. : Cond. Matter* **11** 6427.

14. Bose I and Chaudhuri I 1999 Punctuated equilibrium in an evolving bacterial population; *Physica* **A270** 63.

15. Bose I and Chattopadhyay E 1999 Mixed-spin systems : Coexistence of the Haldane gap and antiferromagnetic long range order; *Phys. Rev.* **B60** 12138.

16. Bose I 2000 Lattice animals and percolation model under rotational constraint; in *Percolation theory and particle systems*, (Ed. Rahul Roy) *The Indian Academy of Sciences, Bangalore and Universities Press, Hyderabad* 91.

17. Bose I and Chaudhuri I 2000 Pattern formation in reaction-diffusion systems; in *Nonlinear Dynamics Integrability and Chaos*, (Eds. M. Daniel, K. M. Tamizhmani and R. Sahadevan) Narosa, New Delhi 355.

18. Home D 2000 Modern perspectives on foundations of quantum mechanics; in *Quantum field theory - a twentieth century profile* (ed. AN Mitra), *Indian National Science Academy, New Delhi*.

19. Home D 1999 Determination of when an outcome is actualised in a quantum measurement using DNA-photolyase system in *Quantum Queries* (ed. R Nair), *Kluwer Academic-Plenum Publishers, New York and Dordrecht*.

20. Saha Swapan K, Daehnick W.W, Flamang R.W, Balewski J.T, Doskow J, Mayer H.O, Pollock R.E, Przewoski B.V, Rinckel T, Thoerngren-Engblom P, Haeberli W, Lorentz B, Rathmann F, Schwartz B, Wise T and Pancella P.V, 1999 Spin correlation coefficients in $pp \rightarrow p\pi\pi^+$ from 325 to 400 MeV; *Physics Letters* **B461**, 175.

21. Meyer H.O, Balewski J.T, Doskow J, Pollock R.E, Przewoski B.v, Rinckel T, Thoerngren-Engblom P, Wellinghausen A, Haeberli W, Lorentz B, Rathmann F, Schwartz B, Wise T, Daehnick W. W, Saha Swapan K and Pancella P.V, 1999 Measurement of Partial-Wave Contributions to $pp \rightarrow pn\pi^0$; *Physical Review Letters* **83**, 5439.

22. Rinckel T, Thoerngren-Engblom P, Meyer H.O, Balewski J.T, Doskow J, Pollock R.E, Przewoski B.v, Sperisen F, Daehnick W.W, Flammang R.W, Saha Swapan K, Haeberli W, Lorentz B, Rathmann F, Schwartz B, Wise T and Pancella P.V, 2000 Facility for studying spin dependence of pion production near threshold; *Nuclear Instruments & Methods* **A439**, 117.

23. Thoerngren-Engblom P, Meyer H.O, Balewski J.T, Daehnick W.W, Doskow J, Haeberli W, Lorentz B, Pancella P.V, Pollock R.E, Przewoski B.v, Rathmann F, Rinckel T, Saha Swapan K, Schwartz B, 2000 Wellinghausen A and Wise T, Initial singlet and triplet spin state contributions to $pp \rightarrow pn\pi^0$; *Nuclear Physics* **A663**, 447c.

24. Shannigrahi S.R, Choudhary R.N.P, Acharya H.N and Sinha T.P, 1999 Phase transition in sol-gel derived Na-modified PLZT ceramics; *J. Phys. D: Appl. Phys.* **32**, 1539.

25. Shannigrahi S.R, Choudhary R.N.P, Acharya H.N and Sinha T.P, 1999 Structural, electrical and piezoelectric properties of rare-earth doped PZT ceramics; *Indian J. Pure Appl. Phys.* **37**, 359.

26. Saha Sonali, Sinha T.P and Mookerjee Abhijit, 2000 Structural and optical properties of paraelectric SrTiO_3 ; *J. Phys.: Condens. Matter* **12**, 3325.

Article :

Home D 10 March 2000 **Conundrums in Copenhagen**, The Times Higher Education Supplement, London.

Participation in Conferences/ Symposia/ Training programmes/ Awards and Honours received :

Prof. S.C. Roy worked in the Medical Physics Department of Tom Baker Cancer

Centre, Calgary, Canada as a Visiting Professor for ten weeks during July to September 1999.

Worked in the Department of Physics and Astronomy of the University of Pittsburgh in the cooperative research programme on elastic scattering of photons for two weeks during September-October 1999.

Delivered an invited presentation and a poster presentation in the 4th Topical Meeting on Industrial Radiation and Radioisotope Measurement Applications (IRRMA99) held in Raleigh, North Carolina during October 4-7, 1999.

Attended the Executive Council Meeting of the International Radiation Physics Society held in Pittsburgh during October 8-9, 1999.

Prof. M. H. Engineer was an invited participant in the First Winter Institute on Foundations of Quantum Physics and Quantum Optics, S. N. Bose National Center for Basic Sciences, Calcutta, 1-15 January, 2000.

Was an invited participant at the one day Symposium on Biology at the S. N. Bose National Center on Basic Sciences, Calcutta.

Was invited to visit the Physics Department, Univ. of Pune and the Physical Research Laboratory, Ahmedabad during the first two weeks of January 2000, and gave seminars on his laboratory's work on the generation of nonlinear structures in fluid interfaces by acoustic waves.

Prof. Sibaji Raha acted as the Member Secretary for the National Task Force for Science at High Altitude.

Delivered an invited plenary talk at the International Workshop on Hadron Physics 1999 at Coimbra, Portugal during September 1999.

Visited the Institute of Theoretical Physics, University of Giessen, Germany and delivered a colloquium in September 1999.

Visited the University of Marburg, Germany for collaborative research in September 1999.

Visited the Physics Department, Brookhaven National Laboratory, USA during December 1999 - February 2000 for collaborative research. Delivered a colloquium there in February 2000.

Delivered a colloquium at the Physics Department, University of Pittsburgh, USA in February 2000.

Delivered a colloquium at the Physics Department, University of Connecticut, USA in February 2000.

Visited the Physics Department, Purdue University, USA in February 2000 and delivered two seminars.

Visited the Physics Department, University of Virginia, USA and delivered a colloquium in March 2000.

Served as expert in various assessment / selection committees in other institutions.

Prof. Indrani Bose has been elected a Fellow of the Indian Academy of Sciences, Bangalore.

Has been invited to give talks at the Discussion Session on Quantum Spin Chains and Ladders at the International Centre of Theoretical Physics, Trieste, Italy (July, 1999).

Discussion Meeting on Recent Trends in Non-equilibrium Statistical Physics at I.I.Sc., Bangalore (Nov. 24, 1999).

Discussion Meeting on Strongly Correlated Systems at I.I.Sc., Bangalore (Nov. 27, 1999).

K. S. Krishnan Birth Centenary Celebrations at Bidhan Nagar College, Calcutta (Dec. 3, 1999).

K.S. Krishnan Birth Centenary Symposium on Solid State Physics at I.A.C.S., Calcutta (Dec. 5, 1999).

Condensed Matter Physics Colloquium at S.I.N.P., Calcutta (Dec. 17, 1999).

The SERC School on Field Theories in Condensed Matter Systems (Feb.- March, 2000).

Advanced Solid State Physics M.Sc. Course of Calcutta University (Feb.- April, 2000).

Discussion Meeting on Field Theories in Condensed Matter Systems at the S.N.Bose National Centre for Basic Sciences (March, 2000).

Has served as a member of the Organising Committees of the One Day Symposium on Biology at the S.N.Bose National Centre for Basic Sciences (March, 2000).

Symposium on Bose-Einstein Condensation organised by the West Bengal Academy of Sciences and held at I.A.C.S., Calcutta (Feb., 2000).

The K.S.Krishnan Birth Centenary Celebrations held at I.A.C.S., Calcutta (Dec., 1999).

Prof. Dipankar Home presented an invited talk at the 8th UK Conference on Foundations of Physics, Imperial College, London, 13-18 September, 1999.

Presented an invited talk at the 1st Winter Institute on Foundations of Quantum Physics and Quantum Optics, Calcutta, 1-15 January, 2000.

Visited University of Oxford, Queen's University of Belfast, University of Cambridge, Birkbeck College, London and University of Southampton, during July-November, 1999 under INSA-Royal Society Exchange Programme and delivered a series of lectures on the Foundational Issues of Quantum Mechanics at these Institutes.

Dr. B. Roy presented a paper in the symposium "Condensed Matter Days-99" held at Jadavpur University, during Aug. 26-28, 1999.

Dr. T. P. Sinha participated in the SERC School on 'Electronic Structure and Physics of Materials' at S. N. Bose National Centre for Basic Sciences, Calcutta from 31st October to 19th November 1999.

Presented paper in the meeting "Condensed Matter Days - 1999" held at Department of Physics, Jadavpur University, Calcutta from 27th to 29th August 1999.

Dr. Sanjay Kumar Ghosh visited the Institute of Physics, Bhubaneswar during July 1999 and delivered a talk.

Mala Das participated in the "National Seminars on Nuclear Physics" held at IOP, Bhubaneswar, during July 26 - 29, 1999.

Presented a paper in the symposium "Condensed Matter Days-99" held at Jadavpur University, during Aug. 26-28, 1999.

Sonali Saha participated in the SERC School on 'Electronic Structure and Physics of Materials' at S. N. Bose National Centre for Basic Sciences, Calcutta from 31st October to 19th November 1999.

Presented paper in the meeting "Condensed Matter Days - 1999" held at Department of Physics, Jadavpur University, Calcutta from 27th to 29th August 1999.

GRANTS-IN-AID SCHEMES

Investigator/ Co-Investigator	Title	Financed by
Prof. S. C. Roy	Investigations on photon detector using superheated liquid and development of an educational kit to demonstrate through sight and sound the radiation interaction with matter.	DST, WB.
Dr. T.P. Sinha	Vibronic Coupling Effect on Fe-Ions in Insulators	CSIR

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

Dr. Sanjay Kumar Ghosh (till 3.9.1999), Mala Das, Indranath Chaudhuri (till 30.11.1999), Shibaji Banerjee, Som Shubhro Bandyopadhyay, Sonali Saha, Emily Chattopadhyay, Ujjwal Sen.

SUPPORTING STAFF MEMBERS

N. C. Dey, A. K. Ganguly, K. C. Das, S. P. Singha, Shyam Sundar Mallik, Subhasis Banerjee, Manas Datta, Subrata Das, Tarun Kumar Maji, B. P. Ram, B. Dutta, D. K. Thakur, S. K. Basu, R. K. Mourya, Prabir Halder.

Chemistry

Faculty : Prof. P. Chakrabarti, Prof. P. Bhattacharyya (Chairman from 11.08.99), Prof. Parimal C. Sen (Chairman till 10.08.99), Dr. Manas Chakrabarty, Dr. K.P. Das, Dr. Joyoti Basu, Dr. Manikuntala Kundu, Dr. Pradip Das

HIGHLIGHTS

Enrichment of medically important EPA and DHA has been made from sting Ray oil. Molecular chaperone properties of α -crystallin and its role in cataract formation is being studied. ATP-dependent drug extrusion systems in *M. avium* and *M. tuberculosis* have been characterised. Hepatoprotective role of the herb *Desmodium filmbriatum* has been demonstrated for the first time. A novel reagent has been developed for the N-formylation of (hetero)arylamines. The cholesterol ester of indole-3-acetic acid has been synthesized and its structure has been determined. A beneficial role of BIM in Cat II TB patients has been indicated. Mechanisms of antibiotic resistance in enteric bacteria have been elucidated.

INSTITUTIONAL PROJECT II

A. Analysis of Fatty Acid Composition and Oil Content in Different Tissues of Mango and *Madhuca indica* : Prantosh Bhattacharyya and Aniruddha Aich

Mango and *Madhuca indica* (locally known as Mohua) are non-traditional oil seed plants yielding high percentage of edible seed oil. Mango contains high percentage of stearic acid (~40%) whereas *Madhuca indica* contains high percentage of oleic acid (~40%). The main fatty acids in Mango Kernel is stearic acid (18:0) and oleic acid (18:1). Average total lipid content is gradually increased from 5%-15% (dry weight basis) from immediate stage. Neutral lipid is major lipid (~70%) containing high percentage of Triglycerides.

In other tissues like Bud, Flower, Leaves etc. stearic acid accumulation is very low (4%-6%) and main constituents are palmitic (~40%) and linoleic acid (~20%).

Madhuca indica contains high percentage of total lipid (10%-40%) of which neutral lipid is above 80%.

The fatty acid analysis of *Madhuca indica* Kernel shows palmitic acid (16:0, ~30%), stearic acid (18:0, ~15%), oleic acid (18:1, ~40%) and linoleic acid (18:2, 10%)

The accumulation of oleic acid in other tissues like Bud, Flower, Leaves are very low

than palmitic acid (16:0), stearic acid (18:0) and linoleic acid (18:2).

This shows that oleate rich fat is stored in seed tissue.

B. Enrichment of Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA) from String Ray Liver Oil after Urea Fractionation : Parimal C. Sen and Prasanta K. Pal

The Sting Ray liver oil is found to contain high level of unsaturated fatty acids. The oil was fractionated by varying urea concentration following the established method of the laboratory. It has been found that fractionation led to enrichment of EPA and DHA, two important unsaturated fatty acids of significant medicinal importance.

INSTITUTIONAL PROJECT III

A.1. Phosphorylation of Lens α -Crystallin Protein by Protein Kinase-C : Parimal C. Sen and Bhaswati Samanta in collaboration with K.P. Das

Protein Kinase-C isolated from rat brain cytosol is found to phosphorylate α -crystallin protein of bovine lens. The phosphorylation is stimulated by an endogenous protein present in the soluble fraction of lens extract.

A.2. Regulation of Ion Transporting Enzymes by Amphiphilic Drugs through Protein

Kinase-C : Parimal C. Sen, Atin K. Mandal, Koushik Roy and Subho Ghosh

In our earlier study, we have shown that ion transporting enzymes e.g. Na, K-ATPase, Ca, Mg-ATPase and Ca-ATPase activities were inhibited by two amphiphilic drugs-chloroquine and chlorpromazine both *in vitro* and *in vivo* in different organs of rat. We have also reported the presence of endogenous regulator proteins of these ion transporting enzymes. In an attempt to explore the mechanism of regulation, we have found *in vivo* study that protein kinase C activity is partially affected by these drugs without any effect on the modulator proteins. Since phosphorylation of the enzymes by PK-C are required for their function, it has been postulated that inhibition of enzymes activities are partly affected due to defective phosphorylation by protein kinase during drug treatment.

A.3. Purification, Characterization and Partial Amino Acid Sequencing of a 70 kD Inhibitor Protein of Na⁺, K⁺-ATPase from Goat Testis Cytosol : Parimal C. Sen, Atin K. Mandal and Koushik Roy (in collaboration with Parames C. Sil and Satya P. Yadav, Cleveland Clinic Foundation, U.S.A.)

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 column chromatography. The purified fraction appears as a single polypeptide band on 10% SDS-PAGE of approximate molecular mass of 70 kDa. The concentration at which 50% inhibition (I₅₀) occurs is in the nanomolar range. The inhibitor seems to bind Na⁺, K⁺-ATPase reversibly at ATP binding site. It does not affect *p*-nitrophenyl-phosphatase activity. The inhibitor is found to inhibit the phosphorylation step of the Na⁺, K⁺-ATPase. The enhancement of tryptophan fluorescence and changes in CD pattern suggest conformational changes of Na⁺, K⁺-ATPase on binding to the inhibitor. Amino acid sequence of the trypsinised fragments show some homology with aldehyde reductase.

B.1. Newer Syntheses of Pyrrolocarbazoles : Manas Chakrabarty, Shampa Khasnobis and Nandita Ghosh

The attempt, reported last year, to prepare pyrrolocarbazoles (PCs) through 9-methyl-3-formylcarbazole has progressed and is being pursued to bring it to completion. Additionally, two other routes, viz., DBU-mediated condensation of nitrocarbazole with ethyl isocynoacetate and photo- and palladium(0)-catalysed cyclisation of bromoanilinoindoles are being tried.

In this connection, the bicyclic amidine DBU has been established to be a novel reagent for the *N*-deacetylation and *N*-debenzoylation of *N*-acetyl/benzoyl carbazoles, indoles and nitroanilines.

B.2. A New Synthesis of Indolocarbazoles : Manas Chakrabarty and Ramkrishna Basak.

A new synthesis of the indolocarbazole skeleton is being attempted by using suitably modified indoles as both diene and dienophile in a Diels-Alder reaction. Preliminary studies seem to be encouraging.

B.3. Studies on New Reactions of Heterocycles : Manas Chakrabarty, Ramkrishna Basak and Sandipan Sarkar

The reaction of indole and its *N*- and *C*-alkyl derivatives with a cerium reagent on silica gel is being studied. Reaction with *N*-methyl indole led to three isomeric (MS) products which are being characterized.

B.4. Studies on Exciplex Energy - An Indicator of Dielectric Maximum of Magnetic Field Effect : Manas Chakrabarty and Nandita Ghosh (in collaboration with Dr. Samita Basu of Saha Institute of Nuclear Physics)

Carbazole and a number of its *N*-alkyl derivatives were prepared following standard procedure. These compounds were observed to form exciplexes with 1,4-dicyanobenzene. Magnetic field effect with these exciplexes was studied to find out the importance of different solvent parameters and other physical parameters of exciplexes on the spin-dynamics.

C.1. Studies on Molecular Chaperones: K. P. Das and Sudipa Saha

Mechanism of thermal activation of chaperone function of α -crystallin is being studied. It has been found that preheating of

α -crystallin enhances chaperone ability. Preheating also increases surface hydrophobicity. The number of hydrophobic binding sites and the affinity of binding are being determined by fluorimetric titration and Scatchard analysis. It has been found that most β -sheet proteins have similar thermal response. The thermal unfolding and refolding of some β -sheet proteins including α -crystallin are being studied to throw more light on their thermal behavior.

C.2. Secondary Structure of Aggregated Proteins: K. P. Das

Aggregates of various substrates of α -crystallin, namely, β -crystallin, γ -crystallin etc. are formed by subjecting these proteins to various stress conditions such as heat, strong uv light, oxidative stress, fast refolding from GuHCl etc. The FT-IR spectra of the amide I region of these aggregates are compared to that of the native substrate. The secondary structure of the native and aggregated proteins were determined by curve fitting of the amide I band. It is found that heating beyond 65°C lead to extensive unfolding and irreversible intermolecular hydrogen bond formation. However, aggregates formed by oxidative stress and uv radiation have native-like secondary structures. Aggregates formed by fast refolding from GuHCl retain ~50% secondary structure. Some non-natural substrates of α -crystallin will be subjected to similar study. This may provide clue to the mechanism of discrimination between natural and non-natural substrates by α -crystallin.

D. Structure and Function of the Human Erythrocyte Membrane : Joyoti Basu, Prasun Moitra and Debabrata Mandal

Evidence accumulated over the years suggests that human erythrocyte membrane protein 4.2 is one of the proteins involved in strengthening the cytoskeleton-membrane interactions in the red blood cell. Deficiency of protein 4.2 is linked with a variety of hereditary hemolytic anemia. However, the interactions of protein 4.2 with other proteins of the erythrocyte membrane remain poorly understood. Using gel renaturation assays, we found that protein 4.2 interacts with spectrin. A series of recombinant glutathione-S-transferase fusion proteins were constructed and their

binding with biotinylated spectrin was studied. The spectrin-binding domain of protein 4.2 mapped to a region encompassing residues 269 to 520 of protein 4.2.

E. Modulation of Phagocyte Signalling by Helicobacter pylori : Manikuntala Kundu, Debashis Maiti and Asima Bhattacharyya

Cytokines and chemokines have been proposed to play an important role in *Helicobacter pylori*-associated gastroduodenal diseases, but the exact mechanism of the cytokine or chemokine induction remains unclear. The close proximity of *H. pylori* to gastric mucosa suggests that the organism interacts with mononuclear cells, thereby modulating the inflammatory response. Gastritis involves the antrum, that is characterized by inflammatory cell infiltration with polymorphonuclear cell invasion of the gastric lamina propria and glandular epithelium. *H. pylori* induces expression of proinflammatory cytokines such as tumor necrosis factor alpha and interleukins- 1, 6, and 8. We propose to study monocyte cell signalling pathways and their modulation by *H. pylori* in relation to the secretion of IL 8 and other cytokines.

Akt/PKB is the major downstream target of receptor tyrosine kinases that signal via the PI3-kinase. Akt phosphorylates caspase 9, Bad, forkhead transcription factor and the IKK kinase (IKK) thereby activating NF-KB. Akt also regulates signalling via the MAP kinase pathway by phosphorylating Raf at serine-259. Our initial studies have shown that the water extract (which contains secreted proteins and lipopolysaccharide) of a *cagA*+ *H. pylori* strain, activates Akt kinase in a phosphatidylinositol 3-kinase-dependent manner.

F.1. Lyotropic Polymorphism in F-actin : Pradip Das and J. Roy

This work involving actin filaments shows that in physical gels in which fluctuational networking exists, small solvent molecules such as water may be involved in anisotropic interaction with one another and with the intrinsically mesogenic moieties such as α -helices and β -sheets to give thermal liquid crystal states. This *inter alia* means that polymer crystallization may proceed through a coupling between chain straightening

and intermolecular forces between the mesogenic units. Also, it is clear that small water molecules may be considered from the point of view of Shubnikov antisymmetry, a method that permits one to find a hidden symmetry whose breaking reveals itself directly at the glass transition or gel transition.

F.2. Molecular Theory of the Smectic-C Mesophase : Pradip Das and N. Chakrabarti

The McMillan model of the smectic-A phase with an orientational and a smectic order parameter is extended to the smectic-C phase by introducing a new order parameter. The latter represents the amplitude of a density wave perpendicular to the direction of the nematic preferred axis in the layer plane, in contrast to the density wave amplitude along the nematic preferred axis for the smectic-A phase. Numerical solution of the three self-consistency equations derived from an anisotropic model interaction leads to the order parameters versus temperature profile for several values of the dimensionless interaction strengths Y_0 and Y for the smectic-C phase. The transition temperatures plotted versus Y for a fixed Y_0 yields a theoretical phase diagram which resembles experimental plots of transition temperatures versus alkyl chain length for homologous series of compounds.

F.3. Thermotropic Mesomorphism in the Cholesteryl ester of Indole-3-acetic acid : Pradip Das and S. Basu

The cholesteryl ester of Indole-3-acetic acid has been synthesised. The structure has been determined by IR, NMR, Mass and Fluorescence Spectrometry. The Bravais lattice of the crystal has been determined from the powder diffraction pattern. The liquid crystalline states are being studied right now, as are the single crystals.

INSTITUTIONAL PROJECT V

A. Drug Targets and Drug Resistance Mechanisms in Mycobacteria : Parul Chakrabarti, Joyoti Basu, Manikuntala Kundu, Sanjib Bhakta, Baisakhee Saha

Bose Institute

Chaudhuri, Anuradha Bhaduri and Suman Kumar Singh

(i) Isoniazid accumulation in Mycobacterium avium is modulated by proton motive force-driven and ATP-dependent extrusion systems

M. avium is intrinsically resistant to isoniazid (INH), a frontline, antituberculosis drug. The mechanism of INH resistance is not understood. We demonstrate that INH accumulation in *Mycobacterium avium* is enhanced both upon addition of a proton motive force (pmf) uncoupler, carbonylcyanide *m*-chlorophenylhydrazone (CCCP) and upon addition of *ortho*-vanadate, an inhibitor of ATP-dependent efflux pumps. Taken together, the results provide evidence of the involvement of both pmf- and ATP-dependent extrusion systems in INH efflux in *M. avium*.

(ii) Cloning and overexpression of the components of a putative doxorubicin efflux pump of Mycobacterium tuberculosis

The *drxAB* genes encode DrrA and DrrB proteins which together function as a doxorubicin efflux pump, and share characteristics similar to the P-glycoprotein multidrug transporter. The DrrA protein harboring the nucleotide-binding domain of this ABC-type transporter has been cloned and overexpressed in *E. coli*.

The nucleotide-binding ability of DrrA has been confirmed. Low-level expression of the membrane protein, DrrB has been achieved in *E. coli* using controlled expression in the vector pBAD and arabinose as the inducer. Genetic manipulation to achieve dual expression of DrrA-DrrB from a polycistronic mRNA, is in progress.

(iii) Characterization of the high molecular mass penicillin-binding protein 1 of Mycobacterium leprae

Mycobacterium leprae has two high molecular mass multimodular penicillin-binding proteins (PBPs) of class A termed PBP1 and PBP1*. PBP1-X- β -lactamase fusions generated periplasmic β -lactamase when X (the amino acid residue at the fusion junction) was 314, 363, 407, 450 or 480. Truncation of the N-terminal amino acids upto residue L147 generated a

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penicillin-binding polypeptide which could still associate with the plasma membrane whereas (DM1-R314) PBP1 failed to associate with the membrane, suggesting that the region between residues L147 and R314 harbours an additional plasma membrane association site for PBP1. Truncation of the C-terminus upto 42 residues downstream of the KTG motif also generated a polypeptide retaining penicillin-binding activity. (DM1-R314). PBP1 could be extracted from inclusion bodies and refolded under appropriate conditions to a form capable of binding penicillin with the same efficiency as the full-length PBP1. This is, to the best of our knowledge, the first of a soluble derivative of a penicillin-resistant high molecular mass PBP of class A of binding penicillin. A chimeric PBP in which the penicillin-binding (PB) module of PBP1 was fused at its N-terminal end to the non-penicillin-binding (n-PB) module of PBP1*, retained penicillin-binding activity similar to PBP1, corroborating the finding that the n-PB module of PBP1 is dispensable for its penicillin-binding activity.

B.1. Immunotherapy of Drug Resistance Tuberculosis : Debasis Ghoshal and Prantosh Bhattacharyya (in collaboration with Dr. S.K. Singh and Dr. K.C. Roy, Bagh Bazar Chest Clinic, Bagh Bazar, Calcutta)

Drug Resistance Tuberculosis is on the rise. On an average a DRT patient has to spend Rs.8,000/- p.m. for 12-18 months to treat the disease. The objective of the current work is to provide an Immunotherapeutic protocol that would enhance the immune system of the patient and thereby in presence of Anti Tubercular Drug can mount an effective fight against the bacilli. Out of 1102 patient that attended the out patient department 54.5% were suspected of pulmonary TB and their sputum were tested. Of these 28.3% patient showed presence of AFB. Sixty percent of these patients were virgin and Cat I treatment was introduced. The remaining 40% were either defaulter of Cat I or have relapse of the disease. Approx. 9% of Cat I patient showed failure and Cat II treatment was introduced. After 3 months of Cat II treatment when all these patients still showed AFB in their sputum immunotherapy with BIM via nasal route was introduced. All the patients became sputum negative within 75 days of

introduction of immunotherapy. In contrast 10% of control Cat II patients either on ATD or ATD + nasal vehicle control treatment were sputum AFB positive. The report indicates that immunotherapy with BIM in Cat II TB patients may have a beneficial role. Those patients on BIM and have responded to the therapy showed neutropenia, have eosinophilia and lymphopoiesis. The upsurge of eosinophil seems to be a hall mark of responding DRTB patients on Immunotherapy and ATD drug.

B.2. Immunotherapy of Patients suffering from Carcinoma : Debasis Ghoshal and Prantosh Bhattacharyya

BIM immunotherapy via nasal route along with chemotherapy is underway in patients suffering from carcinoma. In a seminoma patient with metastasis, as observed in abdominal CT scans, the β hCG marker was 9510 IU/ml prior operation. After removal of the tumor and β hCG measured after 7 days post operation the titre was > 9000 IU/ml. Chemotherapy with carboplatin, etoposide and Bleomycin was initiated along with BIM immunotherapy via nasal route. After 6 CT cycle the β hCG level was reduced to < 24 IU/ml and after 4 months of continuous BIM post CT the β hCG was reduced to 0.5 IU/ml. The patient is now doing his normal duties (in collaboration with Dr. Ketaki Moitra).

Two other carcinoma patients, one with Renal Cell Carcinoma with metastasis and another with Colon Carcinoma, are undergoing BIM immunotherapy along with CT. The important improvements observed in these patients, along with all other patient treated with BIM, are (1) suppression of pain, nausea and vomiting (2) lymphocytosis and (3) leukopoiesis.

In a separate set of experiment herbal treatment of the above mentioned disease conditions are being carried out in collaboration with Dr. Joydeb Chattopadhyay, Southern Health Improvement Samity, Bhargar.

C.1. Microbial Transformation of Condensed Heterocycles : Manas Chakrabarty and Subho Ghosh (in collaboration with P.K. Chakrabarty of the Department of Microbiology)

In this D.B.T.-P.D.F. programme, a few C-alkyl and N-alkylcarbazoles were fed to several

bacteria and one fungus. One of the substrates was found to undergo oxidative transformation by the fungus. The metabolite has been identified spectroscopically. Additionally, a particular pyrrolocarbazole, synthesised through a new route, was fed to the aforesaid microbes. There was no change whatsoever of the substrate.

C.2. Studies on the Immunomodulatory Property of an Indian Medicinal Plant : Manas Chakrabarty, Goutam K. Datta and Subho Ghosh (in collaboration with Prof. P.K. Ray, Environmental Science Section and Prof. P.K. Deb Nath, J.B. Roy State Ayurvedic Medical College, Calcutta)

As a part of our ongoing programme on the search for immunomodulators from Indian medicinal plants, the aq. alcoholic extract of the whole plant of the herb *Desmotrichum fimbriatum*, henceforth called the drug, was screened for its hepatoprotective activity under chemical-induced and psychophysiological stress using mice as models. The drug elevated the levels of lipid peroxidase, catalase and glutathione, and also restored the histoarchitecture of the liver of the mice. The hepatoprotective role of the drug was thus experimentally demonstrated for the first time.

D. Antibiotic Resistance Mechanisms in Enteric Bacteria : Manikuntala Kundu, Debashis Maiti and Somesh Baranwal

Clinical isolates of *Shigella dysenteriae* and *Vibrio cholerae* have been obtained from the National Institute of Cholera and Enteric Disease, Calcutta. Antibiotic resistance-conferring plasmids from several of these strains have been transferred to *E. coli*, and the integrons associated with antibiotic resistance are being characterized in detail. One of the isolates of *S. dysenteriae* has shown the presence of a novel integron harboring two beta-lactamase genes, one of which encodes for a SHV-11 beta-lactamase. The *V. cholerae* isolates characterized so far have shown the presence of integrons carrying streptomycin, chloramphenicol and gentamycin resistance.

The quinolone-resistance-determining region (QRDR) of the *gyrA* and *parC* genes which are involved in quinolone resistance of gram-

negative bacteria, have been amplified by PCR from the chromosomal DNA of *V. cholerae* and are being sequenced, in order to analyze mechanisms of quinolone resistance.

PUBLICATIONS

1. Ray S & Chakrabarti P 1999 Altered Lipid Peroxidation and antioxidant potential in human uterine tumors; *Ind. J. Exp. Biol.* **37**, 439-443.
2. Datta S, Sinha S & Bhattacharyya P 1999 Hepatoprotective activity of a herbal protein CI-1, purified from *Cajanus indicus* against b-galactosamine HCl toxicity in isolated rat hepatocytes; *Phytotherapy Research* **13**, 508.
3. Datta S, Sinha S & Bhattacharyya P 1999 Effect of a herbal protein, CI-1, isolated from *Cajanus indicus* on immune response of control and stressed mice; *Journal of Ethnopharmacology* **67**, 259.
4. Datta S, Sinha S & Bhattacharyya P 1999 Effect of a herbal protein, CI-1, purified from *Cajanus indicus*, in models of liver failure in mice; *Drug Development Research* **48**, 76.
5. Bhattacharyya D and Sen PC 1999 The effect of binding of chlorpromazine and chloroquine to ion transporting ATPases; *Mol. Cell. Biochem.* **198**, 179-185.
6. Bhattacharyya D and Sen PC 1999 Interaction of chlorpromazine with low-molecular-mass ion transporting ATPase regulator proteins from rat brain cytosol; *Ind. J. Biochem. Biophys.* **36**, 82-87.
7. Sikdar R, Mandal A, Ray K and Sen PC 1999 Phosphorylation and dephosphorylation of Mg-independent Ca-ATPase from goat spermatozoa; *J. Biosc.* **24**, 317-321.
8. Roy K, Mandal AK, Sikdar R, Majumdar S, Ono Y and Sen PC 1999 Unsaturated fatty acid activated protein kinase (PKx) isolated from goat testis cytosol; *Biochim. Biophys. Acta* **1434**, 161-169.
9. Das T, Sa G, Subhalakshmi V, Subramaniam S, Sen PC, Biswas S and Ray PK 2000 Protein A activated rat splenic lymphocyte proliferation involved tyrosine kinase-phospholipase C-protein kinase C

pathway. *Innopharmacol. Immunotoxicol.* **22**, 75-90.

10. Ghosh S, Sengupta S, Chakrabarty M and Ghosh A 1999 Action of lithium on chromaffin cells of the domestic pigeon (*Columba livia*); *The Nucleus* **42**, 28-33.

11. Chakrabarty M, Khasnobis S, Harigaya Y and Konda Y 2000 Neat formic acid : an excellent N-formylating agent for carbazoles, 3-alkylindoles, diphenylamine and moderately weak nucleophilic anilines; *Synth. Commun.* **30**, 187-200.

12. Bhaduri A & Das KP 1999 Desorption of lysine modified and unmodified proteins from solid surfaces -elution of β -lactoglobulin adsorbed on alumina and silica; *J. Dispersion Sci. Technol.* **20**, 1125-1142.

13. Bhaduri A & Das KP 1999 Proteins at solid water interface - a review; *J. Dispersion Sci. Technol.* **20**, 1097-1124.

14. Bhattacharyya J & Das KP 1999 Molecular chaperone-like properties of an unfolded protein: α_s -casein; *J. Biol. Chem.* **274**, 15505-15509.

15. Bhattacharyya J & Das KP 1999 Effect of surfactant on the prevention of protein aggregation during unfolding and refolding processes - comparison with molecular chaperone α -crystallin; *J. Dispersion Sci. Technol.* **20**, 1163-1178.

16. Das KP, Choo-Smith LP, Petrash MJ & Surewicz WK 1999 Insight into the secondary structure of non-native proteins bound to a molecular chaperone α -crystallin: an isotope edited infrared spectroscopic study; *J. Biol. Chem.* **274**(47), 33209-33212.

17. Ahamed J, Gangopadhyay J & Kundu M 1999 Mechanisms of quinolone resistance in clinical isolates of *Shigella dysenteriae*; *Antimicrob. Agents Chemother.* **43**, 2333-2334.

18. Ahamed J & Kundu M 1999 Molecular characterization of the SHV-11 β -lactamase of *Shigella dysenteriae*; *Antimicrob. Agents Chemother.* **43**, 2081-2083.

19. Das P, Xu J, Roy J and Chakrabarti N 1999 Liquid Crystal Polymorphism in F-actin : Optical Microscopic and Rotatory Dispersion Studies; *J. Chem. Phys.* **111**, 8240-8250.

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

Prof. P. Chakrabarti

Delivered an invited talk on "drug targets in the mycobacterial cell surface" in the 5th Seminar on Biochemistry and Biophysics organised by the West Bengal Academy of Science & Technology in May, 1999 in Calcutta; Delivered an invited talk on "Mycobacterial Lectin" in the INTERLEC meeting in August, 1999 in England; Delivered an invited talk on "Drug targets and Drug resistance mechanisms in mycobacteria" in Schering Plough Research Corporation, New Jersey, USA in September, 1999; Participated on invitation in the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy in September, 1999, in San Francisco, USA; Conducted a Workshop on "Man, Microbes and Environment" organised by Jagadis Bose National Science Talent Search in May, 1999 in Calcutta.

Prof. P. C. Sen

Has been elected Fellow of the National Academy of Sciences, India; Represented Bose Institute in DBT group monitoring meeting of PD Training Programme at Ahmedabad; Acted as an expert at IISc, Bangalore, for the selection of PD Fellows under DBT PD Training Programme; Taught a course at Calcutta University on Enzymology in the orientation course for College Teachers.

Dr. M. Chakrabarty

Dr. Manas Chakrabarty along with Ms. Nandita Ghosh participated in (i) the "International Conference on Chemistry & 36th Annual Convention of Chemists" held at Calcutta during December 11-16, 1999; (ii) "Symposium on Emerging Trends in Organic Chemistry" held at I.A.C.S., Calcutta during January 13-14, 2000; (iii) "4th National Conference of Indian Society of Chemists and Biologists" held at C.D.R.I., Lucknow during January 28-30, 2000 and (iv) the Royal Society of Chemistry "Symposium on Chemistry in the New Millennium" held at I.A.C.S., Calcutta during February 17-19, 2000 and (v) "Workshop on Biomolecular N.M.R. Spectroscopy" held at

Bose Institute during February 14-22, 2000. Dr. M. Chakrabarty have taught as a Guest Lecturer in the (i) B.Tech. 1st Year Class in Polymer Science and Technology at Calcutta University, Calcutta and (ii) M.Sc. Part II Class in Chemistry at Presidency College, Calcutta during 1999-2000. Dr. Manas Chakrabarty acted as (a) an Examiner and Viva-voice Examiner of two Ph.D. dissertations, (b) a Member of an Award Committee of a University, (c) a Referee for the assessment of a Scientist of a CSIR Institute and (d) Examiner of J.B.N.S.T.S. exams and (e) as a Resource Person, delivered a lecture on 'marine molecules' at the Refresher Course of Aead. Staff. College, Cal. Univ. in Feb., 2000

Dr. K.P. Das

Visited the laboratory of Prof. Witold K. Surewicz, Case Western Reserve University, Cleveland, Ohio, USA from November 15, 1998 to May 28, 1999 on invitation as a visiting scientist; Gave an invited talk on "Protein-protein interaction" at the 9th biennial conference of the Indian Society for Surface Science and Technology held at Kalyani University in 2-4 November 1999; Participated in the symposium on protein structure and folding held on 30th August at Bose Institute and gave a talk entitled

GRANTS-IN-AID SCHEMES

Investigator/ Co-investigator	Title	Financed by
Prof. Parul Chakrabarti Dr. Manikuntala Kundu	Molecular Basis of Non-specific Resistance in Mycobacteria : Role of Efflux Pumps	DBT
Prof. Parul Chakrabarti Dr. Joyoti Basu	Drug Permeation through Mycobacterial Cell Wall	DST
Prof. Parimal C. Sen	Amphiphilic drug induced alteration of ion transporting ATPase activities modulated through protein kinases and endogenous regulators	CSIR
Prof. Parimal C. Sen Dr. Kalipada Das	Studies on the role of phosphorylation on the molecular chaperone function of α -crystallin and cataract formation	DST

"Structural features of substrate proteins bound to molecular chaperone α -crystallin".

Dr. J. Basu

Delivered lectures on invitation in the Satellite symposium on Membranes, sensors and cell surfaces held in CCMB, Hyderabad in September, 1999; The Annual Meeting of the Indian Society of Cell Biology in CCMB, Hyderabad in November, 1999; The First Dorabji Tata Memorial Symposium on Tuberculosis at the Indian Institute of Science, Bangalore in February, 2000; Participated in the Gordon Research Conference on Red Cells in Tilton School, New Hampshire in July, 1999.

Dr. M. Kundu

Delivered a lecture in the CSIR project monitoring workshop of Medical Sciences Research in November, 1999.

Ms. Nandita Ghosh presented a paper at the 4th National Conference of I.S.C.B. held at C.D.R.I., Lucknow in January, 2000.

Lecture delivered by visiting scientists :

Prof. Jean-Marie Ghuysen, University of Liege, Belgium delivered a lecture on "Proteomics and bacterial wall peptidoglycan assembly" on February 11, 2000.

Investigator/ Co-investigator	Title	Financed by
Dr. Kalipada Das	Conformational specificity of α -crystallin for its function as molecular chaperone	CSIR
Dr. Joyoti Basu Dr. Manikuntala Kundu Dr. Manikuntala Kundu	Protein palmitoyl transferase : a potential regulator of dynamic protein palmitoylation Role of outer membrane proteins in drug resistance in <i>Shigella dysenteriae</i>	DBT CSIR
Dr. Joyoti Basu Dr. Manikuntala Kundu	Molecular analysis of the functional role of palmitoylation of human erythrocyte membrane protein 4.2	Indo-French
Dr. Joyoti Basu	Structural and functional analysis of a high molecular mass class A penicillin-binding protein in <i>Mycobacterium leprae</i>	CSIR
Dr. Manikuntala Kundu	Mechanisms of beta-lactam resistance in <i>Shigella dysenteriae</i> .	DST

PH.D. AWARDED

Name	University	Year	Title	Supervisor
Sanjay Mukhopadhyay	Calcutta	2000	Studies on Potential Drug Targets Sites of Mycobacterial Cell Surfaces	Prof. P. Chakrabarti
Alak Kanti Kar	Jadavpur	2000	Combating shigellosis through an understanding of the role of and some membrane proteins in drug resistance in <i>Shigella</i> .	Prof. P. Chakrabarti Dr. M. Kundu
Koushik Roy	Jadavpur	1999	Regulation of ion transporting enzymes by protein kinase and regulator protein	Prof. P.C. Sen
Atin K. Mandal	Jadavpur	2000	Interaction of ion transporting enzymes with protein kinase and endogenous regulator protein	Prof. P.C. Sen
Chaitali Bhatta-charjee	Jadavpur	1999	Studies on the structural stability, unfolding and refolding of b-lactoglobulin	Dr. K.P. Das
Raja Bhatta-charyya	Jadavpur	1999	Protein fatty acylation in the erythrocyte membrane	Dr. J. Basu

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

Shampa Khasnobis (till 02.08.99), Atin K. Mandal (till 04.11.99), Nilanjana Chakrabarti, Kausik Ray (till 23.09.99) Debasish Maity, Sanjib Bhakta, Sandipta Bhattacharyya (till 13.04.99), Nandita Ghosh, Baisakhee Saha Chowdhury, Somesh Baranwal, Debabrata Mandal, Suman K. Singh, Bhaswati Samanta, Sudipa Saha, Asima Bhattacharyya, Ramkrishna Basak, Debasish Roy (till 22.02.2000), Dr. Debasish Ghoshal, Dr. Jaya Bhattacharyya (till 30.04.99), Dr. Prasun K. Moitra, Dr. Subho Ghosh, Dr. Anuradha Bhaduri (upto 11.06.99), Dr. Sandip Ghosh (till 29.10.99) Dr. Anupa Bhattacharyya (till 30.04.99).

SUPPORTING STAFF MEMBERS

Nirmal K. Das, Prasanta Pal, Aniruddha Aich, Sukumar Maitra, Gopinath Mukherjee, Paresh Bhattacharyya, Pranab K. Dey, Bhabani Prasad Banerjee, Chittaranjan Ghosh, (till 30.09.99) Debdas Nandy, Rama Mukherjee, Gopal K. Biswas, Tapas Ghosh, Arindam Basu, Dipak Chandra Konar, Subhas Chandra Paul, Amarnath Bhatta, Kodan Das (till 18.05.99), Asoke K. Maity, Sachchidanand Ram, Kalyan Das.

Botany

Faculty : Prof. S. Gupta (Chairman, upto 30-11-1999), Prof. S.N. Ganguly (superannuated on 30-11-1999), Prof. B. Ghosh, Prof. K.K. Mukherjee (Chairman from 1-12-1999), Prof. S. Sen-Mandi, Dr. B.B. Mukherjee, Dr. P.K. Saha, Dr. D.N. Sengupta, Dr. S. Bhattacharya, Dr. T.K. Ghose, Dr. S. Gupta-Bhattacharya

HIGHLIGHTS

ABRE-based promoter has been constructed and to overexpress Samdecarboxylase, the cDNA has been ligated into Ti-based plasmid. Polyamine induced phosphorylation of P42 has been detected from rice seedlings. To inhibit ethylene biosynthesis in fruit transgenic tomato plants have been generated. *Agrobacterium* mediated transformation work on rice has been initiated. Protocols for PCR amplification of aromatic rice genomic DNA has been standardized. A number of *in vitro* grown plantlets of Teak, Kalmegh and *Ambroma augusta* were transferred to The Department of Science and Technology, Govt. of West Bengal. A patent application on tissue culture methodology has been filed. The allergenic protein from pollen of *Catharanthus* molecular weight 15i-200 kDA, have been indentified. Work on use of remote sensing data in understanding coastal erosion have been initiated.

INSTITUTIONAL PROJECT 1

A.1. Salinity Stress and Gene Expression in Rice : **Dibyendu N. Sengupta**, Kakali Banerjee and Abdur Rouf

As reported earlier regarding expression of N-terminal end of OSBZ8 factor which binds to ABA inducible promoter, ABRE, we have continued our effort and purified the fusion protein (GST:N-terminal OSBZ8) from *E.coli* cells by using glutathione-sepharose. The purified protein will be used to generate antibody against partial (N-terminal end) of OSBZ8 factor. The synthetic promoter 4XABRE:35S -40 has been subcloned in Ti based vector pBI121 and transformation of tomato cotyledon explants has been started using *Agrobacterium* LBA4404 .From the last year's PCR amplified rab16A gene, the promoter region was amplified and then subcloned in pBSKS for sequence verification. Both 4XABRE and Rab16A promoter will be used to monitor the expression of GFP in tomato plants in response to abiotic stress.

A.2. Studies on DNA Polymerase from Rice: **Dibyendu N. Sengupta**, Sankar Bakshi, Sailendra Nath Sarkar

In order to identify the presence of DNA polymerase activity in shoot tips of germinated rice seedlings, an assay system was developed by monitoring the incorporation of α -³²P-dAMP into TCA insoluble DNA by S₁₀ using activated DNA as template primer in presence or absence

of DNA polymerase inhibitor ddTTP. This will help us to quickly identify the level of DNA polymerase active enzyme from small amount of tissue. From published partial amino acid sequence of wheat DNA pol b, degenerate oligonucleotide was synthesized and used in RT-PCR along with oligodT as 3'-primer to amplify cDNA for DNA polb from poly A+RNA. A product of 300 bp fragment (unexpected) was obtained. The PCR product will be verified by DNA sequencing.

A.3. Mechanism of Polyamine Action and Molecular Cloning of cDNAs for the Enzymes of Polyamine Biosynthesis : **Bharati Ghosh, Dibyendu N. Sengupta**, Papiya Roy, Saswati Laha, Kamala Niyogi

In DST, Govt of India , Project our goal is to find out the mechanism of polyamine (PA) under abiotic stress.

As previously reported , the membrane bound H⁺- ATPase was found to be inhibited under varying concentration of NaCl stress , and the same was found to be reversed by exogenous application of polyamines . Further work in this area pointed towards marked difference in H⁺- ATPase activity in salt tolerant and salt sensitive rice cultivars . Salt tolerant cultivars like Nonabokra having greater endogenous levels of higher polyamines showed much higher H⁺- ATPase activity as compared to salt sensitive cultivar M-1-48. Reversal of inhibition

under NaCl stress by polyamines (Spd & Spm) was also more effective in tolerant cultivars. Based on these observations, we are planning on electro-physiological studies of membrane bound ion channels of different cultivars under NaCl stress and the effect of polyamines on the same.

Besides the above, a 42kDa protein phosphorylation was detected from the root cytosolic fraction of both sensitive and tolerant rice cultivars. The protein was found to be induced under NaCl stress and polyamine treatment, induction of the 42kDa kinase was stronger in tolerant cultivars than the sensitive one. At present we are in the process of characterising the 42kDa kinase by in-gel kinase assay.

In DBT funded CPMB programme our goal is to overexpress S-adenosyl methionine decarboxylase [SAMDC] and Spermidine synthase [SPDS] in salt sensitive high yielding rice cultivars to make it tolerant. As mentioned in the last report we have amplified the cDNA for the enzyme Samdecarboxylase from total RNA of NaCl treated roots of Pokkali rice plants. The cDNA was cloned in pBSKS and verified by sequencing from two ends. The cDNA of rice SAMDC was subcloned into pBI121 for Agrobacterium mediated transformation.

The cDNA of rice SAMDC has been expressed in *E. coli* by subcloning in the expression plasmid pEZZ and the synthesis of Protein A: SAMDC fusion protein has been detected. The fusion protein was purified in large scale to generate antibody which will be essential to monitor the expression of transgene (SAMDC) when it will be introduced into rice, tomato or tobacco. Attempt is going on to amplify cDNA Spd synthase and Polyamine oxidase.

A.4. Delay of Fruit Ripening by Antisense RNA approach : **Bharati Ghosh, Dibyendu N. Sengupta,** Mausumi Sengupta, Pallav Kundu, Tapan Bera

Previously we have reported that in order to inhibit endogenous ethylene biosynthesis in tomato fruit we have amplified cDNA for ACS2 and constructed the expression system to produce antisense RNA of ACS2 in transgenic plant. Following Agrobacterium mediated DNA

delivery into tomato cotyledonary explants, transgenic tomato plants were obtained, although the frequency of regeneration was low. The young plants grew well in Kan⁺ medium and produced roots. DNA prepared from lamina of one of those lines showed the presence of transgene as revealed by PCR. Transformation experiment will be repeated to obtain more transformed lines and plants will be grown to obtain homozygous lines in R1 generation. Standardization of tissue culture of Banana shoot tips provoked us to perform Agrobacterium mediated delivery of the Kanamycin resistance and GUS marker genes. Experiments will be continued to regenerate whole plants to study delay of fruit ripening.

A.5. Towards Development of Transgenic Plants Expressing Protein of Immunological or Medicinal Importance: **Dibyendu N. Sengupta** and Chaitali Roy

Plants are now being used to produce protein of immunological or medicinal values and edible fruits like cucumber banana expressing such proteins have been produced in USA and found to be effective. Protein A, a membrane protein of *Staphylococcus aureus*, is wellknown as it binds to IgG. Dr.P.K.Ray and his group have shown that Protein A treatment induces production of several cytokines and gamma interferon in spleenocytes of rat, also reduces toxic effects of many organic solvent, drugs and carcinogenicity of many chemicals and tumorigenicity of cancerous cells.

Therefore, a project (in collaboration with Prof.P.K.Ray) has been started with a goal to express Protein A in fruits like cucumber, banana and in tobacco leaves. The partial gene for Protein A, the IgG binding domain has been amplified and subcloned into Ti-based plasmid. The recombinant plasmid has been introduced into *Agrobacterium* LBA4404 and tobacco leaf transformation has been done to check the ability of the construct to produce Protein A in tobacco leaves. Twenty different lines of plantlets regenerated and rooted plants are growing in Kanamycin containing medium. Tissue culture of Cucumber to get regeneration of whole plants from cotyledonary explants is under standardization, so that Agrobacterium containing same

DNA construct could be used to produce transgenic cucumber expressing Protein A in cucumber fruit.

The full length ORF of Gamma Interferon cDNA has been amplified by PCR using total cDNA mix of Human Spleen (Clonetechn) and subcloned into pBSKS to verify the authenticity by DNA sequencing from two ends. Gamma Interferon will also be expressed in transgenic fruit, as it is a wellknown immunomodulator.

B.1. Cytogenetical investigations on Beta spp. and Chenopodium spp.: **K. K. Mukherjee,** S. K. Mitra, G. Gangopadhyay, S. Das.

A system for somatic embryogenesis and plant regeneration of Indian spinach from embryogenic callus line was established. Nodular compact embryogenic callus derived from nodal explants of eight day old seedlings was induced on MS basal medium supplemented with NAA (1 mg/lit.) and BAP (1 mg/lit) while leaf derived callus induced on the same medium resulted in soft, friable but non-embryogenic callus. The embryogenic callus line showed slower growth rate in comparison to the non-embryogenic one. Scanning electron microscopy of the embryogenic callus line revealed somatic embryos of all characteristic developmental stages. Thirty one percent embryogenic calli showed regeneration of plantlets only after a treatment with Tri Iodo Benzoic Acid (TIBA), followed by sequential transfer to hormone free media. Seventy five percent of the regenerated plantlets survived after transplanting in humidity tent.

On the basis of morphological characteristics of the four genotypes under study (*C. album*, hexaploid, broad leaved and narrow leaved diploid; and *C. murale*) data were scored on the basis of the collection of the first year. Using the pairing affinity values of morphological parameters dendrogram was computed. Dendrogram showed two clusters, one comprising *C. album*, narrow leaved diploid and broad leaved diploid at similarity index 1.05; other including *C. album* hexaploid and *C. murale* at similarity index 2.47. Tissue cultural parameters were used for an understanding of the genetics of speciation of *Chenopodium* spp. The results indicated that the hexa-

ploid *C. album* (2n=54) shared maximum genotypic commonality with narrow-leaved diploid cytotype of *C. album* (2n=18) followed by broad-leaved diploid cytotype of *C. album* (2n=18) and least with *C. murale* (2n=18), the wild *Chenopodium*. To the best of our knowledge this is probably the first attempt to utilize tissue cultural attributes to character-states for the understanding the relationship between species of *Chenopodium*.

B.2. Phenotypic, genotypic and physiological studies with some species of family Fabaceae : **P. K. Saha** and B. Das

Pure lines of *Tephrosia purpurea* were maintained at Madhyamgram Experimental Farm. Further increase in the homozygosity (95% - 98%) of the seeds of F₄ population were obtained. An attempt has been made to correlate the physical cause with the physiological cause of dormancy in case of *T. purpurea* after investigating through Transmission Electron Microscopy. The cause of hardening of testa (at various degree) during development of seed was also studied in *T. purpurea* by means of peroxidase activity. In case of *Sesbania cannabina* and *Albizia procera*, the seeds were routinely studied to check the changes of seed status and water uptake patterns. Role of Phase A determining the Phase B and early onset of germination has been established in *Albizia procera* seeds.

B.3. Improvement of Rice in relation to submergence tolerance : **S. Gupta** and Sarat Kr. Raj

The androgenic double haploid (DH) submergence tolerant lines developed from our laboratory were maintained at the Madhyamgram Experimental Farm. Replicated yield trial conducted during rainy season shows high yield (≥8t/ha) performance in some of the lines. Agromorphological characterization including disease and pest resistance had been done. Most of the lines show low incidence of three major diseases viz. Bacterial Blight, Blast and Brown spot of Rice. High recovery percentage (more than 80%) has been observed for all the lines subjected to submergence stress (50 cm water depth) in green house tank condition.

To characterize all the lines at DNA level, experiments have been conducted using Polymerase Chain Reaction (PCR). Lines had been screened for five disease resistance genes viz. Xa - 21, Xa - 5, Xa - 10, Xa - 13 (Bacterial Blight of Rice) and Pi - 2 (Blast of Rice) using molecular markers linked to the particular diseases. To analyse the transfer of major locus for submergence tolerance (QTL) mapped on rice chromosome '9' from the source genotype (FR-13A & *O. rufipogon*) into the androgenic lines, RAPD markers linked to the QTL have been successfully amplified in the respective tolerant and susceptible parents. Selection of lines using these molecular markers are in progress. DNA fingerprinting of these lines using microsatellite (SSR) primers and by PCR based RFLP (PBR) are on going.

B.4. Generation & Demonstration of Tissue Culture Raised Planting Materials from *Andrographis paniculata* and *Tectona grandis*: **S. Gupta**, G. Gangopadhyay, R. Poddar and **K. K. Mukherjee**.

Protocols developed for clonal propagation of *Tectona grandis* and *Andrographis paniculata* as reported earlier were used for in vitro multiplication of large number of elite plantlets. A number of tissue culture raised plantlets of each species were transferred to the soil (in earthen pots). Both in vitro grown plantlets in culture vessels and plantlets in earthen pots of Teak and *Andrographis* were supplied periodically to W. B. Science and Technology.

B.5. Clonal propagation of medicinal plants: **Sabita Bhattacharya**, Rajasri Bhattacharyya and Sudipta Datta

A large no. of hardened micropropagated plants of *Withania somnifera* and *Coleus forskohlii* have been transferred to field to study their field performance. *In vitro* culture of *Phyllanthus amarus* (a medicinal herb used for treatment of jaundice), has been started. Of different types of explants, "shoot apex" showed best regenerating potential. Within a single culture period 15 plants/explant were obtained in optimum cases. Root culture of *P. amarus* was established following liquid shake culture method. Experiments were undertaken to test the efficiency of the micropropagated *P. amarus*

plants on in vitro inactivation of HBsAg (Hepatitis B surface antigen). The work is in progress.

To work out a programme on protocol development and demonstration of tissue culture raised *Ambroma augusta*, family Sterculiaceae (a project of WBSCST), seed germinated cotyledonary segments were selected as the explant material. The initial problem of low seed germinability was overcome by treating seeds with conc. H_2SO_4 to increase the germination frequency at 100%. In the preliminary attempt shoot tips were subjected to rooting in suitable rooting medium. Fifty hardened plants were handed over to the funding agency.

B.6. Improvement of Aromatic Rice Suitable for West Bengal: **T.K. Ghose**, **S.N. Ganguly**, **B.B. Mukherjee**, **S. Bhattacharya**, **S. Das**, **S. Gupta-Bhattacharya**, T. Roychoudhury, M. De, S. Biswas and S.R. Dey

A total of 88 rice lines, including Basmati, have been identified to maintain aroma in West Bengal. Of these, 40 lines were characterized in detail and eight lines were identified to be moderately high yielders. A preliminary analysis against the standard chemical confirmed the presence of 2-acetyl-1-pyrroline in cultivars maintaining aroma in Madhyamgram. Grain quality traits (length, breadth, length/breadth ratio of grains and kernels and cooked kernel elongation) of the cultivars have been analyzed.

After screening more than 100 F1s and F2 populations, the combination of IR-72/Pusa Basmati 1 (nonaromatic/aromatic) was selected. A total of 537 F2 plants (1997) from this combination were phenotyped for aroma and other agromorphological traits, and 514 F3 families raised (1998) and progeny-tested for aroma (16 plants per family). The results confirm monogenic recessive control for aroma.

Agromorphological characterization of more than 2000 F3 plants, three plants/family, has been completed. A total of 1608 F4 families were raised in micro-plots and more than 3000 F4 individuals have been selected for advancing. For yield improvement, 363 F4 families have been raised from F3 individuals with more than 30g panicle weight. Preparatory to anther culture, the IR-72/Pusa Basmati-1 hybrid has been produced and vegetatively propagated.

In preparation for aroma-linked marker development, mapped SSLP marker primers for chromosome 8, reported aroma-linked RAPD primers have been obtained from commercial sources. Aroma- and cooked kernel elongation-linked RFLP clones have been obtained from Cornell University. Protocols for PCR amplification of rice genomic DNA has been standardized. AFLP protocols have also been standardized using *Entaboeba* genomic DNA.

B.7. Germplasm Screening And Development Of Molecular Markers for seed Vigour in rice: **S. Sen-Mandi** & Subhra Talai

Studies on Germination performance under (a) favourable condition in the laboratory, (b) stressful field condition and (c) cold stress condition were used to short list rice varieties under high and low vigour categories. The high vigour varieties appear to retain DNA integrity better during post-harvest storage (ageing) of seeds under ambient storage condition.

RAPD analysis of genome revealed the presence of two PCR products that were found to be specifically associated with low vigour varieties. This was reproducible in different harvest seed lots suggesting also that the varieties studied are stable in their genetic complement during the period of study. The specific PCR bands are being used to develop specific primer to be used for germplasm screening in rice.

B.8. DNA Finger Printing And Cataloguing Of Tea Germplasm: Developing Molecular Markers In Elite Tea Clones: **S. Sen-Mandi** & Rajan Kumar Mishra.

RAPD analysis, using arbitrary primers (AP-RAPD), of genomes of tea clones. Reproducibility of DNA fingerprints for use in documentation of tea clones has been demonstrated in random sampling of plants of each clone through standardization of amplification medium and stringent annealing temperature. Out of 148 bands obtained by using 9 primers a study of 9 clones exhibited polymorphism of 128 bands; 20 bands were monomorphic.

AFLP analysis of tea genomes have also been initiated.

Enzymes / Isozyme studies on a β -D glucosidase Superoxide dismutase, Ascorbate

peroxidase in tea leaves have indicated clonal variation. This information is now being used to establish precise biomolecular marker for germplasm screening in tea.

B.9. Genetic Engineering of Indica Rice Cultivars by Agrobacterium-Mediated Method: **S. Sen-Mandi** and Malabika Roy

Several Indica rice cultivars were screened on the basis of physio-morphological data in relation to salt and drought stress tolerance in laboratory condition. Embryogenic calli were developed from different Indica-rice cultivars for transformation purpose. Scutellum-derived embryogenic calli were infected with *Agrobacterium*-containing different plasmid constructs. We used ABA-inducible promoter to over-express the desired genes in Indica rice cultivars. The Agro-infected calli are now growing in selection media.

B.10. Collection and Conservation of Medicinal and Aromatic Plants under the Jai Vigyan National Science and Technology Mission on Conservation of Plant Biodiversity, with NBPGR as Lead Institute: **S. Sen-Mandi**

Medicinal plants together with information on their use, obtained from local communities including the tribals have been collected from parts of Midnapur, Hoogly and 24 Parganas (N). Collected plants have been planted in a shady and moist area in Madhyamgram Experimental Farm for plant establishment.

INSTITUTIONAL PROJECT IV

A.1. Studies on Bengal Basin: **B.B. Mukherjee**, Indrani Bhattacharyya and Amitava Bhattacharya

Erosion in the coastal region of the Bengal Basin was studied in respect to sea level scenario. Initially image processing techniques were applied to derive images from the remotely sensed data. Images thus derived were overlaid on the toposheet of 1967 to identify the vulnerable areas in Bakkhali and in Gangasagar. Ground validation was made Beach survey data were used to ascertain the degree of erosion in aforesaid islands.

A.2. Environmental biopollution in relation to human health: Swati Gupta-Bhattacharya, Indrani Chowdhury, Pampa Chakraborty, Debajyoti Ghosh. (In collaboration with Prof. S. Chanda).

Among the different types recorded in Domjur, *Carica papaya* L. pollen was found to be dominant type. It was already selected for biochemical and clinical investigation. Along with *Carica* pollen, papaya fruit is also found to be important in producing IgE mediated allergy as observed by skin reaction test and ELISA. In IgE specific dot immunoblotting, *Carica* pollen showed remarkable cross-reaction with papain, an enzyme present in latex, fruit and other plant parts. Papain is known to be an allergenic component. The study is going on to identify the common allergenic components between the pollen and food allergen of papaya by different immunochemical techniques.

Pollen allergic rhinitis can be sufficiently treated with subcutaneous immunotherapy with allergen extracts. To study the specific immunological parameters changing during therapy in correlation with clinical outcome, a monitoring programme has been started since January 2000. A double blind placebo-controlled trial is being conducted with a group of adult respiratory allergic patients sensitive to four very common airborne palm pollen grains. These types are: Arecanut, plamyra palm, coconut and date sugar palm. The sensitive patients are being examined during the onset of their therapy schedule, which is being followed by three months intervals. During each visit, serum samples are taken and analysed for the levels of specific IgE and IgG1 & IgG4 subclasses, which are known to play significant role in mast cell degranulation and cross-linking. The reduction in symptom score in the immunotherapy group is being compared with placebo-controlled group for assessment of changing parameters.

Besides the monitoring programme, studies are continuing to identify and isolate the cross-reactive allergic components of palm pollen types mentioned above by using immunochemical methods.

A semi-vivo germination technique was applied with excised anthers of *Catharanthus*

roseus. After a night long incubation, pollen tubes were stained with DAPI and male gametes were localized. In a controlled set TEM study of a t.s. of ovary showed 1st zygotic division. Complete isolation of male gamete is in progress. PAGE and activity gel of the crude pollen extract showed two major bands to be esterases. Esterases are the marker protein for outermost surface of the pollen grains, playing vital role in pollen-stigma interaction leading to post fertilization changes. In *Catharanthus* sp. these enzymes are of 150-200 kDa. Pollen calendar of Madhyamgram area shows the presence of this pollen. The pollen showed sufficient allergenicity. The enzyme esterase in spite of having an extreme biological significance, can cause allergic reactions in susceptible individuals. Few other allergenic band were also identified by IgE-ELISA and IgE-western blot against pooled patient sera.

PUBLICATIONS :

1. Das S. and Mukherjee K K 1999 Cytogenetical Studies in *Ipomoea quamoclit* L. The Nucleus. **42** : 45-48.
2. Bandyopadhyay N, Mukherjee K K, Mandal N C and Mandal S 1999 Chemotaxonomical study of some selected species of Bignoniaceae with reference to phenolic compounds. J. of Hill Research **12** (1) : 5-10.
3. Biswas S, Das B and Saha P K 1997 Dormancy status as influenced by hilum in phenotypically characterised seeds of *Pachyrhizus angulatus* Rich. Ex. Dc. and breaking of dormancy. J. Natn. Bot. Soc. **51**: 53- 60.
4. Bhattacharya A, Das B and Saha P K 1999 Qualitative and quantitative estimation of indole-3-acetic acid (IAA) in the seeds of *Shorea robusta*, Gaertn. f. : J. Tropical Forest Research **11** : 528 - 536.
5. Basu S, Gangopadhyay G, Poddar R, Gupta S and Mukherjee B B 1999 Proline enigma and osmotic stress - tolerance in rice (*Oryza sativa* L.). In: Plant Tissue Culture and Biotechnology - Emerging Trends (Ed. Kavi Kishor, P.B.) University Press, Hyderabad, 275.

6. Sengupta D N, Banerjee K and Rouf A 1999 Abiotic stress Responsive Elements, their Binding Factors and their Utilization for the Improvement of Tolerance to Abiotic Stress in Plants. Journal of Plant Biology, **26**, 179-185
7. Chakraborty P, Chowdhury I, Gupta-Bhattacharya S, Gupta S, Sengupta DN and Chanda S 1999. Clinico immunologic studies on *Phoenix sylvestris* Roxb. Pollen : an aeroallergen from Calcutta, India. *Allergy* **54** 85-89.
8. Adhikari A, Sen M, Gupta-Bhattacharya S and Chanda S 1999. Studies on airborne fungal spores from two cowsheds of suburban and rural areas of West Bengal, India. *Indoor Built Environ* **8** 221-229.
9. Mandal N, Raj, S.K., Gupta. S 1999 Anther culture for Breeding submergence tolerance in Rice (*Oryza sativa* L.) : *Phytomorphology* **49**(1), pp. 21-28.
10. Bharati Ghosh 1999 Polyamines in the regulation of plant senescence. *J.Plant biol* **26**(2), 129-134
11. Bhattacharya, S, Das B and Bhattacharya, S. 1999 Investigation on seed germination of *Nyctanthes arborescens* (Oleaceae) in relation to the total phenol content. *Seed Sci. & Technol.* **27**, 321-327

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

Prof. Bharati Ghosh presented invited talks in the following seminars / symposia - S.M. Sircar Symposium organised by PPF at Bose Institute, 2nd National Conference under special assistance programme in Botany Department, Burdwan University, National Seminar on " Role of plant growth regulators and Biotechnology to improve growth and productivity of plants" in Botany Department, Gujrat University, Ahmedabad, Chairperson in a session of invited papers at the National Symposium on "Frontiers of Research in Plant Sciences " in the Botany Department, Calcutta University, present a poster in the International

Symposia on " Signal Transduction in Plants" at ICGB, New Delhi, Millennium lecture in the area of her specialisation in Bose Institute .

Dr. Papiya Roy, Ms. Mousumi Sengupta and Ms.Kakali Banerjee presented papers at the National Symposium on frontiers of research in plant science in Botany Department, Calcutta University. Papiya Roy, Ms Kamala Niyogi, Saswati Laha , M.K.Chatterjee, D.N.Sengupta and Bharati Ghosh presented a poster entitled "Molecular mechanism of Polyamine action in rice plants in relation to abiotic stress" and the poster was adjudged as the Best Poster Presentation in the National Seminar on Recent Advances on Plant Biology at Kasaragod on 3.2.2000 to 5.2.2000.

Dr. P.K. Saha : Attended the National Symposium on Frontiers of Research in Plant Sciences organized by National Botanical Society, Calcutta during December 1-3, 1999 and presented a paper. He also participated in the National Conference on Plants, Microbes and Environment organized by University of Burdwan during March 11-12, 2000. Acted as Asstt. Secretary and participated in the workshop on Plant Genomics : Emerging Trends & Technology held in Bose Institute, Calcutta during March 24 - 25, 2000.

Dr. D.N.Sengupta has delivered Invited Lectures " National Seminar on Recent Advances on Plant Biology" in Kasaragod, Kerala on Feb3 - 5, 2000.

Delivered an invited lecture in the Refresher Course on Techniques in Biomolecular Research, in Chemistry Dept, Univ of Calcutta, organized by the Academic Staff College on 26th Feb, 2000, attended The International Conference on Signal Transduction in Plants, held at ICGB, New Delhi and presented Poster on " Expression of ABRE-binding protein in Rice Plants in Response to Salinity Stress. Poster entitled "Molecular mechanism of Polyamine action in rice plants in relation to abiotic stress presented by Kamala Niyogi, Papiya Roy , Saswati Laha, Manas Chattopadhyay, Dibyendu N. Sengupta and Bharati Ghosh received Best Poster Presentation award in the National Seminar on Recent Advances on Plant Biology at Kasaragod on 3.2.2000 to 5.2.2000.

Dr. Sabita Bhattacharya attended "National Symposium on Frontiers of Research in Plant Sciences" organised by National Botanical Society, held at Calcutta University, Calcutta on 2-4 Dec. 1999; attended "Workshop on Plant Genomics: Emerging trends and technology" held at Bose Institute, Calcutta, on 24-25 March, 2000.

Rajasri Bhattacharyya presented a poster at the "National Symposium on Frontiers of Research in Plant Sciences". She participated in the "3rd Contact Programme on Plant Biotechnology-Molecular Marker Technology", sponsored by DST, Govt. of India, New Delhi, held at Ch. Charan Singh University, Meerut on March 6-18, 2000. She also attended "Workshop on Plant Genomics: Emerging trends and technology", held at Bose Institute, Calcutta, on 24-25 March, 2000. Sudipta Dutta presented a poster at the "National Symposium on Frontiers of Research in Plant Sciences".

Dr. Swati Gupta-Bhattacharya attended a course on Plant Transformation held at International Center for Genetic Engineering and Biotechnology, New Delhi from November 1-12, 1999; participated and presented paper in the International Conference on Man and Environment on the occasion of Silver Jubilee celebration of the Center for Study of Man and Environment on November 25-28, 1999 at Max Mueller Bhavan, Calcutta. She had participated in the "Workshop on Plant Genomics: Emerging Trends & Technology" on March 24-25, 2000 at Bose Institute, Calcutta. Dr. Indrani Chowdhury, Dr. Pampa Chakraborty and Debajyoti Ghosh participated and presented paper in the International Conference on Man and Environment on the occasion of the Silver Jubilee Celebration of CSME on 25-28th

November, 1999 at Max Mueller Bhavan, Calcutta. Dr. Pampa Chakraborty and Debajyoti Ghosh participated and presented paper in 10th National Conference on Aerobiology and its Application at Andhra University, Vishakhapatnam on 20-22 December, 1999. Dr. Indrani Chowdhury, Dr. Pampa Chakraborty and Debajyoti Ghosh participated in the "Workshop on Plant Genomics: Emerging Trends & Technology" on March 24-25, 2000 at Bose Institute, Calcutta. Mr. Debajyoti Ghosh obtained the bronze medal in the P.H. Gregory Contest for original paper presentation for young scientists in the 10th National Conference on Aerobiology and its Application on 20-22nd December, 1999 at Andhra University, Vishakhapatnam.

Prof. S. Sen-Mandi : Participated in poster discussion at (I) "International Conference on Managing National Resources for Sustainable Agricultural Production in the 21st Century" IARI, New Delhi, February 14-18, 2000, and at (ii) Workshop on Plant Genomics Emerging Trends & Technology, Bose Institute, Calcutta, March 24-25, 2000.

Dr. Malabika Roy attended the Workshop on Plant Genomics : Emerging Trends & Technology, Bose Institute, Calcutta, March 24-25, 2000. Ms. Subhra Talai participated and presented poster in (I) the "International Conference on Managing National Resources for Sustainable Agricultural Production in the 21st Century" IARI, New Delhi, February 14-18, 2000 and in Workshop on Plant Genomics : Emerging Trends & Technology, Bose Institute, Calcutta, March 24-25, 2000. Mr. Rajan Kumar Mishra attended Workshop on Plant Genomics : Emerging Trends & Technology, B. I. Calcutta, March 24-25, 2000

GRANTS-IN-AID SCHEME

Investigator/ Co-Investigator	Title	Financed by
Prof Bharati Ghosh Dr D.N.Sengupta	Use of Antisense RNA to delay fruit ripening	DBT
Prof Bharati Ghosh Dr D.N. Sengupta	Molecular Mechanism of polyamine action in Protection against abiotic stress in Indica rice	DST
Prof Bharati Ghosh Dr D.N.Sengupta	Cloning of molecular markers involved in salt tolerance and their overexpression to enhance salt tolerance in rice plants	DBT-CPMB
Prof Bharati Ghosh (Co-PI)	Polyamine metabolism in relation environmental stress in rice	Indo-US
Prof. K. K. Mukherjee Dr. B. K. Dutta, NIMPITH	Studies on biology and genetic diversity of <i>Nypa fruticans</i> from Sundarban, India.	DBT
Dr. B. B. Mukherjee	Holocene history and biostratigraphy of South Bengal on predicted geoidal changes.	DOD
Prof. S. Gupta	Generation & demonstration of tissue culture raised plant materials.	W.B.S.C.S.T.
Dr. T.K. Ghose Dr. S. N. Ganguly Dr. B. B. Mukherjee Dr. S. Das Dr. S. Bhattacharya Dr. S. Gupta-Bhattacharya	Genetic Approaches for the Improvement of Aromatic Rice suitable for the Agro-climatic Conditions of West Bengal : Mapping of the Aroma Gene(s).	DBT (CPMB)
Dr. Sabita Bhattacharya	Generation and demonstration of tissue culture raised planting materials of <i>Ambroma augusta</i>	W.B. SC & ST
Dr. Swati Gupta Bhattacharya	Environmental biopollution and its impact on human health	Dept. of Environment & Forest, W.B.
Dr. D.N. Sengupta	Cloning of cDNA for Ploymerase Beta from Rice (<i>Oryza sativa</i> L.) For structure : Function analysis	C S I R
Prof. S. Sen Mandi Prof. P. Bhattacharya (Co-PI)	DNA Finger Printing and cataloguing of Tea Germplasm Developing Molecular Markers in Elite Tea Clones	DBT/TEABOARD
Prof. S. Sen Mandi Prof. P. Bhattacharya } (Co-PI) Dr. Nanda Paria (Botany Dept., Calcutta University)	National Agricultural Technology Project Exploration and Collection of Medicinal Plants.	NBPGR (ICAR)

Ph. D. Degree Awarded

Name	University	Year	Title	Supervisor
Sudhiranjan Gupta	Jadavpur University	1999	Molecular Analysis of Expression of Salinity Stress Inducible Genes in Different Organs of Rice (<i>Oryza sativa</i> L.) Plants	Dr. D.N. Sen Gupta
Sangita Basu	Calcutta University	1999	In vitro studies on salt and related stress in some indica variety of rice (<i>Oryza sativa</i> L.)	Dr. B.B. Mukherjee

RESEARCH SCIENTISTS/RESEARCH ASSOCIATE/POST-DOCTORAL FELLOWS/
SENIOR RESEARCH FELLOWS/JUNIOR RESEARCH FELLOWS

Sreejit Kr. Mitra, Sarat Kr. Raj, Biswajit Das, Pampa Chakraborty, Debajyoti Ghosh, Tapan Bera, Rajasri Bhattacharya, Subhra Talai, Abdur Rouf, Saswati Laha, Papiya Roy, Mousumi Sengupta, Pallab Kundu, Sankar Bakshi, Kakali Banerjee, Kamala Niyogi, Rajan Kr. Mishra, Indrani Bhattacharya, Gourab Gangopadhyay, Malabika Roy, Sujit Roy, Sailendranath Sarkar, Indrani Chowdhury, Sudipta Datta, Amitava Bhattacharya, Prasenjit Chakraborty, Tathagata Roychoudhury, Mitu Dey, S. R. De, S. Biswas.

SUPPORTING STAFF MEMEBRS

N.N. Chowdhury, Sankar Bose, Sukumar Majumdar, Ramit Poddar, Chanchal Chakraborty, Jadab Kr. Ghosh, Binoy Krishna Modak, Chaitali Roy, Kaberi Ghosh, Shyamal Kr. Ghosh, Dilip Kr. Chakraborty, Shyamapada Halder, Kanailal Chatterjee, Sukadeb Das, Sudhir Jana, Sasanka Sekhar Bera, Sambhunath Debnath, Dhiren Kumar Das, Rabin N. Roychoudhury, Bipul Kr. Nag, Kanan Singh, Jagannath Das, Munna Turi, Sarama Pradhan, Monimohan Ghosh.

Bose Institute

Microbiology

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

HIGHLIGHTS

SoxA, SoxB and SoxC genes in sulfur oxidizing operon in *Thiobacillus* have been identified by a PCR based genome walking technique.

INSTITUTIONAL PROJECT NO. I

A. Control of bacterial wilt of tomato by antagonistic microorganisms : P. K. Chakrabartty, A.K. Halder and Anasuya Ray

Nine strains of *Pseudomonas solanacearum* causing wilt disease were isolated from diseased specimens of tomato, egg plant and potato plants based on characteristic colony appearance on tetrazolium containing medium. The identity of the strains was confirmed by biochemical characterization and their pathogenicity was tested by plant inoculation tests. All the strains were found to be pathogenic and belonged to biotype 3 as revealed by their ability to oxidise both sugars and sugar alcohols. An antagonistic bacterial strain inhibiting growth of *Ps. solanacearum* was isolated which could possibly be used for biocontrol of wilt disease.

raised against the TCP of a classical strain O395, although it recognized the TcpA protein of strain 10259 in immunoblotting experiment, exhibited considerably less protection against 10259 challenge compared to that observed against the parent strain. Incidentally, the *tcpA* sequences of two other toxigenic non-O1/non-O139 strains (V2 and S7, both belonging to the serogroup O37) were determined to be almost identical to that of the classical *tcpA*. Further, *tcpA* of another toxigenic non-O1/non-O139 strain V315-1 (O nontypeable) was closely related to that of El Tor *tcpA*. Analysis of these results with those already available in the literature suggest that there are at least four major variants of the *tcpA* gene in *V. cholerae* which probably evolved in parallel from a common ancestral gene. Existence of highly conserved as well as hypervariable regions within the sequence of the TcpA protein would predict that such evolution is under the control of considerable selection pressure. Theoretical models of the structure of TcpA protein monomer subunit as well as of the TCP fibre have been generated in collaboration with Dr. R. Chattopadhyaya of Biochemistry Department.

INSTITUTIONAL PROJECT V

Molecular and cellular basis of bacterial and parasitic diseases

A.1. Cholera and *Vibrio cholerae* : A.C. Ghose, R.K. Nandy, B. Nandi and A. Sarkar

A toxigenic non-O1/non-O139 strain of *Vibrio cholerae* (10259) was found to contain a new variant of the toxin-coregulated pilus (TCP) protein gene (*tcpA*) as determined by PCR and Southern hybridization experiments. Nucleotide sequence analysis data of the new *tcpA* gene in strain 10259 (O53) showed it to be about 74 and 72% identical to those of O1 classical and El Tor biotype strains respectively. The predicted amino acid sequence of the 10259 TcpA protein shared about 81% and 78% identity with the corresponding sequences of classical and El Tor TcpA proteins respectively. An antiserum

A. 2.1. Leishmaniasis : A.C. Ghose and A. Mookerjee (in collaboration with Prof. P.C. Sen of Chemistry Department)

The progressive visceral infection caused in golden hamsters by *Leishmania donovani* amastigotes led to gradual impairment of proliferative response of their splenic (SPMC) and peripheral blood (PBMC) mononuclear cell populations to *in vitro* stimulation with mitogen or with a combination of phorbol myristate acetate (PMA) and ionomycin (Io). Therefore, studies were directed towards the evaluation of the status of protein kinase C (PKC), a mol-

ecule known to play a key role in the lymphoproliferative process. The PKC activities were measured in the membrane and cytosolic fractions of SPMC and PBMC from normal and infected hamsters following stimulation with PMA. Results showed that the PKC activities in both the fractions of cells from infected animals were decreased significantly as compared to those of cells from normal controls. However, removal of adherent cells from SPMC and PBMC of infected animals resulted into restoration of the membrane associated PKC activities to a significant level in the nonadherent lymphocyte population. All these results suggest that the observed impairment of the lymphoproliferative response in the infected animals may arise as a result of the lack of activation of PKC in these cells.

A.2.2. Leishmaniasis : Timir B. Samanta, Nilanjana Das, Manika Das and R. Marik

The visceral infection in golden hamsters caused by *Leishmania donovani* amastigotes led to the impairment of cytochrome P450 (Cyp450). The decrease in the level of the enzyme was observed to be about 48% of the normal control. The impairment of Cyp450 was maximum when the parasite load in the host liver was maximum. Thus attempts were made to sort out the causative factor(s) of impairment of Cyp450. The flagellated promastigotes were grown in liquid enriched medium at 25°C for 6 days. Cells were then harvested by centrifugation (clarified filtrate F1), washed with phosphate buffered saline pH 7.4. The washed promastigotes suspended in phosphate buffer (0.1M pH 7.3) supplemented with EDTA and PMSF (2 mM each) were disrupted by sonication followed by centrifugation to furnish the supernatant (F2) and cell debris. The animals (Swiss albino mice) were then pushed with 10 mg protein of each of the preparations viz., F1, F2 and homogenised debris for 3 consecutive days by intravenous route. The level of Cyp450 in each case was measured and compared with the normal control. Preliminary results showed 30 and 23 per cent reduction in liver Cyp450 in mice injected with the culture filtrate (F2) and the homogenized debris respectively.

A.2.3. Signal transduction mechanism in visceral leishmaniasis : An approach to investigate the role of cytokines, chemokines and free radicals in *L. donovani* infected murine macrophages : Subrata Majumdar and Sandip Bhattacharyya

Leishmania donovani, an intracellular protozoan parasite resides and multiplies within macrophage of the vertebrate host and is the causative organism for the disease, visceral leishmaniasis. We observed impaired oxidative response under parasitic stress. Activity study, immunoblot and northern blot analysis collectively converge to the fact that selective impairment of Ca^{+2} dependent PKC isotypes accounts for such attenuated oxidative response in leishmaniasis. It was also observed that IL-10, a potential immunomodulator is responsible at least in part, for such defective signal transduction, which in turn favors the survival of the protozoa within the hostile micro environment.

A.3. Molecular genetics of mycobacteria : Sujoy K. Das Gupta, Shreyasi Chatterjee Abhijit Basu and Mahasweta Mitra

The replication of *E. coli* mycobacteria shuttle vectors derived from pAL5000, a mycobacterial plasmid has been studied to understand the molecular genetics and develop efficient tools for genetic engineering of mycobacteria. The mycobacterial disease, tuberculosis has reemerged as dreaded disease all over the globe. Previously the minimal replication region of this plasmid was determined. We have now expressed the two putative replication proteins of pAL5000 in *E. coli* using various vectors. As the expressed products were found to be incorporated in inclusion bodies, therefore it was necessary to use co-expression of chaperons to obtain soluble forms of the polypeptides. The expressed proteins are being functionally characterized by performing DNA binding studies. In another area, we have developed a minimal vector system for mycobacteria comprising of the pAL5000 origin of replication, a selectable marker and a convenient cloning site. The system does not contain *E. coli* vector sequences which we have demonstrated earlier to be the

cause of instability for *E. coli*-mycobacteria vectors. It has also been shown that promoter sequences present within the mycobacterial origin of replication can be used to express foreign genes in mycobacteria and hence it will no longer be necessary to incorporate additional promoter sequences in the vector for expression purposes. Simultaneously attempts were made to construct a convenient phage based transformation system for mycobacteria. In fact a mutant of mycobacteriophage L1 has been isolated in high yields. The high yielding nature was found to be due to a deletion in the immunity region which resulted in increased burst size as well as increased adsorption. The possible use of this high yielding mutant in the detection of small numbers of mycobacterial particles present in test samples has been demonstrated.

B. Industrially important microbial enzymes

B.1. Biotransformation of organic compounds by microbes : Studies on penicillin amidase : Timir B. Samanta and Anjan Pal

Penicillin G amidase produced by the strain A-13 was purified (29 fold). The purified enzyme had a pH optimum of 8.5 although it was stable over a wide range of pH 4.0-9.0. The amidase was found to be thermostable with temperature optimum at 60°C. Both Fe^{++} and Zn^{++} (10 mM each) were found to enhance the activity of the amidase. Phenyl methyl sulfonyl fluoride (PMSF) and N-bromosuccinimide (NBS) inhibited the enzyme activity. The enzyme has got a broad substrate specificity. It was highly active against penicillin G, semisynthetic penicillin and semisynthetic cephalosporins. However, very poor activity was observed against cephalosporin C. The K_m and V_{max} value of the enzyme against penicillin G were 5.5 mM and 0.6 μ mole/min respectively.

Ca-alginate entrapped cells of A-13 were tested for both hydrolytic and synthetic activity. Activation of Ca-alginate entrapped cells both by nutrients and organic solvents showed differential increase in amidase activity. As co-solvents the lower alcohols and polyols reduced the hydrolytic activity. On the other hand, less polar solvents like DMSO and DMF increased the synthetic activity only.

B.2. Molecular recognition by microbes : Timir B. Samanta and Subhendu Das

In continuation of our studies on molecular recognition by microbes two bacterial strains were isolated. One of the isolated strains SD/S was identified as *Actinobacillus suis* which is a gram negative bacterium while the other SD/B appeared to be a gram-positive *Bacillus* sp. The strains were characterised by the Microbial Type Culture Collection, IMTECH, Chandigarh. Both these strains did possess oxido reductase property. The strain *Actinobacillus suis* (SD/S) transformed $\Delta 4$ -androstene-17 β -ol to $\Delta 4$ -androstene 3, 17-dione. The transformation was found to be stereospecific and substrate specific because other steroid(s) having either 17 β - or 17 α -hydroxyl function remained unaltered.

Bacillus sp. (SD/B) converted testosterone to $\Delta 4$ -androstene 3, 17-dione (AD) and other metabolites which remain to be characterized.

B.3. Microbial starch degrading enzymes : P.K. Chakrabartty and Mithu De

In continuation of our previous study, a bacterial strain (MD 109) isolated from soil and tentatively identified as *Bacillus* sp. produced a thermostable alpha-amylase extracellularly in growth medium. Optimization studies revealed that a combination of 2% peptone, 1% corn steep liquor, 5% soybean meal and 2% dextrin added to the medium resulted in elicitation of highest enzyme activity (18 DUN/ml) by the strain. Incorporation of 2-deoxy-D-glucose in the medium allowed selection of a mutant with 2-fold higher enzyme activity (36 DUN/ml) than that of the wild type. A laboratory strain of *Bacillus amyloliquefaciens* was also found to produce high enzyme activity (475 DUN/ml) in the same medium containing the supplements.

C.1. Identification of a novel sulfur oxidizing operon from a sulfur lithotrophic new bacterium : Pradosh Roy and Chandrajit Lahiri

Working with a poorly characterized genetic system like *Thiobacillus*, a PCR based genome walking technique has been developed to identify a putative sulfur oxidizing (sox) operon, from which a 1124 bp *soxA* gene encoding an ORF with 286 amino acids has been characterized. Detailed sequence analyses revealed the presence of a potential ribosome binding site, pro-

moter region with -10 and -35 like sequences and a signal peptide of N-terminal 26 amino acids along with distinct homologies with gene(s) from phylogenetically diverse organisms like an extremophile *Aquifex aeolicus*, a phototrophic green sulfur bacterium, *Chlorobium limicola* and a sulfur chemolithotrophic bacterium, *Paracoccus denitrificans*.

Identification of such a *soxA* gene with two potential cytochrome c-heme -binding sites (probably one of the 4 subunits of thiosulfate dehydrogenase) was followed by walking down the genome to identify *soxB* and *soxC* genes. The partial sequence analysis of *soxB* and *soxC* indicate 5'-nucleotidase and sulphite dehydrogenase function respectively.

C.2. Colony development of *Thiobacillus ferrooxidans* in association with acidophilic heterotrophs: Pradosh Roy and Anjana Singh

A bacterial cell seems to be independent of the neighbouring cells for its function and development. However, discovery of cell density dependent specific function, quorum-sensing, have changed this view of asocial existence of a bacterial cell.

Acid mine water is the normal habitat of *Thiobacillus ferrooxidans*, a chemolithotrophic autotroph. The acid mine water is virtually a typical inorganic environment. However, continuous life cycle of *T. ferrooxidans* and other acidophilic iron-sulfur oxidizing populations supply a pool of organic compounds. This process of chemosynthesis had helped to develop an environment suitable to evolve and sustain acidophilic heterotrophs. It has been observed that the colony development and colony morphology of *T. ferrooxidans* is dependent on the presence of acidophilic heterotrophs, and colony forming ability of *T. ferrooxidans* is completely diminished in the absence of acidophilic heterotrophs. It seems that certain quorum sensing process between *T. ferrooxidans* and acidophilic heterotrophs is essential for colony development of *T. ferrooxidans* on agar medium.

PUBLICATIONS

1. Nandy RK, Mukhopadhyay S, Ghosh AN and Ghose AC 1999 Antibodies to the trun-

cated (short) form of "O" polysaccharides (TFOP) of *Vibrio cholerae* O139 lipopolysaccharides protect mice against experimental cholera induced by encapsulated O139 strains and such protection is mediated by inhibition of intestinal colonization of vibrios; *Vaccine* **17** 2844-2852.

2. Mandal NC and Chakrabartty PK 1999 Enzymes of carbohydrate metabolism in root-nodule bacteria during growth on acetate; *J Basic Microbiol* **39** 253-256.

3. Mukhopadhyaya S, Sen P, Bhatta-charyya S, Majumdar S, Roy S 1999 Immunoprophylaxis and immunotherapy against experimental visceral leishmaniasis; *Vaccine* **17** 291-300.

4. Das S, Bhattacharyya S, Ghosh S, Majumdar S 1999 TNF- α induced altered signaling mechanism in human neutrophil; *Molecular and Cellular Biochemistry* **197** 97-108.

5. Majumdar S, Ghosh S, Bhattacharyya S, Das S 1999 Role of ceramide in host-pathogen interaction during visceral leishmaniasis; *Molecular Biology of the Cell. Supplement* **10** 334.

6. Roy K, Mandal AK, Sikdar S, Majumdar S, Ono Y and Sen PC 1999 Unsaturated fatty acid-activated protein kinase (PKx) from goat testis cytosol; *Biochim. Biophys. Acta* **1434** 161-169.

7. Nandi B, Nandy RK, Vicente ACP and Ghose AC 2000 Molecular characterization of a new variant toxin-coregulated pilus protein (*TcpA*) in a toxigenic non-O1/non-O139 strain of *Vibrio cholerae*; *Infection and Immunity* **68** 948-952.

8. Mukhopadhyay S, Nandi B and Ghose AC 2000 Antibodies (IgG) to lipopolysaccharide of *Vibrio cholerae* O1 mediate protection through inhibition of intestinal adherence and colonisation in a mouse model; *FEMS Microbiology Letters* **185** 29-35.

9. Mukhopadhyay S, Bhattacharyya S, Majhi R, De T, Naskar K, Majumdar S, Roy S 2000 Use of attenuated leishmanial parasite as an immunoprophylactic and immunotherapeutic agent against murine visceral leishmaniasis; *Clinical and Diagnostic Laboratory Immunology* **7** 2.

10. Das S, Bhattacharyya S, Ghosh S, Majumdar S 2000 Signal transduction mechanisms in neutrophil : comparative study between b and c-protein kinase isotypes; *Molecular and Cellular Biochemistry* **203** 143-151.

11. Mukhopadhyaya PN, Deb C, Lahiri C and Roy P 2000 A *SoxA* gene, encoding a di-heme cytochrome c, and a *sox* locus, essential for sulfur oxidation in a new sulfur lithotrophic bacterium; *Journal of Bacteriology* **182** 4278-4287

12. Chatterjee S, Mitra M and Dasgupta SK 2000 A high yielding mutant of mycobacteriophage L1 and its application as a diagnostic tool; *FEMS Microbiol Letts* **188**(1) : 47-53

Participation in Conferences/Symposia/ Training programme/awards and Honours received

Prof. A.C.Ghose acted as a member of the Scientific Advisory Committee of Rajendra Memorial Research Institute (ICMR), Patna; served as a member of the Ethical Committee of National Institute of Cholera and Enteric Diseases (ICMR), Calcutta; delivered a talk in the Refresher Course for College and University Teachers, Calcutta University; delivered a series of lectures to the M.Sc. (Microbiology special) students of the Biochemistry Department, Calcutta University; served as an external examiner of M.Sc. (Biotechnology), Visva Bharati University.

Prof. Timir B. Samanta acted as an Expert in assessment of scientists at RRL, Jorhat,

Assam; delivered invited lecture in the National Conference on Quality Assurance in Health Care held at Park Hotel, Calcutta; attended the symposium on Emerging Trends in Organic Chemistry held at Indian Association for the Cultivation of Science, Calcutta; acted as a Resource person in Academic Staff College and delivered a lecture in the department of Zoology, University of Calcutta; acted as a convenor, Pasteur Memorial Oration Committee and organised the First Pasteur Memorial Oration Lecture delivered by Professor Jean Louis VIRELIZIER, Institut Pasteur, France, at Bose Institute, Calcutta.

Prof. P.K. Chakrabartty delivered an invited lecture entitled "Biological Nitrogen Fixation" in the S. M. Sircar Memorial meeting held at Bose Institute and organised by Plant Physiology Forum, Calcutta, on 26th February, 2000.

Dr. Subrata Majumdar presented a paper on host-parasite interaction at the 39th Annual Meeting of The American Society of Cell Biology at Washington D.C., USA; delivered an invited talk at the Dental College of Medicine, University of Pennsylvania, Philadelphia, USA.

Dr. S.K. Dasgupta delivered a lecture on "PCR based diagnosis of tuberculosis" at the 13th State Tuberculosis & Chest Disease Workers' conference at Bengal Tuberculosis Association.

Shri Bisweswar Nandi presented a paper at the National Symposium on "Perspective in Environmental Health: Vector and Water Borne Diseases" organised by the National Environmental Science Academy at Bose Institute, December 4-5, 1999.

GRANT-IN-AID SCHEME

Investigator	Title	Financed by
Prof. A.C. Ghose	Molecular characterization of a new variant of toxin coregulated pilus protein (<i>TcpA</i>) of <i>Vibrio cholerae</i> and evaluation of its role in the pathogenesis of cholera	CSIR
Prof. Timir B. Samanta	Studies on protection of impairment of cytochrome P450 during experimental leishmaniasis in golden hamster : Role of nitric oxide synthase	CSIR
Prof. P.K. Chakrabartty	Suppression of bacterial wilt of tomato and related crops caused by <i>Pseudomonas solanacearum</i> (Smith) Smith in India making use of introduced micro organisms in the rhizosphere	ICAR
Dr. P. Roy	Development and evaluation of microbial based arsenic assay kit	DBT
Dr. S. Majumdar	Signal transduction mechanism in Visceral leishmaniasis in mice : Role of TNF in leishmaniasis	DAE
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
Mamta Chawla	Jadavpur University	1999	An analysis of the DNA sequence requirement for replication of Escherichia coli - Mycobacteria shuttle vectors derived from pAL5000, a Mycobacterium fortuitum plasmid	Dr. S.K. Das Gupta

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

Dr. Anup K. Halder, Dr. Nilanjana Das (upto 16.8.99), Dr. Anjana Singh, Dr. Madhumita Sirkar, Dr. Radharani Marick, Sonali Das (upto 17.9.99), Ananda Mukherjee, Ranjan Nandy (upto 30.4.99), Anupama Saha (upto 24.12.99), Anjan Pal (upto 29.2.2000), Shreyashi Chatterjee (upto 31.1.2000), Sandip Bhattacharya, Sanjukta Ghosh, Biweswar Nandi, Shuvendu Das, Chandrajit Lahiri, Manika Das (upto 30.4.99), Abhijit Basu, Mahashweta Mitra, Mithu De, Amit Sarkar, Anasuya Ray

SUPPORTING STAFF MEMBERS

S.K. Das (I) (upto 30.9.99), S.K. Das (II), Dr. Bandana Banik, N.K. Malakar, B.N. De Sarkar, B.N. Mandal, P.K. Chattopadhyay, P. Gupta, S. Gazi, D. Sarkar, Babli Marrick, Prabir Halder, Debashis Majumdar, B. Bahali, N. Patra, J. Prasad, D. Bhattacharyya, R. Prosad, B. Ram, S. Das, Rabin Pal, Sankar Bari, Purnendu Manna, Narayan Patali

Biochemistry

Faculty : Prof. B. Bhattacharyya (Chairman), Prof. N. C. Mandal, Prof. P. Sinha, Dr. P. Chakrabarti, Dr. A. Lohia, Dr. R. Chattopadhyaya, Dr. P. Parrack

HIGHLIGHTS

To understand the physicochemical basis of protein folding and to facilitate protein engineering work, the effect of the side chain on the backbone conformation, structural features of the *cis* peptide bonds and the microenvironment around different protein residues have been elucidated.

INSTITUTIONAL PROJECT NO. I

A.1. An Unique Apyrase from *Mimosa pudica* : **S. Biswas** (Retired) and Mita Sen

The immunofluorescence studies, made to understand the role of motor cells in leaf movement of *Mimosa pudica* plant, showed the apyrase enzyme is mainly localized on the protoxylem inside the metaxylem and the tissues are composed mostly of parenchymatous cells. Microscopic observations revealed that only protoxylem parenchyma and narrow phloem cells are excitable, confirming the involvement of apyrase enzyme in the perception and transduction process of movement.

This apyrase enzyme is capable of autophosphorylation and also shows protein kinase activity. The results confirm that the specific activity of apyrase enzyme is stimulated by the addition of microtubule both from animal and plant sources. Electron microscopic studies show the association of apyrase enzyme with microtubule structures. The studies are now going on to identify the specific sites of phosphorylation using specific inhibitors and also antibodies specially for tyrosine kinase. The phosphorylation of tubulin protein by the apyrase enzyme indicates its direct involvement in the downstream network of signal transduction process.

INSTITUTIONAL PROJECT NO. III

A.1. Studies on Developmental Regulation in *Mycobacteriophage L1* : **N.C.Mandal**, Prajna Mandal, Chandrani Chattopadhyay, Rajat Bhattacharyya

Starting from sonicated extract of heat-induced lysogen of L1c1ts391 in mc²6 (5 g fresh

wt.), a phage-coded deoxyribonuclease was purified to more than 90% homogeneity. The purified enzyme could be stored in presence of 10 mM EDTA and 10% glycerol at 0°C for quite a long time without any appreciable loss of its activity. The enzyme activity was inhibited completely by 10 mM EDTA and 50% by 20 mM PO₄³⁻ or 40 mM K⁺. At its extremely low concentration, the enzyme could convert CCC-form of plasmid DNA first to relaxed circle and then to linear form. The enzyme could also degrade single-stranded circular form of M13 DNA.

In an attempt to understand the regulation of late gene expression of L1 phage, it was reported earlier that the two genes, G23 and G25 of L1 are essential for the transcription of two different sets of its late genes. To confirm this further, a physical map of L1 DNA for XbaI (2 sites), PvuII (2 sites), SstII (1 site), and MluI (6 sites) and a preliminary transcription map have been constructed which have identified different regions of L1 DNA wherefrom early, delayed early and late transcriptions occur. Using this information, the above two late regulatory genes G23 and G25 have been shown to be required for the transcription from two different but adjacent segments within the left half of L1 DNA where the late genes map. Among the 5 known genes essential for phage DNA synthesis, the G20 gene is also essential for the synthesis of several structural proteins of the phage (all are late gene products). To further characterize this gene, the wild-type G20 gene has been cloned in a 2.5 kb DNA fragment. The transcription of late genes of L1 was found to be resistant to rifampicin. This property of the phage is dependent on the presence of delayed early gene product(s) which possibly contain a component that either makes the host RNA polymerase resistant to rifampicin

or the component may itself have the drug-resistant RNA polymerizing activity.

In continuation of the characterization of the master positive regulatory gene G27 of L1, the DNA segment located immediate upstream of the coding sequence has been cloned in a promoter cloning vector. By reporter gene expression study, the presence of promoter and operator elements in this DNA segment has been indicated. This promoter functions well in *M. smegmatis* but very poorly in *E. coli*. The putative transcription start site has been identified by primer extension analysis. The promoter appears to have "extended -10 element" with TGN motif at the 5' end of the nonamer sequence. There is no defined -35 elements and one of the three putative operator sequences is located overlapping the -35 region. A stretch of sequence from 59-134 position containing the putative promoter and operators bears 100% homology with *M. fortuitum* and *M. tuberculosis* chromosomal DNAs and pAL5000 plasmid. The whole of the G27 coding sequence from 198 to 602 bp positions is not completely homologous to a similar length of L5 DNA (only a portion shows homology).

A promoter of L1 has been cloned finally in a 500 bp HaeIII DNA fragment which shows promoter activity in both *E. coli* and *M. smegmatis*. It has been sequenced. This promoter has been shown to be active in *in vitro* transcription with *M. smegmatis* RNA polymerase. Determination of its transcription start site is in progress.

A.2. Structure function Relationship of Tubulin- Antimitotic Drug Interaction : **B. Bhattacharyya**, Siddhartha Roy, Suparna Sengupta, Gopal Chakrabarti, Suroopa Banerjee and Suvroma Gupta

Isocolcemid, a colcemid analogue in which the positions of the C-ring methoxy and carbonyl are exchanged, is virtually inactive in binding to tubulin and inhibiting the formation of microtubule assembly. We have found that the substitution of a NBD group in the side chain of the B-ring of isocolcemid can reverse the effect of these structural alterations (at the C-ring) and the newly synthesized NBD-

isocolcemid restores the lost biological activity. It inhibits microtubule assembly with an IC₅₀ of 12 µM and competes efficiently with [³H] colchicine, for binding to tubulin. NBD-isocolcemid has two binding sites on tubulin; one is characterized by fast binding, whereas the binding to the other site is slow. These two sites are independent and unrelated to each other. Colchicine and its analogues compete with NBD-isocolcemid for the slow site. Association and dissociation rate constants for the fast site, obtained from the stopped-flow measurements, are $(7.37 \pm 0.70) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and $7.82 \pm 2.74 \text{ s}^{-1}$, respectively. While the interaction of colchicine and its analogues with tubulin involves two steps, NBD-isocolcemid binding to tubulin at the slow site has been found to be a one-step reaction. This is evident from the linear dependence of the observed rate constant (K_{obs}) with both NBD-isocolcemid and tubulin concentrations. The interaction of NBD-isocolcemid with tubulin does not involved the conformational change of NBD-isocolcemid, as is evident from the unchanged CD spectra of drug. The absence of enhanced GTPase activity of tubulin and the native-like protease cleavage pattern of the NBD-isocolcemid-tubulin complex suggest an unaltered conformation of tubulin upon NBD-isocolcemid binding to it as well.

A.3. Tubulin Substructure and Identification of its Structural, Functional and Regulatory Domains. : **B. Bhattacharyya**, Manami Roychowdhury, Tapas K. Manna, Taradas Sarkar and Sankar Bhattacharyya

The 20 cysteine residues of tubulin are heterogeneously distributed throughout its three-dimensional structure. In the present work, we have used the reactivity of these cysteine residues with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) as a probe to detect the global conformational changes of tubulin under different experimental conditions. The 20 sulfhydryl groups can be classified into two categories; fast and slow reacting. Colchicine binding causes a dramatic decrease in the reactivity of the cysteine residues and causes complete protection of 1.4 cysteine residues. Similarly, other colchicine analogs that bind reversibly initially decrease the rate of reaction;

but unlike colchicine they do not cause complete protection of any sulfhydryl groups. Interestingly, in all cases we find that all the slow reacting sulfhydryl groups are affected to the same extent, that is, have a single rate constant. Glycerol has a major effect on all these slow reacting sulfhydryls, suggesting that the reaction of slow reacting cysteines takes place from an open state at equilibrium with the native. Ageing of tubulin at 37°C leads to loss of self-assembly and colchicine binding activity. Using DTNB kinetics, we have shown that ageing leads to complete protection of some of the sulfhydryl groups and increased reaction rate for other slow reacting sulfhydryl groups. Ageing at 37°C also causes aggregation of tubulin as indicated by HPLC analysis. The protection of some sulfhydryl groups may be consequence of aggregation, whereas the increased rate of reaction of other slow reacting sulfhydryls may be a result of changes in global dynamics. CD spectra and acrylamide quenching support such a notion. Binding of 8-anilino-1-naphthalenesulfonate (ANS) and bis-ANS by tubulin cause complete protection of some cysteine residues as indicated by the DTNB reaction, but has little effect on the other slow reacting cysteines, suggesting local effects.

A. 4. Chromosome Transmission in Yeast :
P. Sinha, Niranjan Das, Kaustuv Sanyal, Atasi Poddar, Santanu Ghosh and Sujata Hajra

Work on *mcm* mutants of the yeast *Saccharomyces cerevisiae* defective in chromosome segregation was continued. We have already shown that *MCM16* - *MCM19*, *MCM21*, *MCM22* genes, cloned and characterized by us previously, are required for kinetochore function. How do these Mcm proteins control kinetochore function? Are they localized at the kinetochore and contribute towards its structure? Or do they regulate kinetochore function by modulating the function of some other kinetochore protein? To answer these questions we have spent last year in trying to identify other genes which interact with these genes. We have also tried to localize the proteins in the cell using the Green Fluorescent Protein (GFP) and c-myc tags.

Identification of interacting genes

We are carrying out the following experiments. (i) Synthetic lethal approach; identifying mutations in other genes which cause lethality in combination with the *mcm* mutation. For example, if another gene XYZ shares an essential function with the *MCM22* gene, a strain carrying mutations in both these genes may not survive. If the function of XYZ is known, that of *MCM22* may be inferred also. (ii) Identification of the suppressor genes. This is being done in two ways. One, we are looking for dosage suppressors; if a gene carries out an overlapping function with, say *MCM22*, the *mcm22* mutation may be suppressed if that gene is present in high copies. In the second approach we are looking for second-site suppressors of the *mcm* mutation; if a mutation in another gene can compensate for the mutation in the *MCM* gene, the products of these two genes are most likely interacting physically with each other. Again, if the identity of the suppressor gene is known, it would throw light on the functioning of the *MCM* gene. (iii) Two-hybrid analyses. We have been carrying out these experiments with different *MCM* genes. While all these studies are still in progress, the results obtained from (iii) are described below.

Two-Hybrid Approach to Detect Protein-Protein Interactions

Very recently we have initiated this approach to detect whether the Mcm proteins physically associate with each other. It is already reported that Mcm18p (which is the same as Ctf19p) and Mcm21 are kinetochore proteins (Hyland *et al.*, 1999, J Cell Biol 145: 15-28; Poddar *et al.*, 1999, Mol Microbiol 31: 349-360; Ortiz *et al.*, 1999, Genes & Dev 13: 1140-1155). We also know that other *MCM* genes like *MCM16*, *MCM17* and *MCM22* are required for chromosome segregation and all are involved in kinetochore related functions. To see whether these genes also code for proteins which localize at the kinetochores, we are investigating the protein-protein interaction by two-hybrid method. If any of these proteins (e.g. Mcm16p, Mcm17p, Mcm22p) interact physically with either with Mcm18p or Mcm21p, we can say these are also kinetochore proteins. Preliminary results show that Mcm16 interacts with Mcm19, Mcm21 and Mcm22 proteins and

Mcm17 interacts with Mcm19. These results are being confirmed by other methods and the nature of these protein complexes is under study.

SUBCELLULAR LOCALIZATION OF MCM PROTEINS

We are concentrating on the subcellular localization of these Mcm proteins. For this purpose we have constructed fusions of GFP (green fluorescent protein) with the Mcm protein in high copy expression vectors. The vector for the expression of GFP-Mcm protein fusions was constructed by us and we used a strong constitutive promoter *PYK1* for driving the expression of these genes (Fig. 4). Using this vector, the Mcm21p was localized to the nucleus (Fig. 5). During the course of these studies, Mcm21 has been localized to the kinetochore (Ortiz *et al.*, 1999, Genes & Dev 13: 1140-1155). Therefore, we are not pursuing the localization studies with this protein anymore.

The Mcm16 protein was localized to the cytoplasm (Fig. 5). Since the vector we used had a strong constitutive promoter *PYK1* to express proteins, the Mcm16 protein was overexpressed which could have resulted in its mis-localization in the cytoplasm. We are now trying the c-myc tag (for which we have acquired monoclonal antibodies) for confirming the cytoplasmic location of Mcm16p produced from its own gene. Work with the localizations of Mcm17 and Mcm22 protein is under progress.

A.5. Identification of stabilizing interactions in protein structures : P. Chakrabarti and Debnath Pal

The native structure of proteins is stabilized by various noncovalent interactions, the hydrogen bonding being one of the most conspicuous one. Searching for examples where two polar atoms can be held together through interactions other than hydrogen bond we found that the methionine sulfur atoms can have direct contact with the carbonyl oxygen atom and with aromatic atoms along the face of the ring. There is no involvement of proton, and the interacting groups have fixed orientations relative to each other, pointing to the stabilizing nature of the interactions. These contacts are found within helices and sheets and also between subunits in oligomeric proteins. Thus

these can be important for the stability of secondary structural elements and affinity in protein-protein complexes.

A.6. Cis peptide bonds in proteins : P. Chakrabarti and Debnath Pal

Cis-trans isomerization of peptide bond plays an important role in protein folding. Using all the known protein structures we have carried out an analysis of the residue preference, local conformation, hydrogen bonding and other stabilizing interactions involving *cis* peptide bonds. This has led to a reclassification of turns mediated by *cis* peptides, and their average geometrical parameters have been evaluated. A comparison of *cis* peptides containing proline and non-proline residues shows differences in conformation and other structural features.

A.7. Analysis of the environment of tryptophan residue : P. Chakrabarti, Uttamkumar Samanta (DIC)

The environment of Trp residues, which are very important for studying protein structures and dynamics using fluorescence spectroscopy, has 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 derived from our analysis. Algorithms are being developed to assess the role of Trp residues at the interface of protein-protein complexes and those found in the binding sites of substrates and cofactors.

A.8. Aromatic-aromatic interactions in and around helices and their use in the design of helical peptides : P. Chakrabarti, Rajasri Bhattacharyya and Uttamkumar Samanta (DIC)

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.9. Crystallographic studies : P. Chakrabarti and Rajasri Bhattacharyya

In collaboration with Prof. A. L. Majumder of PMCG Section a crystallographic analysis of L-myo-inositol 1-phosphate synthase from a salt-tolerant variety of rice, *O. sativa* has been undertaken. Very little is known about the structure of this important class of enzyme and the molecular features responsible for the salt-tolerance. Additionally, the crystal structure determination is in progress for the cII protein of lambda phage in collaboration with Dr. P. Parrack of our department.

A.10. Crystallographic studies of lipase from *Aspergillus niger* : Rajagopal Chattopadhyaya and V.M.H. Namboodiri

It was reported last year that the purified lipase failed to crystallize despite extended efforts. Crystallization trials were performed with the lipase after removal of the covalently bound carbohydrate with endoglycosidase F. The sugar was completely removed, but a portion of the lipase was cleaved by a metalloprotease contaminating the endoglycosidase F. Nevertheless crystallization trials of the inhomogeneous deglycosylated lipase yielded crystals. This crystal diffracted X-rays to 3.2 Å on an image plate but the pattern indicates a disordered crystal. Better crystals are possible with the deglycosylated lipase if homogeneity could be achieved.

A.11. Sequence determination of lipase : Rajagopal Chattopadhyaya and Swati Ray

Sequence determination of the lipase was attempted first by using the N-terminal amino acid sequence of the purified lipase. Based on the codon frequency table generated from the known *Aspergillus niger* gene sequences in the EMBL gene database, a mixture of degenerate primers was designed for multiple oligonucleotide primed amplification of cDNA. PolyA containing mRNA was captured and reverse transcribed into cDNA by AMV reverse transcriptase and oligo dT primers. Amplification of the 3' end of the cDNA was carried out by using degenerate primer. The PCR analysis showed amplification of a 400 bp region, whereas the full length clone of the 300 aa lipase should give a 900 bp cDNA. We attempted to clone the 400 bp region in a T

Vector but it was not successful. Two degenerate primers were designed from the conserved active site of the lipase corresponding to the sequence GHSLG. Specific amplification of a 500 bp region starting from ca. 140 aa position of the lipase was noticed with one of these primers; it could be reamplified. Both cDNA fragments are yet to be cloned into a suitable vector.

A.12. Lambda cI repressor : papain digestion, circular dichroism, dimerisation and autocleavage : Rajagopal Chattopadhyaya and Kaushik Ghosh

The circular dichroism spectra of three different purified carboxy terminal fragments 93-236, 112-236 and 132-236 of the bacteriophage λ cI repressor were measured and compared with those of the intact repressor and the amino terminal fragment 1-92. All three carboxy terminal fragments contain a majority of β-strands and loops, a minor helix content increasing with the size of the fragment, showing that the 93-131 region previously called a hinge is structured. Papain is known to cleave the cI repressor but we showed for the first time that it cannot cleave the operator-bound repressor dimer. For the 132-236 fragment, both the wt and the SN 228 repressors gave similar dimerization properties as investigated by HPLC, yielding K_D of 13 μM and 19 μM respectively. The three Cys residues in the 132-236 fragment were found to be unreactive upon incubation with DTNB. First order rate constants for the autocleavage of cI were measured and the activation energy determined to be 22.2 kcal/mole. Type-II transcleavage previously observed for the LexA repressor was not observed with the λ repressor 112-236 fragment used as "enzyme" and covalently modified intact repressor as substrate.

A.13. Model building studies of TcpA *Vibrio cholerae*, Bundle forming pilus of human enteropathogenic *E. coli* and Colonization Factor Antigen III of human enterotoxigenic *E. coli* : Rajagopal Chattopadhyaya and Ashoke C Ghose

A complete three dimensional model (RCSB001169.pdb code 1qqz) for the *Vibrio cholerae* toxin coregulated protein (TcpA) was deposited at and accepted by the PDB. The

crystal structure of the *Neisseria gonorrhoeae* pilin, available biochemical data about TcpA, variations in the primary sequences among various *V. cholerae* strains, secondary structure prediction, hydrophilicity, surface probability and antigenicity plots for TcpA were used to build our model. Later it was shown that BFP and CFAPIII proteins can also be rationalized by similar three dimensional structure as for TcpA. All three models are available from RCSB (<http://www.rcsb.org>), the new headquarters of PDB. While our manuscript has been submitted, another group appreciatively used and referred to our TcpA PDB entry as part of their own paper (T J Kirn *et al.*, *Molecular Microbiology*, February 2000, **35**, 896-910).

A.14. Structural Basis of Control of Transcription Initiation in *E. coli* : P. Parrack, Jayanta Mukhopadhyay & Rima Chattopadhyay

We have earlier shown that the transcription activator protein CRP (Cyclic AMP Receptor Protein) in *E. coli* adopts different functional conformers at different concentrations of cyclic AMP (cAMP). Such an observation leads to the possibility of an additional level of transcriptional control, mediated by the amount of cAMP. Whether such a control operates in cells *in vivo* is being tested using various approaches, including site specific mutagenesis of the different cAMP binding sites of CRP.

A.15. Role of upstream sequences in promoter recognition by RNA Polymerase : P. Parrack & Runa Sur

A specific upstream region in the gal operon of *E. coli* has been identified, to which RNA polymerase binds and from which transcription initiation occurs both *in vitro* and *in vivo*. This promoter-like element (called P3) has been found to function as a weak promoter *in vivo*. However, its presence contributes to the overall promoter strength of galP1/P2, stimulating transcription to the tune of 1.6 to 1.8-fold, as confirmed from deletion and site specific mutagenesis studies. Further experiments are in progress to elucidate the biological role of promoter P3.

A.16. Structural Studies on the cII protein of bacteriophage lambda : P. Parrack & Ajit Bikram Datta

The transcription activator protein cII of lambda phage activates the P_{RE} and P_{int} promoters 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 the high expression vector (pET3a) under T7 promoter control. The protein expressed from this clone (pAB119) has been purified. Preliminary equilibrium denaturation studies have been carried out with this protein, using circular dichroism and intrinsic tryptophan fluorescence, leading to some interesting observations. Further, the gene for the protein cIII, which plays a role in stabilising cII, has been cloned in pET15b, another T7 based expression vector used to express cIII as a fusion protein containing N-terminal 6X His tag.

Crystallisation of the cII protein, being carried out in collaboration with Dr. P. Chakrabarti of our department, has met with partial success, and further attempts at improving the size and quality of the crystals are in progress.

A.17. An approach to improve the yield of alkaline protease using low cost substrates : P. Parrack & C. Ganesh Kumar

Alkaline proteases find a wide range of applications in food and dairy industries, besides playing a key role in laundry, dishwashing, textile and tannery industries. An alkalophilic *Bacillus* sp. MK5-6, isolated from a soda soil of pH 10.2, produces alkaline proteases for use in ultrafiltration membrane cleaning. In view of the industrial potential of alkaline proteases, studies were undertaken to purify and characterize this protease, especially with a view to optimizing growth conditions. Shake flask studies were carried out to standardize the nutritional requirement of this isolate for optimum enzyme production. Further, in order to use low-cost substrates for medium formulation, soluble starch, potato starch and arrow root starch were screened for their optimum enzyme producing ability in shake flasks. Of the substrates screened, arrow root starch proved better for enzyme production. On the basis of shake flask studies, a new low-cost alkaline medium has

been formulated. Scale up studies of this formulated medium to a bioreactor (5 litre capacity) are in progress.

INSTITUTIONAL PROJECT NO. V

A.1. Molecular genetics of *Entamoeba histolytica* : Anuradha Lohia, Anasuya Ganguly, Suchismita Das, Sulagna Ghatak

Invasion of human intestinal mucosa by *Entamoeba histolytica*, is accomplished by adhesion, phagocytosis and killing of epithelial cells leading to tissue lysis and ulceration. This cytopathic effect is further enhanced after colonization of the gut tissue by proliferation of vegetatively dividing trophozoites. To look for useful anti-amoebic targets we have chosen to study the proteins controlling invasion and proliferation of *E. histolytica*.

A.1.1. Characterisation of Eh Diaphanous-a protein involved in the control of motility and cell division :

It has been shown that adhesion, protrusive motility and cytokinesis are mediated by specialized regions of the actin cytoskeleton. Each of these processes requires the recruitment of different sets of proteins to specific cortical sites resulting in the modulation of actin organization and dynamics. Diaphanous protein and its orthologs having homology with the vertebrate formins have been implicated in the organisation of actin assemblies required for cytokinesis in yeast, mouse and *Drosophila*. We have isolated the Eh Diaphanous gene by screening a cDNA and gDNA library of *Eh*. The predicted ORF codes for a protein of approximately 127 kDa and the transcript size from Northern blots was seen to be around 4kb. BLAST searches of the complete sequence showed 28% identity and 48% similarity to vertebrate (human and mouse) and *Drosophila* Diaphanous. Three major homology domains were identified i) a N-terminal Rho1 binding domain; ii) polyproline repeats similar to the formin homology domain 1 (FH1); and iii) a C-terminal formin homology domain 2 (FH2).

We cloned the proline repeats and the C-terminal end of Eh Diaphanous separately, overexpressed them in *E. coli*, and used them for

raising polyclonal antisera. Both antisera cross reacted with a protein of approximately 130 kDa in the amoeba cell extract. *Eh* cells stained with anti Diaphanous antisera were visualized with a confocal laser microscope. Mostly the Eh Diaphanous protein was found to localize in the uroid suggesting that it is important for cell motility and capping. Formation of uroids occurs due to a redistribution of F-actin and it has been shown to be regulated by p21 RacG. We have observed activated p21Rho binding to Eh Diaphanous. Eh Diaphanous was also seen to form one dense body per cell in the cytoplasm. In contrast multiple dense bodies were seen with anti-Dia antiserum in cytokinesis defective Eh transformants overexpressing RacA(V12). Eh Diaphanous colocalises with actin both in the uroid and in the dense bodies.

Formin homology proteins mediate cytokinesis or formation of membrane protrusions by binding to actin through profilin. Our results suggest that Eh Diaphanous also performs these functions. It is evident that p21Rho/Rac GTPases regulated actin mediated processes are largely responsible for these phenomenon as shown by impaired cytokinesis and cell motility in p21RacA(V12) overexpressing transformants of *Eh*. Accumulation of Diaphanous-actin oligomers in dense bodies along with a decrease in the formation of uroids in RacA(V12) transformants is indicative of their role both in cellular motility and cell division.

A.1.1. Amoeba Mcm proteins do not restrict DNA reduplication in one cell division:

A majority of eukaryotes have developed an intricate machinery for precisely replicating their genome only once per cell cycle. DNA re-duplication is not permitted until mitosis has been completed. This is effected by a group of proteins called 'licensing factors' when they bind to replicated chromatin. Mcm proteins are known to act as licensing factors in yeasts and metazoans. Amoeba cells on the other hand replicate their DNA more than once per cycle leading to a heterogenous population of euploid and polyploid cells in culture. We have demonstrated in synchronised cells that in one cell division cycle, DNA synthesis continues even after the total DNA content has doubled. Since

then, we have looked for the presence of known eukaryotic DNA replication licensing factors and to identify their role during DNA re-duplication in *Entamoeba histolytica*. We have found homologs of MCM2, MCM3 and MCM5 genes in *E. histolytica*. These genes have been completely sequenced and their recombinant clones have been expressed in *E. coli*. Polyclonal rabbit antisera to these proteins have been raised and used to study their subcellular localisation during the cell cycle by Western blot analysis and confocal immunofluorescence. Eh MCM2 and MCM3 are constitutively nuclear proteins, whereas Eh MCM5 is associated with DNA synthesising nuclei alone. Apart from the nuclear localisation, the constitutive cytoplasmic localisation of Eh MCM2 and Eh MCM5 suggests that these proteins may have other biochemical functions in addition to DNA replication licensing. Importantly, the Eh Mcm proteins do not appear to restrict DNA duplication to once per cell division.

PUBLICATIONS

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2. Roy Chowdhuri M, Sarkar N, Manna T, Bhattacharyya S, Sarkar T, Basu Sarkar P, Roy S and Bhattacharyya B 2000 Sulfhydryls of tubulin A probe to detect conformational changes of tubulin. *Eur. J. Biochem.*, **267**, 1-9.
3. Pal, D and Chakrabarti, P. 1999 Estimates of the loss of main-chain conformational entropy of different residues on protein folding. *Proteins* **36**: 332-339.
4. Samanta U, Pal D, and Chakrabarti P 1999 Packing of aromatic rings against tryptophan residues in proteins. *Acta Crystallogr.* **D55**: 1421-1427.
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7. Samanta U, Chakrabarti P, Naskar J P, Hati S and Datta D 1999 Structure and metal binding of a 1H-1,5-enzodiazepine. *Indian J. Chem.* **38A**: 553-557.

8. Maji M, Hossain M, Chatterjee M, Chattopadhyay S K, Puranik VG, Chakrabarti P and Ghosh S 1999 Synthesis, characterisation and reactivity of trans-[Ru(L)(PPh₃)Br₂]; L=2-pyridyl-N-(2'-methylthiophenyl)methyleneimine. Crystal structure of trans-[Ru(L)(PPh₃)Br₂]. *Polyhedron*, **18**: 3735-3739.

9. Maji AK, Ghose, TK and Lohia A 1999 Use of AFLP DNA fingerprinting to differentiate pathogenic strains of *Entamoeba histolytica*. *J. Paras. Dis.* **23**, 77-79.

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11. Chattopadhyaya R and Ghose AC. Bundle forming pilin of enteropathogenic *E. coli*, RCSB001205, PDB code 1QT2, processed 30.06.99.

12. Chattopadhyaya R, Major pilin of pilus colonization factor antigen III of enterotoxigenic *E. coli*. RCSB001294, PDB code 1C4H, processed 27.08.99.

13. Mukhopadhyay J, Sur R and Parrack P 1999 Functional roles of the two cyclic AMP-dependent forms of cyclic AMP receptor protein from *Escherichia coli*. *FEBS Lett.* **453**, 215-218.

Participation in Conferences/Symposia/ Training Programme/ Awards and Honours Received:

Prof. N. C. Mandal gave a course of lecture on Molecular Biology to the M.Sc. students of Biochemistry and Bio-Technology Departments of the Calcutta University; Served as: (i) a member of the Assessment Committee of Indian Institute of Chemical Biology, Jadavpur, Calcutta; (ii) as a member of the Ph.D. Committee in Biochemistry of the Calcutta University; (iii) as

examiner of Ph.D. Thesis of different Indian Universities; (iv) Served as a member of the Programme Advisory Committee of the Department of Science & Technology, Government of India. Participated in the International Symposium on Transcription and Nucleic Acid-Protein Interaction held at IISc, Bangalore; Invited to chair session in a Symposium Organized by IICB, Calcutta; Nominated by the Vice-Chairman, CSIR as a member of the National Search Committee for the Selection of Director of Indian Institute of Chemical Biology, Jadavpur, Calcutta.

Dr. Pratima Sinha (i) Dr. Pratima Sinha participated in the XIX International Conference on 'Yeast Genetics and Molecular Biology' held at Rimini, Italy (May 25-30, 1999) and visited Prof. B. K. ty's Laboratory at Cornell University, U.S.A. (June-July, 1999). (ii) Attended GRC Meeting at Munnar, Kerala (From 17-20th Nov, 1999); (iii) Invited talk at 'All India Cell Biology Conference held at CCMB, Hyderabad (from 27-29th Nov., 1999).

Dr. P. Chakrabarti presented papers at: XVIII International Union of Crystallography Congress and General Assembly, Glasgow, August 4-13, 1999; Frontiers in Structural Biology, Bangalore, August 25-27, 1999; Protein Structural Bioinformatics and Genomics, Bangalore, August 28, 1999; Protein Structure and Folding, Calcutta, August 30, 1999; DIC Coordinators meeting, New Delhi, December, 1999; Reviewed papers for J. Mol. Biol. and Acta Crystallogr. D.

Dr. Anuradha Lohia attended GRC Meeting at Munnar, Kerala (From 17-20th Nov, 1999).

Dr. P. Parrack taught X-ray crystallography to M.Sc. part II students of the Dept. of Biophysics, Kalyani University, Kalyani, W.B.

Dr. P. Parrack, Jayanta Mukhopadhyay and Runa Sur attended the meeting "Transcription Assembly & Nucleic Acid-Protein Interactions" at Bangalore, June 7-9, 1999. P. Parrack delivered an invited lecture entitled "Activation of transcription of *E. coli* galactose operon by the Cyclic AMP Receptor Protein in its different

cyclic AMP dependent conformational states". J.M. and R.S. both presented posters ("Characterization of cAMP induced structural changes among three hinge mutants of the Cyclic AMP Receptor Protein (CRP) from *Escherichia coli*" and "A novel RNA polymerase binding site upstream of the galactose promoter in *Escherichia coli* exhibits promoter-like activity" respectively).

Dr. P. Parrack and Ajit Bikram Datta attended the "XIII International Biophysics Congress Satellite Symposium on Structural Biology and Molecular Recognition" at SINP, Calcutta, 27-29 Sept, 1999. P.P. delivered an invited talk entitled "Diverse structural forms of a prokaryotic transcription factor are functionally competent."

Dr. C. Ganesh Kumar attended the International Conference on Processed Food for 21st Century, January 14-16, 2000 at Calcutta. He also attended the Workshop on "Protein Structures: Analysis, Modelling and Evolutionary Aspects", 9-10 March 2000, Bioinformatics Centre, Bose Institute, Calcutta.

Dr. C. Ganesh Kumar was selected for Young Scientist Award-1999 and delivered talk at the 40th Annual Conference of Association of Microbiologists of India, 22-24 January, 2000 at CIFA, Bhubaneswar.

Mr. Ajit Bikram Datta participated at the symposium "Frontiers in Structural Biology" at the National Center for Biological Sciences, Bangalore, August 25-27, 1999, and presented a poster entitled "Equilibrium denaturation studies on DNA binding protein cII of bacteriophage lambda: Facts and features". He also participated in the NMR workshop organised at Bose Institute, Calcutta, in March 2000.

Mr. Debnath Pal was awarded "Prof. B. B. Biswas Outstanding Student Award" of Bose Institute for the year 1999.

Mr. Debnath Pal of the dept. of Biochemistry has been awarded the INSA Medal for Young Scientists in Biological Sciences for the year 2000. The award carries a bronze medal and cash prize of Rs. 25,000/-.

GRANTS-IN-AID SCHEMES

Investigator/ Co-investigator	Title	Financed by
Prof. S. Biswas	Signal transduction pathways operative in <i>Entamoeba histolytica</i> mediated by inositol phosphates (Terminated on Oct., 1999)	DAE
Prof. S. Biswas/ Dr. S. Roy	Biochemical and biophysical studies on the novel NTP-NDPase (apyrase) linked with the sensitivity of <i>Mimosa pudica</i> leaflets	DST
Prof. N. C. Mandal	Studies on the mechanism of action of gene G23 in post-transcriptional regulation of bacteriophage L1 gene expression	CSIR
Prof. B. Bhattacharyya	Taxol and colchicine binding to tubulin: A mechanistic study to understand tubulin-antimitotic drug interaction (Started on 23.8.99)	DST
Prof. P. Sinha	Characterisation of MCM 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 an crystallography (Terminated on 14.4.98).	DST
Dr. P. Chakrabarti	Analysis of interactions in helices in proteins and their use in the design of helical peptides	CSIR
Dr. P. Chakrabarti	Analysis of protein-protein association in crystals and complexes (Started on 01.04. 2000)	IFCPAR
Dr. A. Lohia	Role of DNA replication licensing factors in regulating cell division in <i>Entamoeba histolytica</i> (Started on 30.6.99)	DST
Dr. A. Lohia	Construction of yeast artificial chromosomes of <i>Entamoeba histolytica</i>	CSIR
Dr. A. Lohia	Transformation of <i>E. histolytica</i> to study the role of proteins involved in the growth, virulence and drug sensitivity of the parasite	US-INDIA
Dr. A. Lohia	Identification of pathogenic strains of <i>Entamoeba</i> by a PCR based diagnostic assay (Terminated on March 1999)	CSIR

Investigator/ Co-investigator	Title	Financed by
Dr. A. Lohia	Role of tocotrienols as anti-proliferative agents against <i>Entamoeba histolytica</i> (Terminated; onetime money)	UNESCO Special grant
Dr. A. Lohia	Studies on the expression of ras superfamily proteins in <i>Entamoeba histolytica</i> (Terminated on Dec., 1998)	CSIR
Dr. R. Chattopadhyaya	Sequence and three dimensional structure determination of lipase from <i>Aspergillus niger</i>	CSIR
Dr. P. Parrack	Structural basis of control of transcription initiation in bacteria by the cyclic AMP receptor protein	CSIR

Ph.D. AWARDED

Name of the Candidate	University	Year	Title	Supervisor
Hirock Jyoti Datta	Calcutta University	1999	Genetic Studies of the temperate Mycobacteriophage L1	N. C. Mandal
Suroopa Banerjee	Jadavpur University	1998	Studies on the Structure-Function Relationship of Colchicine-Tubulin Interaction	Prof. B. Bhattacharyya
Shalmali Chakrabarti	Jadavpur University	1999	Structural Studies on tubulin :Ligand Binding & Conformation	Prof. B. Bhattacharyya
Rinku Ghosh	Jadavpur University	1999	Physicochemical characterization of novel and complex nucleoside triphosphatase from <i>Mimosa pudica</i> and cloning of its gene	Prof. S. Biswas
Samarpan Majumdar	Calcutta University	2000	Expression of reportergene b-glucuronidase (GUS) under seed-specific Arabidopsis thaliana promoter (2S) in Agrobacterium, yeast and plant	Prof. S. Biswas

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

Dr. Jayashri Basak, Dr. Mita Sen, Dr. Niranjana Das, Dr. Sibes Bera, Mr. Niladri Sekhar Kar, Ms. Surekha Mandal, Mr. V.M.H. Namboodri, Ms. Runa Sur, Ms. Anasuya Ganguly, Mr. Ajay Koomer, Mr. Tapas Kumar Manna, Ms. Sujata Hajra, Ms. Sulagna Ghatak, Ms. Shalmali Chakraborty, Ms. Prajna Mandal, Mr. Kausik Ghosh, Mr. Kaustuv Sanyal, Mr. Jayanta Mukherjee, Ms. Chandrani Chattopadhyay, Ms. Indrani Datta, Ms. Atasi Poddar, Mr. Santanu Kr. Ghosh], Mr. Shankar Bhattacharya, Mr. Taradas Sarkar, Mr. Uttam Kumar Samanta, Mr. Debnath Pal, Mr. Ajit Bikarm Datta, Mr. Rajat Bhattacharyya, Ms. Suchismita Das, Ms. Swati Roy, Ms. Lipika Pal, Ms. Suvroma Gupta, Dr. C. Ganesh Kumar, Ms. Rajasri Bhattacharya, Ms. Debasree Dutta, Ms. Rima Chattopadhyaya.

SUPPORTING STAFF MEMBERS

Mr. Sukumar Saha (upto 30.9.99), 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

Faculty : Prof. Asok. Banerjee (Chairman), Prof. S. Roy, Dr. G. Basu

HIGHLIGHTS

The structure of Calotropin DII and Acetanilide - Cox interaction complex are fully characterised. Structure elucidation of a series of small model complexes of amino acids and nucleotides with adequate hydration has been done. A few medically important potential substrate- receptor, drug-receptor, protein-protein interaction at structural level has been characterized by computer modelling and deposited in Protein Data Bank. Molecular basis of operator recognition by λ -repressor elucidated. Molecular basis of tRNA recognition by GluRS characterised. Development of a model for protein electron transfer have been made.

INSTITUTIONAL PROJECT III

A.1.1. Structural elucidation of the catalytic mechanism and interaction scenario at active site of two enzymes: a) Calotropin DII, a cysteine protease b) Acetanilide and cyclooxygenase interaction : Suparna Bhattacharya, Sibani Chakravarty, Asok K. Pal, Sreya Ghosh, Umesh Halder, Soumen Ghosh Bishnu Mukhopadhyay, **Asok Banerjee**

a) Enzymes are involved in various biological processes with wide importance in medicine and industry. Recently proteolytic enzymes are implicated in anti cancer, AIDS and DNA binding activity. We are working on a native plant cysteine protease, Calotropin DII and the crystallographic structure of it has been solved by molecular replacement method. The sequencing and refinement of the Protein progressed with prime objective to visualize substrate specificity and compare it with other papain family members to rationalize a conclusion. The electron density map confirmed that N-terminal is free in conformity with sequence result and contrary to earlier observation that N-terminal is blocked. The structure is completely solved at 2.1 Å resolution. A series of inhibitor complexes with protease have been done via computer modelling and deposited in PDB.

b) One Acetanilide structure, a non-steroid anti inflammatory compound has been crystallographically solved and modelling study of the complex with cyclooxygenase has been

studied. The characterization of the mode of interaction of this series progresses to a receptor based drug design protocol and our results can be accessed from PDB via internet.

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, Asok Pal, S. Chakraborty, B. P. Mukhopadhyay, **Asok Banerjee**

The mode of interaction of Protein and Nucleic Acid is a prime topic in Biology. Their complexation, binding and recognition embrace many aspects of cellular DNA function & genetic expression. The molecular description of their nature of interaction are emerging in several x-ray structural works of trp-repressor/ λ -repressor/lac-rep/pec/cro - protein nucleotide complexes but none at resolution less than 1Å. Our group has succeeded to co-crystallize and solve a series of small model complexes of amino acids and nucleotide structures of i) a complex of L-serine with two Inosine 5'-monophosphate in highly hydrated form 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. These structures, first of its kind reported, throw 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 also look very similar with inherent disciplines of interaction mode. It is unlikely that these structural signatures will not be exploited in biological function by Nature. Critical model building study with structural information reveals insight in recognition phenomena.

A.3. Modelling & Structural studies of potential receptor-substrate, Drug-Receptor and protein-protein interaction : Suparna Bhattacharya, Sibani Chakravarty, Asok K Pal, Asim Bera, Sreya Ghosh, Bishnu P. Mukhopadhyay, **Asok Banerjee**

i) Visualization of potential substrate-receptor, drug-receptor and protein-protein interaction at structural level in various areas of medical importance has been made possible via computer modelling. i) A 28 mer polypeptide chain, Luffa Acutangula, isolated and purified from bitter gourid seeds, is found to be active in AIDS and Cancer diseases. The molecular mechanism of this polypeptide with Trypsin/Chymotrypsin has been characterized by molecular modelling in absence of x-ray data and deposited in PDB. ii) Non steroidal anti-inflammatory drugs (NSAID) are potent inhibitors of synthesis of prostaglandins. The pharmacological target of NSAIDs is Cyclooxygenase (COX, also known as PGH synthase). Difficulties associated with membrane protein crystallization till date has led us to build a computer aided knowledge based model of the Acetanilide-COX complex to understand structure-function relationship and data deposited in PDB. iii) Covalent binding and oxidation of thiol proteases by NAPQI, (N-acetyl-p-benzoquinone imine) a highly reactive intermediate, by p450-mediated N-hydroxylation of acetaminophen, a major metabolite of Acetanilide, lead to irreversible inactivation of the enzyme and is implicated in pathogenesis of hepatotoxicity by depleting normal glutathione level. Computer assisted molecular modelling studies have been initiated to dock NAPQI on Papain and Calotropin DII for deeper understanding. iv) Structural studies of a series of small molecules of biological interest are continuing with a hope to correlate these structures to their functions.

A4. Molecular basis of operator recognition by λ -repressor : Sudip Bose, Atanu Maiti and **Siddhartha Roy**

Using a mutant F235C repressor, we have shown that the λ -repressor is an asymmetric dimer. We have also shown that this asymmetry is crucially important for recognition of natural operators which consists of asymmetric operator sites. We are now engaged in creating semi-synthetic variants of repressor, that can recognize new DNA sequences.

A5. Molecular basis of tRNA recognition by GluRS : Rajat Banerjee, Soma Samadhar and **Siddhartha Roy**

We have studied a mutant GluRS (C100Y) which disrupts the Zn-finger in the N-terminal domain of GluRS. Using spectroscopic techniques we have shown that this loss of Zinc leads to weakening of amino acid binding, suggesting that this Zn-finger is important in amino acid recognition. We have also studied domain-domain interaction in GluRS and have shown that this domain-domain interaction is very tight and is significantly affected by binding of cognate amino acid. This suggests that amino acid binding may cause a conformational change that is transmitted to the distal anti-codon binding domain.

A.6. Studies of Peptide and Protein Structure and Dynamics : Gautam Basu, Manasi Ghose, Lipika Pal and Raja Bannerjee

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 have earlier been 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 α - helical states. Simultaneously, peptide fragments from the protein Lamprey Hemoglobin have been synthesized and NMR / CD studies are ongoing. One paper has been published in Protein Engineering out of this work.

PUBLICATIONS

1. Bera A K, Mukhopadhyay BP, Ghosh S, Bhattacharya S, Chakravarty S and Banerjee Asok 1999. Influences of cooperative hydrogen bonding economy on protein-nucleic acid complexation: Structure of undecahydrated Inosine C5'-monophosphate and L-glutamine (2C10H13N4O8P, C5H10N2O3, 11H2O) cocrystal at atomic resolution. *J. Chem. Crystallogr.* USA **29**(5), pp 531-540.

2. Dey R and Banerjee Asok 1999 Structure of N,N,N-trimethyl-triazole-2,4,6- trione *Indian J. Physics*. **73A**(4), pp 543-551.

3. Bhattacharya S, Chakraborty S, Ghosh S, Bera AK, Pal A and Banerjee Asok 1999. A series of important structural reports are published in Protein Data Bank (1999) available via internet from Protein Data Bank. PDB codes: ID30, 1DF2, IDJJ, IEFF, IC7X

4. Raha T, Chattopadhyay D, Chattopadhyay D & Roy S 1999. A phosphorylation induced major structural change in the N-terminal Half of the P protein of Chandipura Virus. *Biochemistry*. **16**; **38**(7):2110-6.

5. Geanacopoulos M, Vasmatzis G, Lewis DE, Roy S, Lee B, Adhya S 1999 GalR

mutants defective in repressosome formation *Genes Dev* **13**:1251-62

6. Roy, S. 1999 An Exact Solution of Abortive Initiation Rate Equation *Anal Biochem* **271**:86-9.

7. Roy, S. 1999 Multi-functional Enzymes and Evolution of Biosynthetic Pathways: Retro-evolution by jumps. *Proteins*. **1**; **37**(2) : 303-9.

8. Sengupta S, Mahapatra P, Chakrabarti G, Roy S & Bhattacharyya B 2000 NBD-isocolcemid: A probe for colchicine binding sites of tubulin *Biochemistry* **39** 2227-2234.

9. Sakaguchi K, Saito S, Higashimoto Y, Roy S, 2000 Carl W. Anderson1, and Ettore Appella DNA Damage-mediated Phosphorylation of Human p53 Threonine 18 Inhibits Mdm2 Binding *J. Biol. Chem.* **275**: 9278-9283.

10. Deb S, Bandyopadhyay S and Roy S 2000 DNA sequence dependent and independent Conformational changes in Multipartite operator recognition by λ -repressor. *Biochemistry* **39** 3377-83.

11. Pal L, Basu G 1999 Novel protein structural motifs containing two-turn & longer 3_{10} -helices; *Protein Engineering* **12**(10) pp 811-814.

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

Prof. A. Banerjee delivered an invited talk on "Molecular Design of Life and Unity in Biodiversity" at the National Seminar on Environmental Biology held at NEHU, Shillong. 1999.

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GRANTS-IN-AID SCHEMES

Investigator/ Co-investigator	Title	Financed by
Prof. A. Banerjee	Structural basis of differences in catalytic mechanism of different Cysteine Proteases, Calotropin DII by X-ray and modelling	DBT
Dr. S. Roy	A study of structural and molecular basis of co-operative binding of operator sites of repressor.	CSIR
Dr. S. Roy Dr. G. Basu	Molecular basis of recognition of tRNAs by Aminoacyl-tRNA synthetases	DST
Dr. G. Basu	Theoretical studies of peptide and protein dynamics from a collective motion viewpoint	CSIR
Dr.G..Basu	A thermodynamic scale for the Relative preferences of amino acids between 3_{10} and α -helical states.	DST
Dr.G. Basu(with Dr.P.Chakraborti)	Analysis of interactions in helices in proteins and their use in the design of helical Peptides	CSIR

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

Dr.Jayati Sengupta, Mr.Amit Kr Mondal, Mr. Rajat Banerjee, Ms.Sunanda Deb,Ms. Manashi Ghose, Ms.Lipika Pal, Ms.Ruchira Chattopadhyaya, Ms.Suparna Bhattacharya, Ms.Sibani Chakrabarty, Ms.Sanchari Kar, Ms. Soma Samaddar, Mr. Atanu Maity, Mr. Asim Kr Bera, Dr. Sudip Bose, Dr. Soumen Ghosh, Mr. Raja Banerjee.

OTHER SUPPORTING STAFF MEMBERS

A. Bhattacharyya, B. Majumder, B.Marick, B. Munshi, S. Jogsharma

Animal Physiology Section

Faculty : Prof. A. K. Ray (In-Charge) and Dr. G. Sa

HIGHLIGHTS

Basic fibroblast growth factor-mediated neuronal outgrowth requires activation of Stat-1 and Stat-3 transcription factor. Endothelin stimulates differentiation as well as maturation of glial cells through the signaling pathways involving Jak-1 and Stat-1. Curcumin, the active component of turmeric showed anti-tumor activity in Ehrlich's Ascites tumor cell *in vivo* and breast cancer cell (MCF-7) *in vitro*. Some morphological characters to identify triploidy in Catfish in an easy and farmers friendly way have been worked-out. The juvenile rats appear to be more susceptible to phenylhydrazine (environmental pollutant) than the adult rats.

INSTITUTIONAL PROJECT I

A. Environmental Physiology and Hormonal Regulation :

A.1. Carbofuran effects on fish hepatocytes *in vivo* and *in vitro* : Arun K. Ray, Rama Ghosh and Manik Ch. Ghosh

Carbofuran (CF), a carbamate pesticide is used in agriculture world-wide and it shows toxic effects in fish. Treatments with CF to Singi fish (*H. fossilis*) causes elevation of hepatic phospholipid and cholesterol contents by 2 to 3 fold in comparison to the untreated fishes. Cultured hepatocytes from Singi fish, when treated with graded doses of CF in the medium, showed dose-dependent inhibition of the membrane Ca^{2+} -ATPase activity with graded accumulation of hepatocyte membrane cholesterol. Conversely, when cholesterol molecules were inserted in untreated hepatocyte membrane by sonication, it also showed gradual decline in the membrane Ca^{2+} -ATPase activity depending on the degree of cholesterol insertion in the hepatocyte membrane. Both the *in vivo* and *in vitro* experiments state that CF causes accumulation of membrane lipids with concomitant fall in the Ca^{2+} -ATPase activity. Such behavior of hepatocytes could be one of the defensive cellular responses to the toxic effects of CF.

A.2. Production of triploid and unisex fish : Morphometric characteristics of triploid Singi fish : Arun K. Ray and Basant K. Tiwary

In course of our study we have reported production of a triploid (3n) variety of cat fish (*H. fossilis*) having 2.5 times higher growth rate than the normal diploid (2n) variety. We have identified triploidy by the chromosomal analysis and estimating the cellular and nuclear diameter of erythrocytes. We have observed certain morphometric characters specific of triploidy and those can be used very easily by the fish farmers in the land. The ratio between post-orbital length and pre-orbital length has been found to be 71% higher in triploids. The ratios between standard length and head width, and body length and body depth were significantly lower in triploids compared to the diploids.

A.3. Role of estrogen in ovarian development of freshwater prawn (*M. rosenbergii*): Arun K. Ray, Anuradha Chaudhuri and Sharmila Chatterjee

Yolk proteins from mature eggs of freshwater prawn, *Macrobrachium rosenbergii* were purified by selective precipitation and Ultrogel AcA 34 gel filtration, and HA-ultrogel affinity chromatography by which yolk protein of sufficient purity was obtained. Antibodies raised against purified yolk protein cross reacted with hepatopancreatic cell extract in western blot analysis. Proteins bearing antigenic similarity to yolk protein were detected only in mature female hemolymph. Evidence obtained indicate hepatopancreas to be a site of extraovarian source of egg protein vitellin precursor in the freshwater prawn.

Annual Report

A.4. Thyroid hormone regulation of mammalian brain function : Arun K. Ray and Nilkanta Chkraborty

During our investigation to understand the non-genomic actions of thyroid hormone (TH) on adult brain functions, we have demonstrated relationships with synaptosomal membrane Ca^{2+} -ATPase activity, nitric oxide synthetase (NOS) activity and acetylcholine esterase (AChE) activity in TH induced condition in rat. It appeared that Ca^{2+} influx was the main factor relating the cascade of incidents that expressed TH action. In an *in vitro* study using cerebrocortical synaptosome it has been demonstrated that a transient rise in intra-synaptosomal $[\text{Ca}^{2+}]_i$ concentration occurs within 4 sec. of T_3 application and that the dose-dependent rise in $[\text{Ca}^{2+}]_i$ occurs within 0.1-100 nM T_3 doses in depolarized condition only. Therefore, it appears that the T_3 action on $[\text{Ca}^{2+}]_i$ is related to the voltage-dependent Ca^{2+} entry through synaptosomal membrane.

A.5. Phenylhydrazine hydrochloride (PHH)-thyroid hormone interaction in rat : Arun K. Ray and Mitali Pramanik

During our investigation on the toxic effects of PHH and its protection by thyroid hormone (TH) we have demonstrated a novel nature of TH-PHH interaction on amelioration of most of the PHH effects at the subcellular level in erythrocytes in juvenile male rats. We were very much interested to compare the TH responsiveness to the PHH-induced toxicity in juvenile and adult stages in rat. As a prerequisite to this the severity of PHH effects has been checked in rats of both the age groups. After treatment of PHH to both juvenile and adult rat a significant fall in RBC number, Hb content, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration was noticed in both the cases with remarkably higher degree of PHH effects in juvenile rats. Although, the adult rats recovered totally from the toxic PHH effects on haematological parameters on 16th day after treatment, the juvenile animals could not.

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

B.1. Topical application of estradiol-17 β appears to be effective in transport of this hormonal steroid to silkgland : Arun K. Ray and Bela Keshan

In our earlier studies we used injections of estradiol-17 β (E_2) as a mode of application of the hormone to demonstrate its actions in fat body and ovary of silkworm (*Bombyx mori* L.). In order to make it more simpler and farmers friendly, experiments have been conducted with topical application of the hormone to the silkworm pupae and its efficiency has been examined on accumulation of this hormone in silkgland cytosol. We have observed that posterior silkgland contained an endogenous level of E_2 which showed a variation in its content on different days of fifth larval instar. Topical application of a dose of 1.0 $\mu\text{g/g}$ of E_2 demonstrated a rise of about 12-fold of the hormone in the silkgland in comparison to the untreated control. Therefore, this topical mode of application of the hormone may be used as a simpler farmers friendly procedure to induce growth and development of silkgland.

C. Cell Signaling

C.1. Basic fibroblast growth factor stimulates sis-inducing factor-DNA binding activity : the causative role in neuronal cell differentiation : Gaurisankar Sa, Tanya Das (Immunotechnology Section) and Suman Pal

In contrast to our knowledge of signal transduction pathway mediating cell proliferation, little is known about the pathways of nuclear signaling responsible for Fibroblast growth factor (FGF)-induced cellular differentiation. We have demonstrated that basic FGF elicit a distinct signaling mechanism by which it mediates neonatal rat neuronal cell differentiation. It has been shown that unlike fibroblast cells neuronal cells differentiates as a result of basic FGF stimulation. Upon receptor activation basic FGF stimulates tyrosine phosphorylation of Stat-1 and Stat-3 isoforms of signal transducer and activator of transcription (Stat) proteins. The tyrosine phosphorylated Stat-1 and Stat-3 then heterodimerizes forming sis-inducible factor (SIF) and translocates to the

nucleus. The SIF is then additionally phosphorylated at the serine residue of the C-terminal end of Stat-1 and Stat-3 by activated Erk-1. The phosphorylated and activated SIF then binds to sis-inducible element (SIE), which can induce transcription of differentiation-specific genes.

C.2. Molecular mechanisms of endothelin-induced differentiation and maturation of microglial cells, the resident macrophage of brain: **Gaurisankar Sa**, Tanya Das (Immunotechnology Section) and Tathagata Chaudhury

Although our understanding of many components is unclear, endothelin (ET) peptides appear to evoke transcriptional activation of previously quiescent genes which are responsible for the differentiation as well as maturation of glial cells to microglial cells, the resident macrophages of brain. For the first time, we have demonstrated that ET-1 elicits a distinct signaling mechanism by which it mediates glial cell 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 non-receptor tyrosine kinase) through tyrosine phosphorylation. Phosphorylated and activated JAK-1 then tyrosine phosphorylates p91 Stat-1 (Signal transducer and activator of transcription) protein. The tyrosine phosphorylated Stat-1 is then homodimerized forming a sis-inducible factor (SIF) and translocates to nucleus. The SIF is then additionally phosphorylated at the serine residue by Erk-1, activated by ET-1 through a protein kinase C-dependent signaling pathway. The phosphorylated and activated SIF binds to sis-inducible element (SIE), which alone is sufficient, under certain circumstances to mediate endothelin-induced transcriptional activation of differentiation specific genes.

D. Apoptosis

D.1. Curcumin: a new look to selective apoptosis of tumor cells: **Gaurisankar Sa**, Tanya Das (Immunotechnology Section), Suman Pal and Tathagata Chaudhury

It has been established that, the active component of turmeric, curcumin (diferuloylmethane)

has an anti-tumor property. Our laboratory is actively engaged to find out the mechanisms of anti-tumor activity of curcumin using Ehrlich's ascites tumor cells (EAC) in the peritoneal cavity of mice as a model. Analysis of morphological features and cell cycle phase distribution as well as nuclear fragmentation and Br-dUTP incorporation assays reveal an induction of apoptosis in EAC by curcumin administration *in vivo*. Curcumin blocks the transition of G1 to S phase of cell cycle indicating the growth arrest of EAC. In contrast, curcumin stimulates cell cycle progression in splenic lymphocytes of the tumor-bearing mice. The mechanisms of EAC apoptosis and splenic lymphocytes activation involve differential regulation of pro-proliferative protein Bcl-2 and pro-apoptotic proteins p53 and Waf-1. In EAC, level of Bcl-2 decreases whereas level of p53 and Waf-1 increases significantly after curcumin treatment. On the other hand, in splenic lymphocytes curcumin induces the Bcl-2 expression keeping the levels of p53 and Waf-1 as low as untreated cells. Thus, it appears that there may be a cross-talk between Bcl-2, p53 and Waf-1 gene expressions and such cross-talk may be important factor determining whether a cell would switch on the apoptotic phenomenon or not. Caspase-3, the downstream of p53, is also activated by curcumin in EAC but not in splenic lymphocytes. The levels of free radical degenerating enzyme like superoxide dismutase (SOD), catalase, peroxidase and glutathione S transferase (GST) activities are also increased in liver of tumor bearing mice indicating the antioxidant and detoxifying property of curcumin. Further studies are in progress.

D.2. Curcumin: Molecular mechanism(s) of cell cycle arrest and apoptosis: **Gaurisankar Sa**, Tanya Das (Immunotechnology Section), Tathagata Chaudhury and Suman Pal

Curcumin (diferuloylmethane), the active component of turmeric has an anti-inflammatory and anti-cancer property. Our laboratory is actively engaged to find out the mechanism(s) of anti-cancer activity of curcumin using breast cancer cell (MCF-7) as an *in vitro* model. It has been shown that curcumin inhibits nuclear translocation of cyclin A and expression of cyclin D1 level in cell there by blocking the progres-

sion of cell cycle from G0/G1 to S phase. Curcumin also stimulates the expression of p21 Waf-1 and p27 Kip-1 levels in breast cancer cells but not in normal breast epithelial cells, thereby prevents the cyclin/cdk complex formation in breast cancer cells. As a result of these inhibitions cells are arrested in G0/G1 phase of cell cycle. Further works are in progress.

E. Aging

E.1. Molecular regulation of cell growth and death: An approach to control aging: **Gaurisankar Sa**, Tanya Das (Immunotechnology Section) and **Prasanta K. Ray** (Immunotechnology Section)

Aging as a phenomenon in the life span of organisms has intrigued mankind from time immemorial. It is characterized by a set of complex endpoints, which appear as a series of molecular events. It is now recognized that apoptosis plays an important role in many physiological processes. Of particular importance is its both positive and negative role in aging. Therefore, the regulation of apoptosis may pose to be an inviting target for therapeutic intervention in aging. Protein A of *Staphylococcus aureus* has been found to possess anti-oxidant, anti-tumor and immunostimulatory properties. During aging the balance shifts from pro-proliferative one to pro-apoptotic one ultimately leading to cell death. Aging also has a direct relationship with the cellular status of reactive oxygen intermediate (ROI) including oxy, peroxy, and hydroxy radicals. Thus we studied the $\text{Cu}^{2+}/\text{Zn}^{2+}$ SOD as an anti-oxidant and free radical quencher in the Protein A-treated mice. Further studies are being done which will highlight the telomere and telomerase activity during this process.

PUBLICATIONS

1. Tiwary BK, Kirubakaran R & Ray AK 1999 Altered body shape as a morphometric indicator of triploidy in Indian Catfish, *Heteropneustes fossilis* (Bloch); *Aquaculture Research* **30** 907-910.

2. Sa G & Das T 1999 Basic FGF stimulates phospholipase A₂, Phospholipase C- γ 1 and phospholipase D through distinguishable signaling mechanisms; *Mol. Cell. Biochem.* **198** 19-30.

3. Das T, Sa G, Sinha P & Ray PK 1999 Induction of cell proliferation and apoptosis: dependence on the dose of the inducer; *Biochem. Biophys. Res. Commun.* **260** 105-110.

4. Das T, Sa G & Ray PK 1999 Mechanisms of Protein A superantigen-induced signal transduction for proliferation of mouse B cells; *Immunol. Lett.* **70** 43-51.

5. Ghosh AK, Sinha P, Das T, Sa G & Ray PK 1999 *S. aureus* superantigen protein A expand CD4+ / CD19+ / CD34+ cells in mice - a potential immunorestorator; *Biochem. Biophys. Res. Commun.* **256** 142-146.

6. Sinha P, Ghosh AK, Das T, Sa G & Ray PK 1999 Protein A of *S. aureus* evokes Th1 type response in mice; *Immunol. Lett.* **67** 157-165.

7. Amiya K. Ghosh, Srikanta Jana, Tanya Das, Gaurisankar Sa, Nripen Mondal and Prasanta K. Ray 1999 Protection by protein A of apoptotic death caused by anti-AIDS drug zidovudine. *Biochem. Biophys. Res. Commun.* **264** 601-604.

8. Chakraborty N and Ray AK 2000 Rise of intrasynaptosomal Ca^{2+} level and activation of nitric oxide synthase in adult rat cerebral cortex pretreated with 3-5-3'-L-Triiodothyronine; *Neuropsychopharmacology* **22** 36-41.

9. Chaudhuri A, Krishnan N & Ray AK 2000 Induction of subcellular malic dehydrogenase activity in fat body cells of diapausing pupae of wild tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera; Saturniidae) by 17 β estradiol; *Current Science* **78** 179-184.

10. Das T, Sa G, Subbulakshmi V, Subramaniam S, Sen PC, Biswas S & Ray PK 2000 Protein A-activated rat splenic lymphocyte proliferation involves tyrosine kinase-phospholipase C-protein kinase C pathway; *Immunopharmacol. Immunotoxicol.* **22** 75-90.

Participation in Conferences / Symposia / Training Programme / Awards and Honours Received

Prof. Arun K. Ray delivered talks in National Seminar on Environmental Biology and Fish Biology at Visva-Bharati during April 6-7, 1999, National Symposium on Trends in Environmental Biology at North Eastern Hill University, Shillong from June 22-24, 1999, 5th Indian Fisheries Forum at Bhubaneswar from 17-20th January, 2000, 12th Refreshers' Course in Zoology at University of Burdwan in November 1999, Refreshers' Course in Physiology, Calcutta University, in March, 2000 and in North-East Regional Entomologist Meet at Tripura University from March 22-23, 2000.

Prof. Arun K. Ray received Foundation Day Award on 30th November, 1999.

Ph.D. AWARDED

Name of the student	University	Year	Title	Supervisor
Ms. Bela Keshan	Calcutta	1999	Responses of silkgland to Estradiol-17 β in silkworm, <i>Bombyx mori</i> L (race Nistari)	Prof. Arun K. Ray

GRANT-IN-AID SCHEME

Investigator/ Co-Investigator	Title	Financed by
Dr. G. Sa	Intracellular signaling mechanisms that mediate endothelin-induced transcription of immediate early genes.	DST
Dr. G. Sa	Intracellular signaling mechanisms of basic fibroblast growth factor-stimulated early gene transcription.	CSIR
Dr. G. Sa	Proliferative signaling and topoisomerase II α regulation.	NIH, USA

Dr. Gaurisankar Sa visited San Jose, USA for Special Training Programme on Flowcytometer in 1999. He also visited and worked in the Cleveland Clinic Foundation, USA in the field of cell cycle control of mammalian cells during 1999-2000. Dr. Sa delivered invited lecture in the Dept. of Molecular Biology, Cleveland Clinic Foundation, USA in 2000.

Dr. Mitali Pramanik attended 15th National Training Programme in Electron Microscopy for Scientific Investigators (Basic and Advance) at All India Institute of Medical Sciences, New Delhi, from 7th to 29th February, 2000. Sri Manik Ch. Ghosh presented a paper in the 5th Indian Fisheries Forum from 17th-20th June, 2000, at Bhubaneswar. Sri Suman Pal and Tathagatha Choudhuri presented papers in XXVI Annual Conference and Symposium on Cancer Immunology in the New Millennium, from January 13-15, 2000, at Mumbai.

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

Dr. Mitali Pramanik (From 27.5.99), Mr. Manik Ch. Ghosh, Mr. Suman Pal and Mr. Tathagatha Choudhuri

SUPPORTING STAFF MEMBERS

Dr. A. K. Dasmahapatra (upto 03.02.2000), Mr. I. Ray, Mr. S. K. Ghosh, Mr. A. K. Santra and Mr. N. C. Jana

Plant Molecular & Cellular Genetics

Faculty : Prof. A.N. Lahiri Majumder (Head), Dr. Amita Pal, Dr. Sampa Das, Dr. S.R. Sikdar.

HIGHLIGHTS

The cytosolic inositol synthase(s) from *Oryza* and *Porteresia* have been completely sequenced. A full length cDNA for cytosolic FruP₂ase from *Porteresia* has been sequenced and submitted to Gene Bank (Accession number AF 218845). Current study elucidate that the variation in the genome duplication pattern of two cotyledon types determines the mode of differentiation in *Vigna radiata*. Lectins with insecticidal property have been characterized. Genetic transformation protocol for a number of crop species has been standardized.

INSTITUTIONAL PROJECT I.

A.1. Inositol metabolism in relation to salinity stress: Implications in the chloroplast functions: **A.N. Lahiri Majumder**, Nitai Chand Hait and Manoj Majee

Activation of the chloroplast inositol synthase during salinity stress in salt-tolerant varieties of rice has been found to be a two-step procedure involving (i) light-dependent cleavage of a precursor 80 KD subunit protein to a 60 KD protein (ii) phosphorylation of the 60 KD subunit protein by a light/salt-induced protein kinase. Both the processes are operative during light/dark growth of the seedlings under salt-environment, signalling increased synthesis of the osmolyte, inositol.

Full length cDNA for cytosolic inositol synthase(s) from *Oryza* (RINO) and *Porteresia* (PINO) earlier obtained in this laboratory, have been completely sequenced. Analysis of the sequences reveal differences in the coding region between 174-320 amino acids in an otherwise identical gene sequence. The two cDNAs were expressed in *E. coli* as pET-20b constructs and the enzyme protein, overexpressed in the inclusion bodies, purified upon solubilization. Biochemical characterization of the purified expressed enzyme protein revealed that the PINO is salt-tolerant *in vitro* upto 400 mM NaCl, RINO being salt-sensitive. Attempts are being made to look for possible correlation between salt sensitivity/tolerance and the structure of the gene/protein.

A.2. Regulation & expression of Fructose-1,6-bisphosphatase during salinity stress in rice: **A.N. Lahiri Majumder**, Shilpi Ghosh & Sanghamitra Bhattacharyya

Following the earlier observations that cytosolic and chloroplastic Fructose-1, 6-bisphosphatase(s) (FruP₂ase) in rice are affected by salinity stress, the purified enzyme(s) have been studied indepth along with the expression of the protein through Northern & Western blots during salt-growth. A full length cDNA for cytosolic FruP₂ase from *Porteresia* has been cloned through RT-PCR, sequenced and submitted to Gene Bank (Accession number AF 218845). A partial cDNA for the chloroplast FruP₂ase from *Porteresia* has been isolated through similar procedure. Attempts are being made to obtain full length cDNA for chloroplast FruP₂ase (s) for both *Oryza* and *Porteresia* for *in vitro* expression and indepth structural studies of the expressed protein(s) which differ in salt-tolerance properties.

A.3. Standardization of regeneration protocol for *Oryza* & *Porteresia* : **A.N. Lahiri Majumder** & Sitakanta Pattnaik

A number of indica varieties of *Oryza* are used for establishment of a callus culture and plant regeneration protocol. Proliferated embryogenic calli were used for plantlet regeneration. High frequency plant regeneration was achieved for Pusa Basmati 1 and Matla cultivars. Shoot development from axillary buds was induced for *Porteresia*. Efforts are now being made for induction of multiple shoot development and enhanced rooting of the regenerated shoots.

Annual Report

A.4. Phylogenetic analysis of L-myo-inositol-1-phosphate synthase: **A.N. Lahiri Majumder**, Anirban Chatterjee and Sanghamitra Bhattacharyya

Previous work from this laboratory demonstrated the ancient nature of the L-myo-inositol-1-phosphate synthase gene/protein. In order to trace the origin of the chloroplast inositol synthase gene, cyanobacterial sources were looked for. A 60 to 70 KD protein, cross-reactive to the *Euglena/Entamoeba* inositol synthase, was detected in Western blots of proteins from *Spirulina*, *Anabaena* and *Synechocystis*. The enzyme was purified to homogeneity and characterized from all three sources. Analysis of the completely sequenced *Synechocystis* genome revealed at least four unidentified ORFs coding for hypothetical proteins as putative synthase ORF by using a number of computational programmes.

A.5. Pathway engineering of fatty acid biosynthesis in Brassica: **A.N. Lahiri Majumder**, **Sampa Das** and Shilpi Ghosh

Although most oil-yielding crop plants can produce alpha-linolenic acid (18:3), production of a nutritionally important gamma-linolenic acid (GLA) from linoleic acid (18:2) by the δ 6 desaturase reaction, is restricted to only a few plant species and the cyanobacterium like *Spirulina*. Hence, introgression/expression of δ 6 desaturase from such sources to oil-yielding crop plants like *Brassica* have tremendous biotechnological implications. Keeping this in view, a full length δ 6 desaturase gene from *Spirulina* has been PCR amplified and cloned in pGEM-T vector and the clone being confirmed by DNA sequencing. Attempts are currently made to express the cloned gene into bacterial expression vector.

B.1. Analysis of agromorphological traits in MYMV susceptible T9 cultivars of *Vigna mungo* and three resistant lines : **Amita Pal**, **Tapas Kumar Ghose**, Sabyasachi Kundagrami and Jolly Basak

The Mungbean Yellow Mosaic Virus (MYMV) susceptible T9 cultivar of *V. mungo* and three resistant lines, VM -1, VM- 4 and VM - 6, were analyzed for different agromorphological characters. No appreciable changes in plant

height, 100 seed weight, seed yield/plant, number of seeds/ plant and pod length were observed amongst these susceptible cultivar and resistant lines. However, significant decrease ($P > 0.5$) in branch number was observed in resistant lines as compared to susceptible cultivar. This decrease in branch numbers was compensated in the resistant lines by significant increase ($P > 0.5$) in number of pods/ plant and seeds/ pod. Of the three resistant lines, VM -1 possessed the highest number of pods/ plant, branch number and seeds/ pod.

The susceptible cultivar T-9 (female) was crossed with two resistant lines, VM-1 and VM-4 (male), and F₁s raised. Seeds from these F₁s would be used to produce F₂ populations segregating for MYMV reaction.

B.2. Cytogenetical analysis of differential behaviour of two cotyledon types in *Vigna radiata* : **Amita Pal**, Malay Das and Shamik Das

Different stages of zygote formation and cotyledon development under *in vivo* condition were studied and the days after fertilization were scored (Figs. DAF 1, 2, 4-7). This is in continuation of our earlier work, based on this information different stages of Cot and Cot E explants were collected and nuclei were isolated. The DNA contents of the two types of cotyledonary explants of *V. radiata* were measured and analysed by flow cytometry using FACS -CALIBUR. The variation in the genomic duplication pattern of Cot and Cot E explants at the different stages of zygotic embryo development was analysed. This study provides an insight into what is clearly one of the crucial differences between these two cotyledon types, which determines direct shoot differentiation in one under the influence of cytokinin, while in other, callus preceded shoot differentiation.

C. Development of insect resistant plant varieties with potential lectin genes:

C.1. Identification, extraction and purification of lectins from different plant species : **Sampa Das** and Santanu Banerjee

As part of the crop improvement programme generation of insect resistant crop variety has been taken as a challenging tool. Keeping this in view, different plant lectins have been isolated and purified from different plant parts of *Al-*

lium sativum, *Allium cepa* and *Arum* spp. through standard procedures. Those lectins have been characterised through agglutination assay with rabbit erythrocytes. Their binding affinity have been monitored through agglutination inhibition assay using different concentration of sugars. An elaborate insect bioassay has been conducted with different concentration of those lectins supplemented in artificial diet on first and second instar of two aphid spp. and one model homopteran insect pest. Determination of the efficacy of those lectins on insect growth and fecundity have been established. Coding sequence of garlic leaf lectin has been cloned and its transfer to target plant is underway.

C.2. Standardization of efficient protocol for transforming plant species like mustard, chickpea and pigeonpea : **Sampa Das**, Krishna Roy and Indrajit Datta

Engineering and expression of agronomically important traits into plant varieties necessitated the standardization of efficient regeneration and transformation protocols. Monitoring of the transformation efficiency of chickpea, pigeonpea and mustard have been carried out with the help of *gus* reporter gene. Cotyledonary nodal explants or the hypocotyl explants have been infected with *Agrobacterium* strain LBA 4404 containing the chimeric *gus* reporter gene and hygromycin gene and incubated in MS medium for cocultivation. The infected explants were then transferred to MS media with supplementation of plant hormones for initiation of multiple shoots. The emerging shoots have been selected in presence of hygromycin. After two to three months of selection individual shoots have been transferred to elongation media and then to root generation media for root initiation. *Gus* assay have been carried out with the leaves and other parts of the hygromycin resistant presumptive transgenics. In case of chickpea and pigeonpea the frequency of transformation has turned out to be around 0.2 - 0.3% whereas it appeared to be around 1% in case of mustard.

D.1. Protocol standardization for development of somatic hybrid between *Brassica juncea* and *Rorippa indica* : **S.R. Sikdar** and Palash Mandal.

To introgress summer growing character and aphid resistance property of *Rorippa indica* to

cultivated oil yielding *Brassica juncea*, protoplast fusion programme was undertaken. After PEG-mediated fusion, somatic hybrid calli were isolated through micropipetting and grown upto macrocalli stage. Till date 3 somatic hybrid shoots have been regenerated; efforts are being made to develop more somatic hybrid plants from the fusion programme.

PUBLICATIONS

1. Pal A 1999 Production of Insecticides. In: Biotechnology : Secondary Metabolites (Ed. K.G. Ramawat and J. M. Merillon) Science Publishers, Inc., U.S.A. and Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp. 111- 122.
2. Pal A 1999 Microstructure of pyrethrin containing oil glands in leaves and petioles of *Chrysanthemum cinerariaefolium* Vis. Phytomorphology **49** 463- 468.
3. Banerjee S, Patra NK & Pal A 1999 Karyotypic and Biochemical analysis of spontaneous triploid of *Solanum nigrum* complex. J. Cytol. Genet. **34** 101- 109.
4. Paul S & Sikdar SR 1999 Expression of *npt II* marker and *gus* reporter genes and their inheritance in subsequent generations of transgenic *Brassica* developed through *Agrobacterium*-mediated gene transfer; Curr. Sci., **76** 1569-1573.
5. Nandi A, Basu D, Das S & Sen SK 1999. High level expression of soybean trypsin inhibitor gene in transgenic tobacco plants failed to confer resistance against damage caused by *Helicoverpa armigera*. J. Bioscience **24(4)** :445-452.

Participation in Conference/Symposia/Training Programme/Awards and Honours :

Prof. A.N. Lahiri Majumder worked as a Rockefeller Biotechnology Career Fellow in the Department of Biochemistry, University of Arizona, Tucson during June/July, 1999. Participated as an invited speaker in International meeting on Rice Biotechnology in Phuket, Thailand and in the workshop "Protein structures : analysis, modelling and evolutionary aspects" organized by Bioinformatics Centre, Bose Institute.

Dr. Amita Pal has served as guest faculty to teach various topics of Plant Biotechnology and Biodiversity for the final year M.Sc. students at the B. C. Guha Centre for Biotechnology and Genetic Engineering, Calcutta University, Calcutta. Also served as paper setter and external examiner for M.Sc. Biotechnology Course of Calcutta and Visva Bharati University.

Dr. Sampa Das attended the National Conference on Plants, Microbes and Environment, Dept. of Botany, the University of Burdwan and presented a talk entitled "Plant Protein as Insect Control Element", 11-12 March, 1999.

Dr. Samir R. Sikdar was on deputation for 3 months (From 26th May to 31st August, 1999) in the laboratory of Dr. Swapan K. Datta at International Rice Research Institute (IRRI), Manila, Philippines, as "On-the-job-trainee" to get training on rice transformation. This training programme was sponsored by Rockefeller Foundation.

Mr. Santanu Bandyopadhyay attended the 68th annual general meeting of the Society of Biological Chemists (India) and symposium on Current Trends in Biotechnology, Bangalore, December 1999, and presented a poster.

Ms. Krishna Roy and Ms. Jolly Basak participated in the 25th Mahabaleshwar Seminar on Modern Biology, Functional Genomics and Plant Biotechnology, during February 6-12, 2000.

Symposia/Seminar held

Plant Molecular & Cellular Genetics in collaboration with the Centre for Plant Molecular Biology and the Department of Botany, organized a two-day workshop on "Plant Genomics : Emerging Trends and Technology" during March 24-25, 2000 held at Bose Institute. All faculty members and research scholars of PMCG section participated in the workshop.

GRANT-IN-AID SCHEMES

Investigator/ Co-investigator	Title	Funding Agency
Prof.A.N. Lahiri Majumder	Role of inositol and polyamine metabolism in relation to osmotic stress in rice (<i>Oryza sativa</i>)	Indo-US
Prof.A.N. Lahiri Majumder	Metabolic engineering of inositol pathway in rice: Implications in the chloroplast functions.	DBT (CPMB Phase II)
Prof.A.N. Lahiri Majumder	Plant Fructose-1,6-bis-phosphatase(s): Regulation and expression during salinity stress in rice.	CSIR
Dr.Amita Pal/ 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
Dr. Amita Pal/ Dr T. K. Ghose	Development of PCR- based DNA-marker (s) linked with MYMV- resistance in <i>Vigna mungo</i>	DBT
Dr. Amita Pal	Micropropagation of commercially important bamboo species and in vitro induction of precocious flowering	CSIR
Dr. Sampa Das	Cloning of a new lectin gene from <i>Allium</i> sp in order to develop transgenic aphid resistant plants.	DAE

Ph.D. AWARDED

Name	University	Year	Title	Guide
Ms. Rajalakshmi Venkataraman	Jadavpur University	1999	Development of the restoration system in mustard [<i>Brassica juncea</i>] in the context of Barnase and barstar strategy for development of nuclear male sterility.	Dr. Sampa Das
Ms. Soma Paul	Calcutta University	1999	Towards manipulation of the genetic make up of the oil yielding <i>Brassica</i> sp. through non-conventional methods.	Dr. S.R. Sikdar

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

Dr. Pinaki Chowdhury, Dr. Shilpi Ghosh, Dr. Sabyasachi Kundagrami, Dr. Sanghamitra Bhattacharyya, Dr. Sitakanta Pattanaik, Ms. Krishna Roy, Santi Ranjan Dey, Nitai C. Hait, Shamik Das, Debabani Chakraborty, Tathagata Roychoudhury, Sanjoy Guha Roy, Palash Mandal, Anirban Chatterjee, Mitu De, Sudarsana Biswas, Indrajit Dutta, Santanu Bandopadhyay, Manoj Majee, Jolly Basak and Malay Das.

SUPPORTING STAFF MEMBERS

Puspita Mukherjee, Ashim Kumar Nath, Motilal Singh, Pravash Basu Roy, Ganesh Chandra Dey, Arup Kumar Dey, Sk. Eklas Ali and Subal Basak.

Immunotechnology Section

Faculty : Prof. P. K. Ray and Dr. Nripendranath Mandal

HIGHLIGHTS

Protein A (PA), the cell wall protein of *Staphylococcus aureus*, has been found to initiate pro-proliferative signals to the immunocytes and pro-apoptotic signals to the tumor cells of the same host. Two peptides, 16 and 20 mer as derived from PA by protease digestions, have been found to mimic many of these functions of the mother protein.

INSTITUTIONAL PROJECT V

A1. Protein A-induced Ehrlich's ascites tumor cell killing is via cell cycle arrest and apoptosis :
Prasanta K. Ray, Tanya Das and Gaurisankar Sa (Animal Physiology Section)

Protein A (PA) of *Staphylococcus aureus* has already been reported as a potent antitumor agent. This investigation has been undertaken to find out the effect of PA on EAC cell cycle. Flowcytometric analysis of cell cycle pattern of Ehrlich's ascites tumor cells (EAC) revealed that the effects of this protein were associated with an increase in the number of EACs in the G0/G1 cell cycle phase. The existence of an aneuploid peak suggests that PA at the dose used (1 µg/20 g mice) causes apoptosis in the tumour cell population. Further studies indicated that the level of cyclin D1, an inducer of G0/G1 to S transition, decreased as a result of PA treatment. Another cell cycle stimulatory cyclin, cyclin A, that drags cells from S to G2/M phases, was also found to be inhibited by PA treatment. On the other hand, level of p21Waf1, a cyclin-dependent kinase inhibitor that works downstream of p53, was increased upon PA inoculation. These results together signify that by causing reduction in the levels of cell cycle stimulating cyclins as well as increase in the level of cycle inhibitory p21, PA is causing cell cycle arrest and ultimately apoptosis. These findings may be of great help in designing immuno-therapeutic measures for cancer treatment.

A2. Molecular mechanism(s) of Protein A-induced EAC killing : involvement of soluble apoptogenic factors from immunocytes :

Prasanta K. Ray, Tanya Das and Gaurisankar Sa (Animal Physiology Section)

It is well accepted that for inducing signaling reactions, any agonist must bind to or intrude the cell membrane. Our flowcytometric analysis using FITC-PA showed that PA has high binding affinity for splenic lymphocytes but none for EAC of the same host. These findings were further reflected in the observation that when PA was directly added to EAC culture, it failed to cause either any apoptosis of EAC. Interestingly, when EAC were co-cultured with PA-treated splenic lymphocytes, or the cell free culture medium of the same was added to EAC culture, EAC apoptosis was again observed. These results led us to conclude that PA is inducing the release of different apoptogenic factors from splenocytes which in turn initiate cell cycle arrest and apoptosis in EAC. Previously we reported PA-induced production of cytokines and nitric oxide (NO) in the cell free splenocyte culture medium as well as in mice serum. All these soluble biomodulators are known apoptogenic factors. We thus next planned to identify the apoptogenic factors responsible for PA-induced EAC killing via immunomodulation. When NMMA, the NOS inhibitor, was added to the splenocyte culture medium prior to PA addition or neutralizing anti-TNF-α antibody was added to the PA-treated splenocyte culture medium, % apoptosis of EAC in culture was decreased significantly. These results indicate that TNF-α and NO (as released by NOS) are two important apoptogenic factors released by PA-treated splenocytes and are responsible for EAC apoptosis. However, when both TNF-α and NOS are inhibited, 100% inhibition in EAC

apoptosis could not be observed. Thus the possibility of involvement of other factors in this process can not be overruled.

A3. Large scale production of interferon- γ (IFN- γ) and interleukin-2 (IL-2) using engineered cells : **Prasanta K. Ray**, Tanya Das and Gaurisankar Sa (Animal Physiology Section)

Cytokine therapy is one of the most potent techniques in the medical science. These biomodulators are of central importance in the regulation of immunity, inflammation, tissue remodelling, embryonic development etc. IFN- γ and IL-2 are two important cytokines that generally act in both paracrine and autocrine fashions and their production being transient and subject to complex and strict control. As soluble mediators of immunity, inflammation and cell growth, these cytokines are being used with increasing success in the treatment of malignant diseases, chronic viral and parasitic infections and also in immuno-deficiency caused by AIDS, cancer, chemotherapy, radiation therapy etc. Thus, the large-scale production of these cytokines in functionally active state is of utmost importance to increase their availability as well as to decrease their price for the benefit of mankind. We have standardized the assay method of IFN- γ and IL-2 in the laboratory and shown that using proper biological response modifier, production of these cytokines can be increased significantly.

For the over-expression of IFN- γ and IL-2, we have cloned IFN- γ and IL-2 cDNAs from the cDNA library in PGX3X vector. The right constructs were sub-cloned in pcDNA3 plasmid containing PCMV promoter for eukaryotic expression. Further works are in progress to transfect these clones in mammalian cell lines, e.g., NIH3T3, Cos-7 and Jurkat for the large scale production of these cytokines in functionally active condition.

A4. Protein A induced activation of anti-tumor responses : Involvement of nitric oxide : **Prasanta K. Ray**, Sreya Chattopadhyay, Tanya Das and Gaurisankar Sa (Animal Physiology Section)

Protein A (PA) of *Staphylococcus aureus* has been shown to reduce tumor burden and simultaneously to stimulate the intrinsic defense

machinery of the host, particularly the immunological defense system. The role of Nitric Oxide (NO) in the immunological defense mechanism and in apoptotic signaling cascade has been well documented. We wanted to find out whether NO is an effective mediator in the pathway of Protein A-induced tumor killing and immunocyte proliferation. NO exhibits contradictory effects in the regulation of apoptosis. This biological signaling molecule possesses an enormous range of important functions in the organism. The enzyme Inducible Nitric Oxide Synthase (iNOS) or NOS2 is activated in the immune cells, especially in the macrophages in response to Protein A. PA treatment *in vitro* of mouse peritoneal macrophages in culture increases NO levels significantly. PA was also shown to increase the cytotoxicity of the macrophages against tumor targets in *in vitro* conditions. This cytotoxicity was abolished when the macrophages were pre-treated with NMMA (N^G-monomethyl-L-arginine), which indicates a positive correlation between NO release and PA induced target toxicity. It has been observed that in the splenocytes and the thymocytes of normal mice, PA causes increase in the levels of the proto-oncogene bcl-2, which is pro-proliferative and anti-apoptotic in nature and a decrease in the levels of p53 and bax, both pro-apoptotic gene products. These effects of PA are also mediated by NO, as evidenced by the fact that pre-treatment with NMMA causes diminution of these effects.

A5. Protein A induced differential stress responses in Ehrlich's ascites carcinoma cells and splenocytes of tumor bearing mice : **Prasanta K. Ray**, Sreya Chattopadhyay, Tanya Das and Gaurisankar Sa (Animal Physiology Section)

Recent results from several laboratories have made it clear that Hsp70 (a 70kD member of the Heat Shock Protein family) protects cells not only from heat, but also from most apoptotic stimuli. Hsp70 is abundantly expressed in malignant tumors. *In vivo* accumulation of Hsps in stationary tumor cells can be a part of an endogenous protective device against apoptotic death induced by anticancer agents. Hsps are also expressed in the immunocytes of the tumor bearing host, probably to counter the immense

stress caused by the tumor burden on the host. Boosting the immune system by way of immunomodulators like Protein A might tune up the stress response of the immune cells of the host. Studies in our laboratory have indicated that Protein A treatment can cause an elevation in the expression of Hsp 70 in the splenocytes of the tumor bearing host while causing a depression of their expression in the tumor cells of the same host. This differential regulation of the expression of Heat Shock Proteins in the host and the tumor cells may be part and parcel of the anti-tumor activity of Protein A. Further explorations on the role of Protein A in controlling differential Hsp expression are in progress.

A6. A combinatorial approach towards IDS-management : Studies on molecular signalling : **Prasanta K. Ray**, Amiya Kr. Ghosh, Tanya Das and Gaurisankar Sa (Animal Physiology Section)

Previous reports from our laboratory have already proved that Protein A can ameliorate the toxicity of Zidovudine, the FDA approved drug that is widely used in the treatment of HIV infected patients. Studies have been conducted to find out the molecular mechanism(s) of AZT-induced toxicity of immune cells and its reversal by PA. It has been found that PA itself increases the level of endogenous phosphorylation in splenic lymphocytes. Further studies revealed that in these cells PKC mainly resides in the cytosol which translocates to the membrane as a result of PA treatment indicating the activation of PKC by PA. AZT alone reduced the activity by 40%. Interestingly, when cells were incubated with PA+AZT combination, reversal of PKC inhibition by AZT was observed. Flowcytometric analysis showed that in the level of the proliferative protein Bcl-2 was low in control splenocytes which increased in PA treated system indicating the mitogenic effect of PA in these cells is via Bcl-2. However, AZT has no effect on Bcl-2 expression and thus does not affect cell proliferation through Bcl-2 pathway. Interestingly, when PA was treated in combination with AZT, profound increase in Bcl-2 expression was observed. This information might help developing strategies as to how AIDS therapy involving AZT can be made more effective.

A7. 16-MER and 20 MER peptides derived from protease cleavage of *Staphylococcus aureus* protein A : **Prasanta K. Ray**, Pratima Sinha and Jayati Sengupta

Protein A (PA) of *Staphylococcus aureus* has various array of biological functions such as antitumor, antitoxic, anticarcinogenic, immunomodulatory, antifungal and antiparasitic. Our hypothesis is that PA, being a foreign protein, may be subjected to proteolytic cleavage *in vivo*. It is thus possible that such degraded products might be functionally operative *in vivo*. Our previous molecular modeling studies qualitatively showed that two peptides of PA, 20-mer and 16-mer, may retain Fc binding ability from interaction energy point of view. In the present study, we have selected 16-mer peptide derived from chymotrypsin digestion (theoretical) and 20 mer peptide derived from tryptic fragment (theoretical) of protein-A having IgG-binding property. Our aim was to understand whether these theoretically predicted fragments could be formed in real experiment and whether these peptides can mimic the biological activities of PA. We verified the IgG binding capacity of 16-mer peptide by capillary electrophoresis. We also observed that 16-mer peptide induces production of TNF α and IL1 α in mice serum. The peptide also activates macrophages causing *in vitro* EAC tumor cell cytotoxicity. The 20-mer peptide when injected to EAC tumor bearing mice increases the % survival of the host. We found that this peptide increases the levels of IFN γ , TNF α and IL2 whereas IL4 and IL6 were down-regulated. The ratio of IFN γ to IL4 clearly indicates TH1 response in mice. Thus this 20-mer peptide executes its antitumor property by shifting T cell response to TH1 type as Protein A. We also observed that 20-mer peptide activates macrophages and induces *in vitro* cytotoxicity against EAC tumor. Above observations suggest that these peptides may be obtained by protease cleavage and each of these peptides possesses some of the biological properties of PA, such as IgG binding, cytokine release, *in vivo* antitumor effect as well as *in vitro* cytotoxicity against EAC tumor.

INSTITUTIONAL PROJECT III

A1. Solution structure determination of short synthetic peptides having sequences of Protein-A using CD and NMR techniques : **Prasanta K. Ray**, Jayati Sengupta and Gautam Basu (Dept. of Biophysics)

It is well established that short synthetic peptides are able to induce antibodies that recognize, with high frequency, the cognate sequence in the folded protein. This phenomenon is difficult to explain unless the peptide is able to adopt a highly preferred conformation, either free in solution or on the B-cell receptor, approaching that which it adopts in the native protein or else that protein itself approaches disorder through conformational fluctuations. In the present study, conformational preferences were investigated in synthetic 20-mer peptide, derived from the B domain of Protein-A, using ^1H NMR (1D and 2D) and CD spectroscopy.

Our results indicate that the structure of 20 residue peptide is basically a flexible one in water with two low populated helical turn like structures (b turn or nascent helix) at N- (residue 4-9) and C- (residue 16-18) terminal and weaken in the centre (near proline). These observations are consistent with the conformations of this portion of the peptide in the native protein.

A1. Cloning and expression of protein A gene into mammalian system : **Nripendranath Mandal**, Srikanta Jana and Selvamani Vijayalingam

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 will be 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 focus 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. Different construct of truncated gene of Protein A has been developed into a suitable expression system. More experiments are in progress to study the expression of protein A and truncated version of protein A gene.

A2. Cloning and Characterisation of a novel protein purified from mice splenocyte and thymocyte : **Nripendranath Mandal**, Srikanta Jana, Selvamani Vijayalingam and Eliza Chakraborty

There are two arms of acquired immunity that have different sets of participants and different sets of purposes but with one common aim; to eliminate the antigen. Of these two arms of the acquired immune response. One set is mediated mainly by B-cells and circulating antibodies hence it is termed humoral immunity. The other set mediated by T cells, instead of synthesising antibodies, release various cytokines that affect other cells to mediate the others. Hence, this arm of the acquired immune response is termed cellular or cell mediated immunity.

Immunocompetent lymphocytes combine with the foreign antigen, or a portion of it, termed epitope, by virtue of their surface receptors. They are stimulated under appropriate conditions to proliferate and differentiate into clones of cells with the corresponding identical receptors to the particular portion of the antigen, termed antigenic determinant or epitope. As with B cells, each T cell bears on its surface a clonally distributed receptor that interacts with antigen. This is known as T-cell receptor (TcR).

We have purified a novel single band protein (44kd) from mice splenocyte and thymocyte having affinity with protein A. This protein has different molecular weight than IgG as determined by Gel-electrophoresis. These proteins were present on the membrane of both CD4 and CD8 cells that confirmed by FACS

analysis. More experiments are in progress to study the molecular cloning and characterisation of these newly found proteins.

A3. Quantification of the cytokines and their receptors mRNA based on competitive PCR : **Nripendranath Mandal**, Srikanta Jana and Selvamani Vijayalingam

The lymphokines or cytokines, the hormone-like growth factors and differentiation factors, provide a network of coordination for T cells, B cells and macrophages to get activated during immune responses. Cytokines mediated immune and inflammatory responses can be modulated at two stages; i.e., at the activation of Cytokine genes and at the effector phase involving Cytokine-receptor interaction. The structure and biology of cytokines, the regulation of Cytokine genes and their receptors, signal transduction and hematopoietic growth factors have been well studied during the last two decades.

Cytokine detection and quantification at the protein level is hampered by their low production and by the lack of monoclonal antibodies for each Cytokine or receptor subunit. In addition, the sensitivity of assays such as flowcytometry or various binding assays using receptor-specific antibodies or the ligand for the receptor itself is low. Consequently, Northern blot analysis, RNase protection assays and mRNA phenotyping have been widely used for the study of short-lived, low-copy number mRNA transcripts. It has wide spread applications in genetic disease diagnosis, disease susceptibility and cancer diagnosis. However, even these techniques require at least 105-106 molecules per sample and also suffer from lack of sensitivity. Reverse transcription (RT)-PCR is an extremely powerful method for mRNA analysis. However, it can be difficult to obtain quantitative information with this technique.

Recently we are developing an assay system that will allow us to quantitate mRNA of cytokines at very low level (fg level). We have constructed a plasmid that will allow us to generate standard RNA for quantification of several cytokines mRNA under different experimental condition. More experiments are in progress.

PUBLICATIONS

1. Mishra A, Dwivedi PD, Verma AS, and Ray PK 1999 Protein-A activates membrane bound multicomponent enzyme complex, NADPH oxidase in human neutrophils. *Immunopharmacology Immunotoxicology* **21**: 683-94.
2. Das T, Sa G and Ray PK 1999 Mechanisms of protein A superantigen-induced signal transduction for proliferation of mouse B cell. *Immunol Lett.* **70**: 43-51.
3. Ghosh AK, Jana S, Das T, Sa G, Mandal N, and Ray PK 1999 Protection by protein A of apoptotic cell death caused by anti-AIDS drug zidovudine. *Biochem Biophys Res Commun.* **264**: 601-4.
4. Ray PK 1999 Stress genes and species survival. *Mol Cell Biochem.* **196**:117-23.
5. Verma AS, Dwivedi PD, Mishra A and Ray PK 1999 Glutathione reduces the toxicity associated with antitumor therapy of ascites fluid adsorbed over *Staphylococcus aureus* Cowan I in tumor bearing mice. *Toxicol Lett.* **1**:106(2-3):119-27.
6. Sinha P, Sengupta J, and Ray PK 1999 Functional mimicry of protein A of *Staphylococcus aureus* by a proteolytically cleaved fragment. *Biochem Biophys Res Commun.* **260**:111-6.
7. Das T, Sa G, Sinha P, and Ray PK 1999 Induction of cell proliferation and apoptosis: dependence on the dose of the inducer. *Biochem Biophys Res Commun.* **260**:105-10.
8. Sinha P, Ghosh AK, Das T, Sa G, and Ray PK 1999 Protein A of *Staphylococcus aureus* evokes Th1 type response in mice. *Immunol Lett.* **67**:157-65.
9. Sinha P, Sengupta J, and Ray PK 1999 A minimized Fc binding peptide from protein A induces immunocyte proliferation and evokes Th1-type response in mice. *Biochem Biophys Res Commun* **258**:141-7.
10. Verma AS, Dwivedi PD, Mishra A, and Ray PK 1999 Ehrlich's ascites fluid adsorbed over protein A containing *Staphylococcus aureus* Cowan I produces inhibition of tumor growth. *Immunopharmacol Immunotoxicol.* **21**:89-108.

11. Ghosh AK, Sinha P, Das T, Sa G, and Ray PK 1999 *S. aureus* superantigen protein A expands CD4(+)/CD8(+)/CD19(+)/CD34(+) cells in mice: a potential immunorestorator. *Biochem Biophys Res Commun.* **256**:142-6.

12. Sa G and Das T 1999 Basic fibroblast growth factor stimulates cytosolic phospholipase A2, phospholipase C-gamma1 and phospholipase D through distinguishable signaling mechanisms. *Mol Cell Biochem.* **198(1-2)**:19-30.

13. Das T, Sa G, Subbulakshmi V, Sen P, Biswas S, and Ray PK 2000 Protein A-activated rat splenic lymphocyte proliferation involves tyrosine kinase-phospholipase C-protein kinase C pathway. *Immunopharmacol Immunotoxicol* **22(1)**:75-90.

Participation in Conferences/Symposia/ Training Programme/Awards and Honours received

Dr. Tanya Das went to The Cleveland Clinic Foundation, Ohio, USA, during August 1999 to February 2000, as an Exchange Technologist in a NIH grant to get acquainted with most so-phisticated Imaging Technologies required to study cell cycle and cell migration. Dr. Das delivered invited lecture in the Dept. of Molecular Cardiology, Cleveland Clinic Foundation, USA in 1999.

Mr. Amiya Kr. Ghosh presented a poster in the XXVI Annual Conference and Symposium of Indian Immunology Society on Cancer Immunology in the New Millennium, held in Mumbai, January 13-15, 2000.

GRANTS-IN-AID SCHEMES

Investigator/ Co-investigator	Title	Financed by
Prof. P.K. Ray	Development of an Enzyme Linked Immunosorbent Assay for Organochlorine pesticides.	DBT
Prof. P.K. Ray	Production of Biological Response Modifiers Using engineered cells	DST

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

Dr. Tanya Das, Dr. Pratima Sinha, Dr. Jayati Sengupta, Dr. Elisa Chakraborty, Dr. S. Vijayalingam, Amiya Kr. Ghosh, Srikanta Jana, Sreya Chattopadhyay.

SUPPORTING STAFF MEMBERS

Uttam Kr. Ghosh, Tapan Mandal, Rina Das, Amartya Sen, Ranjit Das.

Bose Institute

Environmental Sciences Section

Faculty : Prof. P. K. Ray

HIGHLIGHTS

Bioaccumulation of aflatoxin from liver of farm animals in the consuming individual is possible and continuous accumulation of the toxin may be detrimental.

A.1 Environmental Impact Assessment and Environmental Management Plan for Proposed River Front Development Scheme for Calcutta (Phase-1).

P.K. Ray, A. Dalal, A. Adak, J. Datta, S. Bid, T. Maji

Calcutta Port Trust in collaboration with Calcutta Metropolitan Development Authority and Calcutta Municipal Corporation has undertaken a massive river front beautification programme for a total stretch of about 9.7 kms. of the river bank on the Calcutta side and about 56 kms. on Howrah side of the river Hooghly. We have been entrusted with the responsibility of making an Environmental Impact Assessment (EIA) for the entire stretch. The first phase (Phase-1) involves the section between Princep Ghat and Fairlie Place on the Calcutta side of the Hooghly only. The objective of the riverside beautification plan is to attract more people during vacation time, for pleasure trips etc. By making it an enjoyable and aesthetically charming place that also provides younger people with an opportunity to learn.

This work involves baseline data collection in respect of EIA for the winter season. A detailed characterisation of the status of the various components of the environment along the river Hooghly around the project site viz, its air, noise, water, land, biological and human aspects has been done. Evaluation of impacts has been carried out within the study area and predictions have been made.

INSTITUTIONAL PROJECT 1

A.2. Development of an immunotherapeutic approach to control aflatoxin mediated death in poultry :

P.K. Ray, P. Bhattacharyya, Debashis Ghosal and Sushmita Bid

It has been suggested that aflatoxin toxicity is a major factor in hepatocellular diseases. Aflatoxin produced by *Aspergillus flavus*, particularly during its growth in various types of fatty food, is carcinogenic. To ascertain the probable mechanism of action of aflatoxin as an inducer of hepatocellular damage mice were fed on diet contaminated with aflatoxin. The animals were observed to concentrate the toxin in the liver. When their livers were heat cooked and fed to other rats along with their regular diet, aflatoxin became further concentrated in the livers of the second set. Prolonged accumulation of the toxin in the liver appears to cause hepatocellular damage and may induce cancer.

A.3. Comprehensive Environmental Study and Preparation of Environmental Management Plan for Calcutta Dock System.

P.K. Ray, A. Adak, A. Dalal, J. Datta, S. Bid, T. Maji

Calcutta Dock system of Calcutta Port Trust is a deep-water dock system which covers the area including Khidirpur Dock-II, Netaji Subhas Dock and Budge Budge oil terminal. These areas handle bulk commodities like petroleum oil products, edible oils, containerised cargo, fertilizers, engineering goods, logs, dry bulk cargo like food grains etc. They are also supposed to promote port based and port related industries in the region. Offshore litherage operational studies at Sagar/Sandhead & Diamond Harbour are also required to be carried out. This study covers all operations carried out at the Calcutta Dock system. Environmental damage and ecological imbalance occur due to ship breaking operation carried out at old coal berths of Khidirpur Dock-II. The Budge Budge oil terminal is meant for unloading of POL

products, petroleum fractions, which are highly inflammable and toxicity of these products may impair the live processes in the micro and macro ecological structure depending on meteorology and physical state of the environment in case of any accident. The objective of the present study is to identify the present status of the environment and to formulate mitigation plan for controlling environmental pollution, if any, including preparation of Environmental Management Plan.

A.4. Development of waste water treatment technology of Enpee Wire Industries, Barasat

P.K.Ray, P. Bhattacharyya, P.K. Chakrabartty, A. Dalal, S. Bid, A. Adak

Enpee Wire Industries located at Barasat, has a manufacturing unit which produces galvanized wire. The process involves annealing, pickling, cleaning & chloride zinc processing of mild steel wire in turns. The volume of water consumed

during the rinsing process results in discharge of a large volume of waste water, i.e., about 28000 lits. per day. Analysis of this effluent shows very low pH, high value of total suspended solid, metals, oil and grease in non permissible levels. The work responsibility of this project is development of baseline data, evaluation of the existing problems, carrying out laboratory work for suggesting necessary rectification and adaptation of the same in the field condition.

A.5. Societal Programme:

Environmental Sciences Section has also carried out consultancy work regarding quality control, monitoring and control of Environmental pollution at the local and national level. In this connection this year our section has audited more than 130 industries/foundries at Howrah and Calcutta, worked at Santaldih Thermal Power Station, Purulia and Bandel Thermal Power Station, Hooghly.

GRANTS-IN-AID SCHEMES

Investigator/ Co-Investigator	Title	Financed by
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 Impact Assessment Study and Preparation of Environmental Management Plan for the Proposed River-Front Beautification Work (Phase-1) From Princep Ghat to Fairlie Place on Calcutta River Side.	Calcutta Port Trust
Prof. P.K. Ray	Comprehensive Environmental Study And Preparation of Environmental Management Plan for Calcutta Dock System.	Calcutta Port Trust
Prof. P.K. Ray	Environmental Monitoring Control or 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)
Consultancy projects from various other industries		Amount earned is approx. Rs.6.0 lakhs.

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

Dr. Jharna Datta, Ms. Sushmita Bid, Mrs. Sreya Chatterjee.

SUPPORTING STAFF MEMBERS

Mr. Anandamay Adak, Mr. Asis Kumar Dalal, Mr. Sudam Chandra Jana, Mr. Siddhartha Ray, Mr. Tanmay Maji, Mr. Dwijendra Mukherjee and Mr. Biswajit Das.

**AUXILIARY AND
SERVICE SECTIONS**

Madhyamgram Experimental Farm

Dr. T. K. Ghose, Dr. S. R. Sdkdar (Jt. Scientists-in-Charge)

Experimental Cultivation :

During the year a total of 71,952 sq. ft. (2 acre) of land was used for growing experimental plants which included rice, mustard, mung, arhar, tomato, spinach and a number of medicinal plants.

Demonstration Garden for Medicinal plants :

A demonstration garden for medicinal plants, funded by ICAR, with approximately 100 species has been established (Prof. S. Sen-Mandi, Botany).

Establishment of Micropropagated plants

A large number of micropropagated plants has been established in the field including *Clerodendron*, *Dendrocalamus*, *Withania*, *Pelargonium* & *Psoralea*.

Revenue Generation :

A total of Rs. 30,857/- revenue was generated.

SUPPORTING STAFF MEMBERS

Sri Pulak Kummar Roy, Sri Biaml Chandra Modok, Sri Kachi Ram Kole, Sri Basudeb Jana, Sri Rakhal Chandra Jana, Sri Dilip Pramanick, Sri Chittaranjan Mallick, Sri Sukumar Das, Sri Subhas Senapati, Smt. Sabita Baisya, Sri Jagobandhu Nayak, Sri Rabin Talukdar, Sri Laxmi Kanta Prodhan, Sk. Inal Ali, Sri Bhanu Kisku, Sri Mahesh Dasgupta, Sri, Nodiran Kayal, Sri Rajkishor Lenka.

Library

Ms. R. Mitra (Librarian, Acting)

The Library primarily serves the scholars, faculty members and staff of the institute. Researchers and scientists of other institutes can also be member of the library with an annual membership fee. Library has two wings, serving in each campuses with 13 staff members. Ms Reba Bhattacharya, Technical Officer(B) retired on superannuation on 29 February 2000, after serving the library for quite long time. The collection consists of both biological and physical sciences. Library subscribed 80 journal titles in 1999, both foreign and Indian. Internet facilities are available for internal use only.

Acquisition

Library contains 32,961 volumes of books and bound journals upto 31.3.2000. Library purchased 58 books during 1999-2000. 14 theses have been deposited during that period.

Photocopying services

Library provides photocopying facilities to institute as well as outside users on payment

basis, 1,46,067 copies in the Centenary Building Library and 42,638 copies in the Main Campus Library were supplied during the period. Under Inter-Library loan system, photocopies were also supplied to INSDOC.

Participaiton in Conferences/Symposia/ Training Programme/Awards and Honours received

Pradip Kumar Sarkar participated in the XXII All India IASLIC conference held at Dr. B. R. Ambedkar University (formerly Agra University) Agra, from 28-31 December, 1999.

Rukmini Mitra attended a day long seminar on "Changing vision of library and information services", organised by the Dept. of Library & Information Science, Jadavpur University on 28 March 2000.

SUPPORTING STAFF MEMBERS

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.

Workshop

Mr. A. Mukherjee (Superintendent)

The Workshop of Bose Institute consisting the following units for maintenance, repair and fabrication of different instruments as per the requisitions. The units are as follows :

Units	Requisition receipts	Job discharge	Continuing job
Mechanical	120	80%	20
Electrical	1100	85%	15%
Carpentry	55	100%	Nil
Refrigeration	110	85%	15%
Glass blowing	50	100%	Nil
Drafting	120	90%	10%
Electronic	86	100%	Nil

Revenue earned : Rs. 2,500/- for making and supplying one camera stand to Calcutta University.
Additional responsibility : Sri S. Ghosal

(Technical Officer-B) has been discharging the overseer's job (Main Campus) in addition to his normal duties of drafting section.

SUPPORTING STAFF MEMBERS

Main Campus :

Mrinal Ch. Roy, Ashutosh Sarkar, Samar Dutta, Sisir Kr. Ghosal, Sithil Mukherjee, Tarapada Das, Dipen Sen, Bankim Dutta, Subhas Chakraborty, Sk. Md. Ehia Hossian, Basanta Kr. Shit, Bholanath Saren, Sourendra Nath Chakraborty, Abdul Rahaman Molla, Panchanan Santra, W.D. Rozaria, Panchugopal Karak, Rajkumar Das, Pranab Banerjee, Subrata Basak, Lochan Shee, Gopal Ch. Das, Sairoom Begum, Sk. Md. Farruck, Kishori Mohan saha, Kalachand Sen, Bramdeo Prasad, Thakur Gond, Kalipada Das, Alok Kundu.

Centenary Campus :

Mr. Dipak Dutta, Pitambar Patra, Anil Sengupta, Tapan Kr. Mondal, Jogendra Nath Das, Ashit Banerjee, Sk. Md. Raffick, Murari Mohan Shee, Baidya Nath Murmu & Sanjoy Santra.

Regional Sophisticated Instrumentation Centre

Dr D. K. Mukherjee (**Scientist-in-Charge**) and Dr. Siddhartha Chaudhuri

The Regional Sophisticated Instrumentation Centre continued to provide analytical instrumental and consultancy services to the scientific communities in the region. Scientists from different universities and research organisations in the region, and R&D personnel from industries have availed of these services. With many of the older instruments having become obsolete, it has been extremely difficult to maintain continuity of services in some cases. It is now absolutely important to replace some of the oldest instruments which still continue to be in great demand. Efforts are being made to procure funds necessary for the purchase of a new Scanning Electron Microscope to replace the existing instrument which is already more than twenty years old.

In addition to the normal day to day activities, RSIC personnel have helped in the maintenance of analytical instruments in various departments of the Institute.

Some members of the staff of RSIC have given courses at different organisations including Calcutta and Jadavpur Universities.

PUBLICATIONS

1. Adhikari N, Chaudhuri S, Butcher RJ and Saha N, Synthesis and spectroscopic characterisation of copper(II) complexes with 5-methyl-1-(2'-pyridyl) pyrazole-3-carboxamide (MPyPzCA) : X-ray crystal structure of $[\text{Cu}(\text{MPyPzCA})_2(\text{H}_2\text{O})](\text{ClO}_4)_2$. *Polyhedron*, **18**, 1999, 1323-1328.

2. Sen A, Microprocessor controlled transistor lead identifier. *Electronics for You*. January, 2000, 51-58.

3. Sen AK, Resonance type L-C meter. *Electronics for You*, March, 2000, 46-50.

Participation in Conferences/Symposia/ Training Programme/Award and Honour received

Ms Tanima Modak (Dhar) attended a two-part "Workshop-cum-Training Course on Repair and Maintenance of General Purpose Electronic Instruments" organised by USIC, Calcutta University, in June and November, 1999.

SUPPORTING STAFF MEMBERS

Mr Arabinda Biswas, Mr Tapan Datta, Mr Shyam Sundar Kundu, Mr Gobinda Das, Mr Amit Kumar Mahindar, Mr Jaideb Kumar Chattopadhyay, Mr Pathik Ranjan Ray, Mr Alokanda Sarkar, Mr Arup Kumar Sen, Mr Samir Kumar Mukherjee, Mr Pradip Kumar Karmakar, Mr Jagannoy Guin, Mr Subhas Chandra Maikap, Ms Tanima Modak (Dhar), Ms Manisha Banerjee, Mr Ranjit Kumar Ghosh, Mr Debabrata Polley, Mr Sankar Santra, Ms Saraswati Sen.

J. C. Bose Unit and Museum

Dibakar Sen (**In-Charge**)

During the year a good number of visitors, including dignitaries, both from India and abroad visited the Museum. Additionally the Museum furnished information regarding Acharya J. C. Bose and his works to eminent persons and common man on request. Special display in Museum was arranged on the occasion of the Foundation Day of the Institute 30th November and National Science Day on 28th February. Arrangements are being made to display more works of Acharya J. C. Bose.

Film Shooting in the Museum on J.C. Bose :

Siddherth Kak Production on May 1999, Sarasij Bose Production on August 11, 1999, Bangla Telefilm, Bangladesh on January 07,

2000 and Dr. Michael Tehiriatiev, St. Petersburg, Russia on behalf of International Centre of the Roerichs on February 16, 2000.

Participation in Conferences/Symposium/ Training Programme/Award and Honour received:

Dibakar Sen delivered lectures on "Life and Works of Acharya J.C. Bose" in Academic Science and Promotion Society at Bose Institute Lecture Hall on July 11, 1999. "Electrical Reponse in Plants - Studies by J.C. Bose" on September 17, 1999 at Saha Institute of Nuclear Physics Lecture Hall, Calcutta, "Life and Works of Acharya J.C. Bose" in Academic Staff College, University of Calcutta on December 29, 1999.

SUPPORTING STAFF MEMBER

Shri N. Das.

Distributed Information Centre

Dr. P. Chakrabarti (Scientist-in-Charge)

Distributed Information Centre, Bose Institute was inceptioned in 1988 with Genetic Engineering and Molecular Modelling as the two major thrust areas. Since then the Centre is functioning as a repertoire of information related of Bioinformatics. The Centre works as the backbone of the research and development activities in various fields like, Genetic Engineering, Biocrystallography, Biocomputing, Molecular Modeling, etc.

Bioinformatics Resource Created :

CUCG: A non-redundant codon usage database from complete genomes has been developed. GC percentage at the coding region as well as the three different codon positions was tabulated for each organism. Relative synonymous codon usage (RSCU) values for each organism were also included in this database. The World Wide Web provides a user-friendly interface for this database. The dataset of all the genomes are available at <http://www.boseinst.ernet.in/dic/CUCG.html>.

USE OF DIC :

The Centre has expanded its userbase significantly over the last one 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,

Workshops organised :

A workshop on "Protein structures: analysis, modelling and evolutionary aspects" was organized by the Bioinformatics Centre during March 9-10, 2000. The workshop covered areas on (i) the depiction of some recently determined protein structures using X-ray crystallography, (ii) analysis of protein structures to identify stabilizing interactions and derive structural principles of protein folding, (iii) modelling of protein structures from

sequence, (iv) evolution of protein structures and (v) computer demonstration of various sequence analysis methods and molecular modelling. Prof. P.K.Ray, Director, Bose Institute inaugurated the workshop. Twenty participants from all over the country attended the workshop

Project Supervision :

2 D.C.A. students from RCC (Jadavpur) had completed their projects in DIC under the supervision of DIC personnel.

Hardware / Software Augmentation :

- i) GCG Wisconsin package for sequence analysis, version 10.0
- ii) SPSS version 10.0
- iii) CD/DVD Server
- iv) Color postscript laser printer
- v) 9 GB hard disk for Decalpha
- vi) Hard disk and memory upgradation for Indigo II
- vii) CD Writer

PUBLICATIONS :

1. Compositional correlation studies among the three different codon positions in 12 bacterial genomes. Majumdar S, Gupta SK, Sundararajan VS and Ghosh TC. 1999 *Biochem. Biophys. Res. Commun.* 266, 66-71
2. CUCG: A non-redundant codon usage database from complete genomes. Gupta SK and Ghosh TC 2000 *Current Science* 78, 28-29.
3. Studies on the relationships between the synonymous codon usage and protein secondary structural units. Gupta SK, Majumdar S, Bhattacharya TK and Ghosh TC. 2000 *Biochem. Biophys. Res. Commun.* 269, 692-696.
4. Studies on codon usage in *Entamoeba histolytica*. Ghosh TC, Gupta SK and Majumdar S 2000 *Int. J. Parasitology* 30, 715-722

Participation in Conferences/Symposia/Traning Programme/Award and Honour received

Mr. Sanjib K. Gupta attended and delivered a talk on "Bioinformatics resources at Web" in the workshop on current concepts in

Bioinformatics held at Bioinformatics Centre, Pondicherry University on February 2-4, 2000.

Dr. T.C. Ghosh attended International Centre for Co-operation in Bioinformatics ICCBnet meetings and courses on "Theory and Practice of Bioinformatics", held at Baroda on February 27 to March 2, 2000.

SUPPORTING STAFF MEMBERS

Dr. T.C.Ghosh, Mr. V.S.Sundararajan, Mr. T.K.Bhattacharyya, Ms. S Majumdar, Mr. S.K. Gupta, Mr. U.K.Samanta (RA) and Mr. J.Mandal.

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, Seminars Training Programmes; and works of Sir J. C. Bose. Beside these Publication Section provides photocopying facilities to the staff members of both the Campuses of the Institute.

M/s. Das Gupta & Co. Private Limited, 54/3, College Street, Calcutta 700 073 has been appointed as sole distributor of our publications. The following books are available for sale from their counter.

J. C. BOSE AND MICROWAVES -		
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OTHER ACTIVITIES



A workshop on "Plant Genomics : Emerging trends and Technology" was organized jointly by the Centre for Plant Molecular Biology, Plant Molecular and Cellular Genetics and the Department of Botany during March 24-25, 2000. Prof. A. K. Sharma inaugurated the workshop and delivered the key-note address wherein he discussed the development of the discipline from early days of genetic research. Among several other speakers were Prof. Asis Datta, Prof. S. C. Maheswari, Prof. Alok Bahattacharya, Prof. P. K. Gupta, Prof. Akhilesh Tyagi and Prof. K. P. Gopinathan who spoke on various aspects of this emerging area of Plant Science research and biotechnology. The need for pursuing research in this area by the Plant Biologists with inputs from computational biology, structural biology and allied disciplines as well as increased interaction among agricultural scientists and molecular biologists has been emphasized in the concluding session chaired by Prof. S. C. Maheswari.

Foundation Day Celebration and Acharya Jagadis Chandra Bose Memorial Lecture 1999



Dr. R. A. Mashelkar delivering the 61st Acharya Jagadis Chandra Bose Memorial Lecture.

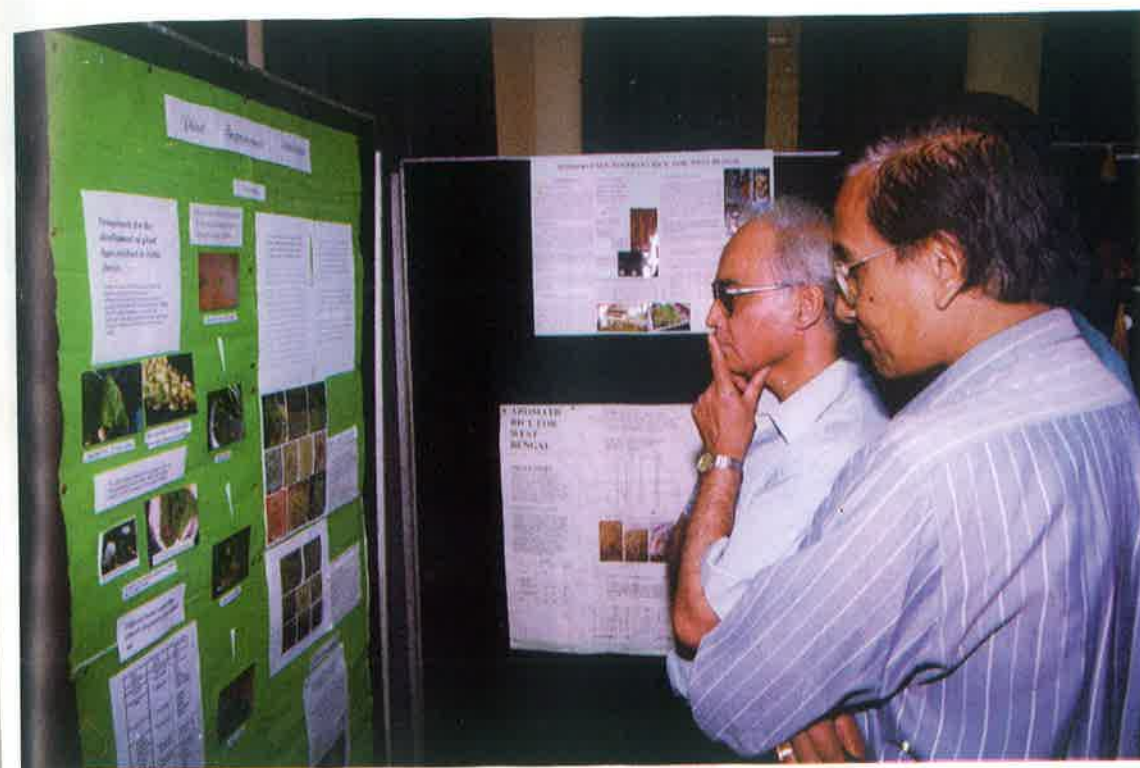


Prof. A. K. Ray receiving the Foundation Day Award 1999 from Dr. A. R. Mashelkar.

The 83rd Foundation Day of Bose Institute was celebrated on 30th November, 1999. Dr. R. A. Mashelkar, Director General CSIR, Delhi kindly graced the occasion as Guest of Honour and delivered the 61st Acharya Jagadis Chandra Bose Memorial Lecture on **"Intellectual Property and Innovation : The New Challenge for India"**.

In this occasion Prof. A. K. Ray of Animal Physiology Section, Mr. Amiya Ghosh of the Immuno-technology Section and Mr. Debnath Pal of the Biochemistry Department received the Foundation Day Award, Sri Nilratan Sirkar Award and Prof. B. B. Biswas Award, respectively. All the awardees delivered lectures on the respective areas of research. The celebration ended with a cultural evening.

National Science Day, 2000



National Science Day was celebrated on February 28, 2000 in a befitting manner in Bose Institute, Calcutta on the focal theme **"Recreating interest and excitement in basic Sciences"**. In this connection, various scientific activities like popular lectures, exhibition, etc., related to the focal theme and also "open house" were organized. Eminent personalities like Prof. R. K. Mandal delivered interesting talks. Visitors from different walks of life, besides students from schools and colleges enjoyed the day's activities with keen interest and visited different laboratories and Sir J. C. Bose Museum.

Minister of Science & Technology, Bangladesh visited Bose Institute

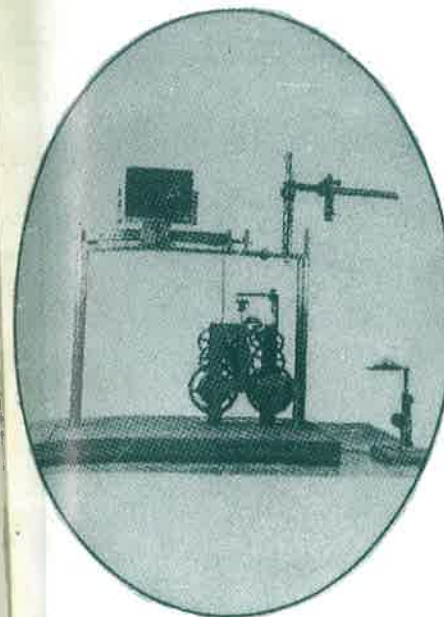


Hon'ble, H.E. Lt. Gen. Md. Noor Uddin Khan,
offering floral wreaths to the Samadhi of
Acharya J. C. Bose

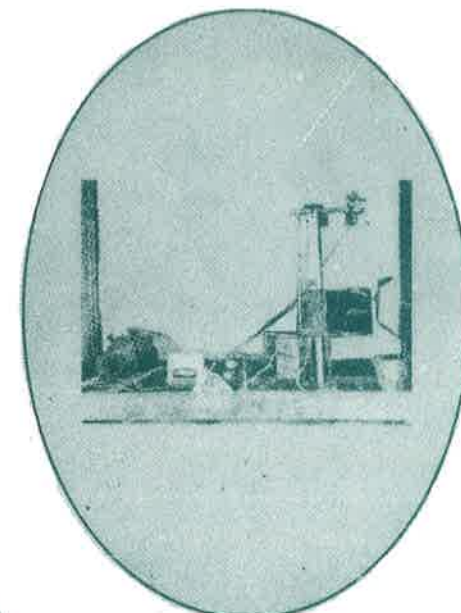


Delegates visiting Informatic Centre

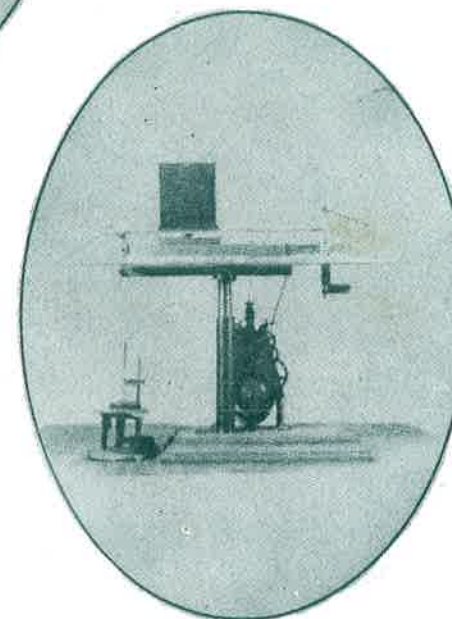
On invitation from Prof. M. M. Joshi, Hon'ble Minister for Science & Technology and HRD, Govt. of India, eight members delegation led by H. E. Lt. Gen. Md. Noor Uddin Khan, Minister of Science & Technology, Peoples' Republic of Bangladesh visited India during March 2, to 12, 2000. As part of their India visit the team visited Bose Institute on March 9, 2000. Increased Scientific co-operation between Bangladesh and India was the major topic of discussion between them and Scientists of Bose Institute.



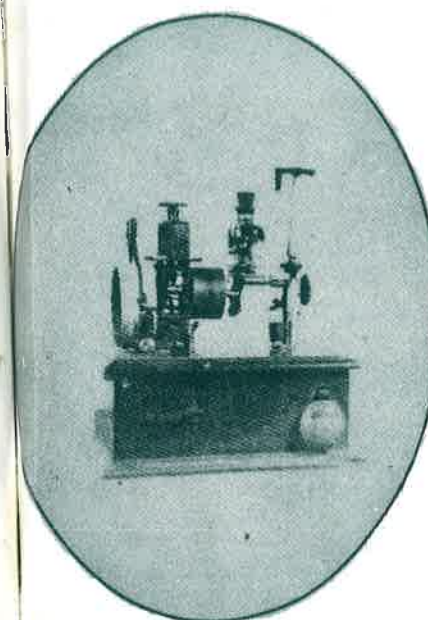
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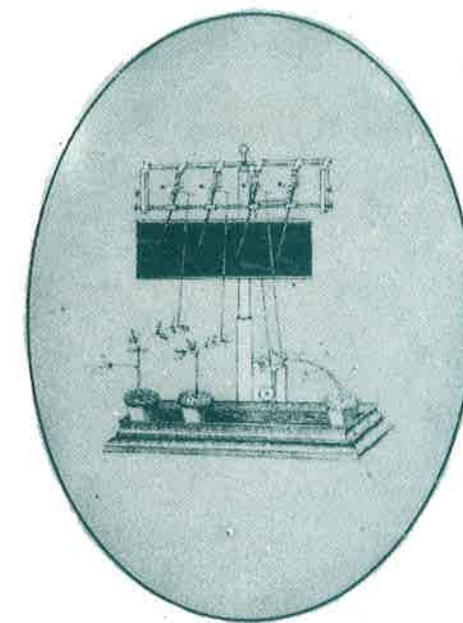
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