REPORT

OF

THE BOSE INSTITUTE

FOR

1964–65

BOSE INSTITUTE

93/1, ACHARYA PRAFULLA CHANDRA ROAD, CALCUTTA-9

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CONTENTS

REPORT OF THE BOSE INSTITUTE
1964-65

The Bose Institute was founded by Jagadis Chandra Bose in 1917 for 'the fuller investigation of the many ever opening problems for the nascent science which includes both life and non-life'. The Bose Institute provides facilities for fundamental and applied research in a wide range of scientific subjects. Besides the main laboratories and workshops of the Institute situated at 93/1, Acharya Prafulla Chandra Road, Calcutta-9, the Institute maintains experimental stations at (i) Mayapuri—Darjeeling (High Altitude Station), (ii) Falta—24-Parganas, and (iii) Shamnagar—24-Parganas.

The Institute has at present four main departments of research, Physics and Biophysics, Chemistry including Plant Chemistry, Physical and Biochemistry, Botany, including Plant Physiology, Plant Biochemistry, Cytogenetics, Plant Breeding; Microbiology including Soil Microbiology and Genetics of Microorganisms. The income of the Institute is derived from endowment funds, from Government of India grants and from grants-in-aid for support of different research projects. The management of the properties and investments of the Institute is vested in a Governing Body, a Board of Life Trustees; and the management of the affairs of the Institute is vested in a Council, in which some representatives of the Governing Body, nominees of the Government of India, Government of West Bengal, Director General, C.S.I.R., representative of the Lok Sabha, Accountant General, West Bengal and some representative Scientists are included.

Obituary

The Bose Institute suffered a grievious loss by the passing away on November 11, 1964, of Shri Abani Nath Mitter, one of the senior-most members. Shri Mitter was amongst the band of workers, who were initiated by Acharya Jagadish Chandra on the Foundation Day of the Bose Institute. He was a member of the Governing Body since its foundation in 1917. After his retirement from the post of Superintendent in 1948, he was elected a member of the Council. Even after his retirement, Shri Mitter was intimately associated with the Bose Institute Governing Body as its Assistant Secretary and the two J. C. Bose Trust Funds as Secretary. He was also entrusted with the honorary supervision of the repairs and construction works of the Bose Institute. Shri Mitter was the last surviving member of the devoted group of men and women who helped Acharya Jagadis Chandra to put into concrete form his many nation building ideas. He served the interest of the Bose Institute with ability and conscientiousness.

Governing Body

Three meetings of the Governing Body were held during 1964-65.

The Governing Body nominated six of its members to the newly constituted Counci of the Bose Institute.

Approved the Budget Estimates for 1964-65.

Transferred the assets of the Bose Institute Provident Fund, till now under management of the Governing Body, to a newly formed Provident Fund Accounts under the control of the Council of the Bose Institute.

Elected Dr. J. C. Sen Gupta, President, Board of Secondary Education, West Bengal, as member of the Governing Body in the vacancy created by the death of Dr. S. K. Mitra.

Passed a condolence resolution on the death of the Abaninath Mitter, Assistant Secretary, Governing Body who passed away on November 11, 1964.

Council

Three meetings of the Council of the Bose Institute were held during the year.

The first meeting of the newly constituted Council* with the following members was held on 15th December 1964.

*12(I)(a)	Seven members of the Governing Body.
12(I)(b)	The Director, Bose Institute.
12(I)(c)	Deputy Director.
12(I)(d)	The Secretary to the Government of India—the Ministry dealing in Science.
12(I)(e)	Director-General, Council of Scientific & Industrial Research or his nominee.
12(I)(f)	One Representative of the Lok Sabha.
12(I)(g)	Two Scientists from out-side West Bengal to be appointed by the Government of India in consultation with the Council.
12(I)(h)	One Central Government nominee with knowledge of financial matters.
12(I)(i)	Accountant-General, West Bengal.
12(I)(j)	One West Bengal Government nominee from the Department of Education.
12(I)(k)	One representative of the Calcutta Corporation.

Some of the important items considered by the Council during the year are given below:

The Council on the recommendations of the duly constituted Selection Committees made the following appointments:

1. Dr. P. K. Bose, D.Sc., F.N.I., as officiating Joint-Director, Bose Institute with effect from 1 May 1964.

2. Prof. S. R. Khastgir, D.Sc., F.N.I., as Head of the Department of Physics, Bose Institute with effect from 1 May 1964.
3. Dr. Amitabha Sen, M.Sc., Ph.D. (Leeds), as Head of the Department of Chemistry with effect from 1 May 1964.

The Council accepted the decision of the Governing Body to transfer to the Council Account the assets of the Provident Fund so long managed by the Governing Body.

Grants

The following grants were received during the year 1964-65:

(*a*) *Non-recurring grants:*	(i) Government of India, Ministry of Education	Rs.	3,16,666.00
(*b*) *Recurring grants:*	(i) Government of India, Ministry of Education	Rs.	9,60,000.00
	(ii) Government of India for Research Training Scholarship	Rs.	22,313.58
	(iii) Governing Body, Bose Institute ..	Rs.	40,000.00

(*c*) *Grant-in-aid Schemes:*

Sponsoring Body	*Schemes*		*Amount*
Council of Scientific & Industrial Research.	Biosynthesis of Soluble RNA ..	Rs.	10,000.00
-do-	Enzymatic Synthesis of RNA ..	Rs.	6,023.01
-do-	Phytin Synthesis of Nuclear acid etc. ..	Rs.	6,587.10
-do-	Gas chromatography to the analysis of fatty acid etc.	Rs.	5,000.00
-do-	Effect on ultraviolet light on DNA transformation	Rs.	4,404.59
-do-	Studies on semi-large scale production of antibiotics	Rs.	550.00
-do-	Study of the frequency power spectrum of lighting discharge	Rs.	14,903.93
-do-	Studies of the sporadic E-region of the ionosphere	Rs.	21,227.15
-do-	Chemical examination of Indian medicinal plants etc.	Rs.	5,267.00
-do-	Physical chemical investigations on milk proteins	Rs.	9,100.00

Sponsoring Body	*Scheme*		*Amovnt*
Council of Scientific & Industrial Research	Studies on the chemical constitution of some antitiotics isolated from Streptomyces ..	Rs.	8,467.00
-do-	Senior Research Fellowship —Sri B. K. Burman	Rs.	3,150.00
-do-	Senior Research Fellowship —Sri R. Chowdhury	Rs.	1,050.00
-do-	Jr. Research Fellowship —Sm. Malati Majumder	Rs.	4,020.00
-do-	Pool Officer—Contingent grant ..	Rs.	769.71
Indian Council of Agricultural Research.	Studies on the occurrence of Rhizobium etc.	Rs.	8,621.00
-do-	Investigation of the role of Liopic acid etc. ..	Rs.	12,453.19
Dept. of Atomic Energy, Govt, of India.	(1) Studies with radioactive tracers etc. ..	Rs.	10,252.00
-do-	(2) Nuclear Physics & Neutron Generator..	Rs.	44,880.00

Visitors

The following visited the Institute during the year: (i) Dr. E. Lennox, Salk Institute, San Diego, California, U.S.A., (ii) Dr. Harris B. Steward, Chief Scientist of the U.S. Coast and Geodetic Survey Ship 'Pioneer', (iii) Dr. R. D. Brock, Chairman, Division of Genetics, Commonwealth Scientific and Industrial Research Organisation, Australia, (iv) Prof. B. L. Horecker, Department of Molecular Biology, Albert Einstein College of Medicine, U.S.A., (v) Dr. N. B. Eddy, Consultant, National Institute of Health, Washington, D.C., (vi) Dr. Mark Vahrman, Director of Research, Rexo Research and Development Company, Mansfield, U.K., (vii) Dr. D. L. Fuller, Science Attaché, American Embassy, New Delhi, (viii) Prof. Arne Muntzing, Professor of Genetics, University of Lund, Sweden, (ix) Prof. T. Caspersson, Karolinska Institute, Sweden, (x) Prof. L. G. Lighter, Manchester, U.K., (xi) Dr. & Mrs. E. Ralph Singleton, University of Virginia, U.S.A., (xii) Dr. J. N. Narlikar, King's College, Cambridge, U.K., (xiii) Prof. I. Hranb, Koln, Germany.

Acharya Jagdis Chandra Bose Memorial Lecture

The twenty-sixth Lecture of the series entitled "The threshold of the break-thorugh: technological and social transformation in India" was delivered by Shri Asoke Mehta, Deputy Chairman, Planning Commission, Government of India on 30 November, 1964. The Lecture is being published in the Transactions of the Bose Institute.

Acharya Jagadis Chandra Bose Endowment Lecture

The thirteenth to fifteenth Endowment Lectures of the series were delivered during the year:

(i) Prof. J. Straub, Director, Max-Planck Institute for Plant Breeding and Genetics, Koln, West Germany "The Mendelian factors and future of Biology" on 5 January 1965.

(ii) Prof. Arne Muntzing, Professor of Genetics, University of Lund, Sweden, "Inbreeding degeneration and heterosis" on 28 January 1965.

(iii) Prof. R. Ramanna, Chairman, Physics Advisory Board, Department of Atomic Energy, Government of India "Research Reactors and their utilization" on 9 February 1965.

Special Lectures

(i) Prof. B. L. Horecker, Department of Molecular Biology, Albert Einstein College of Medicine, New York, on "The Nature of the active sites of Aldolase" on 29 September 1964.

(ii) Under the auspices of the Indian Chemical Society, Dr. R. C. Brasted, Professor of Inorganic Chemistry, University of Minnesota, on "The anomalous behaviour of Resolvable Inorganic complexes in the presence of Resolved species—The Pfeiffer effect" on 10 July 1964.

Degree Awarded

The following were awarded the D.Phil. degree of the Calcutta University.

1. Shri Amalendu Nath—Department of Physics.
2. Shri Kalachand Das—Department of Chemistry.
3. Shri Sheela Chowdhury—Department of Chemistry.
4. Shri Ranjit Kumar Roychowdhury—Department of Chemistry.
5. Sm. Latika Bose—Department of Chemistry.
6. Shri Saroj Bandhu Ghosh—Department of Microbiology.
7. Shri Ajit Kumar Medda—Department of Animal Physiology.
8. Shri Sankar Lal Chakraborty—Department of Microbiology.

Study abroad and deputation

1. Dr. R. K. Mandal, Senior Research Fellow, C.S.I.R. has been awarded a post-doctorial fellowship by the University of Pittsburg, U.S.A.

2. Dr. R. K. Sinha, Research Fellow in the Department of Microbiology has been awarded a post-doctorial fellowship by the North Western University of Evanston, U.S.A.

3. Shri Bijoy Kumar Maity, Research Scholar in the Department of Microbiology has been awarded a Associateship by the Albany Medical College, New York, U.S.A.

4. Dr. B. B. Biswas, Reader, in Plant Biochemistry attended an International Conference in Photo-biology in Oxford and the International Conference in Botany in Edinburgh.

Grants for study abroad

Grants of Rs. 2,000.00 each out of Sir J. C. Bose Trust Fund were made available to Dr. R. K. Mandal, Dr. R. K. Sinha and Dr. B. B. Biswas in partial defrayal of their costs of passage abroad.

New appointment and awards

The following appointments were made during the period under review.

A. *Readers:*

(i) Shri A. M. Ghose, M.Sc., in the Department of Physics with effect from 1 October 1964.

(ii) Dr. A. K. Barua, M.Sc., D.Phil., in the Department of Chemistry with effect from 1 January 1965.

(iii) Dr. B. B. Biswas, M.Sc., D.Phil., in the Department of Botany with effect from 1 January 1965.

(iv) Dr. Sudhamoy Ghosh, M.Sc., D.Phil., in the Department of Chemistry with effect from 1 January 1965.

B. *Research Fellows:*

(i) Dr. A. K. Medda, M.Sc., D.Phil., in the Department of Animal Physiology, with effect from 1 February 1965.

(ii) Shri P. V. Manoranjan Rao, M.Sc., in the Department of Physics with effect from 1 February 1965.

(iii) Dr. Ashalata Chandra, M.Sc., D.Phil., in the Department of Microbiology with effect from 10 March 1965.

C. *Research Assistant:*

(i) Shri Arun Kumar Chatterjee, M.Sc., Department of Microbiology with effect from 28 December 1964.

D. *Government of India Research Training Scholars:*

(i) Shri Panchu Gopal Roy, M.Sc., Department of Microbiology with effect from 24 October 1964.

(ii) Shri Nirmal Kanti Shaw, M.Sc., Department of Microbiology with effect from 21 August 1964.

(iii) Shri Priti Sadhan Basu, M.Sc., Department of Chemistry with effect from 2 December 1964.

(iv) Shri Kali Krishna Ghosal, M.Sc., Department of Botany with effect from 16 December 1964.

(v) Shri Asis Dutta, M.Sc., Department of Chemistry with effect from 11 February 1965.

(vi) Sm. Ujjani Chowdhury, M.Sc., Department of Microbiology with effect from 1 July 1964.

E. *Research Scholars:*

(i) Shri Bijoy Krishna Chowdhury, M.Sc., Department of Chemistry with effect from 1 September 1964.

(ii) Shri Shib Prasad Dutta, M.Sc., Department of Chemistry with effect from 16 January 1965.

(iii) Sm. Roma Barat, M.Sc., Department of Microbiology with effect from 1 February 1965.

(iv) Sm. Shila Sen, M.Sc., Department of Botany with effect from 1 April 1964.

(v) Sm. Kalyani Bhattacharjee, M.Sc., Department of Microbiology with effect from 15 May 1964.

F. *D.A.E. Research Projects:*

(i) Shri Samir Kumar Mitra, M.Sc., Scientific Officer with effect from 1 October 1964.

(ii) Manjusri Bal, M.Sc., Scientific Assistant with effect from 17 August 1964.

G. *Research projects financed by the Council of Scientific and Industrial Research:*

a) *Senior Research Fellows:*

(i) Dr. Kalachand Das, M.Sc., D.Phil., Department of Chemistry with effect from 1 June 1964.

(ii) Shri H. Bhattacharjee, M.Sc., Department of Physics with effect from 4 July 1964.

(iii) Shri Deb Kumar Mukherjee, M.Sc., Department of Chemistry with effect from 16 December 1964.

(iv) Dr. Ranjit Kumar Roychowdhury, M.Sc., D.Phil., Department of Chemistry with effect from 2 July 1964.

(v) Dr. Parul Chakrabarty, M.Sc., D.Phil., Department of Chemistry with effect from 1 June 1964.

b) *Junior Research Fellow:*

(i) Shri Dipak Kumar Neogi, M.Sc., Department of Physics with effect from 11 November 1964.

c) *C.S.I.R. Pool Officer:*

(i) Dr. Purnima Sinha, M.Sc., D.Phil., Department of Physics with effect from 2 January 1965.

RESEARCH ACTIVITIES—NON-TECHNICAL SUMMARY

PHYSICS

I. Nuclear Physics

(*a*) *Investigations on Gamma Radiations*

(*i*) Angular distribution of photons scattered by atomic electrons in the Compton process has been measured in two steps. In the first step, the relative values of scattered intensities have been measured. Absolute values are then determined by measuring the total number of photons which are scattered through more than a given angle. The measurements agree with the predictions of Klein-Nishina formula for relativistic photons.

(*ii*) A theory has been developed to investigate the absorption and scattering of gamma rays during their passage through moderately large shells of absorbers. The theory has been adapted to form the basis of a new method for the measurement of photo-electric cross-sections of heavy atoms. Measurements have been carried out for shells containing lead and mercury. The experimental results are in agreement with the results obtained by other workers using other methods.

(*b*) *Investigations on Neutron Physics.*

Total (n, p) and (n, α) cross-sections of a few low atomic number elements have been determined for 14 Mev neutrons obtained from the Institute's Cockcroft-Walton accelerator. Systematic errors in (*i*) flux measurement, (*ii*) assignment of proper neutron energy, (*iii*) timing and (*iv*) β-activity measurement in radio-active product nuclei, have been very carefully determined and the cross-section data of nuclei ranging between atomic weight 16 and 55 have been obtained, correct to $\pm 10\%$ (even better in a few cases). Special techniques have been developed for the measurements of short half-lives, of the order of a few seconds, from neutron irradiated samples. Both β and γ measurements were performed in most of the samples, for consistency in such activation analysis data. (n, 2n) cross-section in P^{31} in this near-threshold of energy has been determined. Experimental data have been compared with the calculated values from different nuclear model theories.

II. Self diffusion in liquids

A new method has been developed for the determination of self-diffusion in liquids. The method is based on the separation of space dependent parts from the time-dependent functions in the solution of restricted diffusion equation. The method has the advantage of eliminating the zero-time correction which introduces considerable error in the conventional methods. Measurements have been carried out in phosphoric acid and thallous sulphate solutions. The results indicate that the method is capable of being quite precise and accurate, when normal precautions are taken.

III. Radiocarbon Dating.

Several 'dead' and 'modern' samples of toluene have been systhesised. A special counting unit is under construction.

IV. Abiogenic molecular evolution.

Work has been initiated of the study of abiogenic molecular evolution, with emphasis on the effect of absorption of the components of biological molecules like purines, pyrimedenes, nucleotides, nucleosides and amino acids by clay minerals, by testing the state of orientation of biomolecules inside mineral lattices, principally with the help of X-ray diffraction. Work is in progress to equip the X-ray laboratory for such biophysical work, along with other physical investigations on X-ray spectroscopy and solid-state studies.

V. Radio wave Propagation and Atmospherics Unit.

Theoretical investigations on the magneto-ionic theory have settled a controversy of some years' standing regarding the polarization characteristics of the ordinary and the extraordinary modes by fully working out the coupled wave-equations. Simpler geometrical methods of finding the dispersion and the polarization parameters of the electromagnetic wave travelling through the ionosphere have been given. In the field of atmospherics, a theory of the 'hook'-field components, observed at times after the return stroke, has been developed. An estimate of the angle of inclination of the equivalent lightning channel has been made from the waveforms of some reflected types of comparatively near origin. The nature of the change of the pulse-waveform with the increasing order of ionospheric reflection has also been theoretically investigated.

On the experimental side, a radio pulse-transmitter and an automatic atmospherics waveform-recorder have been constructed. The reflections from the sporadic E-region have been recorded and the analysis of the records has revealed the characteristics of the region. A study of the ionospheric absorption has also been made from the direct pen-recorder charts for the first-order and the second-order echoes. For the study of the frequency power spectrum of the lightning discharges and VLF-propagation, various electronic units have been constructed and are under test.

CHEMISTRY

Protein Chemistry:

In the course of investigations on the occurrence of haemoglobin polymorphism in the Indian zebu cattle it has been observed that while the law of genetic equilibrium holds good with four out of six breeds examined, in the cases of the remaining two breeds and the total population a significant excess of heterozygotes has been found. A similar finding made elsewhere with the Indian Gir breed was ascribed to the probable existence of heterozygous advantage in that population. Further detailed studies are in progress to see if this observation which has not been noted with the African zebu population, is of any major significance in the case of Indian zebu cattle.

A detailed comparison of the gene frequencies of Bov. A and B haemoglobins of zebu and non-zebu cattle indicates that although the haemoglobin-B gene frequency is highest in the zebu lowest in the non-zebu populations (except in the Jersey breeds), haemoglobin-B is unlikely to serve as a reliable marker of zebu origin in view of the fact that another

typical characteristic of zebu cattle e.g. α-lactalbumin polymorphism, is not shown by any non-zebu breeds with high Bov. Haemoglobin-B gene frequency.

Detailed physico-chemical studies have been carried out on crystalline bovine haemoglobin-B and goat beta-lactoglobulin.

Plant Chemistry:

Structural studies on naturally occurring carbazole derivatives are being made. Synthesis of compounds related to some of these natural products have been accomplished.

Studies on chemical plant taxonomy have revealed the presence of some new carbazole derivatives in a Rutaceous plant. A novel type of furano-lactone has been found in a plant of the family Meliaceae. The application of chemotaxonomic methods to the problems of hybrid studies of agricultural crops is under study.

During the survey of saponin bearing plants three new sapogenins have been isolated from the seeds of *Barringtonia acutangula* commonly known as Hijal. The chemical structure of these sapogenins have been fully elucidated. The plant *Mollugo spergula*, known as gima in West Bengal, is extremely bitter in taste and is used as a green vegetable. The bitter principles have been characterised as saponins. Two new sapogenins have been isolated from this plant in pure state and the complete chemical structure of one of these products has been established. Saponins have also been detected in a number of other plants.

A thorough chemical investigation of the plant *Lantana camara*, which causes severe photosensitization in sheep, has been taken up. From the active lantadene mixture obtained from the petroleum ether extract of the plant, two new compounds have been isolated and much progress has been made in the elucidation of the chemical structure of these compounds.

Chemical investigation of an antibiotic isolated from a *Streptomyces* sp. has been undertaken in collaboration with the Microbiology Department of this Institute. The abtibiotic has been obtained in a pure crystalline state and some of its chemical properties have been studied.

Radiochemistry:

A fraction obtained from disrupted chloroplasts from spinach leaves have been found to catalyse the incorporation of adenosine triphosphate or guanosine triphosphate into polynucleotide chain, a reaction quite distinct from terminal addition of a single nucleotide and also from that catalyzed by polynucleotide phorphorylase.

Ribonucleoprotein particles from the chloroplasts (20S-130S) are found to be the active site for protein synthesis. Phenylalanine incorporation occurs to a great extent by the chloroplast ribonucleoprotein soluble enzyme systems, and in the presence of polyadenylic acid, this is inhibited upto 40% and this inhibition is restricted to hydrobromic and acetic acid extractable material indicating probably the polyuridylic acid directed polyphenylalanine synthesis.

The incorporation of a number of aminoacids by the chloroplast RNP soluble enzyme systems has been found to be affected differentially by ultraviolet light irradiation.

Polyuridylic and Polycytidilic acids after irradiation with U.V. when tested for their messenger activity, serine and leucine incorporation are increased with a decrease in phenylalanine and proline incorporation respectively, indicating probably a change in coding ability of the messenger.

A novel phosphotransferase system has been isolated from germinating mung beans (*Phaseolus aureus*) which can transfer phosphate residue from phytin (Inositol hexaphosphate) to ribonucleoside diphosphates to generate corresponding triphosphates.

The probable role of phosphoprotein and ATPase on the autonomous movement of *Desmodium* leaflets has further been extended.

Histones associated with the hereditary material (DNA) are found to have antibacterial activity.

Biochemistry:

Host-virsus relationship studies with *S. typhimurium* and *E. coli* and their virulent viruses revealed that infection causes almost immediate production of inhibitors, which inhibit the function of induced enzymes already present in the uninfected cells. A new type of virulent phage against *S. typhimurium* has been discovered Its biological properties are being investigated.

Studies on induction of L-arabinose isomerase in *L. plantarum* showed that the half-life of messanger RNA of the above enzyme is much longer thean that of β-glactosidase of *E. coli*.

Studies with S^{35}-lipoic acid, prepared from *A. vinelandii* biosynthetically, and C^{14}--ATP demonstrated that adenyllipoate was the intermediate in the activation and transfer of lipoic acid to enzyme protein.

It has been shown that in the DNA dependent incorporation of gaunylate residue into polynucleotide chain, only one guanylate residue is attached at the end of DNA chain.

The amino sugar acetylgucosamine can be used as only carbon source for the growth of *Candida albicans*, a potential pathogenic yeast. Glucose has been found to repress the metabolism of acetylglucosamine very efficiently in this organism.

Analytical Chemistry:

The technique of thin layer-chromatography has been developed and necessary equipments have been designed and constructed. Successful separation of a large number of coumarin and some triterpenes have been achieved.

An efficient and easy liver function test has been evolved based upon the paper electrophoretic pattern of the serum proteins and serum transaminase activities of the patient. This test with certain modification has also been extended to diagnose and to locate the site of different types of cancer.

The anthocyanin pigments from different varieties of jute plant under investigation in the Dept. of Botany have been extracted and studied by the technique of paper-chromatography. The results reveal a marked difference in the pigment content of two varieties. This difference in pigment content may be used as genetic marker.

The separation of the methyl esters of higher fatty acids with the help of gas chromatography has been studied at high temperature with a number of different columns in order to separate and estimate the fatty acid content of some commercial fats.

The work on different varieties of maize seed proteins is on progress.

BOTANY

Plant Biochemistry

Successful attempts were made to grow single cell cultures of hollyhock (*Altheae rosea*) crown gall tumour tissue. These were grown in a specially constructed flask. The diameter of the single cells varied from 40u to 150 μ with same giant cells upto 30 μ and above. Work with normal tissue is being undertaken. Attempts were made to isolate and establish tissue clones from single cells on agar medium but these were not successful.

Radiation mutation

Comparison of straw stiffness and flowering time of three mutants of rice (viz. Black mutant, Marichbati Awned and White Dular) were made with their respective controls. Slight lateness was found in the mutants namely Black mutant and White Dular. Increased straw stiffness was observed in both the mutants Marichbati Awned and White Dular. A 3:1 segregation was observed in the F_2 generation (for green versus dark pigmented tillers), when Black mutant was crossed with its parental type (Marichbati). Agronomical and cytological studies were also conducted on some of the mutants isolated in Boro paddy.

Chemical mutation

A derivative of ethylene-imine (2 ethyl-2 phenylethylene -imine) was tried on barley and its effect on producing chlorophyll mutants was observed. The frequency of chlorophyll mutation was found to be 5.2% when based on spike progenies and 1.84% when calculated from M_2 seedlings. About 57% of these mutants were found to belong to the viridis class.

Cytology and Cytotaxonomy

Cytological and Cytotaxonomical investigations were continued in the family Malvaceae. Chromosome morphology of four different species in the genus *Hibiscus* have been worked out. Cytological and Cytotaxonomical investigations were also made in about 18 species belonging to different genera and tribes of the sub-family Papilionaceae.

Details of chromosome analysis were also made on some twenty strains of *Pisum stavium* with a view to finding the number of secondary constricted chromosomes. This was found to be four in all cases. The somatic chromsomes of some varieties of *Lathyrus sativa* (Sweet Pea) were analysed. Certain differences in size, morphology and number of constrictions, were found within the varieties.

Meiotic studies of seven radiation induced mutants of rice showed the presence of irregularities in some of the mutants.

Biometry and Plant Breeding

Studies on three different varieties of aman paddy belonging to the early, medium and late types were made, with respect to the relation of certain quantitative (e.g. yield) characters and dates of sowing (June 4th, June 24th and July 14th). It was found that in all sowings the medium type gave the highest yield.

Several species of fodder grass (species of *Paspalum, Panicum* etc.) and fodder legumes (species of *Stylosanthes, Phaseolus* and others) were selected from the previous year's performances and are under observation.

MICROBIOLOGY

Production of antibiotics by microorganisms

A tetraene antibiotic was isolated from a *Streptomyces* strain Ac_2 435. The fermentation studies indicated nonspecific nutrient requirement and the active principle was isolated and purified. The antibiotic has been catagorised as a new one, substantiated by its chemical and antimicrobial behaviour.

Screening of Streptomyces spp. producing broad-spectrum antibiotic.

Soil samples collected from West Bengal and neighbouring states were plated out and 600 isolates were picked up. Preliminary assay procedures indicated that 135 pigmented strains had either antibacterial, antifungal or both antibacterial and antifungal activity. Finally, 9 strains out of these have been sorted out for detailed studies on their growth and antibiotic production.

Pigment antibiotic of a mutant strain MT-10 derived from a *Streptomyces* sp. (Ac_{21} 460).

The pigment antibiotic from MT-10, a mutant obtained from a *Streptomyces* sp. (Ac_{21} 460) reported earlier, was subjected to further chemical tests and it was found to be a new quinonoid type of antibiotic having one active component.

Induced mutation in microorganisms

Experiments were conducted to induce mutations in *Aspergillus regulosus*. Morphological and biochemical mutants were obtained. By auxanographic tests the actual nutritional requirements of the mutants so obtained have been determined. Deficiencies are either for amino acids, vitamins, nucleic acids or for all.

Study of reverse mutation of *A. chevalieri*

Experiments were performed to determine the effect of chemical, physical and combinations of chemical and physical agents to determine the reverse mutations in *A. chevalieri*. Chemical agents included in the study were hydrogen peroxide, formaldehyde and sodium desoxycholate and physical agent UV-irradiation. The frequency of reverse mutation was found to increase in combined treatment of chemical and UV-radiation.

Studies on the nature of reverse mutants were made and most of them showed wild growth without any nutritional requirement. Distinct difference, however, was observed in the color of conidia, reverse mutants had yellowish green conidia while the wild parent had olive green conidia.

Killing and mutagenic effects of UV-irradiation in species of Aspergillus

Experiments were done with *A. aureus*, *A. oryzeae* and *A. avenaceus* with UV-irradiation in order to study the relation between sublethal dose and mutation frequency. Some biochemical and morphological mutants have been scored.

Recovery from UV-irradiation damage by treatment with nucleic acid hydrolysate in *Aspergillus* species.

Species were treated before, during and after irradiation. It was found that the recovery against damage due to UV-irradiation took place by treating the spores during irradiation with nucleic acid hydrolysate.

Further studies to find out whether there was any change in killing rate with the change of concentration of nucleic acid hydrolysate were made. It was observed that the killing rate decreased gradually with the increase in concentration of nucleic acid hydrolysate and at higher concentration complete protection against radiation injury was observed.

Studies on the recovery of radiation damage in *Streptomyces* species

Various inorganic salts e.g. NaCl, KCI, $CaCl_2$, $MgSO_4$ and $FeSO_4$ were used to study their effect on recovery of radiation damage in a *Streptomyces* strain. It was found that the per cent survival increased fairly when the inorganic salts were added to the plating medium. With NaCl and $MgSO_4$ the maximum survival was obtained.

Microbial genetics

Physiological studies on the growth of *Sordaria bosensis* with different liquid media were done. It was found that addition of fumaric acid, sodium fumarate and succinic acid to the minimal A_1 medium, encouraged the growth favourably. It was also found that increase of N_1 source NH_4 NO_3 and also the carbon source i.e. glucose to the minimal medium accentuate quicker and more profuse aerial growth.

Experiments on induced mutations of *Sordaria bosensis*

Irradiation experiments with UV rays yielded several morphological and biochemical mutants. A number of vitamin-requiring mutants have been isolated.

Studies on induced mutations in *Penicillium vermiculatum* by UV irradiation

A number of morphological and biochemical mutations have been raised from irradiated population of *P. vermiculatum*. The mutants differed from the parent type by their colonial habits, texture, color and pigment production. On biochemical tests by the auxanographic method, it was found that some of the morphological mutants behave also as biochemical mutants. The mutants have been classified into different catagories depending on their requirement.

Studies on the genus *Chaetomium*

A survey of *Chaetomium* spp. occurring in soil in and around Calcutta and other places of West Bengal was taken up with a view to isolate the most powerful cellulolytic members from amongst them. Following Eggins and Pugh's quantitative assay technique it has been observed that *C. indicum* is the most powerful cellulolytic member of all the so far isolated species.

Rhizobium bacteriophage and development of resistant strains

The development of phage resistant strains of *Rhizobium* was undertaken and pot culture experiments were designed for a preliminary study of the effect of bacterial inoculation on the seeds of (*a*) *Crotalaria juncea* and (*b*) *Cajanus indicus*. Among the treated plants there was significant difference between the plants treated with phage-resistant bacteria and those with non resistant bacteria. The production of large-sized and increased number of nodules were indications of higher rate of nitrogen fixation.

Ecology of air microrganisms

Seasonal variations of the occurrence of different groups of microorganisms in air have been determined by culture plate exposure method in three localities in and around Calcutta. The culture plates showed lower counts of actinomycetes almost throughout the year. Bacterial populations are found to exceed the fungal ones generally in the summer and rainy seasons. In the winter, however, the counts of fungi surpassed those of bacteria. Systematic studies are being carried out for the specific determination of some of the fungi collected from air. Allergenic tests of some of the fungi collected from air are under progress.

Industrial Fermentation

Extensive screening of strains of *A. niger* resulted in the isolation of six strains producing considerable quantity of gluconic acid.

Studies indicated that the time taken for the conversion of 50% of glucose ranged from 3-4 days in the case of isolated naturally occurring strains while the type strain consumed the same amount of sugar within 36 hours, such specific strains are being selected from efficient gluconic acid fermentation.

Transformation in microorganisms

Attempts were made to transfer the strains of *Aspergillus niger*. DNA from the donor strain of *A. niger* (V_{35}) was isolated after the method of Hotchkiss and the germinated spores of recipient strains (Q_{30} and SG) were treated, Transformation was achieved in btoth the strains.

Transformation of *Staphylococcus aureus* from penicillin sensitivity to resistance was done by growing sensitive strains in medium containing DNA of the resistant strains. It was further observed that the rate of transformation increased with the incorporation of yeast extract to the culture medium.

Physiology of Microorganisms

Studies on the phosphate solubilizing fungi from Rhizosphere and soil

Isolation of fungi were made from the soil samples collected from various parts of West Bengal and Ranchi in Bihar and their phosphate solubilizing capacility determined. It was found that a fairly good proportion of rhizosphere fungi can solubilize insoluble phosphates when incorporated into the substrate. Preliminary observations indicate the role played by soil actinomycetics in dissolving otherwise insoluble phosphates.

Studies on toxic substances in infected paddy samples collected from Nadia District, West Bengal.

Microscopic examination and subsequent isolation in pure culture revealed the presence of various species of fungi on the surface of the paddy seeds. Three pigmented isolates were selected for further work. All of them colonised paddy seeds successfully. Toxicity tests are being made.

A. PHYSICS

A.2. Radio wave propagation and Atmospherics

A.2.1. *Magneto-ionic Theory:*

Theoretical investigations were undertaken on Polarization of Electromagnetic Waves travelling through the Ionosphere. It was shown that Saha, Banerjea and Guha (Proc. Nat. Inst. Sci., India, **17,** 205, 1951) had not correctly labelled the ordinary and the extra-ordinary modes in their wave-treatment. With correct labelling the expressions for the complex wave-polarization for both the modes and the phase-difference between the normal and the abnormal components of the magnetic vector of the wave were found identical in the wave and the ray treatments. The coupled wave-equations were fully worked out and the wave-polarization and the relations between the electric and the magnetic fields were deduced from these equations.

Geometrical methods of finding the refractive index, absorption coefficient and polarization parameters of the electromagnetic wave travelling through the ionosphere were worked out. The methods simplified considerably the procedure for determining refractive index, absorption index and the polarization parameters.

A.2.2. *Electric field-variations during Lighting discharges:*

An interpretation of the 'hook'-field components, observed at times during the c-field change of a lightning discharge was given in terms of streamer formation at some high potential between the return core and the leader sheath. Taking the condenser system, where the return-stroke which is effectively at the ground potential flows along the axis of the leader sheath which is nearly at the same negative potential as the cloud, it was considered that in the corona-discharges from the return-stroke channel which is positive with respect to the surrounding cylinder, streamers similar to those which were found to reappear in the laboratory experiments of Kip at a potential much higher than the onset corona potential would likewise appear giving rise to the observed 'hook'-field components superimposed on the c-field change after the return stroke.

Taking into account the corona currents from the return-stroke channel, an expression for the effective return-stroke current contributing to the observed electric field-change was deduced. It was also possible to derive the change in the radiated field from the time-derivative of the effective return-stroke current.

In the case of waveforms of the reflected type of comparatively near origin, an estimate of the angle of inclination of the equivalent lightning channel was worked out for four typical reflected types of waveforms. An expression for the pulse-waveform multiply reflected from the ionosphere was also deduced accepting the double exponential form of the return-stroke current using Kimpara's values of the constants. It was possible with the help of this expression to explain the reversal in the nature of the pulse-waveform, observed at times, with the increase in the order of reflection.

A.2.3. *Experimental work:*

The two CSIR-schemes on (i) Studies on the sporadic E-region of the Ionosphere and (ii) Study of the Frequency Power Spectrum of Lighting discharges were transferred from the University Science College to the Bose Institute, on May 1, 1964. As the radio pulse-transmitter and some accessories employed in the work of the Scheme on the Sporadic E-region belong to the University of Calcutta and were not placed at the disposal of the Bose Institute, the work of the Scheme was carried out with the permission of the Vice-Chancellor of the University of Calcutta, partly at the University Science College, while the construction of a new radio pulse-transmitter of suitable power and other necessary units was undertaken at the Bose Institute. The transmitter has been completed. The transmitting and the receiving aerial units have been installed. Ionospheric echoes from E_s & F regions have been photographed. At the University Science College, a number of records of the first and the second-order reflections from the sporadic E-region was taken. The analysis of the records indicated the characteristic features of this region. A study of absorption of the radio waves was also made from the *direct* pen-recorder charts of the reflection coefficient of the region. The experimental set-up involved the use of three integrator circuits, two difference amplifiers and a divider circuit.

With regard to the work on Atmospherics, a duplicate automatic wave-form recorder has been constructed. The work on the Frequency Power Spectrum of the lightning discharges is summarized as follows:

Having completed the design and the construction of the wide-band amplifier and a few multivibrators and power-supplies last year, a dozen frequency-selectors have been designed and constructed during the current year.

For the low-frequency range (100 c/s to 5 Kc/s) the frequency-selection has been made by using the Twin-T in a negative feed-back circuit. The Twin-T has been preferred to the Wien bridge because it has a common ground between the input and the output terminals and can thus be more conveniently employed in a feed-back circuit. The parameters of the Twin-T have been adjusted to give a null-frequency corresponding to the frequency to be selected. For each of the frequencies selected, three component parameters of the Twin-T have been adjusted suitably.

The suitably designed Twin-T has then been used as a feed-back loop in a negative feed-back amplifier. Then the negative feed-back occurs at all frequencies except at the null-frequency and the output of the amplifier becomes maximum at the null-frequency which has been chosen to correspond to the frequency selected.

The amplifier used is a two-stage cascode amplifier using two triodes in cascode. A 6SN7 tube has been conveniently used in place of the two triodes. The input signal has been applied to the grid of the upper tube and the grid of the lower tube has been used as the high-impedance load for the Twin-T network. The final output has been taken through a cathode-follower which has allowed higher loads to be employed in the plate of the upper tube. The cascode arrangement with the final cathode-follower has yielded high Q-values even though the tubes used are only triodes.

Above the frequency of 5 Kc/s the selection has been made by employing the usual L-C tuned circuits. The coils necessary for this range are wound on ferrite rods which make it possible to obtain high Q-s with comparatively short coils.

For recording the amplitudes corresponding to all the frequencies selected, a monitor tube (C.R. tube Type 903) has been purchased and its characteristics are being studied. If the intensity is found sufficient for rapid photography, then ten such tubes will be arranged in a circle and the amplitudes of the selected frequencies will be traced on these tubes simultaneously. The traces appearing on these tubes will be photographed by a camera with a large-aperture lens.

A.4. Gamma radiation

A.4.1. *Compton scattering cross-section*

Although the Compton scattering is one of the most fundamental processes through which relativistic photons interact with matter, experimental investigations on the differential collision cross-sections in Compton effect have been comparatively rare. Also most of the experiments reported hitherto deal with the relative collision probabilities and their angular range is usually limited to angles of scattering smaller than 90°. In the investigations carried out in the laboratory, absolute values of the differential collision cross-sections of Co^{60} photons (1.17 and 1.33 MeV) have been measured in the angular range of 13° to 140°, in two steps. First the relative values of the cross-sections have been determined using a uniform spectral-sensitivity photon-counter developed in the laboratory. Errors caused by multiple scattering and absorption in the scatterer have been eliminated by a semi-empirical extrapolation procedure. Corrections have been made for the effects arising from self-absorption in the source and for small variation of the detector-sensitivity with angle. Experimental data unequivocally rule out the distribution formula of Breit, Gordon and Dirac in favour of the Klein-Nishina formula.

The relative measurements were converted to their absolute values by a partial integral type measurement in which the total cross-section for angles 13° to 180° have been measured. Extrapolation necessary for larger angles have been shown to have negligible effect in this determination. The root-mean-square deviation between the experimental and Klein-Nishina values was found to be 1.5% which is well within the mean probable error spread of the experimental points themselves.

A.4.2. *Photoelectric cross-section*

A new method has been developed for the measurement of atomic photo-electric cross-section of high Z elements. This method is based on the measurement of photon-transmission through spherical shells of absorbers. Theoretical calculations have been made to consider the effects of multiple scattering as the thickness of the shell is gradually increased. It has been shown that the absorption in the shell can be approximated by an effective cross-section which can be expanded in a polynomial in terms of the thickness of the shell. The effect of truncating the polynomial by another polynomial of lower order has been evaluated; in particular, the case of linear extrapolation has been explicitly calculated. Formulae for the evaluation of correction factors caused by the finite size of the

source, by the self-absorption in the source, by the non-uniformity of detector-sensitivity, by scattering in the absorber holders, by small deviations from spherical symmetry and by pair-production in the absorber have been derived.

Experimental investigations to test the above theory as well as to measure photo-electric cross-section by sphere transmission have been carried out using a uniform spectral sensitivity photon-counter and a rotation arrangement for averaging out the photon intensities. The measurements have been carried out on Pb and Hg for Co^{60} photons (1.25 MeV) and on Pb for Cs^{137} photons (0.66 MeV). The experimental results have been compared with the measurements of previous workers and also with the theories of photo-electric effect in the relativistic region. It has been found that the experimental results obtained by different workers for Co^{60} gamma-rays show mutual agreement, if the *ratios* of K-shell photo-absorption to total photo-absorption are assumed to have the values given by the experiment of Hultberg. However for Cs^{137} photons these ratios were found to be invalid. There is general agreement between the sphere transmission and beta spectroscopic methods which indicate the soundness of the foundations of these methods.

A.5. Neutron Physics

A.5.1. *Reaction cross-sections of* 14 *Mev neutrons*

A.5.1. (a) *(n,p) (n,α) reaction cross-section determination for* 14 *Mev neutrons in low At. No. elements*

These series of experiments have been concluded. Total σ (n,p) of O^{16}, F^{19}, Na^{23}, Al^{27}, Si^{28}, Cl^{37}, V^{51}, Cr^{52} and Mn^{55} have been determined relative to the (n,2n) cross-section of Cu^{63}, by the activation analysis method.

Various systematic errors associated with such measurements have been thoroughly studied and experimentally minimized or eliminated.

As comparatively thick samples, of the order of 20 mg/cm^2 thickness, were used, chiefly to obtain good counting rate from the neutron-irradiated samples, a method was developed for accurately determining the self-absorption and self-scattering factors in the β-counting by end window counters.

The method was based on Bayhurst and Prestwood's results (Ref: Necleonics **17,** No. 3, (1959)), who showed that the average beta energy was the correct parameter to correlate with the counting efficiency rather than the maximum energy of the β-rays. An empirical equation of the form

$$\epsilon = a(1-e^{-b\bar{E}_\beta})$$

was developed, where ε=maximum efficiency of the counting system, $\bar{E}_\beta$=average energy of the β-rays, and '*a*' & '*b*' two constants. For relative good geometry β-measurements, the value of the constant '*a*' might be set as unity. Hence

$$\epsilon_{rel} = 1-e^{-b\bar{E}_\beta}$$

The value of the constant '*b*' was determined by measuring the activity induced in copper foils of different thickness.

The self-absorption and self-scattering factor were determined in each case of activiation analysis upto an error limit of $\pm 3\%$.

The 14 Mev neutron spectrum was often checked with emulsion plate exposure and analysis of tracks of recoil protons.

The method followed for recording the activity from small half-life products (of a few seconds order) from irradiations of low Z elements, viz., O^{16}, F^{19}, Na^{23}, was the two-scaler method, where one scaler acting as a timer was photographed along with the scaler with the G.M. tube on the same frame and each frame automatically exposed one after another at an interval of 2 seconds.

A method of rapid transfer of irradiated samples to the counter has been developed. The error in timing has been minimized to $\pm 1/50$th of a second.

The flux of neutrons has been measured relatively and the cross-section values within $\pm 10\%$ (in some cases even better) were obtained relative to Cu^{63}(n,2n) Cu^{62} cross-section, whose normalized value at 14.8 ± 0.1 Mev was taken as 530 ± 25mb.

Along with (n,p) cross-sections of O^{16}, F^{19}, Na^{23}, Al^{27}, Si^{28}, Cl^{37}, V^{51}, Cr^{52}, Mn^{55}, the (n, α) reaction cross-sections of F^{19}, Na^{23}, P^{31}, Mn^{55} were determined.

A.5.12. *(n,2n) reaction cross section of* P^{31} *near threshold:*

14 Mev is the near threshold energy of (n, 2n) reaction for P^{31}, and the values of σ(n, 2n) reported earlier had a spread of nearly 100% in-between them.

On irradiation of P^{31} (isotopic abundance 100%) with 14 Mev neutrons, the following activities are expected to generate:

P^{31}(n,p) Si^{31}, decaying with β^- and γ remission with half life 2.62 hours.

P^{31}(n,α) Al^{28}, decaying with β^- and γ emission with half life 2.27 minutes.

P^{31}(n,2n) P^{30}, decaying with β^+ emission only with half life 2.55 minutes.

Thus the two competing reactions (n,α) and (n, 2n) produce the residual nuelci which decay with nearly the same half lives. Hence it is impossible to separate them by means of beta counting alone. Therefore, recourse was taken to γ-ray measurements. Al^{28} emits 1.78 Mev photons where annihilation radiation is obtained from positrons emitted by P^{30}. In the present experiment the two cross sections *i.e.*, (n,α) and (n, 2n) cross-sections have been measured by a special experimental technique and using the values of cross-sections of Si^{28}(n,p) and Cu^{63}(n,2n) reactions. The method is based on the fact that P^{31}(n,α) and Si^{28}(n,p) reactions have the same daughter nucleus, *i.e.*, Al^{28}; and Cu^{62} is a pure positron emitter.

In the method three identical samples of P^{31}, Si^{28} and Cu were simultaneously irradiated in the same neutron flux, using a rotation device to ensure the same geometrical disposition of the targets towards the neutron source. Then from counting, by keeping the single-channel analyser to record the gammas from Al^{28} from both P^{31} and Si^{28} samples, and also by recording annihilation radiation from Cu^{62}, the contribution from P^{31}(n,α) reaction was eliminated. σ(n,2n) of P^{31} obtained was 16.0 ± 1.6 mb. at 14.8 ± 0.1 Mev of neutron energy.

A.5.2. *Comparison of values of reaction cross-sections with those obtained from statistical model calculations.*

Comparison was made of the values obtained with those calculated using parameters from the statistical theory by (i) Erba *et al.* (Nuov. Cim. **22,** (1961), 1237), (ii) Gardner (Nuclear Phys., **29,** (1962) 373; (iii) Levkovskii (JETP Sov. Phys., **18** (1964), 213). Comparisons were also made of the experimental values with the theoretical values calculated by Chatterjee using shell model parameters (Nuc. Phys., **60,** 1964, 273).

The experimental results do not show any quantitative agreement with their theoretical counterpart. In view of the numerous difficulties which all the available theories have to face in this energy region, this result is not surprising. The experimental results partly show trends predicted by the shell-dependent values.

TABLE

Nucleus	(computed values of $\sigma_{n,p}$, (mb)			Experiment value (Present work)
	Erba *et al.*	Gardner	Levkovskii	
O^{16}	—	64	80	40.1± 2.7
F^{19}	—	40	21	23.3± 2.8
Na^{23}	51	64	44	41.8± 3.8
Al^{27}	81	87	72	97.0±10.0
Si^{28}	251	240	240	222.0±12.0
Cl^{37}	—	53	30	21.3± 2.1
V^{51}	16.4	40	42	24.7± 2.2
Cr^{52}	54	96	84	82.8± 5.8
Mn^{55}	—	56	57	59.5± 5.9

A.6. Low intensity Measurement

A.6.1. *Self-diffusion in liquids*

A new method for the determination of the coefficient of self-diffusion in liquids: A simple and accurate method for the determination of self-diffusion in liquids, employing radio-active tracer technique, has been developed. The method exploits the restricted diffusion (*i.e.*, when finite concentration change occurs at the two boundaries. This is in contrast to the usually employed free diffusion method, in which it is assumed that the concentration change at the boundaries is zero. In other words the time of observation is so short that the diffusing column is virtually an infinite system), so that space and time variables occur in the solution for the concentration distribution as separate functions. Essentially the method consists of observation of the count-rate variation of a counter vertically placed above the diffusing column of which the solute and the solvent are of equal length and the time of observation is such that $kt \geqslant 0.25$ (where $k = \pi^2 D/L^2$); L being the total length of the solute and solvent liquids and D the coefficient of diffusion).

The theory of the method has been worked out in detail. It has been found that the variation of counts registered in any fixed interval is given by the relation.

$$N_o - N(t) = A_e - kt$$

where N_o is the counts when the solute (radio-active) and solvent (non-radio active) liquids are uniformly mixed and $N(t)$ is that at any time t such that $kt \geqslant 0.25$. Various correction factors *e.g.*, due to neglecting the higher order terms of the Fourier series and any inequality in the two lengths of the diffusing system have been considered. It has been found that if the time of observation is restricted to $t \geqslant 0.25\ k^{-1}$, then under normal experimental situation, the corrections amount to less than 1%.

To assess and evaluate the general merit and demerit of the method and the accuracy and reproducibility which it is capable of attaining, several experimental measurements have been carried out. The diffusion cell has been designed on the principle of the sliding cell technique. The cross section of the cell was made rather large (diameter 1″) and observation confined to about 0.5 cm. at the centre by properly collimating the radiation to avoid the effect of curvature at the liquid solid contact.

Leakage and vaporisation of liquids were carefully avoided. Temperature was controlled to ±1°C. For the measurement and control of liquid heights, spherometric method working as electrical contact is used. To the central leg of the spherometer a specially made adaptor with fine end point was attached. This served as one terminal of an electrical circuit. The other terminal was connected with a very thin wire partially dipped in the liquid; so that as soon as the central leg touched the liquid surface the circuit was complete and the corresponding observation could be noted.

Measurements were carried out with Phosphoric (P^{32}) acid solution and Thallous sulfate solution (Tl^{204}). More than ten measurements on the diffusion of phosphoric acid solution of 0.1 N strength in water were carried out. A large number of counting data (more than 20) for each run was analysed by the usual least-square-fit method. It was found that the standard deviation of the observed data was in all these cases, within 0.5%. Experimental values were within 1% of the corresponding theoretical value.

It is expected that with better temperature control the accuracy will be considerably increased. Reproducibility of the measured data of the same specimen has been found to be within experimental error.

The greatest advantage of the method is that it eliminates the zero time correction entirely. Since the absolute time t is not required and essentially the difference of time of observation is considered, the time correction does not arise. The method is quite general and equally applicable to all types of radio-active emitters. It may be of particular use to soft betaemitters like C^{14} and S^{35}.

The results of these measurements show that with a normal stable counting system and good temperature control, the method may be expected to compare favourably in accuracy with the current improved optical methods used for measurements of mutual diffusion coefficients in liquids.

A.6.2. *Radiocarbon Dating*

The synthesis of the counting liquid *i.e.*, toluene from cocoanut charcoal has been reported previously.

At present the synthesis of toluene from several modern carbon samples has been done, and spectroscopically pure toluene has been obtained. The overall yield of toluene is about 60–70%.

The spectroscopic purity is attained by repeated distillation under reduced pressure at the final stage.

A dead sample of toluene (from coal distillation) has also been purified upon spectroscopic purity.

The dead sample and samples from modern carbon are ready for counting.

For counting low level radiocarbon activity, a special counting unit has been designed. In this unit, an all-glass cell can be interposed between two vertical photomultipliers in coincidence, by means of a sliding arrangement. The equipment has been so designed that the cell can be cleaned and the active liquid transferred in one position and then can be slided into the counting position through a light-tight arrangement. In this way the voltage on the photomultipliers need not be turned off at any time ensuring stablity of counting.

A.7. Biophysics

A study of abiogenic molecular evolution has been initiated with particular emphasis on the effect of absorption of the components of biological molecules like purines, pyrimedines, nucleotides, nucleosides and amino acids by minerals (clay in particular) on polymerisation, bond-formation and structural orientation produced by the crystal lattice of the solid supporter.

It has been observed that purines and pyrimedenes have preferential absorption over glycerol by montmorillonite. Experiments are being designed to test whether the purines and pyrimedenes are linked by the H-bond inside the clay lattice, as in DNA and to study the role of other minerals in the formation of linkages similar to biomolecules.

Testing the state of orientation of biomolecules inside mineral lattices is to be done by the method of X-ray diffraction, supported by other physical and chemical methods. Work is in progress to equip the X-ray laboratory for such biophysical investigations along with other physical investigations of X-ray spectroscopy and solid-state studies.

B. CHEMISTRY

B.1. Protein Chemistry

B.1.1. *Haemoglobins of Indian Zebu cattle*

Results of investigations on haemoglobin polymorphism in different breeds of Indian zebu cattle were partly reported in the previous Annual Report (1963-64). So far, studies conducted elsewhere with European and African breeds of cattle tend to suggest that haemoglobin polymorphism is not uniformly distributed. While most breeds in Britain and few in Denmark, Holland, France and Africa are homozygous for Bovine A haemoglobin, the presence of Bov. B has been reported in Algerian, certain French, British and American breeds along with Nigerian and East African zebu and Indian Gir breeds.

The occurrence of Bov. B haemoglobin in certain British breeds, particularly in Jersey, has been taken by some workers as a support to the theory of origin of Jersey cattle from *Bos indicus*. The ancestors of Jersey cattle have been assumed to have migrated from Indus valley through Africa to Europe and the occurrence of Bov. B haemoglobin in African and European breeds appear to follow the suggested line of migration. Bovine B haemoglobin has further been suggested to be associated with susceptibility to disease and tolerence to tropical climate and, in the case of Indian Gir breeds, heterozygous advantage in natural selection.

Results presented in the previous Annual Report (1963-64) on haemoglobin polymorphism in Indian zebu cattle have been analysed in the light of the above observations. Application of Hardy-Weinburg law of genetic equilibrium shows that with the exception of Sahiwal and Tharparkar breeds, in all other breeds examined, the observed and expected numbers are in fair agreement. No advantage to the heterozygotes is noticeable in the Gir cattle. In the case of the total population of 317 cattle, however, the equilibrium law does not hold good and there appears to be a slight excess of AB individuals. This excess, however, is much less than that observed with the sample of Gir cattle by Lehmann (Man, **59**, 66, 1959). Further studies are in progress.

A detailed comparison of A and B gene frequencies of different breeds of zebu and non-zebu cattle known to possess Bovine B haemoglobin has shown that there is a gradual decrease of the B gene frequency in the cattle populations as one goes from the Indian subcontinent to Europe through Africa, a fact which is favourable towards the view on the zebu origin of Bov. B haemoglobin as well as that of Jersey cattle. Indian and African zebu cattle, however, also show alpha-lactalbumin polymorphism in milk producing two genetically controlled variants, alpha-A and alpha-B. The occurrence of alpha-lactalbumin-A has been noted to be a characteristic function of zebu cattle and so far, Jersey or any other European breed showing relatively high frequency of Bov. B haemoglobin has not been known to produce it. Therefore, reliability of Bov. B haemoglobin as a market of zebu origin and migration of cattle and associated populations seems doubtful. Further,

high frequency of Bov. B haemoglobin in Jersey as well as in Hariana cattle which thrive well under widely variable environmental conditions indicates the improbability of distribution of Bov. B haemoglobin being determined by environmental selective agencies.

Detailed physico-chemical studies on crystalline Bovine haemoglobin-B are being carried out with the help of electrophoresis, sedimentation, and ultraviolet absorption. Sedimentation analyses have been carried out as a function of protein concentration, pH and ionic strength. The sedimentation coefficient of the protein remains unaltered in the pH range 4.5–10.5 below and above which its values fall indicating dissociation and/or denaturation. Similar effects also occur on increasing the ionic strengths of solutions.

B.1.2. *Studies on milk proteins*

(a) *Purification buffalo and cow alpha-lactalbumins:* Method of purification of alpha-lactalbumins by DEAE column chromatography has been standardized to a fair degree and preparative work is in progress.

(b) *Studies on goat beta-lactoglobulin:* Physico-chemical studies on goat beta-lactoglobulin has been continued. Its sedimentation behaviour has been studied at different protein concentrations and pH values. The sedimentation coefficient of goat beta-lactoglobin has been found to be linearly concentration dependent like other beta-lactoglobulins and its extrapolated value at zero protein concentration has been found to be 2.85×10^{-13} sec.

The partial specific volume and specific refractive increment of the protein have been determined and found to be 0.753 g/ml. and 1880×10^{-6} respectively. In ultraviolet abosprition also goat beta-lactoglobulin shows similarity to other beta-lactoglobulins and has a specific extinction coefficient of 9.35.

B.1.3. *Specific refractive increments of proteins*

During the course of ultracentrifugal studies the value of specific refractive increment, *dn/dc*, of different purified proteins prepared in the laboratory had to be determined. For this a Brice-Phoenix differential refractometer and a Zeiss interforometrer have been used. Determination of *dn/dc* of some proteins whose values have been previously reported in the literature and accepted as standard results have not been satisfactorily reproducible in all cases. Further work is being continued.

B.2. Organic and Plant Chemistry

B.2.1. *Survey of saponin bearing plants of India*

(a) *Barringtonia acutangula Gaertn.* In the previous report the complete structure and stereochemistry of two new sapogenins, barringtogenol C and D have been described (vide Annual Report 1963-64). The sapogenin mixture obtained by hydrolysis of the crude saponin from *B. acutangula* has now been examined by thin-layer chromatography over silica gel G whereby indication for the presence of a very large number of compounds has been obtained. It has now been possible to isolate another sapogenol, m.p. 248–50°, from the above sapogenin mixture. Ultraviolet spectrum of the sapogenol shows maxima at 213 $m\mu$ (ε, 16, 510) indicating the presence of an α,β-unsaturated ester moiety. Hydrogenation of the sapogenol over Adam's catalyst gives a product which does not show any characteristic absorption maxima for an $\alpha\beta$-unsaturated ester. Further work is in progress to establish the complete structure of the new sapogenol.

(b) *Chemical examination of Duranta plumeiri. Duranta plumiri* is a common hedge plant. Preliminary examination has showed the presence of a good amount of saponin. Further work to isolate and characterise the saponin is in progress.

B.2.2. *Chemical examination of Mollugo spergula*

The glycoside mixture isolated from the alcoholic extract of *M. spergula* has been found to contain at least eight different saponins by thin-layer chromatography over silica gel G. The isolation of two new crystalline sapogenins, spergulagenin A and spergulagenic acid, by acid hydrolysis of the above saponin mixture has been reported earlier (vide Ann. Report, 1963-64).

Spergulagenin A has been shown to be a rare type of completely saturated pentacyclic triterpene having three hydroxyl groups and a keto group. Homogeneity of this compound has been checked by thin-layer chromatography. Attempts for partial blocking of the hydroxyl functions by acetylation under mild condition have been fruitless. Borohydride reduction of spergulagenin A triacetate gives a mixture from which a product, m.p. 268–70°, has been isolated in the pure state and is being studied. The optical rotatory dispersion curve, nuclear magnetic resonance and mass spectra of spergulagenin A are being studied for the structure elucidation of the compound.

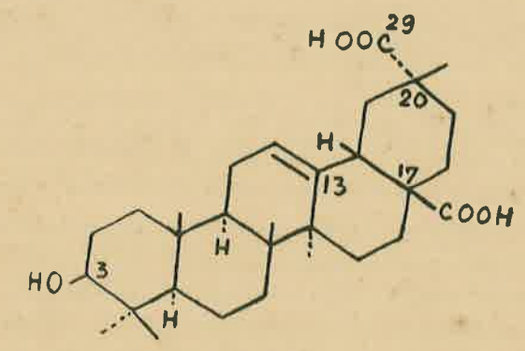
Spergulagenic acid, m.p. 347–50° (dec.), $[\alpha]_D^{26.7°} + 123.7°$ (pyridine) has previously been shown to be a new monohydroxy dicarboxylic acid of the oleanane series having a 12:13 double bond. Dimethyl spergulagenate on reduction with lithium aluminium hydride furnishes a triol, $C_{30}H_{50}O_3$, m.p. 278–80°. It has now been possible to obtain the selenium dioxide oxidation product of monoacetyl dimethyl spergulagenate in a pure state, m.p. 236–39° which shows triple ultra-violet absorption mixima at 242, 250 and 259$m\mu$ (logε, 4.27, 4.32 and 4.13 respectively), characteristic of Δ11:12, 13:18 -dienes of the oleanane series having 12:13 double bond. The molecular rotational difference between dimethyl spergulagenate and its 3-acetyl derivative, is consistent with the values for similar changes occurring in 3β-hydroxylated triterpenes of the oleanane series.

Spergulagenic acid does not undergo easy thermal decarboxylation at its melting point which clearly indicates that the double bond is not located at β,γ-position with respect to any one of two carboxyl groups. This conclusion eliminates position C-14 as a possible site for any one of the carboxyl groups in spergulagenic acid. Spergulagenic acid on treatment with bromine in acetic acid gives a monobromo-γ-lactone, $C_{30}H_{45}O_5Br$, m.p. 295–300°, showing bands at 1705 and 1754 cm^{-1} for a carboxyl group and a γ-lactone ring. The rate of saponification of dimethyl spergulagenate with 10 and 1 per cent ethanolic caustic potash has been studied under standard conditions. On working up the product separately, a dicarboxylic acid monomethyl ester, $C_{31}H_{48}O_6$, m.p. 292–94°, has been obtained. Titration with alkali indicates the presence of one free carboxyl group in the above

compound. The unaffected carbomethoxy group in the dicarboxylic acid monomethyl ester is saponified with caustic potash in ethylene glycol under reflux. The above experiment clearly shows the differential behaviour of the two carboxyl groups in spergulagenic acid. Obviously one of the carboxyl group is less hindered than the other and hence one should be in an angular position and the less hindered one should be at C-20 as the other possible site C-4 has already been considered and rejected (vide Ann. Report 1963-64). The ease of saponification (1 per cent ethanolic caustic potash) suggests the less hindered carboxyl group in spergulagenic acid to be α-equatorial (C-29) rather than β-axial (C-30).

The above analysis leaves only (I) as the most probable structure for spergulagenic acid. Finally spergulagenic acid has been converted to oleanolic acid following the reaction sequence outlined below.

The dicarboxylic acid monomethyl ester on treatment with pyridine and acetic anhydride gives an acetate, $C_{33}H_{50}O_6$, m.p. 260–63°, which is then treated with thionyl chloride. The resultant acid chloride on Rosenmund's reduction furnishes an aldehyde which is directly reduced by Wolff-Kishner method. The reaction product is then esterified with diazomethane and purified by chromatography over Brockmann's alumina when an ester, $C_{31}H_{50}O_3$, m.p. 198–200°, $[\alpha]_D^{32°}+72$, is obtained which forms a monoacetate, $C_{33}H_{52}O_4$, m.p. 218–20°, on treatment with pyridine and acetic anhydride. The physical and chemical properties of the above ester and its acetate agree closely with those of methyl oleanolate and its acetate. The identity has finally been established by direct comparison of mixed melting points with authentic samples. Thus the structure and stereochemistry of the whole molecule excepting the configuration of the carboxyl group at C-20 is established. The ease of saponification of dimethyl spergulagenate to the dicarboxylic acid monomethyl ester requires the carboxyl group attached to C-20 to be α-equatorial (C-29). Spergulagenic acid may thus be represented as (I).



(I)

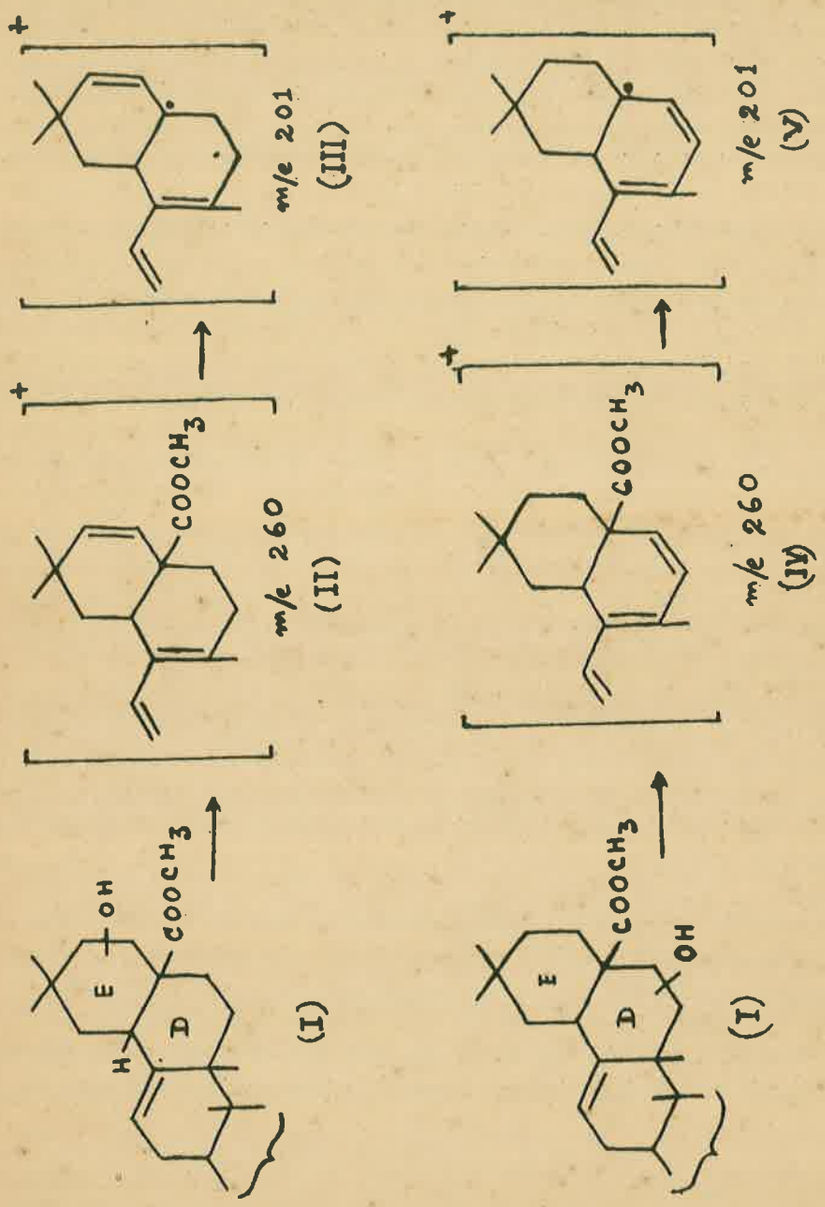
B.2.3. *Chemical investigation of Lantana camara*

A thorough chemical investigation of the plant *L. camara*, which causes severe photosensitization in sheep, has been taken up. The active lantadene mixture obtained from the

petroleum ether extract of the plant has been separated into acidic and neutral fractions. A part of the acidic fraction after esterification with diazomethane, has been subjected to thin-layer chromatography whereby indication for the presence of a tough mixture of a very large number of compounds has been obtained. The above observation probably accounts for the failure of earlier attempts in different laboratories to isolate the active principles in pure state. Attempts made so far in this laboratory for the isolation of individual components from the above latadene mixture, have led to the isolation of four compounds besides lantadene B, of which two, compound A and B, have been reported earlier (vide Ann. Report, 1963-64). Compound A and B both have been isolated as their methyl esters.

Compound A methyl ester (ester A) has the molecular formula, $C_{31}H_{48}O_4$, m.p. 197–98°. It gives pink→violet colour in the Liebermann Burchard test. The I.R. spectrum of ester A shows absorption at 3575 cm^{-1} (hydroxyl group), 1715 cm^{-1} (carbomethoxy group) and at 1365, 1380 cm^{-1} (gem dimethyl groups) and a number of bands in the region 800–900 cm^{-1} which probably indicates the presence of an epoxide or 1:4-oxide ring. The ester A gives a pale yellow colour with tetranitromethane indicating the presence of unsaturation. Hydrogenation of ester A in presence of Adam's catalyst under normal conditions furnishes a product, $C_{31}H_{50}O_4$, m.p. 235–38°, which responds to tetranitromethane colour test thereby proving that the double bond has not been hydrogenated. The above product has been shown to be a diol forming a diacetate, $C_{35}H_{54}O_6$, m.p. 236–39°. It is evident, therefore, that the fourth oxygen function, which is considered as epoxide or 1:4-oxide, has been involved in the hydrogenation process. Potassium borohydride reduction also furnishes the above diol. The diol diacetate on oxidation with selenium dioxide furnishes a compound showing triple UV-absorption maxima at 243, 249 and 260$m\mu$ characteristics of Δ11:12, 13:18-dienes of the oleanane series. Thus compound A appears to be a compound of the oleanane group having a 12:13 double bond. Further work is in progress.

Compound B methyl ester m.p. 227–229°, gives pink→violet colour in Liebermann Burchard test and a pale yellow colour with tetranitromethane. Combustion analysis and molecular weight determination by Rast's method indicates a formula $C_{31}H_{48}O_5$ for ester B. Mass spectrum of compound B methyl ester (ester B) has been studied. The highest mass peak appears at m/e 482 which is most probably the (M-18) peak formed by loss of one molecule of water. If the above assumption is correct then the formula of ester B should be $C_{31}H_{48}O_5$ corresponding to a mass number 500. The mass spectrum of ester B also shows peaks at m/e 260 and 201 which most probably correspond to the ions II or IV and III or V respectively. Formation of the above ions can be explained by only assuming a normal oleanane skeleton for ester B having a 12:13 double bond and a hydroxyl group either at ring D or E as shown below. Figure (I) represents the partial structure of ester B.



Further work on the constitution of compound B is in progress.

B.2.4. *Chemical examination of the fruits of Coccinia indica*

The fruits of *C. indica* (bitter variety) has been chemically examined by Bhakuni *et al.* (J. Sci. Ind. Res., **21B,** 237, 1962) who reported the isolation of β-amyrin, β-amyrin acetate and lupeol. The fruits of the same plant have been re-examined in this laboratory. The residue obtained from the petroleum ether extract of the fruits shows six distinct spots for sterols and triterpenes in the thin-layer chromatogram (silical gel G) and these spots correspond to those of β-amyrin, β-amyrin acetate, lupeol, taraxerol, taraxerol acetate and β-sitosterol. The residue obtained from the petroleum ether extract has been saponified. The non-saponifiable matter on thin-layer chromatography shows four distinct spots corresponding to those of β-amyrin, lupeol, taraxerol and β-sitosterol. From the above non-saponifiable fractions, taraxerol and β-sitosterol have been isolated in pure state and identified by direct comparison of mixed metling point with authenitic samples. As the other three compounds have already been reported, no attempt has been made in this laboratory for their isolation in the pure state.

B.2.5. *Chemical investigation of some antibiotic from Streptomyces sp.*

Chemical investigation of an antibiotic of a mutant strain MT-10 derived from Streptomyces sp (AC_{21} 460) (vide Ann. Report, 1963-64, p. 71) has been undertaken in collaboration with the Microbiology Department of this Institute.

The crude antibiotic has been found to be a mixture of three compounds by paper chromatographic studies. Method has been developed for the separation of components from the above mixture. The major component has been isolated in a pure crystalline state, m.p. 244–46° (decomp.). UV and IR spectra, combustion analysis and other chemical evidences clearly established that the antibiotic is a quinonoid type compound containing nitrogen.

The structure of the above antibiotic is being studied by chemical as well as physical methods like n.m.r. and mass spectrometry.

B.2.6. *Preparation of some potential chemical mutagenic agents*

One potential chemical mutagen has been prepared. Some progress has been made in the synthesis of mutagenic agents with a quinonoid system.

B.2.7. *Studies on the constituents of Murraya koenigii* Spreng (Curry leaf tree)

Phytocarbazoles have been reported for the first time from our laboratory from a Rutaceous plant *Murraya koenigii*. Further studies on different constituents of *M. koenigii* have been carried out. The structural studies on murrayacine are reported here. Further structural studies of previously reported constituents are also being carried out (this report 1963-64, p. 29).

(i) *Structure of murrayacine*

From the petroleum ether extract a new compound has been isolated in a very poor yield. The compound has been named murrayacine. Murrayacine is an optically in active,

paper chromatographically homogeneous, neutral nitrogenous compound. The compound analyses for $C_{18}H_{15}NO_2$, m.p. 243°. It has no methoxy group. The compound furnished a picrate. The UV absorption spectrum of the compound showed λmax at 225, 280 and 301mμ. The IR spectrum of the compound showed peaks at 3250 (NH), 1675 (–CO–) 1640, 1600 (aromatic), 895; 855 and 740 cm^{-1} (substituted benzene derivative).

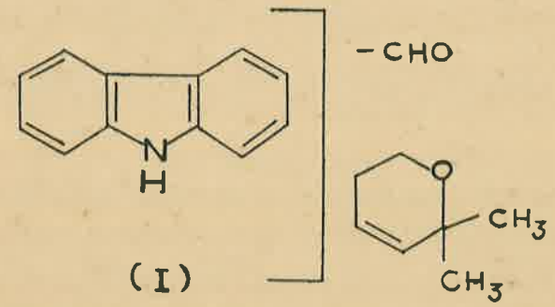
The compound reduced ammonical silver nitrate showing the presence of an aldehyde function. The aldehydic nature of the carbonyl function was also supported by NMR data.

The mass spectral measurements have confirmed the molecular formula when a molecular ion peak at m/e 277 was observed. Other peaks were at m/e 262 (intense), 234 and 210. The high intensity peak at M-15 was suggestive of the presence of a benzo-pyrilium ion as observed in girinimbin, mahanimbine and *iso*-mahanimbine. Further loss of mass 28 could be explained by the loss of –CO– group probably from the aldehyde function. The presence of the pyran ring (I) in murrayacine has been supported by NMR data.

The aldehyde function and the pyran rings accounts for the two oxygen functions of murrayacine. On borohydride reduction a compound m.p. 200°, was obtained. The compound showed UV absorption spectrum very similar to those of pyranocarbazoles like girinimbine, mahanimbine and *iso*-mahanimbine. This further supports the presence of the pyrano-carbazole skeleton in murrayacine.

Murrayacine could be hydrogenated to its dihydroderivative, m.p. 176°.

The data so far obtained suggests that murrayacine has a pyranocarbazole unit with an aldehyde function on it which may be partially represented as follows.



(I)

B.2.8. *Chemotaxonomic work in relation to the Family Rutaceae*

(a) In connection with the chemotaxonomic work in relation to the family Rutaceae and the probable biogenesis of phytocarbazoles which have been considered as new variants derivable from anthranilic acid, (Chakraborty, Proc. Sym. of Plant Sciences, 1964) the root bark of *Glycosmis arborea* was examined. The root bark has previously shown to elaborate some alkaloids which are biogenetically derived from anthranilic acid (Chakraborty and Barman, Trans. Bose. Res. Inst., **24,** 121, 1961). Two new crystalline neutral nitrogeneous

compounds have been isolated. They have been named glycozoline and glycozolidine respectively.

(i) *Glycozoline*—Glycozoline is optically inactive, homogeneous compound having m.p. 181–182°. From analysis and molecular weight determination by the Rast method, the formula $C_{14}H_{14}NO$, has been assigned to the compound. The molecular formula has been confirmed by mass spectral measurements when a molecular ion peak at m/e 211 was obtained. The compound has one methoxyl group and one C-methyl.

NMR data of glycozoline showed, one NH proton (τ 2.2) 2 aromatic protons (centred round τ 2.5), 4 aromatic protons (Multiplets from τ 2.82 to 3.2 444 to 415). It also showed a singlet for 3-aromatic methoxyl protons (τ 6.1) and another singlet for 3 aromatic C-Me protons (τ 7.5). The NMR data therefore confirm the presence of one methoxyl and one C-methyl in glycozoline as indicated by analytical data.

The UV absorption spectrum showed peaks at 227, 252, 264, 304, 345mμ with log ε 4.97, 4.63, 4.71, 3.96 respectively. The spectrum is very similar to that of 3-methoxy carbazole.

The IR data showed peaks at 3500 (NH), 1600, 1595 (aromatic system), 1208 (OMe), 1380 (C-Me) and 813 cm^{-1} (substituted benzene derivative).

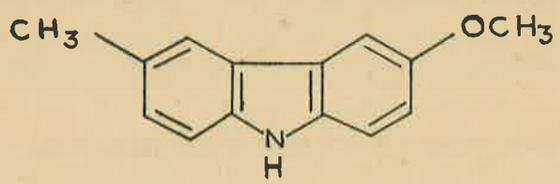
Glycozoline gives a picrate, $C_{20}H_{16}N_4O_8$, m.p. 182°. It could not be hydrogenated neither did it give any N-Me derivative. It did not react with any carbonyl reagent further confirming the absence of any carbonyl function.

Glycozoline on demethylation furnished a phenol $C_{13}H_{11}ON$, m.p. 228–230°. The phenol gave a red colouration with alcoholic ferric chloride. The IR spectrum of the phenol showed the presence of a hydroxyl peak (3400 cm^{-1}) besides the peaks for –NH–, aromatic system and substituted benzene derivative. This phenol on acetylation furnished the monoacetate, m.p. 210°. The UV spectrum of the acetate was very similar to that of 3-methyl carbazole. It proves that the parent hydrocarbon from which the phenol is derived has 3-Methyl carbazole chromophore. Glycozoline on zinc dust distillation furnished a compound, m.p. 203–4°. The degraded product and its picrate melted at 203–04° and 180° respectively when mixed with pure specimens of 3-methyl carbazole and its picrate. The UV spectra of the degradation product and 3-methyl carbazole were superimposable. (λ max 297, 261, 263, 234). Thus the identity of the degraded product with 3-methyl carbazole is established.

The isolation of 3-methyl carbazole by degradation and the UV spectrum of demethylated glycozoline acetate show that glycozoline has a 3-Methyl carbazole skeleton.

Glycozoline on liquid ammonia reduction furnished a compound m.p. 164°. The UV spectrum of the compound showed λmax at 224 and 294mμ showing it to be dihydro-glycozoline. The IR spectrum of the dihydroproduct showed additional band at 1675 cm^{-1} showing the formation of enolether. It shows that the ring containing methoxyl group has been reduced. The NMR data also support that one of the benzene rings have been reduced leaving an intact benzene ring with a C-Me on it.

The data so far obtained could be therefore, expressed by the following (II) probable structure of glycozoline. The isolation of a simple carbazole derivative from the root-bark of *G. arborea* further strengthens the idea that phytocarbazoles are new variants derivable from anthranilic acid. Methoxyl at position 3 is biologically probable as it



(II)

has been shown that the biological hydroxylation results in the production of 3-hydroxy carbazole. This point is being further examined.

(ii) *Glycozolidine*

Glycozolidine is an optically inactive, paper chromatographically homogeneous, neutral nitrogenous constituent, m.p. 160°. It analyses for $C_{15}H_{15}NO_2$. The UV absorption spectrum of the compound shows absorption max at 237, 258–62 and 305 mμ with extinction values log ε 4.47, 4.08 and 4.206 respectively.

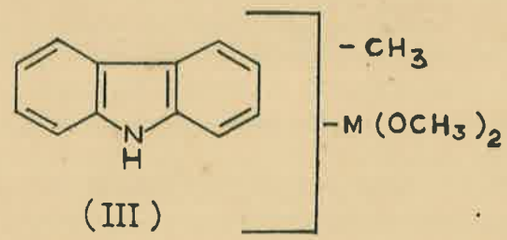
IR data showed peaks at 3450 (NH), 1625, 1575, 1500 (aromatic system), 1205 (OMe) and 812 cm^{-1} (substituted benzene derivative). The NMR data showed the presence of one NH proton (τ 2.27), 5 aromatic protons (τ 2.55 to 2.88, τ 2.98 to 3.032 and τ 3.48). One proton was highly shielded as it showed peak at τ 3.48. This is suggestive that this hydrogen is flanked by two methoxyl group which shields the aromatic proton. The NMR spectrum also showed the presence of aromatic methoxyl groups (τ, 6.10, 6.23) and one aromatic C-Me group. (τ, 7.65). Thus fifteen protons of glycozolidine could readily be accounted for by NMR data. The molecular formula of glycozolidine was confirmed by mass spectral measurements when a molecular ion peak at m/e 241 was obtained.

The NMR and analytical data whereby the presence of two methoxyl groups have been shown, account for all the oxygen functions. The compound is inert to hydrogenation in presence of palladised charcoal in alcohol. It also, did not react with any carbonylic reagents further proving the absence of any carbonyl function. Glycozolidine gave a picrate $C_{21}H_{18}N_4O_9$, m.p. 182°. It gave an N-Me derivative, $C_{16}H_{17}NO_2$, m.p. 140°. On demethylation with HBr gylycozolidine furnished a phenol, m.p. 240–43°. It gave greenish ferric reaction. The phenol could be acetylated in cold in presence of pyridine and acetic anhydride to give an acetate which still retained a methoxy group. It melted at 195–200° and showed an UV absorption spectrum with λmax at 298, 258, 243mμ.

Zinc dust distillation of glycozolidine furnished carbazole, identified by mixed melting point determination and comparison of its IR. spectrum with pure carbazole. The

picrate of carbazole was found to be identical with the picrate obtained from the degradation product. This information together with UV, IR and NMR data of glycozolidine might be taken as a convincing evidence for the presence of a carbazole skeleton of glycozolidine.

NMR data were suggestive that the methoxyl groups are in meta position. From the available informations glycozolidine could be partially formulated as (III).

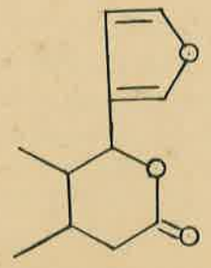


(III)

(b) The taxonomic closeness of the family *Meliaceae* with *Rutaceae* has been reflected in some of the degraded euphol derivatives characterised by a β-substituted furan ring. We were interested in this connection to examine the seeds of *S. mahogani*. Several crystalline constituents have been isolated which are probably β-substituted furan derivatives. Structural studies of one of them named mahoganin are reported here.

Mahoganin a non-bitter constituent of *S. mahogani* $C_{27}H_{34}O_8$, m.p. 225–28° was reported to contain one furan group, one carbomethoxy and one ketonic group and hydroxyl function, besides four C-Me groups.

Mahoganin gives an oxime which further supports the presence of a ketonic function. On acetylation mahoganin furnished a crystalline product $C_{29}H_{36}O_9$ m.p. 200° which still showed hydroxyl band in the IR. On hydrogenation mahoganin furnished anoctahydro acid. This on methylation with diazomethane furnished the ester, m.p. 168–70°. The acid did not show the furan band in IR but the ester had an additional carbomethoxy group.



(IV)

The formation of the octahydroacid readily indicates the presence of the grouping (IV) in mahoganin.

All eight oxygen functions of mahoganin are accounted for.

Mahoganin on mild alkaline hydrolysis furnished an acid $C_{26}H_{32}O_8$, m.p. 246–47°. This compound on methylation furnished the ester m.p. 241–43°.

Mahoganin on reduction with $LiAlH_4$ furnished a crystalline product. Further studies on the compound are being made.

(c) Besides the members within the order Rutales members of plant families related to *Rutaceae* are being examined for their alkaloidal constituents. In this connection the members of the order of Indian *Celastrales* and *Teliales* have been examined. Alkaloids have been detected in both the orders. The order *Teliales* was so far not reported to yield any alkaloid. The UV spectrum, paper chromatographic behaviour and colour reactions of the alkaloid isolated from *Celastrus paniculata* are suggestive that it may be indolaceous. This finding has some taxonomic implication so far the occurrence of variants of anthranilic acids in the families related to *Rutaceae* is concerned. Further studies are in progress.

(d) Alkaloids of *Nigella sativa: Nigella sativa*, a common Indian spice, belongs to the family *Ranunculaceae*. In connection with our chemotaxonomic studies we were interested to examine the alkaloids of the seeds of *Nigella sativa*. One petroleum ether soluble and another benzene soluble alkaloids have been isolated from the plant.

B.2.9. *Synthesis of carbazole derivatives*

Pyrano-carbazoles oxygenated at the position 2 of carbazole nucleus are being synthesised.

B.2.10. *Biochemical properties of natural products*

(a) *Antifungal action of natural products.* Constituents of the root-bark of *G. arborea* have been examined for their antifungal action against some human pathogenic fungi. Some of the compounds are fairly active against *M. adouni* and *T. rubrum*.

(b) *Gonadal activity of coumarin derivatives.*—Several coumarins have been examined for their gonadal activity. None have been found more active than psoralene.

B.2.11. *Physical properties of natural products*

(a) Thin layer chromatographic studies with carbazoles and coumarins have been made in collaboration with the analytical section of the Department.

(b) In connection with the theoretical aspect of the biological action of carbazole derivatives, UV absorption spectra of 15 different substituted carbazoles have been studied. 1,–2,3–,4–methoxy carbazoles have diagnostic spectra which have been very helpful in our structural studies.

B.3. Radiochemistry

B.3.1. *Enzymatic synthesis of polyribonucleotides*

In continuation of the work reported last year, the characteristics of the enzymatic reaction leading to the synthesis of poly guanylate have been studied further with special

reference to the primer requirement of the reaction. It was reported previously that incorporation of GMP requires the presence of a preformed RNA primer. It has now been substantiated that the primer is somewhat specific, all polynucleotides other than the RNA extracted from the enzyme preparation itself being inhibitory. The nearest neighbour frequency analyses indicate, that the primer is presumably a ribopolynucleotide with a UMP residue at the acceptor end. So it might be expected that poly U would enhance GMP incorporation. But it has not been found to be the case. It seems probable that the nucleotide composition and arrangement of the primer may be additional factors in determining specificity. Participation of DNA in this reaction has been ruled out by the facts that DNA (native as well as denatured) is highly inhibitory to the reaction when added to non-preincubated or preincubated enzyme, the incorporation is not DNase sensitive and that actinomycin D does not inhibit GMP incorporation. The synthesis of poly G was previously shown to be inhibited by ATP and ITP. Further studies have indicated that the inhibition is of competitive in nature, the Ki for both, ATP and ITP being 4.3×10^{-5} M whereas Ks for GTP is 0.9×10^{-4} M.

The effect of this poly G product was earlier shown to promote the incorporation of glycine by ribosomes-soluble enzyme systems derived from both chloroplasts and goat liver nuclei. This observation has been extended by investigating the effect of this polynucleotide on the incorporation of a number of other amino acids, especially those with at least one G in the corresponding codon. These studies have revealed that of these several amino acids including proline, valine, tryptophan, phenylalanine, leucine, lysine, alanine, arginine, methionine, aspartic acid, glutamic acid and serine, the incorporation of only tryptophan is stimulated, although to a lesser degree.

Several procedures were tried to purify the ATP-incorporating activity, but only 3–4 fold purification has been achieved in some cases. The product of ATP incorporation has been isolated and characterized. This has been found to be highly susceptible to T_2-RNase and to some extent to pancreatic RNase but resistant to T_1RNase and also to DNase. Inhibition caused by the pretreatment of the enzyme extract can also be restored fully by the addition of poly A or more effectively by oligonucleotides isolated from poly A. Finally the reaction product can be trapped in poly T-cellulose column. All these evidences taken together suggest that the reaction product is of poly A in nature.

Next UTP and CTP incorporation has further been studied with the chloroplast extract. UTP and CTP incorporating activity has been found to be associated with the stroma fraction. UTP and CTP incorporation has also been decreased appreciably in the presence of other three nucleoside triphosphates. The actual substrate has been found to be the triphosphates and not mono or diphosphates. The optimal pH for UTP incorporation is near about 8.0 and for CTP near about 9.0. Lower or higher pH is very effective for both incorporating activities. Optimal incorporation has been observed within 20 min. and the incorporations are linear upto 3.5 mg. of protein in case of UTP and 4.0 mg. of protein in case of CTP incorporation. For maximum incorporation 5μ mole of magnesium ions were most effective in both cases. The Ks value for UTP and CTP has been found to be more or less same and approximates to 1.1×10^{-3} M. UTP incorporation has been

found to be increased with the addition of poly U after pretreatment of the enzyme for 20 min. The incorporation increases gradually with increasing concentration of poly U and 80% activity can be restored at a concentration of 100 μg/ml. Higher concentration of poly U has a retarding effect on the incorporation and similar in the case with CTP system when poly C is added. But RNA extracted from the enzyme also has the ability to restore activity fully in both the cases. Other polynucleotides appear to have no promoting effect. It seems that at least three types of additions occur, (1) terminal additions, (2) internal additions at the end of the pre-existing chain, and (3) additions to synthesize free poly U and poly C. Demonstration of the presence of poly U or poly C in vivo has not yet been met with success, except the indication that the reaction product of UTP, incorporating system when passed through poly A cellulose column at least 30% of it can be trapped. The indirect evidence regarding the presence of poly U has been adduced in the case of protein synthesis by chloroplast (B.3.4.).

B.3.2. *Studies on histones*

In the last year's report (1963-64) some results on the biosynthesis of histones by goat liver nuclei as well as isolated ribosomal soluble enzymes system were presented. Further studies on this aspect of histones have substantiated our earlier contention that the nuclei are fortified with the pre-requisites for histone biosynthesis. This conclusion is supported by the following evidences:

(*i*) Incorporation of amino acids by nuclei into different histone fractions is susceptible to chloromphenicol, a potent inhibitor of protein synthesis.

(*ii*) Experiments with isolated nuclear ribosomes-soluble enzymes system clearly demonstrate that incorporation of amino acids into basic proteins exhibits the same requirements as are known for the synthesis of the bulk of cellular proteins.

(*iii*) Specific activity of the isolated radioactive basic proteins has been found to increase with purification indicating thereby true incorporation rather than physical adsorption. Properties of nuclear histones have also been studied. It has been shown that the histones interact with polymerised DNA in solution and precipitate it. The different histone fractions somewhat differ in their ability to precipitate DNA under identical conditions. All these histone fractions have also been shown to exhibit RNase activity by degrading yeast RNA. Optical conditions for this degradation by different histone fractions are not identical, but the RNase activity in most cases is enhanced by urea, EDTA and NaCl and is reduced by $MgCl_2$. The histone fractions inhibit *in vitro* synthesis of RNA by a partially purified RNA polymerase from *Azotobacter vinelandii* at a histone/DNA ratio of 1.

Studies concerning the biosynthesis of histones have now been extended to germinating Mung bean also. Preliminary results indicate that when these seedlings are incubated with C^{14}-amino acids mixture followed by extraction of basic proteins with acid (0.3N HCl) and fractionation by carboxymethyl cellulose columns, all the histone peaks are radioactive, though the specific activity of different histone fractions are not similar to what have

been reported for the corresponding fractions of goat liver nuclei. Investigations to elucidate the probable significance of differential synthesis of different types of histones with the progress of germination and seedling growth are now being carried out.

B.3.3. *Studies on ribonucleoprotein particles*

The RNP particles of goat liver nuclei have been studied with regard to their composition and stability. They were found to undergo spontaneous degradation under certain conditions presumably by RNase associated with them. This autodegradation is enhanced by the absence of Mg^{++} and presence of substances like EDTA and urea which cause the RNA and protein moieties to separate. A portion of the RNA of RNP is degraded by pancreatic RNase at 0°C even in absence of urea and EDTA. The degradation becomes more extensive when either of these substances is present. These results indicate that the RNA moiety of RNP is not fully protected by its association with the protein part. Nevertheless, the rate of degradation of RNP is 1/3rd to 1/4th that of ribosomal RNA alone in presence of pancreatic RNase.

Active incorporation of amino acids into protein by these particles has also been demonstrated. C^{14}-amino acids have been found to be transferred from enzymatically synthesized C^{14}-aminoacyl-s-RNA complex to proteins by nuclear RNP soluble enzymes system and this transfer has been shown to be promoted by a goat liver nuclear RNA fraction having a high turnover rate and a composition similar to that of homologous DNA.

B.3.4. *Synthesis of protein in chloroplasts*

It was reported in the last year that chloroplast can synthesize proteins. It was observed that phenylalanine incorporation occurred to a great extent in comparison to other amino acids tested. The reaction product after incubating chloroplast RNP particles with C^{14}-phenylalanine was isolated and found that at least 60% of the count was associated with polyphenylalanine like component. It was demonstrated that in presence of poly A- the synthesis of polyphenylalanine was reduced and in conjunction with the synthesis of polyphenylalanine like component other type of protein containing phenylalanine might also be synthesized in the chloroplast.

B.3.5. *Effect of UV on protein and RNA synthesis by chloroplasts*

Photoreactivating system has been demonstrated with the chloroplasts. Under the influence of UV the synthesis of RNA is decreased and RNA species enriched in purine content have been found to be accumulated. The incorporation of a number of amino acids by the chloroplast RNP soluble enzyme systems has been found to be affected differentially by UV irradiation. Among the amino acids tried phenylalanine incorporation is highly sensitive.

To see the effect of UV on poly U with the RNP and soluble enzyme system the solution of poly U has been irradiated with a total dose of 36×10^4 ergs/mm^2. A marked depression in the incorporation of phenylalanine has been observed. Similar is the case with poly C and proline. But poly A directed lysine incorporation seems to be more resistant.

Poly U and poly C after irradiation with UV when tested for their messenger activity serine in the case of former and serine and leucine incorporation in the latter case are increased with a decrease in phenylalanine and proline incorporation respectively indicating probably a change in the coding ability of the messenger.

B.3.6. *Studies on action of plant growth substances*

In the previous report evidence has been presented to show that the nucleus of a cell is the initial target of growth substance action and that, such an action is exerted through regulation of DNA-dependent RNA synthesis. Short term experiments with C^{14}-amino acid reveal that nuclear RNA synthesis is followed by a parallel stimulation of nuclear protein synthesis. It thus appears that the transmission of hormonal stimulus follows the usual pattern of message transfer from DNA-RNA-protein.

The exact mechanism by which such a process is governed, is not as yet known. However, it has been observed that these growth substances interact with genetic material as revealed by studies on binding of C^{14} or tritium labelled growth substances with DNA. In these experiments the DNA preparations obtained from onion roots were incubated with NAA-1-C^{14}, IAA-H^3 and Kinetin-H^3. The DNA preparations were then thoroughly washed with a solvent in which all these substances were highly soluble but not the DNA. When the washings were completely free of all radioactivity and the DNA plated and counted, it was found to imbibe considerable radioactivity. Depending on the concentration of labelled growth substances used, about 15-20$m\mu$ moles of growth substances were found to bind per mg of DNA. The binding was enhanced if a cell extract was added.

Spectrophotometric studies also confirm these observations. When individual spectra (220–340 $m\mu$) of different growth substances, namely IAA, kinetin, gibberellic acid and calf thymus DNA were compared with DNA+growth substances spectra, a shift in the absorption maximum and minimum, was observed. The effect was most marked with IAA and less with kinetin. With gibberellic acid no difference could be obtained due to interference by gibberellic acid with DNA spectrum. It has further been revealed that under the influence of IAA no change occurs in the capacity of aminoacyl s-RNA synthesis but a consistent change with RNP particles so far as amino acids incorporation is concerned is discernible. When analyzed for the RNA content of the ribosomal pellet it has been observed that there is a 12–13% increase in the case of treated ones over the control. If 3–4% of the total RNA is messenger RNA and 40% is the ribosomal RNA, then in terms of rRNA, mRNA will be approximately 9–10% which may partly be correlated with the increase of RNA from treated ribosomes provided all the mRNA synthesized in the IAA treated tissue is in association with the ribosomes. In terms of the chemical mechanism of protein synthesis a question that might be posed is that of which ribosomal constituents are responsible for variation in the activity of ribosomes? It has also been observed that the nucleotide composition of the ribosomal RNA does not change significantly throughout the incubation phase but a consistant variation in the RNA protein ratio of the ribosomal pellet can be found. The change in the rate of synthesis of RNA suggests that the messenger RNA content of the ribosome may differ. Such a condition could also lead to a variation of the polysome content of the cell and these questions are now being investigated.

B.3.7. *Investigations on the protein make up of germinating seeds and growing seedlings*

Pea seeds (*Pisum sativum* Linn.) were germinated at 18–20°C and from the germinating seeds and growing seedlings proteins have been extracted and fractionated by differential extractions procedures, in order to study their protein make up at different stages of growth. Non basic proteins were extracted by homogenizing the fresh tissue with 0.1M Tris buffer at pH 7.3 followed by centrifugation. The residue was extracted with 0.3N HCl to obtain the basic protein. The residue after this extraction could be further fractionated into alkali soluble and alkali insoluble protein components. The results so far obtained seem to indicate that there is very little change in each fraction (when expressed in mg protein/g. in fresh wt.) during the varying period of germination. The ratio of DNA and basic protein throughout the germination stages has been found to be almost same. Attempts are now being directed towards characterising these various protein components including the histones so as to elucidate the role of histones on growth and development of higher plants.

B.3.8. *Phytin and synthesis of nucleic acids*

In continuation of the work on the relationship of phytin and nucleic acid synthesis an enzyme system has been detected from germinating mung beans which can transfer phosphate from phytin to nucleoside diphosphate to synthesize corresponding triphosphate. When further fractionated GTP synthesis has been found to increase indicating probably the presence of different enzymes for four nucleoside diphosphates. Without Mg^{++} this phosphate transfer cannot occur. When RNA-polymerase from the same source has been coupled with this novel phytin phospho-transferases along with C^{14}-labeled nucleoside diphosphates RNA synthesis may continue. It seems that in the germinating seedlings these transferases play an important part in regenerating the substrates for RNA synthesis. GTP generating system is of special interest so far as protein synthesis in the seedlings are concerned. Further purification and characterization of these systems are now in progress.

B.3.9. *Mechanism of movement of desmodium leaves*

Probable phosphorylation of protein, reported previously has been studied more thoroughly. From P^{32}-fed *Desmodium* leaves labelled protein along with nucleic acids have been isolated and after treatment with RNase and DNase about 10–15% of the radioactivity has been retained with the ninhydrin reacting and trypsin susceptible product indicating probably that it is of protein in nature. When this fraction has been treated with acid 80% of the radioactivity has been found to be labile and 20% acid stable. There is also an indication that an enzyme system can transfer phosphate from this phosphorylated protein to ADP to synthesize ATP. This is now being characterized. High ATPase and phosphatase activity have been detected previously in the *Desmodium* leaves. This has further been fractionated by ammonium sulfate into four fractions (0–30%, 30–50%, 50–70% and 70–100% saturation). In all the fractions ATPase activity has been detected but 30–50% fraction exhibiting the maximum activity. pH optimum has been found to be at neutral side. Further characterization and purification of ATPase and phosphatase are now in progress.

B.3.10. *Mechanism of action of plant growth substances*

In the previous report evidence was presented to show that the plant growth substances IAA, Gibberellic acid and kinetin all stimulated the synthesis of nuclear RNA in growth promotive concentrations. The release of nuclear RNA into cytoplasm was re-regulated largely by the concentration of the growth substance used. It has been observed since then that the stimulation of nuclear RNA synthesis is accompanied by a promotion of the incorporation of several amino acids into nuclear protein. Higher concentrations which inhibit growth, inhibit amino acid incorporation. The growth substance induced stimulation of nuclear protein synthesis can be curtailed or completely abolished by DNase, RNase, actinomycin D, porfiromycin, puromycin and chloramphenicol. The inhibition due to actinomycin D alone can be removed by higher concentrations of growth substances, even when such concentrations by themselves do not promote growth. All these inhibitors reduce a partially or completely the auxin component of growth stimulnation. The antibiotics chromomycin A_3 and mitomycin C_2 which also inhibit nucleic acid synthesis inhibit growth.

Auxin stimulation of nuclear protein synthesis can be located within very short periods in nuclear ribosomes and a fraction sedimentable between 1,000–41,000 x g, presumably containing the nuclear fragments. Time course experiments indicate that stimulation in the latter fraction precedes the enhancement of amino acid incorporation into nuclear ribosomes.

The above observations are in agreement with the conclusion that plant growth substance action is concerned primarily with a regulation of the synthesis of nuclear RNA which in turn leads to a stimulation of nuclear protein synthesis. The enhancement in the incorporation of P^{32} into the RNA of ribosomes together with an acceleration of ribosomal protein synthesis may be interpreted to imply an auxin stimulation of the synthesis of nuclear ribosomes. There is evidence to indicate that the ribosomes are assembled within the nucleolus. IAA also affects the synthesis of nuclear histones. C^{14}-labelled Naphthelene acetic acid and tritium-lavelled kinetin bind with DNA. IAA does not bind with actinomycin D but can remove small amounts of actinomycin D bound to DNA as evident from the difference spectra. It is possible that the interaction between DNA and growth substances involves certain regions of the DNA molecule which are a small fraction of the total sites, to which actinomycin D can attach.

B.4. Biochemistry

B.4.1. *Host virus relationship*

In continuation of the studies reported last year a phage causing the complete lysis of *Salmonella typhimurium* has been isolated from the lysogenising strain PLT22. Physicochemical and biochemical studies with the temperate as well as the virulent viruses have been initiated. L-Arabinose isomerase, an enzyme inducible in the host *S. typhimurium* when grown on L-arabinose, becomes 100% inhibited after infection with lytic phage for 20 minutes. The total period required for lysis is about 55 minutes. Similar situation was found in case of *Escherichia coli* when infected with the virulent phage T2h. It is suspected that the viruses produce inhibitors which inhibit the function of the enzymes already produced by the host cell. The phage strains have been characterised regarding their burst sizes, latent periods etc. DNA's have been prepared from the phages isolated by ultracentrifugation and their physical and chemical properties have been studied in detail. Their behaviour on the methylated serum albumin column has also been studied. Attention is being focussed on the differences between the DNA's of the closely related temperate and virulent bacteriophages.

B.4.2. *Induction and repression of enzymes in Lactobacillus plantarum*

As reported previously, indication was obtained from the effect of actinomycin D on the induction of the enzyme L-arabinose isomerase, regarding the half life of the messenger RNA of L-arabinose isomerase. Different methods are being employed to determine the half life of the messenger RNA. The differential rate of enzyme production was studied after the inhibition of the induction by two types of inhibitors, one type which inhibits protein synthesis *e.g.*, chloramphenicol, toluene, puromycin and the others which inhibit messenger RNA production but allow the already synthesised m-RNA to continue its function. These results also indicate that unlike the messenger RNA of β-galactosidase of *Escherichia coli* the messenger RNA of L-arabinose isomerase has longer half life. The half life is estimated to be as 15 minutes.

B.4.3. *Metabolism of lipoic acid*

Last year a new biosynthetic method of preparation of S^{35}-labelled lipoic acid from *Azotobacter vinelandii* was reported. S^{35}-lipoic acid has been extensively used to study the mechanism of activation of lipoic acid and its transfer from the free pool of the protein-bound state. The reversibility of the lipoic acid activation reaction was previously demonstrated. That the adenyl lipoate is the true intermediate in the activation reaction has now been demonstrated with S^{35}-lipoate and cold ATP in one experiment and C^{14}-ATP and cold lipoate in another. In both the cases radioactive adenyl lipoate has been isolated and identified as an intermediate. The subsequent transfer of the activated lipoate from the free pool to the protein-bound state has also been demonstrated with S^{35}-lipoic acid. Thus the mechanism of activation and transfer of lipoic acid to protein seems to be similar to that of biotin.

B.4.4. *DNA-dependent incorporation of guanylate residues into polynucleotide chain*

In continuation of the studies previously reported it has now been established that only one guanylate residue is attached at the end of the each DNA chain. The phosphodiester linkages involving deoxyribose and ribose residues are insensitive to the action of alkali. The properties of the enzyme responsible for the synthesis of ribonucleotidyl-DNA have been studied and the physiological function of this enzyme is being sought for.

B.4.5. Amino sugars have been found to be widely distributed in the living systems, *e.g.*, in the connective tissues of animals and in the cell-wall of capsular materials of microorganisms. During studies on amino sugar metabolism of bacteria and of yeasts, it has

been found that the utilization of amino sugars as carbohydrate source and their subsequent enzymatic degradation or their transformation to higher energy level for the synthesis of bio-polymers containing amino sugar are completely under complex metabolic control systems. For example, glucose grown *Candida albicans* cells does not metabolize acetyl-glucosamine from the medium as long as glucose is present, although transport of acetyl-glucosamine may remain partially operative. Metabolic control phenomena as far as amino sugars are concerned are now being throughly studied.

B.5. Analytical Chemistry & Instrumentation

B.5.1. *Thin layer chromatography*

(i) *Development of the technique.*—Thin layer chromatography (T.L.C.), a very recent analytical technique has been developed in the analytical chemistry laboratory. Spreaders for making both 10×20 cm. and 20×20 cm. layers of different thickness alongwith a plastic aligning table have been constructed. As commercial solid adsorbents required for T.L.C. is not available in our country, methods have been devised in the laboratory to produce suitable adsorbents from available materials. In order to check the activities of these prepared adsorbents, thin layer chromatograms of certain dye stuffs were made with these adsorbents as also with imported adsorbents. The activities of the prepared adsorbents were found to be comparable with those of the imported adsorbents.

(ii) *Thin layer chromatography of coumarins.*—Twenty different coumarins obtained from plant sources have been used in this study. They were psoralene, *iso* psoralene, xanthotoxin, marmin, murayin, herniarin, luvangetin, byakangelicin, ayapin, tri-o-methyl wedelolactone, dalbergin, *nor*—dalbergin, coumarin, bergaptan xanthyletin, wedelolactone, aesculetin dimethyl ether, umbelliferone and mesuol. About a microlitre of 1% solutions of the coumarins in alcohol were applied. 1 cm apart on the 10×20 cm thin layer plates along a straight line 1.5 cm. from one of the 10 cm. edges. The plates were then placed in suitable developing chambers filled to the depth of 0.5 cm with the required developing solvents. The run was continued until the solvent front reached a height of 15 cm from the starting line. The plates were then removed from the chamber and dried at room temperature. The spots were detected by observing the fluorescence of the compound under ultraviolet light. The R_f values were noted. Silica gel and aluminium oxide (basic) were used as adsorbents. A number of solvent systems have been used with both the solid adsorbents. The solvent systems were (i) water, (ii) water and ethanol, (iii) water and ethylene glycol, (iv) water and pyridine, (v) water and acetic acid, (vi) water and dioxane, (vii) water ethanol and ethyl-methyl-ketone, (viii) water, formamide and ethyl-methyl-ketone, (ix) water, formamide and ethyl acetate, (x) water and ethyl-methyl-ketone, (xi) methanol, acetic acid and water, (xii) chloroform, (xiii) benzene, (xiv) hexane, (xv) benzene and acetone, (xvi) benzene and alcohol, and (xvii) hexane and ethylacetate. The best separation was obtained using benzene and acetone as the solvent system on silica gel plate. This work is done in collaboration with the Plant Chemistry Unit.

(iii) *Studies of some triterpenes by thin layer chromatography.*—The possibilities of separation of some triterpenes by T.L.C. has been studied. The samples of triterpenes obtained from the plant chemistry unit were barringtogenol 'C', barringtogenol 'C'-acetate, barringtogenol, 'C'-benzoate, barringtogenol 'D' acetate, spergulagenin acetate, spergulagenin benzoate. The procedure are similar to that used in case of coumarins. 200μ thick aluminium oxide (basic) plates were prepared. The plates after preparation were allowed to dry at room temperature for 3 to 4 hours and then were activated by heating at 130° to 140°C for 1½ hour. The activated plates were cooled in a desiccator and samples applied. The run continued for 2 hours with each system of developing solvents. After the run the plates were air-dried and then heated to 130 to 140°C for 10 min. After cooling the plates were sprayed with a mixture of 10% acetic anhydride 10% conc. H_2SO_4 in absolute alcohol. After spraying the plates were again heated to 130°C for 20 mins. when brown to black spots of the triterpenes were obtained. The R_f values were measured in each case. The developing solvent systems used were: (1) petroleum ether (40–60°C), (2) benzene, (3) cyclohexane, (4) benzene. chloroform, (5) benzene, methyl alcohol, (6) carbontetrachloride, methyl alcohol, (7) petroleum ether (40–60°), alcohol, (8) benzene, alcohol, (9) cyclohexane, alcohol, (10) carbontetrachloride, alcohol, (11) benzene, alcohol, chloroform. Best separation was obtained using a mixture of benzene and chloroform.

B.5.2. *Gas chromatography*

(i) *Separation of methyl esters of fatty acids by gas-chromatography.* A number of chromatographic columns of different cross sections containing different grades of "Gas-chrom" solid support coated with 5% & 10% "Apiezon L" was prepared. Pure methyl esters were used for standardisation, satisfactory separation was obtained with 10% columns. Other columns containing poly-esters are also being tried. A mixture of methyl esters of lauric, palmitic, myristic, stearic, caporic and oleic acid was used.

(ii) *Investigation on the fatty acid content of commercial butter samples.* Three different samples of commercially available butter were used for this study. The samples were saponified by refluxing with alcoholic caustic potash for 6 to 7 hours. After the saponification was complete most of the alcohol was removed by distillation. The solid mass of potassium salts of the fatty acids left was washed with petroleum ether to remove unsaponifiable matters. To the washed mass petroleum ether was added and the fatty acids were liberated by adding hydrochloric acid solution (1:4). The fatty acids liberated remain dissolved in the petroleum ether layer and was removed. This solution of fatty acids was washed with water to remove mineral acid and then dried over anhydrous sodium sulphate. The petroluem ether was removed by distillation and the fatty acids recovered. A part of the fatty acid mixture was methylated to the corresponding methyl esters by diazomethane. The mixture was purified by fractionation and kept ready for gas chromatography.

B.5.3. *Studies on Maize Seed Proteins*

(i) *A comparative study of the amount of extractable proteins by different solvents under different conditions of extractions.* As the previous work on the soluble proteins of maize seed (vide Annual Report, 1963-64) has been extended to 8 different varieties, of maize it has become important to establish an optimum condition of extraction of the soluble protein from the seeds. It has been attempted to compare the amount of proteins extracted in terms of

the amount of nitrogen using different solvents and varying the duration and temperature of extraction. As regards solvents water and phosphate buffers of pH7 with increasing amount of added common salt have been used. For each solvent system chosen extractions were made at 25°C and 3°C for 3 hrs, 12 hrs and 18 hrs. The nitrogen content of each extract was measured by micro-kjeldahl method. The total nitrogen contents of the different varieties of the seeds were also measured. The result so far obtained indicated that water without any added salt was a very proor solvent for extraction of protein. Work with other solvent system is in progress.

(ii) *Paper electrophoretic study of the globulin fraction of Sugar Corn variety of Maize seed after dialysis.* Globulin fraction obtained from 'Sugar Corn' was dissolved in 0.1 M phosphate buffer of pH 7 and was dialysed against water with frequent change of water at 4°C for 72 hrs. A precipitate was found to form in the bag which was recovered by centrifugation. Solution of this precipitate in veronal buffer of pH 8.6, ionic strength 0.05 was subjected to paper electrophoresis for 6 hrs. at 180 volts in veronal buffer pH 8.6 ionic strength 0.05. This result was compared with the paper electrophoretic study of the globulin fraction done prior to dialysis. In the post dialysis case the slowest moving band was found to be missing. The supernatant obtained after centrifuging the precipitate from the dialysis bag was also subjected to paper electrophoresis and all the pre-dialysis bands were obtained. These results show that one of the fractions of the globulin obtained from the seeds of sugar corn can be partially separated from the other fractions by dialysis.

B.5.4. *Studies on paper electrophoresis of serum proteins*

(i) *Study on the comparative changes in the activities of glutamate-oxalacetate and glutamate pyruvate transaminases in blood and liver tissues in liver diseases.* In study of liver diseases (vide Annual Report 1963-64), it has been observed that the activities of transaminases (S.G.P.T. & S.G.O.T.) are increased. This increase indicates that the storage capacity of these enzymes in the liver is disturbed. This disturbance is brought about by the micro-structural changes of the liver tissue due to the pathological condition and leads to uncontrolled release of these enzymes in peripheral blood in different stages of the disease. On the basis of this assumption, the stimultaneous study of transminases activities in blood and liver tissues has been made. In 20 cases of different liver diseases, liver tissue was taken by liver biopsy and the tissue was macerated in 0.1 molar Na-phosphate buffer and the transminases activities were measured in usual way. The transaminases activities in disease liver tissues is much less than that present in normal liver tissues where this study has also been made in the ten normal cases. The transaminase activities were expressed in terms of transaminase units per mgm. of protein present in the extract. The extractable total protein in the tissues has also been measured.

(ii) *Study on paper electrophoresis of blood serum and some enzymes activities in cancer.* This study is being carried out in different types of cancer. In 50 cases of liver and gall bladder cancer, the paper electrophoresis of serum has been done along with the estimation of total protein, transaminases activities and phosphatase activities in blood. From the results obtained uptil now have fairly helped to locate the site of primary diseases and its spread

in other organs. The results in some cases have ruled out the neccessity of biopsy and have been also helpful to give a definite line of treatment and to study the progress of the case. In 20 cases of different types of blood cancer, these investigations have also been done in order to assess the spread of the disease. In cancer of the prostrate gland the observed increase in acid phosphatase activities in blood was typical. The study of transaminase activities and alkaline phosphatase activities may spot out the spread according to disturbance of specific enzyme-storage organs. This work is in progress.

B.4.5. *Studies on the Pigment content in the Bark of Jute Plant*

(i) *Paper chromatography of the anthocyanin pigments in jute plants.* In connection with a genetical studies of different varieties of jute by the Department of Botany it was of interest to study the different anthocyanin pigments present in a different varieties of jute plants. In this study the anthocyanidin content of the barks of four different varieties of jute namely T.M., D.T.M., R26 and D154 was analysed by the method of paper-chromatography. These plants were supplied by the Dept. of Botany. In order to extract the anthocyanins the barks were cut into small pieces and treated with methanol containing 1% HCl. The anthocyanins (glycosides) were hydrolysed to the respective anthocyanidins (aglycones) by mixing the filtered extracts with equal volumes of (N) HCl and heating the mixture for 30 min. on a water bath. The anthocyanidins formed were extracted with *isoamyl* alcohol. These extracts were directly chromatographed on Whatman No. 1 filter paper using water, acetic acid and HCl (10: 30: 3) as solvent for five hours. The developed chromatograms were air dried and then sprayed with a 1% alcohlic solution of $AlCl_3$. The spots were detected from their fluorescence under ultraviolet light. A number of spots exhibiting different colours under ultraviolet light were observed for each variety of jute. The R_f values are noted. The results are presented in the form of a table given below:

TABLE

Variety of jute	Colour of the spots under ultraviolet light	Rf values
T.M.	(i) Yellow (ii) red (iii) value (iv) brown	(i) 3.39 (ii) 0.47 (iii) 0.73 (iv) 0.98
D.T.M.	(i) green (ii) value (iii) brown	(i) 0.41 (ii) 0.80 (iii) 0.97
R 26	(i) value (ii) brown	(i) 0.83 (ii) 0.98
D 154	(i) blue (ii) brown	(i) 0.85 (ii) 0.97

The results revealed a marked difference in the pigment content of two varieties of jute namely T.M. and D.T.M. Attempts are being made to identify the spots by extracting anthocyanidins from flowers known to contain specific anthocyanin pigments.

C. BOTANY

C.1. Plant Biochemistry

C.1.1. *Studies on tissue cultures and single cell-cultures*

C.1.1.1. *Normal Tissue.*—In continuation of the experiments carried out to verify the report of Braun & Wood (1962) that the normal tissues could be grown profusely without an exogenous supply of auxin if the basic medium is fortified with increased levels of KCl, $NaNO_3$, NaH_2PO_4 and inositol it was found that *Coreposis lanceolata* and *Vicia faba* normal tissue could be grown only for 5 and 3 successive two-week transfers, respectively.

C.1.1.2. *Single cell culture.*—An attempt was made to isolate and obtain single cell culture of hollyhock (*Althaea rosea*) crown gall tumour tissue. For this study a special type of shaker with flasks, each with six projecting nipples around the circumference as culture vessels, was used. The procedure followed was to transfer 28-day old tissues grown in liquid medium to one litre culture vessels and allowed to grow for 30 days. One ml. suspension culture was then transferred under aseptic conditions to 250 ml. culture vessels and subsequent sub-cultures were made at 30 days interval. The suspension contained single cells and cell clumps with varying cell number. Considerable variation in cell shape and size was observed. The single cells were generally of three types: oval, elongated and round. The diameter of the single cells varied from 40μ to 150μ with some giant cells upto 300μ and above. Cell count in different flasks varied between 2000 to 9000 cells per ml. As compared to the suspension culture obtained with reciprocating and wrist action shaker, damage and injury to the cells was negligible. No sign of differentiation of single cells in suspension culture was observed during seven successive transfers. In case of cells from normal tissues differentiation and even formation of plantlets have been reported. Work is being undertaken with normal tissue. Attempts made to isolate and establish tissue clones from single cells on agar medium have not been successful.

With the method used it has been demonstrated that it is possible to cultivate freely floating crown gall tumour cells in continuous suspension culture. So far, successful cultivation of cells from normal tissues only have been reported.

C.1.1.3. *Source of Iron in culture medium.*—In studies on single cell culture iron was supplied in the form of iron-chelate, in place of iron sulphate to avoid precipitation in the medium with alkaline drift in pH with time. It has been suggested recently that iron chelate complex may have a cumulative 'poison' effect when supplied continuously. No diminution in growth rate of hollyhock crown gall tissue was observed after 14 successive bi-weekly transfers. The experiment is being continued. Along with the experiment utilisation of different iron sources by gall tissue is being studied.

C.2. Plant Physiology

C.2.1. *Electric variation at different parts of the leaflet of Desmodium and its correlation with the rhythmic movement.*

In previous report it has been mentioned that an electrical impulse is executed in other parts of the leaflet beyond the pulvinule during its downward movement, but it is of opposite sign shown by the pulvinule. This electrical impulse is, however, of very low E.M.F. and it has only been detectable by the introduction of a photographic recording arrangement with twenty times greater amplification than that obtained with original Pen Oscillograph. Extensive study has been made in this direction during the year by taking synchronised records of the movement of the leaflet and electrical impulse at different regions.

As the speed of the recording plate of the Phytograph is much less than the speed of the drum of the photographic recorder the record of the mechanical movement of the leaflet had to be later photographically enlarged to requisite extent, in order to make it coincide with the electrical record and have a synchronised effect. Electrical reactions of different regions were studied individually. Simultaneous recording of the electrical impulse of the pulvinule, for comparison, was discarded to avoid complications of artifact arising out of close vicinity of two electrode connections.

The pattern of the electrical impulse in the midrib and lamina is a sharp positive wave of 2 to 5 m.v. followed by quick return towards the base line. The quickness and over-shooting of the return wave indicates that it is not mere recovery to normal potential but positive followed by negative. Synchronised mechanical and electrical records show that these electrical changes occur in the midrib and lamina at the beginning of the downward movement of the leaflet. Forced stoppage of mechanical movement to the leaflet did not interfere with the occurrence of electrical impulse at those regions at regular intervals as when the leaf was freely pulsating. It may be mentioned here that in the pulvinule also the electrical phenomenon continued in such condition of forced stoppage of leaf-movement.

The pattern of the electrical impulse bears some similarity with the biphasic impulse obtained in Mimosa and with the second impulse of the paired responses of Biophytum. There also the pattern of response was an initial positive followed by a negative. In *Mimosa* and *Biophytum* simultaneous occurrence of this impulse at different points leads to the assumption that it was the outcome of a hydrostatic impact produced by the contractile cells of the pulvinus. Here also such an explanation seems plausible. If the down movement of the leaflet causes a negative pressure in the pulvinule, there will be a positive pressure elsewhere. The initial positive indicates such a possibility. If the positive pressure is dissipated from the region a drift toward negativity may follow.

Experiments were also conducted by electode connection at different points on the main petiole, below the pulvinule. In a large number of experiments only a few indicated a pattern of response similar to that obtained in the midrib and lamina. The cause of this discrepancy may be due to the presence of normal suctional flow opposing the hydrostatic

impact produced by the negative pressure in the pulvinule. This may also happen if there is a preferential path toward the preriphery normally existing in the system. It may be recalled that such a preferential path was detected in *Mimosa* in the outflow of pulvinar sap during its contraction.

C.2.2. *Diametric changes of the pulvinule during the movement of the leaflet of Desmodium.*

In a previous investigation attempts were made by a micro-Potometric study to detect if there is any displacement of sap from the pulvinule through the main petiole, during its downward movement, similar to that occurring in *Mimosa* with the contraction of the pulvinus. But the suctional rate did not indicate any such change with the movement of the leaflet. It was assumed that either the method employed was not sensitive enough to detect the minute change or the pulvinule does not undergo any total contraction during the rhythmic movement of the leaflet.

Later, study of electrical changes in the pulvinule and other regions of the leaflet suggested that there might be a change of sap pressure in the pulvinule during its movement. In order to obtain a more definite proof of the assumption, study was undertaken on the diametric changes of different parts of the leaf during its movement. It has been reported previously that for this purpose Bose's Plant Sphygmograph with suitable modification has been employed. By photographic method, arrangement has been made to record the changes in high magnification. The movement of the leaf was recorded simultaneously by a Phytograph to observe its correlation with the diametric change if any.

At first the main petiole was subjected to study by the Sphygmograph, expecting that the downward movement of the leaf might induce expansion in the petiole. A number of experiments were performed—by placing different points of the petiole in the feeler of the Sphygmograph but no definite change in diameter could be ascertained.

Next, experiments were undertaken by making sphygmographic contact in the pulvinule. The pulvinule showed periodic expansion and contraction with the movement of the leaflet. By photograpihically synchronising the records of the leaf movement and the diametric changes of the pulvinule it has been found that the pulvinule undergoes diametric contraction with the downward movement of the leaf and expansion during the up movement. The photographic records of the diametric changes of the pulvinule obtained with magnification of 12,000 times showed variations of 2 to 4 c.m. in different specimens; the actual changes taking place was therefore, 2 to 3μ. The results of this experiment definitely shows that the turgor pressure in the pulvinule is diminished during the downward movement of the leaflet.

Arrangement has also been made to study the diametric changes of the midrib and its correlation with the pulsation of the leaflet. In this case the leaf blade being fixed the mechanical pulsation of the leaflet is to be recorded in a reverse way from the movement of the petiole which is kept free.

C.2.3. *A device for application of electrical stimulus in plant without disturbance to the electronic circuit of the Pen Oscillograph.*

In the study of action wave propagation with Pen Oscillograph only thermal stimulation could be applied, so long, in producing excitation at a point. But the ideal form of

stimulation, the intensity of which can be kept constant for successive experiments, is the calibrated electrical stimulus. This, however, could not be used with the electronic apparatus because it created electronic imbalance. Sibaoka has described an electrical circuit to eliminate this imbalance. But it may take considerable time to construct the same here. In the mean time we have devised a simpler method which can be profitably used in some study.

In this device the plant (*Mimosa*) is enclosed in a specially made metal box with a part of the leaf protruding out through a slit, so that the leaf blades are exposed outside. A measured shock from an induction coil can be applied to the protruding portion and the lead electrodes are kept inside the chamber. Preliminary trial has shown that this method may be successfully used in such experiments without creating electronic imbalance.

C.2.4. *An attempt for further amplification of Pen Oscillograph*

An attempt has been made to achieve further amplification of Pen Oscillograph by introducing a separate optical lever to the pen arm for detection of cellular pulsation of ordinary plants with more details. By this device the amplification could be increased by about 70 times to that of the Oscillograph. But on trial it was not found to be suitable for the intended purpose. With such high amplification the minute artifacts of the apparatus make their appearance in enlarged form and obscure detection of the wanted electrical changes of the plant.

C.3. Plant Breeding and Biometry

C.3.1. Species of fodder grasses (*Paspalum dilatatum*; *P. commersonii*; *Panicum maximum*; *Melinis minutiflora*; *Cenchrus oiliaries*) and of fodder legumes (*Stylosanthes gracilis; S. humilis; Phaseolus atropurens; Glycine javanica; Desmodium uncinatum*) were selected from the previous year's introductions on the basis of their performances under local conditions. Grasses were sown in an available area of approximate 3822 sq. ft. at Bose Institute Experimental Farm, Shyamnagar on January 4-5, 1965 in replicated small plots for further observations on their productivity based on cutting experiment and for the purpose of seed multiplication. The available seeds from the selected fodder legumes were also sown for the purpose of seed-multiplication in an area of approximate 390 sq. ft. The work is still in progress.

C.3.2. *Studies on Aman Paddy*

C.3.2.1. Three varieties of aman paddy, belonging to early flowering (Badkalamkati) medium flowering (Rupsail) and late flowering (NC 1281) types were sown on three different dates—June 4, June 24, and July 14 (at 20 days intervals) in order to study their relative performances with respect to different quantitative characters on three different dates of sowing. Plants were transplanted approximately 30 days after sowing and in each sowing a randomised block design with nine replications was used. In each replication there were three plots, each consisting of three rows of 25 plants each. The three

TABLE 1

MEAN PERFORMANCE OF QUANTITATIVE CHARACTERS OF EARLY, MEDIUM AND LATE TYPES OF *Aman Paddy*

	First sowing (4-6-1964)			Second sowing (24-6-1964)			Third sowing (14-7-1964)		
	Early	Medium	Late	Early	Medium	Late	Early	Medium	Late
Mean flowering time (days) ±S.D.	128.9±7.06	138.4±1.12	155.3±1.12	109.9±6.47	118.7±0.96	135.2±0.88	97.2±4.75	104.5±1.28	117.2±1.38
No. of plants	150	150	150	150	150	150	150	150	150
Mean flowering date	(574) Oct. 11	(638) Oct. 20	(477) Nov. 6	(656) Oct. 12	(670) Oct. 21	(663) Nov. 6	(658) Oct. 19	(648) Oct. 26	(624) Nov.8
Mean height (cm)	150	153	143	150	150	147	137	136	133
		S.E. = 1.68			S.E. = 0.95			S.E. = 1.38	
Mean No. of panicles	6.1	6.9	4.9	6.5	7.3	4.8	6.0	6.3	4.7
		S.E. = 0.33			S.E. = 0.35			S.E. = 0.21	
Mean length of panicle (cm)	27.3	27.7	28.7	27.1	27.9	29.4	26.5	27.2	29.2
		S.E. = 0.36			S.E. = 0.19			S.E. = 0.22	
Mean grain yield/plot (kg)	0.67	1.11	0.74	0.68	1.34	1.18	0.95	1.05	0.96
		S.E. = 0.095			S.E. = 0.044			S.E. = 0.072	

varieties were randomly allocated among the three plots in each replication. Spacing given was 9″ between plants and 9″ between rows.

All plants in each row were taken into consideration when recording the number of days taken to flower (*i.e.*, from date of sowing to date of ear emergence). At harvest time, two plants were chosen at random from each row and the average height, number of panicles and length of penicle of six plants in three rows were considered as the representative values of each plot. Bulked grain yield was recorded for each plot. Table 1 shows the results obtained after analysis of the data. The mean time of flowering in all the varieties decreased when sowing time was delayed. When the mean time of flowering was converted to dates it was found that Badkalamkati, Rupsail and NC 1281 flowered respectively on October 11-12, October 20-21 and November 6 in the first two sowings. A slight variation was noticed in the third sowing. Mean flowering date was delayed by seven days in Badkalamkati, by five days in Rupsail and by two days in NC 1281. Height of early and medium varieties while not different amongst themselves, were significantly taller than the late type in the 1st sowing. In the other two sowings not much variation in height was noticed. In all sowings mean length of panicles increased with increase in flowering time of the varieties. Among all types the medium type gave highest grain yield in all three sowings. This difference in yield was not significant in all cases when compared to the late type.

C.4. Radiation Mutation

C.4.1. *Studies on Aus Paddy*

Several mutants were isolated from the variety Marichbati after treatment with X-rays and ^{32}P in the year 1958.

A comparison of flowering times of the different mutants were made with their respective parents. The results obtained have been given below:

TABLE 2

MEAN STRAW STIFFNESS (GMS) AND FLOWERING TIME (DAYS) OF MUTANT LINES AND THEIR CONTROL

Types	Mean straw stifness ±S.E.	Mean time of flowering ±S.E.
Marichbati (control)	217.72±11.10	78.89±0.22
Dular (control)	195.22 7.94	77.00 0.34
Black Mutant (S.1)	94.22 13.90	80.90 0.19
Marichbati-awned mutant (S.236)	226.12 11.95	79.60 0.60
White Dular (S.240)	336.25 17.06	80.72 0.16

The mutant line Black mutant (S.1) and white Dular (S.240) shows 2.01 and 3.72 days late in the meantime of flowering respectively when compared to their respective

parents. No differences were noticed between the mutant line Marichbati awned (S.236) and its parent Marichbati variety.

C.4.1.2. *Breeding result:*—The Black Mutant (S.1) was isolated in P_1 generation (1958) after 15 μc/seed treatment of ^{32}P. The selection S.1 which maintained its character for anthocyanin pigmentation in seeds and tillers (black colour) was reciprocally crossed with its parental variety Marichbati; in the F_1 generation it was noticed that the deep purple leaf sheath was dominant over the green leaf sheath of the Marichbati variety. In the F_2, the sedlings with deep purple leaf sheath and seedlings with green leaf sheath segregated out in the ratio of 3: 1.

C.4.1.3. *Agronomical behaviour of the twin seedlings:*—From the seedlings of selection S.221 every year a small number of twin seedlings were isolated. These twin plants were tagged as right hand side plants and left hand side plants. At maturity the agronomical behaviour of these tagged seedlings were analysed individually and compared with the mutant line S.221 and the results can be seen in the table below:

TABLE 3

COMPARISON OF AGRONOMICAL CHARACTERS OF THE TWIN PLANTS AND SELECTION S.221 (MEAN OF 25 PLANTS)

Selections	Mean height of plants (cm)	Mean number of tillers	First flowering time (days)	Last flowering time (days)	Range of flowering (days)
S. 221	100.20	7.30	77	92	15
Twin plants (R.H.S.)	58.21	2.07	78	114	36
Twin plants (L.H.S.)	57.61	2.10	77	113	34

From the above table it is noticed that plant height, and number of tillers is lower but range of flowering time was longer in the twin seedlings than in selection S.221.

Studies on Boro Paddy:—The selection S.369 was again grown with its control Chinsura Boro II and the agronomical data were statistically analysed. The results so far obtained can be seen below:

TABLE 4

COMPARISON OF AGRONOMICAL CHARACTERS OF THE SELECTION S.369 AND CHINSURA BORO II—YEAR 1963

Character	Chinsura Boro II (control)	S.369	Diffence±S.E.
Height (cm)	101.20	98.30	2.90±1.70*
No. of tillers	10.90	8.30	2.10±1.78**
No. of panicles	11.00	8.70	2.30±0.78**
Length of panicles (cm)	19.70	19.10	0.60±0.38
Grain yield (gm)/plant	13.80	11.60	2.20±0.30*
Mean time of flowering (days)	109.10	101.87	7.32±0.43**
Flag leaf length (cm)	26.30	21.30	3.00±0.44

From the above comparison of agronomical characters, it is noticed that selection S.369 is lower in plant height, number of tillers, number of panicles, length of panicles, length of flag leaf, and yield of grains (per plant) than the Chinsura Boro II (Control).

The selection S.369 flowers 7 days earlier than the parental variety Chinsura Boro II (Control).

C.4.1.4. *Cytological studies:*—Cytological studies of the selection S.369 and Chinsura Boro II (control) were made from both the root tips and flower buds. The results obtained (mean of 4–5 cells) are summarised in the table below:

TABLE 5

COMPARISON OF CHROMOSOME CHARACTERS OF CHINSURA BORO II (CONTROL) AND THE SELECTION S.369

Characters	Chinsura Boro II	S.369
Chromosome number ($2n$)	24	24
Chromosome number (n)	12	12
Total length of long arm (μ) of the chromosome	17.68	17.28
Total length of short arm (μ) and satellited chromosome (μ)	9.04±0.96	8.80±0.96
Total length of chromatin material (μ)	27.68	27.04

The mitotic chromosome number and the meitotic chrmosome number was $2n = 24$ and $n = 12$ respectively. There were no significant changes in the total chromosome length or in the length of short arm and long arm of the chromosomes.

Cytogenetical studies on other isolated mutants of Aus and Boro Paddy are in progress.

C.5. Chemical Mutagenesis

C.5.1. A derivative of ethylene-imine, 2-ethyl-2-phenylethyleneimine (fig. 1) was recently tried on barley (*Hordeum vulgare*) and some of the results are presented below:

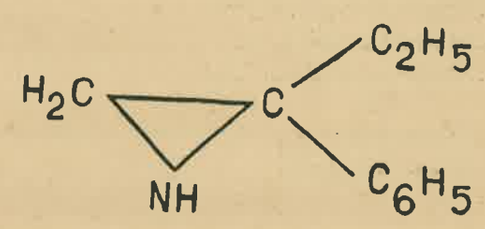


FIG. 1.

A concentration of 5 parts of the chemical in 1000 parts of water (5 p.p.t.) was found to be effective in producing chlorophyll mutants. Higher concentrations (*e.g.*, 7 p.p.t.) produced lethal effects. The frequency of chlorophyll mutation was found to be 5.2% when based on spike progenies and 1.84% when calculated from M_2 seedlings. The frequency in control population was estimated to be 0.3% and 0.1% respectively. The maximum number of mutants that were scored in the treatment belonged to the viridis class (c.a. 57%). The percentage of xantha was 18% and that of albina 10%. 2 ethyl-2-phenyethyleneimine was found to be an effective mutagenic agent but its use is limited by its high lethal effects at concentrations a little higher than that described above.

C.6. Cytological and Cytotaxonomical studies

C.6.1. *Cytological and Cytotaxonomical studies in some genera of Malvaceae*

C.6.1.1. During the year under report (1964-65), cytological and cytotaxonomical investigations were carried out on a single species of the genus *Althaea*. Linn., and one species of the genus *Abelmoschus* and three species of the genus *Hibiscus* Linn. under the family Malvaceae as a continuation of observations made and reported in previous years.

In the present investigations two very commonly cultivated species of India and three imported species were taken up. Both the cultivated species showed differences in chromosome number with reports of earlier workers which may be due to the existence of polyploid characters in those species. Extremely small chromosomes as well as their large number per cell may also lead to some inaccuracy in determining the correct number.

These are 1. Genus: *Althaea*. Linn.
1) *Althaea rosea*. Linn.

2. Genus: *Abelmoschus* Medic.
1) *A. esculentus* W. & A.

3. Genus—*Hibiscus*. Medik.,
1) *Hibiscus diversifolius*, Jacq.
2) *Hibiscus ludwigii*. Linn.
3) *Hibiscus pedunculatus*. Linn.

C.6.1.2. Genus—*Althaea*. Linn.
1) *Althaea rosea*. Linn.

A biennial herb, erect pubescent branched: leaves large, long petioled acutely 5 to 7 lobed: flowers large, varied colours, short pedicelled, forming long terminal racemes; calyx 5 cleft, downy: petals very broad, waxy, variously coloured: staminal tube short: anthers pale yellow: ovary many celled, each cell 1—ovuled. Plants yield althein, a

pigment belonging to anthocyanin group. Seeds, flowers, leaves and roots are used for medicinal purposes.

This species reveals a $2n$ complement of 42 chromosomes which is quite in agreement with the observation of Skovsted (1941) but differs with that of Suguira (1936b) who reported the number as 52. Primary constriction varies between median to sub-median. Range of variation in length between longest to the shortest pair of chromosomes is not much and the chromosomes are short. Three pairs of chromosomes with satellites on the shorter arms were observed. Feulgen Fast-green technique confirmed that these satellited chromosomes are of nucleolar origin.

Meiotic studies showed 21 bivalents. Chiasmata formations are mostly end to end.

Abnormalities like bridges both in somatic and meiotic anaphase were found.

Pollen grains are round and about 13μ in diameter. Pollen sterility in different coloured flowers reveals that white flowered variety has the highest percentage of sterility (6.27%) while the violet flowered variety has the lowest (4.11%). and that of red and other allied coloured flowers are in intermediate positions. Detailed studies of this character are in progress.

C.6.1.3. Genus. *Abelmoschus* medic. Linn.

C.6.1.3.1. *A. esculentus* W. & A.

A tall herb covered with rough hairs: leaves coarsely toothed: petioles more or less bristly: flowers yellow with a crimson centre: staminal-tube antheriferous throughout: fruits pyramidal to oblong, glabrascent: cells 5–8 seeded. Pods are used as pot herbs and also occasionally for medicinal purposes. Cultivated throughout India and in all tropical countries.

This species has been found to have 72 very small chromosomes having median to sub-median constrictions. Teshima in 1941 reported the same number in its $2n$ complement but Purewal and Randhawa in 1947, Joshi and Hardas in 1953 and Medvedeva in 1936 have reported the number as 120, 130 nand 132 respectively. During the present investigation root tip cells having 116 to 130 chromosomes were also observed occasionally, along with the normally occurring numbers. Due to extreme shortness and high number of chormosomes it was generally very difficult to get a well spread metaphase plate revealing the details of chromosome morphology. Difference in number of chomosomes observed here with that of the reports of the earlier workers may be attributed to its polyploid nature attained during cultivation in different localities. Chromosomes were found to have sub-median to median primary constrictions. Four pairs of only satellited chromosomes were found to occur. The eight chromosomes attached to a nucleolus at early prophase and eight small nuceoli at early telephase observed with help of Feulgen Fast-green technique confirm that the satellited chromosomes are of nucleolar origin.

Abnormalities like hyperploid number in metaphase and somatic bridges or laggards in anaphase were recorded.

C.6.1.4. Genus. *Hibiscus* Linn.

C.6.1.4.1. *H. diversifolius. Jac.* It is a tall herb or under shrub: petioles and nerves of leaves are armed with conical prickles: leaves variable, usually cordate: sepals

linear-lanceolate: corolla 3 to 4 times the size of calyx: capsule ovoid, pointed. Seeds smooth: Distribution-Burma, Tropical Africa and Australia.

2*n* complement of this species has been found to consist 106 chromosomes. Occasionally cells with 144 chromosomes have also been observed. Skovsted in 1941 reported the number as 144 and ca. 180. No cell with 180 chromosomes has been recorded in the present investigation. Chromosomes are extremely small with median to sub-median primary constrictions. Six pairs of satellited chromosomes have been recorded. One nucleolus with 12 chromosomes attached in early prophase has been observed following Feulgen Fastgreen technique.

Various types of abnormalities like laggards and somatic bridges in anaphase and appearance of hypo- and hyper-ploid number in mitotic metaphase have been observed.

C.6.1.4.2 *H. ludwigii. Linn.*

A hairy annual, much branched: leaves 5–7 lobed: flower axillary 3–1 together: calyx deltoid: corolla whitish: seed kidney shaped, grey, about 2 to 2.5 mm. long. Distribution-throughout Oriental and Mediterranean regions and S. Africa.

This species has a 2*n* complement of 40 chromosomes having median to sub-median primary constrictions. Length of chromosomes ranges from 3 to 5μ. Three pairs of satellited chromosomes were recorded. Three pairs of small nucleoli at telophase and six chromosomes attached to a nucleolus at early prophase observed through Feulgen Fast-green technique confirm the origin of the nucleoli from the satellited chromosomes.

Abnormalities like laggards and somatic bridges in the anaphase stage and hyperploid number of chromosomes in metaphase were recorded.

C.6.1.4.3 *H. pedunculatus*. Linn.—Erect herb or undershrub, branched: leaves 5–7 lobed, pubescent: flowers axillary: calyx lobed: corolla larger than the calyx: seeds pubescent, black, kidney shaped about 1.5 to 2 mm. long. Distribution—Tropical Africa and Australia.

It has a 2*n* complement of 30 chromosomes having median to sub-median primary constrictions. Three pairs of satellited chromosomes of which one pair with comparatively bigger knobbed satellites were observed. Length of chromosomes varies between 3 to 5μ. Occurrence of six small nucleoli at telophase and six chromosomes found attached to a nucleous at prophase showed their relationship with the satellited chromosomes.

Somatic bridges were occasionally observed as abnormality in anaphase stage.

C.6.2. *Cytological investigations on Papilionaceae*

C.6.2.1. During the year 1964-65, detailed cytological investigations were carried out on a number of members of the sub-family Papilionaceae under the family Leguminosae.

The investigated members with their chromosome numbers have been tabulated below:

Name of Taxa	*Somatic chromosome number*
I. Tribe—Vicieae	
1. *Lens esculenta*	
var. i) *macrosperma*	14
ii) *microsperma*	14
2. *Lathyrus sativus*	14
3. *Cicer arietinum*	14
var. i) Kabulicum	16
ii) B–75	16
iii) B–98	16
II. Tribe—Hedyserae	
4. *Desmodium gangeticum*	22
*5. *D. pulchellum*	22
*6. *D. cephalotes*	22
III. Tribe—Trifolieae	
7. *Melilotus alba*	16
8. *M. indica*	16
9. *M. parviflora*	16
IV. Tribe—Galegae	
10. *Indigofera tinctoria*	16
*11. *I. frutescens*	16
*12. *Psoralea pinnata*	22
V. Tribe—Podalyrieae	
*13. *Podalyria calyptrata*	18
VI. Tribe—Genistae	
*14. *Aspalathus sarcodes*	18
*15. *Crotalaria capensis*	16
16. *C. juncea*	16
*17. *C. rotundicarinata*	16
VII. Tribe—Phaseolae	
18. *Clitoria ternaea*	
var. i) Double White	16
ii) Single White	16
iii) Double Blue	16
iv) Single Blue	16

Chromosome numbers for plants with (*) asterisks are reported for the first time.

For the study of the somatic chromosomes, root-tips of the materials were pretreated with saturated solution of paradi-chlorobenzene for 40 minutes to 1 hr. 20 minutes at 16°C. and fixed in acetic alcohol (1 : 3). Of the other pretreating chemicals tried, 8-oxyquinoline (.002 mm.) for 1 hour 45 minutes at 16°C. gave satisfactory results in some cases. After fixing the root-tips were stained with Propiono-orcein following the usual schedule.

Karyomorphological studies revealed that there exists a gross similarity in chromosome morphology within a genus between species and varieties. Inspite of this, their detailed chromosomal study presents sufficient characteristic interspecific and inter-varietal differences.

In *Lens esculenta* differences exist in the two varieties *macrosperma* and *microsperma*; the former contains one pair of chromosomes with secondary constrictions while the latter has two in the diploid complement. Besides, the total chromatin length of a haploid set in *macrosperma* is greater than that of *microsperma*.

Lathyrus sativa as studied, shows large chromosomes with three pairs of chromosomes with secondary constrictions in its diploid chromosome complement.

In *Cicer arietinum* the two varieties B-75 and B-98 have three pairs of chromosomes with secondary constrictions while the other variety *kabulicum* have only two pairs. But all the varieties have their longest satellited chromosome very conspicuous.

From the cytological studies on the three genera of Vicieae, some common features were observed in the two genera *Lens* and *Lathyrus* while *Cicer* showed much dissimilarities and thus inclusion of the genus *Cicer* along with *Lens* and *Lathyrus* under Viceae does not seem well justified.

The diploid chromosome complement of the species of *Desmodium* investigated shows differences in their nature of secondary constrictions. *D. cephalotes*, *D. pulchellum* and *D. gangeticum* have 1, 2, and 3 pairs of secondary constricted chromosomes respectively.

The genus *Melilotus* shows that each of its three species of the present study contains three pairs of chromosomes with secondary constrictions in their diploid somatic complement. *M. alba* has the longest chromosomes with a total chromatin of 38.67μ in its haploid set; while *M. indica* has a chromatin length of 30.34μ and *M. parviflora* with comparatively smaller chromosomes has a length of chromatin matter of about 23.90μ.

The two species of the genus *Indigofera* showed variation in their size and nature of secondary constrictions of chromosomes. I. *frutescens* has larger chromosomes and two pairs of chromosomes with secondary constrictions while I. *tinctoria* has comparatively smaller chromosome and three pairs of chromosome with secondary constrictions in its diploid complement. *Psoralea pinnata*, the other member of the tribe Galegae, though not investigated in details, has been found to contain 22 chromosomes in its diploid complement and which is a first report.

Podalyria calyptrata with two pairs of chromosomes having secondary constriction in its diploid chromosome complement has certain characteristic chromosomes not found in any other tribe.

Aspalalthus sarcodes a new report for its chromosome number, has two pairs of chromosomes with secondary constrictions. All the chromosomes are highly acrocentric.

All the species of the genus *Crotalaria* investigated here have two pairs of chromosomes with secondary constrictions. *C. juncea* shows more similarities in chromosome forms with *C. capensis* and *C. rotundicarinata* than that of between the latter two. Their variation in chromatin content is not very wide, varying from 32.02μ in *C. capensis* to 29.04μ in *C. rotundicarinata* with 29.25μ in *C. juncea* as intermediate.

Clitoria ternatea representing the tribe Phaseolae provides interesting data for inter-varietal cytological study. Their strikingly different chromatin content as well as lack of structural uniformity of chromosomes are so pronounced as to enable one differentiate one variety from another. Single White and Single Blue have chromatin content higher than Double White and Double Blue respectively. But Single White has the highest chromatin content of all the four varieties studied and Double White has a longer chromatin matter than Double Blue.

Meiotic studies were made on *Lens*, *Lathyrus*, *Clitoria* varieties and *Cajanus*. All showed regular type of 1st and 2nd divisions.

Mitotic and meiotic behaviour and morphology of chromosomes of different species of the present study suggest that structural alternations of chromosomes have played an important role in speciation.

C.6.3. *Cytological studies on Pisum sativum*

C.6.3.1. A review of literatures concerning the somatic chromosomes of *Pisum sativum* reveals that all authors are in agreement regarding 14 as the chrosomsome number in a diploid cell, but there are widely divergent points of view regarding the number of secondary constricted chromosomes in the chromosome complement of the species. The present study was also undertaken to ascertain the number of secondary constricted chromosomes as well as to study the structure of the nucleolar chromosomes in detail in twenty cultivated strains of *Pisum sativum*.

C.6.3.2. For the study, the following twenty strains of *Pisum sativum* collected from different centres were used:—

Phenomenon, Laxton Progress, Kelvendon Wonder, Show Perfection, Early giant, Globe's Evergreen, White Marofat, Early Badger, Bonnivilli, Thomas Laxton, Acclimatised Round Seeded, Alderman, Blue Bantam, Supreme, Little Marble, Sutton's Pioneer, American Wonder, N.P. 23, T.56, and T.61.

For the study of somatic chromosomes of all the strains mentioned above, root-tips were pretreated in 0.003 M oxyquinoline for 2½ hours at 14°–16°C, then hydrolysed and stained in a mixture of 2% Propiono-orcein and N/Hcl (9: 1). Smearing was done in 1% Propiono-orcein.

The detailed karyotypic analysis of the different strains reveals a striking similarity amongst the different chromosome complements. On the basis of gross morphological features, the following six chromosome types can be morphologically recognized.

Type A—Represents long chromosomes (4.35μ–7.65μ), each with nearly sub-median to median primary constriction.

Type B—Comprises long chromosomes (4.28μ–6.53μ) with large satellites (0.66μ–0.90μ), each with primary constriction sub-median to nearly median in position and the satellite on the longer arm of the chromosome.

Type C—Long chromosomes (4.05μ–6.08μ) with small satellites (0.45μ), each with primary constriction sub-median to nearly median in position and the satelllite on the longer arm of the chromosome.

Type D—Consists of chromosome (4.50μ–6.08μ), each with a sub-median to nearly sub-median primary constriction.

Type E—Chromosomes (4.28μ–5.85μ), each with a sub-terminal primary constriction.

Type F—Chromosomes (3.60μ–5.40μ), each with a nearly sub-median to median primary constriction.

The karyotypes of all the twenty strains can be formulated as A_2, B_2, C_2, D_2, E_2 and F_4. In spite of general basic similarities, minute differences exist in the details of the chromosome, such as in the length of different arms of chromosomes, in the total amount of chromatin matter. Table 6 shows the arm ratio (short arm/long arm) of the different types of chromosomes, number of secondary constricted chromosomes and total amount of chromatin matter in a haploid complement of each strain.

The number of secondary constrictions in all the strains has been found to be four. Feulgen and Light green staining were employed for counting the number of nucleoli and the maximum number of chromosomes attached to the nucleolus or nucleoli in the early prophase nucleus of the root-tip cells. In all the strains studied, generally one or two nucleoli were observed in maximum frequencies, but occasionally three or four nucleoli were found, but never more. At the same time four chromosomes were also found to be attached to a nucleolus or nucleoli. Persisting nucleoli at metaphase and anaphase and presence of extra-nuclear bodies in the cytoplasm of the somatic cells which were previously recorded in this material were not observed in any of the strains during the present course of investigations.

C.6.4. *Cytological studies in the different varieties of Lathyrus odoratus*

C.6.4.1. Cytological studies were made of the following eight varieties of *Lathyrus odoratus*.

(1) Royal blue, (2) Black Prince, (3) Shirley temple, (4) Multiflora deep rose, (5) Madonna, (6) Mixed, (7) White giant, (8) H. Daphne. The varieties differ morphologically in the colour of the flower, size of leaf and size of tendril.

For somatic studies, the seeds were germinated on the petri dish and roots were pretreated in paradichlorobenzene and stained in (1%) 9:1 propiono-orcein: Hcl.

Chromosome numbers were found to be $2n = 14$ in all cases (Latter 1925, Datta, 1956). It was found that though each variety possesses the same number of chromosomes they differ in their morphology. The primary constrictions of the chromosomes may vary from

TABLE 6

Strain	Short arm/Long arm ratio of different types of chromosomes						Number of secondary constricted chromosomes	Total amount of chromatin matter in a haploid complement (in microns)
	A	B	C	D	E	F		
Phenomenon	.54	.43	.52	.43	.20	1 : 1	4	35.71
Show Perfection	.53	.61	.49	.57	.22	1 : 1	4	34.65
Little Marble	.57	.60	.60	.66	.18	1 : 1	4	29.26
Early giant	.55	.50	.50	.51	.18	1 : 1	4	39.97
T–56	.63	.49	.49	.50	.15	1 : 1	4	36.53
T–61	.58	.46	.46	.53	.15	1 : 1	4	38.93
American Wonder	.51	.52	.53	.71	.20	.83 : 1	4	27.28
Globe's Evergreen	.45	.53	.47	.64	.23	1 : .81	4	38.87
White Marofat	.54	.50	.57	.50	.20	1 : 1	4	40.73
Early Badger	.50	.53	.46	.60	.19	1 : .83	4	39.61
Bonnivilli	.54	.50	.50	.57	.20	1 : .83	4	41.22
N.P. 29	.50	.46	.48	.46	.18	1 : 1	4	38.32
Kelvendon Wonder	.55	.53	.53	.66	.22	.81 : 1	4	34.43
Laxton Progress	.62	.49	.49	.57	.22	.83 : 1	4	35.97
Thomas Laxton	.52	.56	.61	.60	.22	.81 : 1	4	36.54
Acclimatised Round seeded	.57	.53	.53	.57	.26	1 : 1	4	38.50
Alderman	.45	.50	.57	.68	.18	1 : .80	4	40.74
Blue Bantam •	.45	.47	.57	.62	.20	.83 : 1	4	39.83
Supreme	.56	.66	.66	.62	.22	1 : 1	4	35.73
Pioneer	.57	.56	.56	.62	.20	1 : 1	4	37.38

median to subterminal positions and on the basis of the position of primary and secondary constrictions and their relative sizes, the chromosomes can be divided into 12 types. The number of chromosomes with secondary constriction found in the different varieties and the total chromatin length are given in Table 7.

TABLE 7

Name of variety	No. of secondary constricted chromosomes	Total chromatin length (in haploid set) (μ)
Multiflora deep rose	Six	48.0
Royal blue	Six	47.1
White giant	Six	40.5
Mixed	Six	40.4
H. Daphne	Four	40.4
Madonna	Two	34.3
Black Prince	Six	32.4
Shirley temple	Four	29.7

C.6.5. *Cytological investigations on rice (aman) mutants*

C.6.5.1. During the year under report, cytological investigations on radiation induced mutations in rice (*aman*) were undertaken. The mutants namely, (1) red husk with red tiller, (2) red husk with awn, (3) deep red husk with awn, (4) small fruit (grain) with awn, (5) small fruit (grain) without awn, (6) purple apiculus with purple tiller and (7) purple apiculus with green tiller were isolated from the variety Patnai following treatments with S^{35}. The first five were isolated by Dr. R. K. Basu in the year 1955 and the two latter by Shri A. K. Basu in 1958.

Pollen mother cells were collected from flower buds fixed in glacial acetic acid and absolute alcohol mixture (1 : 3) and smeared with acetocarmine. Pollen grains were staining collected from the same plants which were used in the study of meiosis and examined after with iodine.

Meiosis from diplotene onwards was studied in the control and all the mutants in order to make a comparative survey of the pairing behaviour and chromosomal abnormalities, if any, in the mutants.

The behaviour of the chromosomes during meiosis, in most of the materials, was normal. However, certain irregulareties were observed in mutants (2) and (7). The separation of bivalents during anaphase I was found to be slightly irregular. The irregularities included non-disjunction of bivalents (1.5% in mutant 7), laggards (2.1% and 1.6% in mutants (2) and (7) respectively) and early separation of one or two bivalents (14.4%) and 2.0% in mutants (2) and (7) respectively.

Compared to the control significant ($P<0.01$) difference in pollen grain sterility was observed in mutants (1), (2) and (7). The increase in total pollen grain sterility in the mutants could not be attributed to chromosomal abnormalities, even assuming that pollen mother cells with abnormal behaviour of chromosomes during meiosis led to the formation of sterile pollen grains.

D. MICROBIOLOGY

D.1. Production of antibiotics by microorganisms

D.1.1. *Studies on the production of a new antifungal polyene antibiotic by Streptomyces strain $Ac_2$435.*

Studies on the fermentation of the antifungal antibiotic showed that the *Streptomyces strain Ac_2*435 could produce sufficient quantity of the active substance in synthetic media. The problem of isolating and purifying the active principle has been dealt with by the following methods. Using cellulose powder as the adsorbent, an inactive pigment fraction has been separated from the antibiotic. Ion exchange resins have been used but the results were not promising. At the next step paper chromatography and countercurrent distribution techniques have been employed in purifying as well as in testing the homogeneity of the antibiotic fraction. Finally, a method was adopted in which the active principle was adsorbed on activated charcoal (Darco G-60) and *n*-butanol, which has been employed in isolating the polyene antibiotics in general, was used for eluting the antibiotic. Purification of the antibiotic into brown amorphous powder was achieved by repeated precipitation with ether, chilling and drying the precipitate.

The antibiotic which was acidic and hygroscopic in nature, was highly soluble in methanol, pyridine, *n*-butanol and formamide. Stability of the antibiotic under different conditions was studied. As is common with the polyenes, the antibiotic due to its unsaturated nature, was found to be unstable being sensitive to light and temperature. The activity of the antibiotic was not markedly influenced by the change of *p*H from 2–10. In 2% methanol it has been found to exhibit an optical rotation of $(\alpha)_D^{27} = +40$. Its melting point was found to be 160°C (Decomposition). Microanalytical investigations carried out at the Micro-analytical Laboratory, Oxford, England indicate that the antibiotic has the following elemental composition:

C — 43.01%
H — 7.68%
O — 49.31%

Chemical tests indicate the absence of nitrogen and the presence of a carbohydrate moiety. Ultraviolet absorption spectrum showed maxima at 288, 305 and 320 mμ indicating the presence of a conjugated tetraene structure. The infrared spectrum indicates the unsaturated nature of the compound and the presence of a conjugated double bond system. It is also suggestive of the presence of a polyhydroxy compound and a—COOH grouping.

The antimicrobial activity of the antibiotic has been studied against a large number of test fungi including pathogenic and saprophytic forms. Determination of the minimum inhibitory concentrations using the agar-cup method has been found to vary from 4.5–30.5 μg/ml. The complete inhibition of the colonies of *Saccharomyces cerevisae* at a concentration of 25 μg/ml. in 24 hours gave an indication of the fungicidal property of the antibiotic.

Toxicity tests of the antibiotic were performed at the laboratory of Boots Pure Drug Co. Ltd., Nottingham, England on experimental laboratory animals. Mice dosed with 200 mg/kg. S.C. remained completely healthy; 50% of those receiving 400 mg/kg. died

after a few days immediately while all those receiving 800 mg/kg. were dead. The S.C. LD_{60} therefore was 400 mg/kg. The chemical composition of the tetraene antifungal antibiotic under investigation has been compared with other known antibiotics of similar nature. The substance was compared with authentic samples of some of the known tetraene compounds on the basis of paper chromatographic studies and its antimicrobial properties indicate differences with those of the known compounds. The antibiotic, therefore, has been found to be a new one, as substantiated by a comparative study of the infra-red spectra of the polyene compounds already described in literature.

D.1.2. *Survey of antibiotic-producing actinomycetes from Indian soils.*

Screening of broad-spectrum pigment-forming strains of *Streptomyces* was undertaken for the study of genetics in relation to pigment and antibiotic production. As the distribution of broad-spectrum antibiotic producers is not very frequent in the soils examined in the present survey, an attempt was made to undertake plating of sixty samples collected from different localities of West Bengal and neighbouring states. The usual procedure was to employ Norris's agar medium which specifically supports growth of *Streptomyces* in culture plates. The aqueous suspension of the soil samples was made in sterile tap water in steps of 10. Dilutions 10^{-3} to 10^{-5} were generally useful for the isolation of most Actinomycetes. The plates were prepared in quintuplicates and random isolations were made after 10 days of incubation at 28°C.

While selecting the isolates special attention was given to pigment-producing strains. They were subcultured in M_{12} agar medium and stored at 4°C. until they were screened against bacterial and fungal test organisms. The total number of isolates were above 600 *i.e.*, on average ten isolates per soil samples. Preliminary assay tests showed that there are 135 pigmented strains producing either antibacterial and antifungal or both. In the final screening 9 strains were selected out of the lot and their growth and antibiotic production has been studied. Table I shows the results of the assay tests of the selected strains against six test organisms. The nutritional requirements of strains are being studied as pre-requisite for semi-large scale production of antibiotics.

TABLE 1

SCREENING TEST OF ISOLATES AGAINST FUNGI AND BACTERIA

Streptomyces strain	Pigment		C.lunata		A.solani		F.oxysporium		A.niger		C.albicans		S. aureus		E.coli	
	Surface	Reverse	S	C	S	C	S	C	S	C	S	C	S	C	S	C
Ac_7760	White	Yellow	35	20	24	20	—	—	—	—	—	—	10	—	—	—
Ac_7766	Grey	Orange	24	13	—	20	—	—	—	—	24	—	37	—	34	—
Ac_7767	Olive green	Grey	30	20	15	17	—	—	—	—	20	—	—	—	—	—
Ac_2784	Grey	Orange	11	20	22	20	—	—	—	—	18	—	40	20	35	15
Ac_4789	Grey	Yellow	35	25	45	25	—	—	—	—	30	—	10	—	—	—
Ac_1797	Slate Colour	Yellow	36	15	38	22	—	15	—	—	30	—	20	—	—	—
Ac_8798	Grey	Yellow	20	27	20	20	—	12	—	—	13	—	25	20	25	—
Ac_1805	Grey	Orange	25	17	20	15	—	18	—	—	16	—	—	—	—	—
Ac_2806	White	Orange	20	30	30	30	—	12	—	13	15	19	—	—	20	—
Ac_2820	Ash	Yellow	22	35	11	25	—	15	—	—	15	—	30	14	25	—
Ac_2821	Grey	Yellow	—	12	—	—	—	—	—	14	—	11	—	—	—	—

S = Streak assay C = Cup assay

D.1.3. *Studies on the taxonomy of the antibiotic-producing strain of Streptomyces sp.* Ac_5 658.

The *Streptomyces* strain Ac_5 658 showed a wide range of antibiotic activity against Gram positive and Gram negative test organisms. As the antibiotic spectrum (Table 2) shows promising characters it was thought necessary to study the morphological and biochemical characters of the strain aiming ultimately to identify the isolated strain.

TABLE 2

ASSAY OF ANTIBIOTIC Ac_5 658

Test organism	*Zone of inhibition (in mm)*
1. Eb. typhosa ..	20.0
2. E. coli ..	10.0
3. Sal. typhosa ..	20.0
4. Str. albus ..	21.0
5. Staph. aureus ..	30.0
6. Str. faecalis ..	19.0
7. B. cereus ..	15.0
8. B. subtilis ..	16.0

To characterise the isolated strain of *Streptomyces sp.* Ac_8 658 the procedure described in Bergey's Manual of Determinative Bacteriology was followed. The growth, colour of aerial mycelium and formation of pigment were studied in different media as proposed by Waksman (1957). The following media were used.

1. Starch agar
2. Yeast glucose agar.
3. Yeast peptone agar.
4. Egg albumen agar.
5. Melanin pigment formation medium.
6. Soil extract agar.
7. Czapek agar.
8. Glycerine asparagine agar.
9. Nutrient salt agar.
10. Glucose asparagine agar.
11. Nutrient agar.
12. Oat-meal agar.
13. Carbon nutrition medium.
14. Ca-malate agar.
15. Straw infusion agar.
16. Potato plug.
17. Carrot plug.

To study the biochemical properties, following tests were carried out.

1. Reduction of nitrates.
2. Coagulation and peptonisation of milk.
3. Hydrolysis of starch.
4. Hydrolysis of gelatin.
5. Acid production in different carbohydrate media.

The results of the studies on growth characters as well as biochemical reactions were compared and it was found that the strain Ac_5 658 could be related to *Streptomyces diastaticus* as described in Bergey's Manual of Determinative Bacteriology (7th Edition) but there are several differences which warrant its inclusion as a different species.

D.1.4. *Studies on the pigment antibiotic of a mutant strain MT-10 derived from Streptomyces species* Ac_{21} 460.

The mutant strain MT-10 isolated by irradiating *Streptomyces* strain Ac_{21} 460 with ultraviolet rays (2537 Å of 100 ergs/mm²/sec.), acquired certain specific character, *e.g.*, the formation of a diffusible yellow pigment found to be antibiotically active. This active principle was extracted by the solvent extraction method using ether and was finally purified by the adsorption chromatography using Brockmann's alumina. The crude antibiotic mass was dissolved in methanol and poured into the alumina column. Elution was carried out with ether. The etheral extract was then allowed to evaporate solwly in vacuum when a solid orange-yellow crystalline substance was isolated.

Homogeneity of the pigment antibiotic was studied by paper chromatography method. One dimensional descending chromatography using the paper strip technique with a solution of the antibiotic substance having concentration 100 μg/ml in methanol was followed. The following table shows that corresponding to a given R_f value, only one active spot was detected by bioautography. It appears, therefore, that the pigment antibiotic contains only one active component.

TABLE 3

R_f VALUES OF THE PIGMENT ANTIBIOTIC IN DIFFERENT SOLVENT SYSTEMS AND BIOAUTOGRAPHY

Solvent system	R_f value
1. Butanol: acetic acid: water (2: 1: 1)	0.94
2. Butanol saturated water	0.92
3. Ethanol: water (3: 1)	0.85
4. Butanol: methanol: Water (4: 1: 2)	0.97
5. Methyl-ethyl-ketone: propionic acid: water (75: 25: 30)	0.96
6. Butanol: water+0.25% para-toluene sulphonic acid	0.92
7. Pyridine: acetic acid: water (50: 35: 15)	0.89

Activity of the pigment antibiotic against a number of test fungi.

To determine the spectrum of the antibiotic assay tests were done against a number of fungi as the activity was highly antifungal. The medium used for the growth of the test organisms was Czapek Dox agar. Minimum amount of the antibiotic required for inhibition was studied by the agar cup method of assay. The results are expressed in μg/ml and given in Table 4.

TABLE 4

ACTIVITY OF THE PIGMENT ANTIBIOTIC AGAINST THE FOLLOWING TEST ORGANISMS

Test organisms	μg/ml
1. Curvularia lunata	12.5
2. Alternaria solani	15.0
3. Fusarium vasinfectum	20.0
4. Aspergullus niger	125.0
5. Helminthosporium oryzeae	16.0
6. Candida albicans	25.0
7. Epidermophyton floccosum	32.0
8. Trichophyton sulphureum	30.0

D.2. Induced mutation in microorganism

D.2.1. *Study of reverse mutation in a specific gene of A. chevalieri*

The strain U_7Ar_{12}, a biochemical mutant derived through UV-treatment from the wild parent *A. chevalieri* was selected for the present study. The mutant strain showed spreading growth, characteristic of the wild type (*A. chevalieri*) with heavy sporulation. The colour of conidia is olive green and it forms small distinct colonies on the agar surface when plated out. It did not produce antibiotics and was deficient in arginine. During the experiment, following media were used—complete medium (CM), minimal medium (MM) in which case washed and purified agar was used, and minimal medium supplimented with arginine. The mutagenic chemicals used in this experiment were hydrogen peroxide, formaldehyde and sodium deoxycholate.

Two sets of experiments were performed with each chemical—(i) chemical treatment only (ii) chemical treatment followed by UV-irradiation, and (iii) UV-irradiation only.

(i) *Chemical treatment*—In this experiment freshly prepared spore suspension of the strain was centirfuged, washed and rejecting the mixture of Ringer's solution and 'calsolene' solution, the treatment with chemicals was given for 1,2,3 and 4 hours respectively. During treatment, the mixture of the spore suspension containing the chemical was placed on the shaker for thorough mixing of the spores with the chemical. At the end of the treatment the spores were washed thrice and plated out at different dilutions in CM and MM. The culture plates were incubated for 2–7 days at 30°C. and colonies were counted.

(ii) *Chemical+UV treatment*—For this purpose spore suspension was treated as above and after chemical treatment the suspensions were finally centrifuged and washed thrice with sterilized water to free from chemicals. The spores were resuspended in water and exposed to UV radiation for 4 minutes. The irradiated suspension of spores was plated out on CM and MM agar at different dilutions. The plates were then incubated as usual and counts were made.

(iii) *UV-irradiation*—Freshly prepared spores were exposed to UV-irradiation for 2,4,6 and 8 minutes respectively and after irradiation, spores were plated out on CM and MM agar medium and incubated as usual. The results are given in Table 5.

TABLE 5

STUDY OF REVERSE MUTATIONS BY TREATMENT WITH DIFFERENT CHEMICALS IN CONNECTION WITH UV RADIATION TREATMENT

Treatment	Spore concentration ($\times 10^6$/ml)	Exposure time in minutes	Per cent	
			Survival	Reverse mutation
UV-irradiation	28.2	0	100.0	—
		2	1.5	0.003
		4	1.2	0.05
		6	0.39	0.24
		8	0.15	0.27
H_2O_2 (0.1%)	34.8	0	100.0	—
		60	64.9	0.0001
		120	46.2	0.0005
		180	27.8	0.002
		240	8.8	0.003
H_2O_2 (0.1%) + UV	34.8	0	100.0	—
		60	19.4	0.004
		120	3.8	0.03
		180	0.2	0.74
		240	0.03	0.9
Formaldehyde (0.1%)	24.2	0	100.0	—
		60	37.5	0.0002
		120	59.5	0.0006
		180	42.9	0.001
		240	26.4	0.003
Formaldehyde (0.1%) + UV	24.2	0	100.0	—
		60	50.4	0.001
		120	6.5	0.01
		180	0.75	0.2
		240	0.08	0.8
Sodium desoxycholate (0.5%)	24.4	0	100.0	—
		60	92.0	—
		120	58.4	—
		180	37.4	0.0005
		240	34.6	0.0003
Sodium desoxycholate (0.5%) + UV	24.4	0	100.0	—
		60	10.0	—
		120	1.2	—
		180	0.19	0.11
		240	0.07	0.9

— indicate no scoring of mutants.

From Table 5 it will be seen that effect of combined treatment of chemicals and UV showed more injurious effect on the survival of the spores. With increasing time period of treatments, the survival percentage gradually decreased. The frequency of reverse mutation was found to increase in combined treatment of chemicals and UV radiation. In the case of sodium desoxycholate treatment, no reverse mutation was observed. Reverse mutants scarcely appeared in case of combined treatment of sodium deoxycholate and UV radiation.

The nature of the reverse mutants are also studied. Most of the reverse mutants showed wild growth without any nutritional requirement like the wild parent *A. chevalieri* in MM. The colour of the conidia in the reverse mutants are yellowish green as distinct from the wild parent and most of the reverse mutants showed slightly antibiotic activity.

D.2.2. *Killing and mutagenic effects on Ultraviolet radiation in three species of Aspergilli.*

The strains of fungi selected for the experiment are *Aspergillus aureus* (50567), *Aspergillus oryzeae* (17299) and *Aspergillus avanaceous* (16140) belonging to the groups *A. niger*, *A. flavus* and *A. wentii* respectively. The strains of the organisms selected, were first purified by single conidium micromanipulation. For each organism spore suspension was made from 8 days old culture in sterile aqueous calsolene (1 : 10,000) solution and Ringer's fluid and exposed to ultraviolet radiation for different time periods. The wavelength of the UV source was 2537Å. The irradiated spore suspension was diluted by sterile Ringer's fluid and plated out in complete medium (CM) immediately. Colonies were counted after 3 days incubation at 28°C. Colonies growing in CM were transferred to minimal medium (MM) for determining per cent biochemical and per cent morphological mutations. The results are given in Table 6 to 8.

D.2.3. *Recovery from Ultraviolet radiation damage by treatment with nucleic acid hydrolysate in Aspergillus spp.*

To show the modifying effects of nucelic acid hydrolysate on ultraviolet radiation in fungi, experiments with *Aspergillus niger* (V_{35}), *A. aureus* (50567), *A. oryzae* (17299), *A. avanaceous* (16140) and *A. terreus* (16043) were performed. The spores of the selected strains were treated by nucleic acid hydrolysates (concentration—1*ml* DNA+3*ml* RNA) per 100 ml MM) before, during and after irradiation. (a) *Pre-irradiation treatment*—The solution of

TABLE 6

ORGANISM—*A. aureus* (50567)

Incubation temperature—25°C; UV dose—100 ergs/mm²/sec.

Time of exposure to UV in mins.	0 (Control)	1	2	3	4	5	6
No. of viable spores	8×10^6	7×10^6	4×10^6	14×10^5	3×10^5	10×10^4	6×10^4
Per cent survival	100	86.4	50	17.5	3.75	1.2	0.75
No. of colony transferred on MM agar	112	112	112	112	112	112	112
Per cent biochemical mutant	0	0	.892	0	1.784	.892	1.784
Per cent morphological mutant	0	6.24	11.6	26.76	22.186	21.298	16.946
Total per cent mutation	0	6.24	12.5	26.76	23.97	22.19	18.73

nucleic acid hydrolysate in the required concentration was added to the spores and kept shaking for 6 hrs. at 28°C.

TABLE 7

ORGANISM—*A. oryzae* (17299)

Incubation temperature—25°C; UV dose—100 ergs/mm²/sec.

Time of exposure to UV in mins.	0 (control)	1	2	3	4	5
No. of viable spores	6×10^6	4.3×10^6	4.5×10^5	4×10^4	5.4×10^3	3×10^3
Per cent survival	100	71.7	7.5	0.66	.09	.05
No. of colony transferred on MM agar	112	112	112	112	112	112
Per cent biochemical mutant	0	0	.892	2.67	0	1.784
Per cent morphological mutant	0	4.46	13.38	19.624	11.59	8.92
Total per cent mutation	0	4.46	14.27	22.29	11.59	10.704

TABLE 8

ORGANISM—*A. avanaceous* (16140)

Temperature—25°C; UV dose—100 ergs/mm²/sec.

Time of exposure to UV secs.	0 (control)	30	60	90	120	150	180
No. of viable spores	6×10^6	3.75×10^6	6×10^5	20.4×10^4	7.2×10^4	3×10^4	12×10^3
Per cent survival	100	62.5	10.0	3.4	1.2	0.5	0.2
No. of colony transferred on MM agar	112	112	112	112	112	112	112
Per cent biochemical mutant	0	0	.892	.892	.892	0	0
Per cent morphological mutant	0	10.704	14.268	12.488	14.268	17.84	15.16
Total per cent mutation	0	10.704	15.16	13.38	15.16	17.84	15.16

After 6 hrs. the spores were washed with normal saline three times by centrifugation and then irradiated for the required time period, resuspending in sterile Ringer's fluid. The spore suspension was finally diluted and plated out in CM and incubated at 28°C for 2 to 3 days.

(b) *Treatment during irradiation*—The spores were centrifuged twice, Nucleic acid hydrolystate in desired concentration was added to the spores and irradiation was given for the required time period. The treatment fluid was rejected by centrifugation and spores were resuspended in sterile Ringer's fluid and diluted for plating in CM. The plates were incubated as usual.

(c) *Post-irradiation treatment*—The spore suspension in this case was subjected to UV irradiation for required time period and the nucleic acid hydrolysate in the required concentration was added. The flaasks were left as such in a shaking condition for 6 hrs. at 28°C. The spores were washed thrice by centrifugation and then after dilution plated out in CM. Besides these treatments two sets of control were set up (i) without UV irradiation and nucleic acid hydrolysate treatment and (ii) with irradiation but without any chemical treatment. The spores growing in CM were transferred to MM plates for determining the per cent mutation. The result is summarised in Table 9

From the above data it was found that the recovery against the damage due to UV radiation took place by treating the spores during irradiation with nucleic acid hydrolysate. So the second part of the experiment was planned to find out whether there was any change in the killing rate with the change of the concentration of nucleic acid hydrolysate. For this part of experiment nucleic acid hydrolysate solution was made at the following concentration per 100 ml. MM.

TABLE 9

Incubation temperature—26°C; UV Dose—100 ergs/mm²/sec.

Organism	A. niger	A. aureus	A. oryzae	A. avanaceous	A. terreus
Time of irradiation in mins.	12	4	3	2	1.5
			Per cent survival		
Treatment					
Nil	2.5	2.14	2.0	2.2	2.2
Six hrs. pre-irradiation	2.6	3.1	2.9	2.3	2.5
During irradiation	100	100	100	100	100
Six hrs. post-irradiation	2.5	2.1	2.9	1.89	1.64
			Per cent mutation		
Treatment					
Nil	15.2	14.046	16.604	11.784	14.678
Six hrs. pre-irradiation	17.136	18.596	18.98	12.784	15.568
During irradiation	14.16	12.244	14.46	11.784	10.0
Six hrs. post-irradiation	13.468	11.676	18.812	11.0	10.892

(1) Solution—0.025 ml [illegible]+0.075 ml RNA hydrolysate (stock)
(2) ,, 0.05 ml DNA+0.15 ml RNA
(3) ,, 0.1 ml DNA+0.3 ml RNA
(4) ,, 0.2 ml DNA+0.6 ml RNA
(5) ,, 0.4 ml DNA+1.2 ml RNA
(6) ,, 0.8 ml DNA+2.4 ml RNA

For each concentration, the solution of biochemicals was added to the spores, irradiated and then plated out after dilution in steps of 10. Colonies were counted after 2 or 3 days incubation at 28°C; per cent mutation was also determined. The results are given in Table 9.

The part of experiment showed that the killing rate decreased gradually with the increase in the concentration of nucleic acid hydrolysate and at higher concentrations complete protection against radiation injury was observed. Hence the next experiment was planned to show whether RNA is responsible for this recovery from radiation damage or DNA, or the effect was due to both of them combined.

The spores were irradiated separately with RNA stock soln. Conc—0.05 ml in 100 ml MM and DNA stock soln. Conc—0.05 ml in 100 ml MM and RNA+DNA Conc.—0.15 ml RNA+0.05 ml DNA in 100 ml MM. After dilution the spores were plated out on CM and incubated at 28°C. The results are given in Table 10.

From the above data it was observed that though DNA is little more active in recovery from radiation damage, survival percentage rose higher with the combined action of DNA and RNA.

TABLE 10A

Incubation Temperature—26°C; UV Dose—100 ergs/mm²/sec.

Organism		A. niger	A. aureus	A. oryzae	A. avanaceous	A. terreus
Time of irradiation (min.)		12	4	5	2	1.5
Conc./100 ml mm		Percent survival				
DNA (ml)	+ RNA (ml)					
Nil	Nil	2.3	1.95	2.15	1.94	1.95
.025	.075	2.8	4.2	4.27	4.33	4.1
.05	0.15	3.05	17.9	17.95	17.22	20.2
0.1	0.3	3.5	30	30.2	25.0	32.2
0.2	0.6	13.8	80	75.7	88.8	70.0
0.4	1.2	46.6	100	100	100	100
0.8	2.4	100	100	100	100	100
		Percent mutation				
Nil	Nil	17.136	18.0	15.892	16.932	15.352
.025	.075	18.028	21.384	17.678	18.73	18.46
.05	0.15	14.46	18.7	14.80	12.488	16.24
0.1	0.3	12.244	14.0	10.24	10.0	15.35
0.2	0.6	10.892	7.0	7.676	8.028	10.784
0.4	1.2	13.56	3.4	3.5	5.352	5.7
0.8	2.4	14.46	.892	0	3.7	0

TABLE 10B

Incubation Temperature—26°C; UV Dose—100 ergs/mm°/sec.

Organism	A. niger	A. aureus	A. oryzae	A. avanaceous	A. terreus
Time of irradiation (mins)	12	4	3	2	1.5
	Per cent survival				
Conc./100m/MM					
No chemical	2.5	2.14	2.0	2.1	2.2
.05ml/DNA	2.9	3.5	3.8	4.2	4.02
.05ml/RNA	2.0	2.1	2.2	2.5	2.3
0.15ml/RNA	3.1	10.1	12.18	10.9	11.56
0.5ml/DNA+ 0.15ml/RNA	4	19.2	18.9	17.6	20.0
	Per ce[illegible]tation				
No chemical	15.2	14.046	16.604	11.784	14.678
.05ml/DNA	16.568	15.164	17.84	12.48	11.92
.05ml/RNA	12.0	11.596	16.948	11.784	10.0
0.15ml/RNA	12.892	12.488	16.056	10.784	13.568
.05ml/DNA+ 0.15ml/RNA	16.568	16.056	12.5	13.38	13.38

D.2.4. *Studies on the recovery of radiation damage in Streptomyces sp.*

There are several factors which could modify the effects of UV irradiation in microorganisms *e.g.*. the presence of protective chemicals, the composition of the recovery medium, post-irradiation treatments of light, temperature etc. Besides these external factors, the physiological state of the organisms, the different phases of its growth cycle also control the extent of radiation response. In the present study an attempt was made to show that both the lethal and mutagenic effects of Ultraviolet irradiation (2537Å) in the *Streptomyces* strain can be reversed partially by the addition of chemicals to the plating medium.

The inorganic salts used were—NaCl, KCl, $CaCl_2$, $MgSO_4$ and $FeSO_4$ which were of analytical grade. The experiments were done in two ways by adding inorganic salts—(a) in the spore suspension to be irradiated and (b) in the recovery medium as reactivating agents. These two treatments were however, made independent of each other.

(a) For studying the effect of salts during irradiation, the spore suspensions were made in 10μ g/ml solution of each salt in double distilled water. The spore suspension prepared in the salt solution were then irradiated by Ultraviolet light at 2537Å (100 ergs/mm²/sec) for 20, 40, 60 and 80 seconds respectively. Plating was done in the (complete medium) CM medium,

(b) To determine the effect of these salts in the post-irradiated recovery medium, the original spore suspension was prepared in distilled water and after irradiation the salts at the concentration of 10μg/ml were added separately to the plating medium. The plating medium in all cases was CM prepared in distilled water.

Both the irradiated and control samples were incubated at 28°C for 8 days, after which the colony counts were made. Besides the lethality factor, the percentage of morphological and biochemical mutants were also studied by the usual method of "auxanography". The percentage survival and the mutation rate are represented in the following tables. From the results it was found that percentage survival increased fairly when the inorganic salts were added to the plating medium (Table 11). With NaCl and $MgSO_4$ maximum survival was obtained. When the above salts were added at the same concentration to the spore suspension before irradiation, percentage survival was also seen to increase with NaCl, KCl, $CaCl_2$ and $MgSO_4$. But with $FeSO_4$ no rise in the percentage survival was noticed, on the contrary lethality was observed. (Table 12). The rate of mutation, in general, was decreased with the rise of percentage survival.

TABLE 11

EFFECT OF SALTS ON UV IRRADIATION OF STREPTOMYCES STRAIN Ac_{21} 460 WHEN ADDED TO THE PLATING MEDIUM

Time of irradiation in seconds	CM		CM+NaCl		CM+KCl		CM+$CaCl_2$		CM+$MgSO_4$		CM+$FeSO_4$	
	Per cent											
	survival	mutation	survival	mutation	survival	mutation	survival	mutation	survival	mutation	survival	mutation
20	12.91	6.35	18.27	5.41	17.00	5.11	17.41	5.0	19.0	4.6	16.83	5.28
40	1.93	8.73	2.69	7.13	3.21	7.5	2.75	7.62	2.64	7.14	1.99	6.5
60	0.24	11.66	0.36	4.45	0.32	6.24	0.33	6.24	0.28	7.71	0.27	6.0
80	0.028	10.46	0.035	5.90	0.029	8.02	0.032	6.03	0.030	3.12	0.028	4.01

TABLE 12

EFFECT OF SALTS ON UV IRRADIATION WHEN ADDED TO THE SPORE SUSPENSION OF STREPTOMYCES STRAIN Ac_{21} 460.

Time of irradiation in seconds	Control		NaCl		KCl		$CaCl_2$		$MgSO_4$		$FeSO_4$	
	Per cent											
	survival	mutation	survival	mutation	survival	mutation	survival	mutation	survival	mutation	survival	mutation
20	12.91	6.35	15.21	5.34	14.21	4.0	14.17	4.16	13.97	4.01	11.10	6.65
40	1.93	8.73	2.30	6.64	2.22	6.87	2.32	6.0	2.44	6.66	1.88	7.66
60	0.24	11.66	0.26	7.19	0.27	5.64	0.24	6.34	0.25	5.80	0.16	8.17
80	0.028	10.46	0.030	7.34	0.028	6.24	0.027	6.5	0.028	7.81	0.018	11.45

D.2.5. *Induced UV mutation in Aspergillus regulosus*

A strain of *Aspergillus regulosus* was subjected to UV-irradiation with a view to develop mutant strains. The growth of the organism was luxuriant and the spore production was heavy in the culture medium. The culture became greenish-grey within 6–8 days after the production of conidial heads. Perithecia are large in size which form red-coloured ascospores.

Suspension of spores form 8 day old flask cultures (250 cc. flask with 50 ml. of liquid Czapek Dox medium) was prepared in aq. soln. of calsolene (1 : 10,1000). The suspension was then shaken for 30 minutes to break up spore clumps. The spore suspension was irradiated with a 4-watt G.E. germicidal lamp emitting 90% of the energy in the wave length 2537Å. Five ml. of the spore suspension was taken for irradiation for each lot. The time of exposure for irradiation was adjusted to 1,2,3,4 and 5 minutes after serveral experiments were done at different dosages. After irradiation the spore suspension was diluted in Ringer's solution and plated out in complete medium (CM). The colonies were counted after 2 days incubation at 28°C. Table 13(a) and 13(b) show the results of irradiation at different doses.

Total isolation. Biochemical mutants were isolated by the total isolation method of Fries (1948). The survivors developing on complete medium after UV-treatment were picked up by a forked needle and the inoculum was transferred onto minimal agar medium. Before inoculation the agar plates were placed on a piece of paper with intersecting parallel lines at equal distances. The inoculum from complete medium was carefully transferred at each intersect and the plates were kept at 28°C. Strains having nutritional deficiences failed to grow on minimal medium; colonies not growing in minimal media were transferred to complete media for further study. Strains differing from parent in morphology (form, colour, size etc.) were transferred to CD agar slants. The biochemical mutants could, however, be characterised by their failure to grow in minimal media.

TABLE 13(A)

EFFECT OF UV IRRADIATION ON *Aspergillus regulosus*

Exp. I	Time of irradiation in min.	Colony count	Survival per cent
	0	47×10^{-5}	100.0
	1	24.8×10^{-5}	52.7
	2	5.9×10^{-5}	12.6
	3	1.0×10^{-5}	2.1
	4	40×10^{-4}	0.7
	5	35.6×10^{-4}	0.7

TABLE 13(B)

EFFECT OF UV IRRADIATION ON *Aspergillus regulosus*

Exp. II	Time of irradiation in min.	Colony count	Survival per cent
	0	13×10^{-5}	100.0
	1	74.5×10^{-4}	57.3
	2	18×10^{-4}	13.8
	3	20×10^{-3}	1.5
	4	56×10^{-2}	0.4
	5	44.7×10^{-2}	0.3

Ten biochemical mutants were isolated and tested for the determination of their growth requirements for amino acids, vitamins and nucleic acids. The results are given in Table 14.

TABLE 14

Biochemical mutants and their growth requirements	*Index of Strains*
(1) Amino acid	U_6A
,,	U_3A_2
,,	U_3A_3
,,	U_7A_4
,,	U_7A_5
,,	U_7A_6
(2) Vitamin mixture	U_3V_1
,,	U_3V_2
(3) Nucleic acid hydrolysate	U_6N_1
,,	U_7N_2

The biochemical mutants were further tested for the determination of exact growth requirements of a specific compound either amino acids or pure constituents of nucelic acids.

TABLE 15

SPECIFIC GROWTH REQUIREMENTS OF MUTANTS

(1) *Amino acid*	*Index of strain*
DL-phenylalanine	U_6A_1
DL-methionine	U_3A_2
L-lysine HCl	U_3A_3
DL-phenylalanine	U_7A_4
L-lysine HCl	U_3A_5
L-cystine or l-arginine	U_7A_5
DL-methionine	U_7A_6
(2) *Nucleic acid components*	
Adenine & hypoxanthine	U_6N_1

In Table 15 it could be seen that most of the mutants isolated required amino acids for their growth and specific vitamin requirements are not characterised in the present test.

D.3. Microbial Genetics

D.3.1. *Physiological studies on Sordaria bosensis*

Cultural studies in liquid media with various modifications of the basal medium have been continued. The nature of growth response in culture tubes have been observed so that the media encouraging quickest growth could be used while isolating the probable mutants from large scale culture transfers of irradiated population.

Growth studies with A_1 medium with addition of fumaric acid 1.32 gm/l, sodium fumarate 1.82 gm/l, succinic acid 0.5 gm/l and increasing the quantity of glucose to 50 gm/l and NH_4NO_3 to 2 gm/l separately and together and various modifications of the same have revealed interesting comparative data.

Ten culture tubes with 5 ml liquid medium in each were prepared and kept at about 30°C after inoculation. The cultures were observed through 15 days.

It will be seen in Table 16 that all the media in Group I have responded well, in as much as, they have given compact surface growth. In Group II with 50 gm glucose in

TABLE 16

GROWTH RESPONSE OF SORDARIA BOSENSIS IN DIFFERENT LIQUID CULTURE MEDIA.

Group No.	Media	Degrees of growth	Remarks
I	A_1	+++	Compact
	A_1 + Fumaric acid 1.32 gm/l	++++	,,
	A_1 + Sodium fumarate 1.82 gm/l	++++	,,
	A_1 + Succinic acid 0.5 gm/l	++++	,,
II	A_1 with 50 gm glucose	++++	,,
	A_1 ,, ,, ,, + Fumaric acid 1.32 gm/l	+++	Submerged leaky
	A_1 ,, ,, ,, + Sodium fumarate 1.82 gm/l	+++	,, ,,
	A_1 ,, ,, ,, + Succinic acid 0.5 gm/l	++++	Compact
III	A_1 with 2 gm NH_4NO_3	++++	Compact
	A_1 ,, ,, ,, + Fumaric acid 1.32 gm/l	+++	Leaky
	A_1 ,, ,, ,, + Sodium fumarate 1.82 gm/l	+++	Medium growth
	A_1 ,, ,, ,, + Succinic acid 0.5 gm/l	++++	Compact
IV	A_1 with 50 gm glucose and 2 gm NH_4NO_3	++++	Compact
	A_1 ,, ,, ,, ,, + Fumaric acid 1.32gm/l	+++	Submerged
	A_1 ,, ,, ,, ,, + Sodium fumarate 1.82 gm/1.	++	,,
	A_1 ,, ,, ,, ,, + Succinic acid 0.5 gm/l	++++	Compact

++++ indicates wild type growth.
+++ medium growth.
++ poor growth.

common to all the media only A_1+50 gm glucose and also the one with succinic acid gave the best results others grow less, being submerged and leaky. In Group III the medium having 2 gm NH_4NO_3 also grew well. In this group fumaric acid and sodium fumarate give leaky and less vigorous growth respectively but succinic acid stimulates profuse growth. The last group *i.e.*, Group IV comprises of media having 50 gm glucose and 2 gm NH_4NO_3 and the other ingredients. The increased amounts of sugar and NH_4NO_3 give growth comparable to those of group I. Fumaric acid and sodium fumarate in group IV retard growth of mycelia which show a tendency of being submerged.

All the media with succinic acid prove to be favourable for compact surface growth as is found in media belonging to Group I. It is evident that succinic acid is a favourable ingredient in the medium encouraging heavy mycelial growth in all combinations so far tried.

From quantitative growth studies in flask cultures it has been previously found that although A_1 medium with 2 gm NH_4NO_3 and also succinic acid exhibit quick and profuse aerial growth, but give lesser mycelial weight as compared with those of other media having fumaric acid and sodium fumarate.

Experiments on induced mutations of Sordaria bosensis

Experiment on irradiation with UV rays has been done with 60,70,80 and 90 minutes treatments. Following the filtration enrichment technique large number of colonies were raised from time to time on A_2/48 complete medium and the colonies transferred to minimal liquid medium in tube twice consecutively to get them free from any trace of the ingredients of the complete medium. Maximum number of mutations have been found in 80 and 90 minutes of UV treatments. A detailed assay of the mutants is being done.

Amongst the mutants isolated previously from X-rayed ascospores it was observed that many of them responded to vitamins particularly biotin. But a tendency of reversion of the mutants to wild type has been noticed.

D.3.2. *Studies on UV-induced mutation in Penicillium vermiculatum*

Experiment on mutation by UV radiation has been continued with treatments of 2, 4, 6, 8 and 10 minutes with efficient shaking of spore suspensions in the specially designed vibratory shaker during irradiation. Maximum killing of spores has been observed at only 2 mins. duration of exposure to UV. The treated and control spores were plated on the complete medium and a 'total' transfer of colonies was done on the minimal medium devoid of any organic nutrient ingredients. Those inoculated cultures which grew very little or failed to exhibit growth were selected as probable biochemical mutants. By auxanographic tests the actual nutritional requirement of the mutants were determined. Some colonies grew on the minimal medium but had different phenotypic characters. These mutants differed from the parent type by their colonial habits, texture, colour and pigment production. On biochemical tests it has been found that some of these morphological mutants behave as biochemical mutants too.

D.3.2.1. *Classification of mutations*

(a) *Biochemical mutations*

(i) Mutations requiring more than one amino acid
(ii) ,, responding to more than one nucleic acids
(iii) ,, having alternate requirement for amino-acids or nucleic acids or vitamins
(iv) ,, having alternate requirement for amino acids or nucleic acids
(v) ,, having alternate requirement for amino acids or vitamins
(vi) ,, having alternate requirement for nucleic acids or vitamins

(b) *Morphological mutations*

(i) Mutations having buff organic, avellaneous, pink, grey, black and green shades
(ii) ,, showing scaly growth and albino character
(iii) ,, with irregular flucculent growth
(iv) ,, with crumpled colonial growth
(v) ,, with raised colonies
(vi) ,, producing various pigments such as yellow, brown and peach red

(c) *Biochemical mutations having altered morphological characters*

These mutations differ from the parent strain in their character for spore colour or texture but at the same time they have deficiencies in their nutritional abilities.

Detailed biochemical and physiological assay is in progress.

D.3.3. *Studies on the genus Chaetomium*

With a view to isolate the ascomycetous group of cellulolytic fungi namely members of the family Chaetomiaceae occurring in soil, a survey of soil from localities in and around Calcutta and other places of West Bengal was done. Following Ames's isolation technique with some modification, about seven species of *Chaetomium* have been isolated so far. Identification of a few of these species could not have been done as yet. However, out of these unidentified spp. two species exhibit interesting characteristics; one with broad osteolar neck having slender base of the perithecium, the terminal hairs of which are simple and sinuous, and the other with a net work of pseudodichotomous branching of terminal hairs which at maturity drop down thereby enveloping the perithecial body.

Following Eggins and Pugh's quantitative assay technique it has been notably observed that *Chaetomium indicum* is the most powerful cellulolytic member of all the isolated chaetomia.

For further studies all the available species of *Chaetomium* will be taken up with an aim to deriving out cellulolytic enzymes and the phenomenon of cellulolysis will be studied in relation to the genetical set up of the organisms.

D.4. Rhizobium bacteriophage and development of resistance strains

D.4.1. *Pot culture experiments on legume inoculation with phage-resistant Rhizobium strain*

The development of phage-resistant strains of *Rhizobium* was demonstrated and pot culture experiments were designed to undertake a preliminary studies of the effect of bacterial inoculation of seeds of (a) *Crotalaria juncea* and (b) *Cajanus indicus* both belonging to the cowpea group. The seeds of the legumes were tested for germination and it was noted that 96–98% of the seeds germinated and heathy plants were developed.

Soil from cultivated plots was collected and sieved after air-drying. The sieved soil was used for pot culture in all experiments. The pH and N_2 content of the soil was tested before the experiment was started. Surface sterilization of the seeds was done before sowing in each case. The phage resistant cultures of *Rhizobium* sp. for *Crotalaria juncea* and *Cajanus indicus* were freshly grown and applied to the roots of young plants which were 5 days old. Both resistant and non-resistant strains were applied and the methods followed were similar. The plants grown in pots were watered as often as it was necessary. The population was thinned out at the early stage and ten healthy plants were finally allowed to grow in each pot. Each experimental set had 5 pots and the data were collected from each pot by random sampling. The mean number of nodules and their mean sizes were recorded in each case and the morphological characters of the nodules were compared. The results obtained are given in Tables 17 & 18.

TABLE 17

TREATMENT OF HOST PLANTS WITH RESISTANT AND NON-RESISTANT NODULE BACTERIA IN *Crotalaria juncea*

Treatment	No. of plants examined	Mean number of nodules	Mean size of nodules	Morphological characters
Control (No inoculation).	50	6.2	(6×4)—(14×12)	Simple pink
Treated—phage resistant bacteria	50	12.8	(6×5)—(20×15)	Complex, pink colour
Treated—phage resistant bacteria	50	10.5	(6×4)—(16×12)	Complex, pink-rose colour
Treated—bacteriophage only	50	Nil	Nil	Nil

TABLE 18

TREATMENT OF HOST PLANTS WITH RESISTANT AND NON-RESISTANT NODULE BACTERIA IN *Cajanus indicus*

Treatment	No. of plants examined	Mean number of nodules	Mean size of nodules	Morphological characters
Control No. inoculation.	50	2.2	(3×2)—(5× 4)	Simple, elongated, round, pink.
Treated—phage resistant bacteria	50	4.9	(4×2)—(18×10)	,,
Treated—non-resistant bacteria	50	4.6	(3×2)—(14×10)	,,
Treated—bacteriophage only	50	Nil	Nil	Nil

It will be seen from the tables that among the treated plants there was significant difference between the plants treated with phage-resistant bacteria and those with non-resistant bacteria or control. The treatment with bacteriophage was in both cases, however, detrimental to nodule formation. The production of large-sized and increased number of nodules was an indication of higher rate of nitrogenfixation.

D.5. Ecology of air microorganisms

The monthly variations of different groups of microorganisms occurring in air have been determined by the culture plate exposure method and the medium used was malt extract agar (1.5%). The spores of organisms trapped on agar plates were incubated at the room temperature. For generic and specific identification, fungal isolates were subcultured and details of growth were worked out. Six's maximum and minimum thermometer and a hydrometer were employed for determining the daily temperature and relative humidity respectively. The accurate meteorological data including rainfall, wind velocity etc., were compiled from the records of the Meteorological office, Alipore, West Bengal. In Shyamnagar and Falta Experimental Stations the daily temperature records (maximum and minimum) were available.

It has been found, that in all the three localities, the culture plates showed lower counts of actinomycetes throughout the year, except in April 1965, when due to some other factors they were found to comprise a higher percentage of the total count of the colony in culture plates. However, it seems probable that the actinomycetes are not frequently distributed in air as the fungi and bacteria. On the other hand, the bacterial colonies are found to exceed the fungal ones specially in the summer and rainy months. In winter however, they follow just the other way, *i.e.*, the count of the fungi surpassed that of bacteria. In the report of the previous year the counts of the fungi were found to lower with the amount of the rainy season and they tend to rise during the winter months. The fungi newly recorded are *Beltrania* sp., *Aspergillus clavatus*, a form producing coremia, and some others whose generic identifications has been progressing. Systematic studies are being carried out for the determination of species of some fungi, whose identification upto genus has already been made. *Aspergillus* spp. were found to be very common during the summer and the rainy seasons but they gradually decrease as the temperature tends to lower. *Cladosporium* spp. follow just the opposite way *i.e.*, they are totally absent during the summer months and at lowering of temperature they would appear on the culture plates. *Penicillium* spp. were found to occur in more or less irregular frequency throughout the year. Although *Curvularia* spp. occurred throughout the year, the number was high during the winter months, *e.g.*, December and January while *Alternaria* spp. in January and continued upto April. *Nigrospora* spp. appeared intermittently first in October and after a pause of two months, reappeared in January persisting generally until the end of March. *Spicaria* spp. was commonly found in April, but it was restricted only to the sites of the Bose Institute, Calcutta. The remaining isolates did not follow any noticeable seasonal frequency. The sterile forms of fungi were common throughout the year, but they were usually found to predominate in the rainy season. The dead or diseased leaves and stems of the vegetation

in the localities of the different exposure sites, were collected and kept in moist chambers for a few days and were examined microscopically for the presence of the fungi, with a view to establish correlation between the isolated fungi of air *e.g.*, *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp. Among the isolated only *Alternaria* sp. was found to be present in the plant debris in large numbers even when they were absent or rarely occurred in the exposed plates.

D.5.1. *Determination of cellulose-decomposing ability of certain fungi isolated from air samples*

Ceratocystis paradoxa, an Imperfect fungi, was tested for its cellulolytic activtity. It was found that it could utilize cellulose as carbon source though the growth was not so pronounced. The growth remained restricted when it was grown in synthetic medium (CD) without the addition of any growth promotor. Detailed studies on the enzymatic activity of other fungi were carried out.

D.5.2. *Physiological studies of fungal isolates from air*

Ceratocystis paradoxa and *Penicillium* sp. (No. 11) were found to be biochemically deficient in some of the growth factors. Perhaps they are naturally occurring mutants. *Ceratosystis paradoxa* had been found to be deficient in amino acids and responded to amino acid mixture while *Penicillium* sp. (No. 11) required vitamin B complex for growth.

D.5.3. *Fungi as allergens*—Air-borne fungal spores are widely known to cause allergic conditions in susceptible persons. These spores produce allergic irritation when inhaled and thus cause asthma. For the determination of allergic properties, the spores of fungi occurring in air are trapped and studied. The fungi, which can grow on exposed Petri dishes containing agar medium are tested for allergenic principles. The following were isolated from exposed plates and they are tested for allergenic principles. (1) *Alternaria* sp., because of the large size of the spores and of high antigenic property, plays an important rôle in allergy. (2) *Cladosporium* sp. with its vast number of spores in air is equally significant in fungal allergy. (3) *Aspergillus* sp. is one of the first fungi considered in the etiology of allergy. (4) Reports are available for numerous skin reactions to *Aspergillus fumigatus*. (5) Skin reactions to *Penicillium* sp. are fairly common.

D.6. Industrial fermentation

D.6.1. *Surface fermentation of gluconic acid by strains of A. niger.*

Gluconic acid had been reported previously as a product of metabolism of *A. niger*. Specific strains of the organism would only produce this organic acid. Strains of *Penicillium chrysogenum* and *A. fumaricus* were also reported to have the capacity to produce gluconic acid in sugar media.

As a result of extensive screening, six strains of *A. niger* (black mould) were isolated which produced a reasonable quantity of the above organic acid. The type strain No. 67

of *A. niger* was grown along with the new isolates for a comparative study. The medium used contained the following:

Commercial Glucose	200—250 gms
$NaNO_3$	3.0 ,,
KH_2PO_4	0.30 ,,
$MgSO_4$, $7H_2O$	0.25 ,,
$CaCO_3$ (added later)	20.0 ,,

The time taken for conversion of 50% of glucose ranged from 3–4 days in the case of the isolated naturally occurring strains while the type strain 67 consumed the same amount of sugar within 36 hours. This indicates that there has been strain specificity and selection of a better strain would be an essential pre-requisite for success in gluconic acid fermentation.

The following Table (Table 19) will show some of the important results obtained in preliminary experiments.

TABLE 19

(GLUCOSE 25 GM/100ML) MEDIUM 100 ML IN A FLASK

Strain (A. niger)	Sugar consumed 50%	Gluconic acid produced*
Type culture 67	(Hours) 36	9.2 gm
40 (K)	56	5.2 ,,
112	72	6.2 ,,
106	72	6.9 ,,
502	96	4.6 ,,
763	92	5.0 ,,

* Calculated from calcium gluconate produced.

D.7. Transformation in microorganisms

D.7.1. *Transformation in A. niger*

Attempts have been made to transform the strains of *A. niger* with varying colour of aerial mycelium and conidia. Of the recipient strains which are mutants of *A. niger* V_{35} one is avellaneous (Q 30) and the other serpentine green (SG). They normally grew in minimal medium and no colour variation was noticed. From the donor strain *A. niger* (V_{35}) DNA was extracted by the method developed by Hotchkiss. The concentration of the DNA solution was tested before use and the stock preparation was stored at 5°C. The activity of the solution retained for 2 months.

The spores from the recipient strains were harvested and germinated in minimal medium at 30°C for 12 hours. The DNA was added at this stage, the concentration of which was 50μ g/ml. as determined spectrophotometrically. The flasks were incubated for another 6 hours and the material was repeatedly washed and centrifuged. The treated spores were then plated in minimal medium. The plating had to be continued for sometime as large number of plates were required to detect the transformants. The Table 20 shows the results.

TABLE 20

TRANSFORMATION OF *A. niger* STRAIN S.G. AND Q30

Recipient strian	No. of spores treated (per ml.)	DNA conc. µg/ml.	No. of transforments (scored)	% Transformation
(I) S.G.				
(a) Control	3×10^3	0	0	0
(b) Treated	3×10^3	50.0	18*	0.6
(II) Q 30				
(a) Control	3.3×10^3	0	0	0
(b) Treated	3.3×10^3	50.0	3**	0.09

* Colour (1) White and yellow (2) Light yellow.
** Colour (1) White (2) Light brown.

D.7.2. *Transformation of Staph. aureus from penicillin sensitive to penicillin resistance*

Resistant strains of *Staph. aureus* were developed by combined treatment of UV-irradiation and penicillin treatment. The resistant strains could grow in medium with 200 units of penicillin. The character for penicillin resistance was not altered in subsequent studies. The resistant mutants were grown for 20 successive generations without penicillin but changes in the character were not found to occur.

TABLE 21

DNA CONC. 125 µg/ml COUNT OF RECIPEINT *S. aureus* (SENSITIVE)

Hours of incubation	No. of transferred colonies (average) per plate (0.1 ml culture added)	Per cent transformed
0	Nil	Nil
6	4	.04
18	14	0.14
36	10	0.1
72	10	0.1

Of the resistant mutants of *Staph. aureus*, two strains were selected and from one DNA was extracted by the method of Marmur. The concentration of the extracted DNA was determined spectrophotometrically and experiments were conducted to grow the sensitive strains (inhibited at 0.5 unit of penicillin per ml) in medium containing DNA of the resistant strains. The cultures were incubated at 37°C and mutrient broth was used as medium all through the experiments. The results are presented in Table 21.

As it was difficult to reproduce the results, the experiments were repeated many times and it was ultimately found that the competence of the strain was variable. This was due to cultural conditions and critical incubation temperature. The rate of transformation in *Staph. aureus* has been increased in subsequent experiments using yeast ext. as growth factor with culture medium.

D.8. Physiology of microorganisms

D.8.1. *Studies on phosphate solubilising fungi from rhizosphere and field soil.*

Recently phosphorus problem has drawn attention of soil scientists and ways and means are being worked out to increase the availability of added or the native phosphates present in soil. The addition of organic and living matter has produced some effect though the real problem has not been solved.

Attempts have been made in India and other countries to define the role of micro-organisms in solubilizing fixed and native phosphates in soil. The information that *Bacillus megaterium* var phosphaticum can solubilize and render otherwise or available phosphates, available to plants, yet its exact role under field conditions remains to be defined. As for fungi, the picture is still obscure. However, it is evident from the works done that fungi can play a role in phosphate solubilization but thorough investigations on the phenomenon has to be worked out.

The present work was undertaken to study the indegenous and zymogenic fungi capable of solubilizing insoluble inorganic and organic phosphates and the mechanisms of solubilization and their possible role in enhancing crop yield.

Soil and plant root samples were collected from the following Experimental farms:— (a) Chinsurah, (b) Kalyani, and (c) Berhampur. Soil samples from Kanke, Ranchi, (Bihar) were received through the courtesy of the members of the staff to the Ranchi Agricultural College. Isolations of fungi were carried out according to the standard procedure on glucose-peptone agar medium supplemented with streptomycin 20μg/ml. to control bacterial growth. So far 140 fungal isolates have been isolated and 80 tested for phosphate solubilizing activity. Phosphate solubilizing capability was determined after the modified method of Katznelson and Bose (1959). Out of 80 isolates 16 have shown clearance zones, indicative of phosphate solubilization. Though studies so far are not conclusive, it indicates enzymatic activity of fungi solubilizing insoluble phosphates in soil. It is of interest to note that besides bacteria and fungi, actinomycetes are also capable of dissolving insoluble phosphates and their possible role in this important soil process remains to be worked out.

D.8.2. *Studies on toxic substances in infected paddy samples collected from Nadia District, West Bengal.*

It was reported from the authorities of the said district that persons taking dark brown rice with a distinct smell died and several families were severely affected. The following studies were made.

(a) Microscopic examination of the teased samples of paddy revealed the presence of fungal structure (spores and mycelium).

(b) Moist chamber studies showed that within 40 hours, fungal growth was evident and microscopic examinations indicated that the organisms belonging to the genus *Aspergillus* and members of *Dematiaceae* groups were present in culture plates.

(c) Isolation of fungi in pure culture were made in Sabouraud's agar with streptomycin (20μ g/ml.) from whole paddy seeds, husks and grains. All the colonies were isolated and observations are made till fifteen days of incubation. At this stage particular emphasis was laid on pigment producing isolates. Three such isolates belonging to *Dematiaceae* were selected out for further studies.

(d) In the study of colonization of paddy seeds and rice grain it was seen that all the three isolates colonized paddy seeds and rice grains fairly well and rendered them blackish and deep yellow.

E. ANIMAL PHYSIOLOGY

E.1. Physiological studies on the liver and thyroid of tadpoles (Rana tigrina and Bufo melanostictus)

E.1.1. *Histochemical studies on the liver of tadpoles*

E.1.1.1. *Alkaline and acid phosphatase activity in the liver of tadpoles at various stages of development.*

Histochemical studies on the activity of the enzymes, alkaline and acid phosphatases in the liver of tadpoles at various stages of development were made. Attempts were made to correlate the activity of the enzymes with the gradual progress of the tadpoles towards metamorphic stages.

The tadpoles of *Rana tigrina* and *Bufo melanostictus* at various stages of development were selected. The seven different stages of tadpoles as used in the present as well as in the following experiments and their corresponding stages described by Taylor and Kollros are given in Table I.

TABLE 1

DIFFERENT STAGES OF TADPOLES USED AND THEIR CORRESPONDING STAGES (AFTER TAYLOR AND KOLLROS)

Stages designated		Stages in larval development of *Rana pipiens* (after Taylor and Kollros)	
No.	Corresponding stage number according to the classification of Taylor and Kollros	General designation	Stage number
N_1	V	Limb-bud stages	I—V
N_2	IX or X	Paddle stages	VI—X
N_3	XII or XIII (with small hind limbs)	Foot stages (Premetamorphic stages)	XI—XVII
N_4	XVII (Full-grown hind limbs)		
N_5	XX (with four limbs)	Metamorphic stages	XVIII—XXV
N_6	XXII or XXIII (with four limbs and partially retracted tail)		
N_7	XXV (Completely metamorphosed tadpoles; froglet).		

The results of alkaline and acid phosphatase activities in different stages of development of tadpoles are shown in Table 2. Similar pattern of distribution of particular enzyme

was observed in both the species of tadpoles under study. The result of all individual animals at a particular stage was also approximately similar.

TABLE 2
ALKALINE AND ACID PHOSPHATALE ACTIVITY IN LIVER OF TADPOLES AT VARIOUS STAGES OF DEVELOPMENT

Stage	Alkaline phospahtase activity	Acid phosphatase activity
N_1	+++	++++
N_2	++++	+++
N_3	++	++
N_4	+	+
N_5	+++	+++
N_6	++	++++
N_7	+++	++++

++++ = Maximum phosphatase activity.
\+ = Minimum phosphatase activity.

It is apparent from Table 2 that the activity of either alkaline or acid phosphatase in the liver of tadpoles was not at the same level during the different stages of larval life. When the tadpoles overcame the limb-bud stages (stage N_1) and reached the paddle stages (stage N_2), alkaline phosphatase increased but acid phosphatase was reduced. This decrease of the latter enzyme was found to be more and more prominent during the growth of hind limbs (stages N_3 and N_4), and reached the lowest value when the hind limbs appeared to be fully grown (stage N_4).

The alkaline phosphatase also started to decline from the beginning of the premetamorphic stages (stage N_3) and attained the minimum level at the end of these stages (stage N_4). There occurred an enhancement of the level of both the enzymes at the time of emergence of fore limbs (stage N_5). The acid phosphatase showed this gradual rise during the metamorphic stages (stages N_5–N_7) and reached the maximum when the tadpoles completely metamorphosed (stage N_7). It appears, therefore, that this enzyme was more or less at the same level of activity at the limb-bud stages (stage N_1) as well as at the later period of metamorphic stages (stages N_6 and N_7). But in case of alkaline phosphatase a fall was again noticed during the regression of the tail (stage N_6), followed by a rise when the tadpoles just developed into tiny froglets (stage XXV of Taylor and Kallros). Maximum alkaline phosphatase activity was observed at the end of paddle stages (stage N_2).

E.2. Protein, RNA and DNA contents in liver of tadpoles at different stages of larval life

Protein, RNA and DNA contents of liver of tadpoles vary at different stages of development. The results are given in Table 3. The individual tadpoles of the two species at a particular stage showed similar results.

TABLE 3
PROTEIN, RNA, AND DNA CONTENTS OF LIVER OF TADPOLES AT VARIOUS STAGES OF DEVELOPMENT

Stages	Protein	RNA	DNA
N_1	+++	+++	++
N_2	+++	+++	++
N_3	++	++	++
N_4	+	+	+++
N_5	+++	+++	++++
N_6	+	++++	++++
N_7	+++	+++	++++

\+ = Minimum activity
++++ = Maximum activity

Protein was found to be more or less at the same level at limb-bud stages (stage N_1), paddle stages (stage N_2), and at metamorphic stages (stages N_5 and N_7) excepting during the regression of tail (stage N_6) when a sharp fall was noticed. It started to decrease from the beginning of foot stages (stage N_3) and reached the minimum level at the end of these stages (stage N_4), which was similar to that found at stage N_6. In case of RNA nearly similar pattern of changes as protein was noticeable throughout the period of larval life of tadpoles, the important difference between the two being that instead of the lowest value of RNA at stage N_6 as observed in case of protein, this nucleic acid was found to be maximum during the regression of tail (stage N_6). DNA was at the same level up to the beginning of foot stages (stages from N_1 to N_3) and started to rise from the end of these stages (stage N_4). The maximum level of DNA was attained at the time of emergence of fore limbs (stage N_5) and maintained till the time of complete metamorphosis (stage N_7).

E.3. Level of glycogen in liver of tadpoles at various stages of development

The glycogen in liver of tadpoles was not at the same level of distribution at various stages of development. Glycogen was found to be more or less at the same level up to the end of premetamorphic stages, excepting at the beginning of foot stages (stage N_3) when a slight rise was observed (Table 4). But at the time of appearance of fore limbs (stage XX of Taylor and Kollros) it showed a marked rise (stage N_5). The sharp fall of glycogen was found during the retraction of tail (stage N_6), followed by a sharp rise, when the tadpole exhibited complete metamorphosis (stage N_7). It appears that both the species of tadpoles store glycogen in liver till the emergence of front legs and only during the resorption of tail, the stored glycogen is rapidly utilized. Maximum level of glycogen in liver was found when the tadpoles showed just emergence of fore limbs (stage XX) as well as complete metamorphosis (stage XXV of Taylor and Kollros).

TABLE 4

LEVEL OF GLYCOGEN IN LIVER OF TADPOLES AT VARIOUS STAGES OF DEVELOPMENT

Stages	Glycogen level
N_1	++
N_2	++
N_3	+++
N_4	++
N_5	++++
N_6	+
N_7	++++

++++ = Maximum glycogen level
\+ = Minimum glycogen level

E.4. Melanin and tyrosine contents in liver of tadpoles at various stages of development

Similar pattern of changes were observed in case of both melanin and tyrosine. These two substances gradually decline during the growth of hind limbs and reached the lowest level at the time of emergence of fore limbs. Thereafter, melanin and tyrosine began to rise and became maximum when metamorphosis was completed. Further studies are being made.

E.4.1. *Histochemical studies on the liver of tadpoles under penicillin, vitamin B_{12}, thyroxine and thiourea treatments*

Histochemical studies on the alkaline and acid phosphatases, protein, RNA, DNA, glycogen, melanin and tyrosine contents in liver of tadpoles under the influence of penicillin, vitamin B_{12}, thyroxine and thiourea treatments are being made. Preliminary results indicate that thyroxine and vitamin B_{12} cause increase of alkaline and acid phosphatases, and thiourea and penicillin cause a fall of these enzymes. Glycogen is gradually stored in the liver up to the time of emergence of fore limbs, as found in normal stages of development, but it is rapidly utilized during the regression of tail. The various results show that the variations of the level of these substances at various stages of development of treated tadpoles are in the same pattern as found in normal stages of larval life. The interesting point which is revealed from the histochemical studies in normal and treated conditions is that any fluctuation of the above substances in liver of tadpoles is largely dependent upon the stage, which the tadpole has attained.

E.4.2. *Histometric studies on the liver of tadpoles at various stages of development*

The cell area and the nuclear area of liver of tadpoles at various stages of development were measured. The exact area of μ^2 of each cell and nucleus was determined. The

mean results show that as the stages of tadpoles are advanced towards metamorphic stages, both the cell area and the nuclear area gradually increased. There is a definite relationship between the cell area and the nuclear area. The percentage of nuclear area to total cell area is roughly 10 per cent. It is interesting to note that between the time of emergence of fore limbs and the complete absorption of tail, the rate of rise of nuclear area is very marked. This rapid rate of rise of nuclear area during the regression of tail is accompanied by a rise of RNA and DNA and also acid phosphatase. The work is in progress.

E.4.3. *Histological studies on the activity of thyroid glands of tadpoles*

It has been reported previously that the activity of thyroid glands of tadpoles gradually increases as the tadpoles advance towards metamorphic stages. Penicillin treatment causes depression of activity of thyroid glands and vitamin B_{12} stimulates the activity of these glands. The metamorphosis of tadpoles was inhibited by thiourea treatment because the thyroid glands of these larvae became atrophied by such treatment. Considering the inhibitory action of thiourea on the thyroid glands and the metamorphosis inducing action of thyroxine, studies have been undertaken on the activity of these glands in tadpoles under various doses of thiourea and thiourea plus thyroxine treatments.

F. LIBRARY

The library contains 7343 volumes (3421 books and 3922 bound periodicals). During the year under review 824 volumes (359 books and 465 bound periodicals) were added to the stock as against 481 volumes during the previous year. The total number of periodicals received was 264 out of which 147 were subscribed and the remaining 117 were received either in exchange of Institute publications or as gifts. The library also received a fairly good number of technical pamphlets, circulars, reports, etc. from various institutions and governments, both Indian and foreign.

The library was kept open for 61 hours a week from 9 a.m. to 8 p.m. on weekdays and from 9 a.m. to 3 p.m. on Saturdays.

According to rules the library is primarily meant for use of its research staff; but it is frequently consulted by many research workers and scientists from other institutions. As the number of outside readers are increasing provision has been made to admit, on the basis of reader's card, bonafide outside research workers and scientists for use of its Reading Room only.

The Library grant received during the year was Rs. 60,000.00 (Rs. 30,000.00 as recurring and Rs. 30,000.00 as non-recurring). To meet the pressing demands of readers for more books and journals the recurring grant was ear-marked for subscriptions to journals, annual reviews and binding of books and periodicals. The non-recurring grant was spent on purchasing of books, back volumes of journals and important reference work like the sets of "Handbuch der Physik" and "Landolt-Bornstein".

A library Committee was formed with the Heads of Departments as its members and the Librarian as Secretary for the purpose of advising the Librarian on all matters connected with the library; three meetings of the Committee were held during the year.

PUBLICATIONS

During the year under report Vol. 25, No. 4 (December 1962 issue) and Vol. 26, Nos. 1 & 2 (March and June 1963 issues) of the Transactions of the Bose Research Institute were published.

Several important books and monographs of Jagadis Chandra Bose are out of print for some time. Following a plan of reprinting them, the popular accounts written by Jagadis Chandra of his investigations "Plant Autographs and their Revelations" and "Response in the Living and Non-Living" have already been published. It is now proposed to take up the reprint of the monograph "Comparative Electrophysiology". A collection of four lectures delivered under the auspices of Jagadis Chandra Bose Endowment Lectures, Series II (1962-63), has already been published.

Other publication during the year was "Report of the Bose Institute for 1963-64".

G. WORKSHOP

During the year under report about 600 requisitions were received from the different departments of the Institute. Most of them were completed. The following is a list of some major works.

1. Conical lead filters and holders of high active gamma sources
2. Glass apparatus for the measurement of self-diffision coefficient in liquids
3. Photo multiplier mounting assembly with perspex light guide, voltage divider, shield etc.
4. Modification of some components of the 14 Mev neutron Generator
5. Components of Gas phase chromatography apparatus
6. Thin layer chromatography apparatus
7. Improved design of Nossal shaker
8. Paper Electrophoresis apparatus
9. Vacuum locks
10. Special type Gas burners
11. Some spares of Refrigerators and Air conditionears

Besides these, the workshop was heavily engaged in repair of costly and precision imported instruments such as Serval Centrifuge, Warburg Apparatus, Refrigerated Centrifuge, Microscopes, Recorders, Vacuum pumps, Air Compressors, etc.

In addition to the maintenance of Refrigerators, Air Conditioners and other refrigerated equipments, the Refrigeration Section has completed the installation of on 0°/21°C Cold Rooms for the Chemistry Department.

The new Electronic Section is also maintaining the electronic instruments of the Institute.

The Electrical Section is maintaining all the electrical installations including the repairs of Electrical equipments of the laboratories.

In glass blowing section many special types of glass apparatus were made and repairing works were done as usual.

In the Carpentry Section special types of furniture and fittings for laboratories were made.

Usual maintenance and check up of Diesel Engine pumping units at Shamnagar and Falta Experimental Stations and Electrical pumping unit at Birati Experimental Station were carried out.

APPENDIX A

I. The Govrning Body

Dr. Debendra Mohan Bose.
Dr. Sunando Bose.
Shri Kedar Nath Chatterjee
Shri Saibal Kumar Gupta, I.C.S. (*Retd.*)
Prof. Satis Ranjan. Khastgir
Shri Sudhir Kumar Mandal
Shri Amiya Nath Mitra
*Shri Abani Nath Mitter
Shri S. C. Mukherjee, I.C.S. (*Retd.*)
Dr. Basanti Dulal Nagchowdhuri
Prof. Bhupati Mohan Sen, I.E.S. (*Retd.*)
Dr. Jatish Chandra Sengupta

II. The Council

Shri K. N. Chatterjee
Shri S. K. Mandal
*Shri A. N. Mitter
Dr. B. D. Nagchowdhuri
Prof. B. M. Sen
Dr. J. C. Sengupta
} Nominees of the Governing Body

Dr. D. M. Bose, Director, Bose Institute (*Ex-officio*)
Secretary to the Government of India, Ministry of Education or his nominee.
Director-General, Council of Scientific and Industrial Research or his nominee
Shri P. R. Ramakrishnan, M.P.
Prof. P. Parija, Vice-Chancellor, Utkal University
Prof. G. N. Ramachandran, Madras University
} Scientists from outside West Bengal

Shri K. N. Channa, Financial Adviser to the Ministry of Education
Accountant General, West Bengal
Prof. P. C. Mukherjee, Director of Public Instruction, Govt. of West Bengal — Representative of the Govt. of West Bengal
Shri N. D. Sreemany (Representative of the Calcutta Corporation)

* Died on 11th November 1964.

III. The Finance Committee

Prof. B. M. Sen (Representative of the Council)
Accountant General, West Bengal
Shri K. N. Channa, Financial Adviser to the Ministry of Education
Dr. D. M. Bose, Director (Ex-officio)
Registrar, Secretary.

IV. The Building Committee

Dr. D. M. Bose, Director (Ex-officio)
Shri K. N. Chatterjee (Representative of the Council)
Shri A. N. Mitter (Representative of the Governing Body)
Registrar, Secretary.

APPENDIX B

Director: Dr. D. M. Bose, M.A., Ph.D., F.N.I.

Jt. Director: Dr. P. K. Bose, D.Sc., F.N.I.

Registrar: Shri N. Das Gupta, B.Sc., B.L.

Department of Physics

Head of the Dept.: Dr. S. R. Khastgir, Ph.D., D.Sc., F.N.I.

Reader: Shri A. M. Ghose, M.Sc.

Research Fellow: Dr. T. C. Bhadra, M.Sc., D.Phil. (on study leave)
Shri P. V. Manoranjan Rao, M.Sc.

Research Scholar: Shri A. K. Chatterjee, M.Sc.
Mary & Richard
Keating Scholar: Shri Amitava Ghosh, M.Sc.

Department of Chemistry

Head of the Dept.: Dr. A. Sen, M.Sc., Ph.D. (Leeds)

Reader: Dr. A. K. Barua, M.Sc., D.Phil.
Dr. Sudhamoy Ghosh, M.Sc., D.Phil.

Research Fellow: Shri J. Dutta, M.Sc.
Dr. D. P. Chakrabarty, M.Sc., D.Phil.

Research Assistant: Shri H. C. Chakrabarty, B.Sc.

Research Scholar: Shri Debdutta Roy, M.Sc.
Shri Bejoy Kr. Chowdhury, M.Sc.
Shri Deb Kr. Mukherjee, M.Sc.
Shri Shib Prosad Dutta, M.Sc.

Govt. of India Scholar: Shri Asoke Ch. Ghosh, M.Sc.
Shri Basudev Das, M.Sc.
Shri Asis Dutta, M.Sc.
Sm. Sujata Roy, M.Sc.

C.S.I.R. Pool Officer: Dr. Maharani Chakravarty, M.Sc., D.Phil.
Dr. M. K. Pal, M.Sc., D.Phil.

Guest Workers: Prof. S. N. De, M.B., D.T.M., Ph.D. (Lond.), D.Sc. (Lond.)
Dr. P. N. Das, M.B.B.S.
Dr. D. P. Burma, M.Sc., D.Phil.

Sir J. C. Bose Fellow: Dr. S. R. Ghoshal, M.B.B.S., D.C.H. (Calcutta University)

Attached Workers: Shri Sudhir Kumar Ghosh, M.Sc.
Dr. S. D. Bhattacherjee, M.Sc., D.Phil
Sm. Anima Dutta, M.Sc.

Department of Botany

Head of the Dept.: Dr. Sunando Bose, M.Sc. (Cal.), Filosofic Doctor (Lund.)

Reader: Dr. B. B. Biswas, M.Sc., D.Phil

Research Fellow: Dr. R. K. Basu, D.Sc.
Dr. M. C. Basu, M.Sc., D.Phil
Dr. V. N. Gadgil, M.Sc., D.Phil

Statistician: Shri A. K. Roy Chowdhury, M.Sc.

Research Assistant: Shri A. T. Guhathakurta
Shri B. K. Dutta
Shri A. K. Adhikari, M.Sc.

Research Scholar: Shri B. R. Banerjee, M.Sc.
Shri S. S. Sarkar, M.Sc.
Sm. Sheela Sen, M.Sc.
Sm. Susweta Biswas, M.Sc.
Sm. Anima De, M.Sc.

Govt. of India Scholar: Sm. Pratima Roychowdhuri, M.Sc.
Shri Kali Krishna Ghoshal, M.Sc.
Shri Pritisadhan Bose, M.Sc.

Pool Officer: Dr. J. G. Bhowal, M.Sc. D.Phil

Department of Microbiology

Head of the Dept.: Dr. P. N. Nandi, M.Sc., Ph.D. (Lond)., D.I.C.

Research Fellow: Shri K. L. Chowdhury, M.Sc.
Dr. A. K. Mishra, M.Sc., D.Phil (on study leave)
Dr. R. K. Sinha, M.Sc. D.Phil

Research Assistant: Shri S. L. Chakraborty, M.Sc.
Shri A. K. Chatterjee, M.Sc.

Research Scholar: Sm. Kalyani Sen, M.Sc.
Sm. Jharna Mitra, M.Sc.
Sm. Roma Barat, M.Sc.
Shri B. R. Maity, M.Sc.

Birla Scholar: Sm. Gita Nanda, M.Sc.

Govt. of India
Scholars: Sm. Ujjaini Chowdhury, M.Sc.
Shri Nirmal Kanti Shaw, M.Sc.
Shri Panchu Gopal Roy, M.Sc.

Department of Animal Physiology

Fellow Research: Dr. A. K. Medda, M.Sc., D.Phil
Research Scholar: Sm. Roma Ghosh, M.Sc.
Attached Worker: Shri G. C. Bhattacherjee

Workshop

Superintendent: General—Shri M. L. Chatterjee
-do- : *Electrical and Refrigeration*—Shri S. R. Ghosh

Library

Librarian: Shri P. K. Chakraborty
Asst. Librarian: Sm. Ashoka Das Gupta, B.A.
Library Assistant: Sm. Protima Chakrabarty

STAFF APPOINTED UNDER GRANT-IN-AID SCHEMES

A. Department of Atomic Energy, Govt. of India

Scheme I: Nuclear Physics investigations with Neutron Generator
Investigator-in-charge: Shri A. M. Ghose, M.Sc.
Shri B. Mitra, M.Sc.
Junior Scientific Officer: Dr. Amalendu Nath, M.Sc., D.Phil
"Biosynthesis of Polyneucleotides in Microorganisms"
Investigator-in-charge: Dr. D. P. Burma, M.Sc., D.Phil
Research Assistant: Sri Samir Kr. Mitra and Sm. Manjushree Bal

B. Indian Council of Agriculture Research

1. The role of lipoic acid in plant metabolism with radioactive traces"
   Investigation-in-charges: Dr. D. P. Burma, M.Sc., D.Phil
   Research Assistant: Shri Sujit Kr. Chowdhury (upto 31.3.65)
2. Studies on the occurrence of Rhizobium bacteriophage and its effect on host legumes grown in Indian soils.
   Investigator-in-charge: Dr. P. N. Nandi, Ph.D. (Lond.)
   Junior Research Assistant: Sm. Itu Sanyal, M.Sc.

C. Council of Scientific and Industrial Research

1. "Biosynthesis of Soluble RNA"
   Investigator-in-charge: Dr. D. P. Burma
   Junior Research Fellow: Sri Utpalendu Sekhar Maitra, M.Sc. (upto 28.2.65)
2. Application of Gas chromatography to (1) the analysis of fatty acid....mixture"
   Investigator-in-charge: Shri J. Dutta, M.Sc.
   Junior Research Fellow: Sm. Anita Ghosh, M.Sc.
3. "Physical chemical investigation on milk proteins etc".
   Investigator-in-charge: Dr. A. Sen, M.Sc., Ph.D. (Leeds)
   Senior Research Fellow: Sm. Sheela Chowdhury
4. Chemical Examination of Indian Medicinal Plants belonging to the families etc."
   Investigator-in-charge: Dr. D. P. Chakraborty, M.Sc., D.Phil.
   Senior Research Fellow: Dr. Kalachand Das, M.Sc., D.Phil.
5 Studies on the chemical constitution of some antibiotics isolated from Streptomyces sp."
   Investigator-in-charge: Dr. P. K. Bose, D.Sc., F.N.I.
   Senior Research Fellow: Dr. (Miss) Parul Chakraborty, M.Sc., D.Phil

6. Studies of the Frequency Power Spectrum of Lighting discharges:
Investigator-in-charge: Prof. S. R. Khastgir, Ph.D., D.Sc., F.N.I.
Junior Research Assistants: Shri Deepak Niyogi, M.Sc.
-do- Shri Janakinandan Maiti, M.Sc.

7. Study of sporadic E-region in the Ionosphere:
Investigator-in-charge: Prof. S. R. Khastgir, Ph.D., D.Sc., F.N.I.
Senior Research Fellow: Shri Himansu Bhattacharya, M.Sc. (till Jan. 31, 1965)
-do- Shri Salil Kumar Banerjee, M.Sc. (Feb. 1965)
Junior Research Fellows: Shri Suman Ganguly, M.Sc.
-do- Shri Dwipal Chandra Mandal, M.Sc.

8. Studies on semi-large scale production of antibiotics from strains etc.
Investigator-in-charge: Dr. P. N. Nandi, Ph.D., (Lond.)
Research Assistant: Shri Sankar Kr. Mukherjee, M.Sc.

9. Studies on the recovery from radiation damage in microorganisms.
Investigator-in-charge: Dr. P. N. Nandi, Ph.D., (Lond.)
Junior Research Fellow: Sm. Malati Mazumdar

10. Effects on Ultraviolet light on Transforming etc".
Investigator-in-charge: Dr. P. N. Nandi, Ph.D., (Lond.)
Junior Research Fellow: Sm. Meena Guha, M.Sc.

11. Phytin synthesis of nucleic acid and differentiation
Investigator-in-charge: Dr. B. B. Biswas, M.Sc., D.Phil
Senior Research Fellow: Sri R. Roychowdhury, M.Sc.

12. "Enzymatic synthesis of RNA and its role in protein synthesis"
Investigator-in-charge: Dr. B. B. Biswas, M.Sc., D.Phil
Junior Research Fellow: Shri A. K. Chakraborty, M.Sc.

APPENDIX C

LIST OF PUBLISHED PAPER 1964-65

Title of papers	Authors	Where published	Reference: Vol. page and year
1. Occurrence of genetic polymorphism in the milk proteins of Indian zebu cattle.	S. D. Bhattacharya, N. K. Sinha and A. Sen	Symposium on polymorphism of serum proteins, Proc. of the V International Congress for Animal Reproduction and Artificial Insemination, September, 1964, Trento, Italy	In Press
2. The constitution of barringtogenol C.	A. K. Barua, Parul Chakrabarti	*Sci. & Cult.*	**30,** 332, 1964
3. Chemical Investigation of Mollugo spergula	Parul Chakrabarti, A. K. Barua	*Ind. J. Chem.*	**2,** 339, 1964
4. The constitution of barringtogenol C.	A. K. Barua, Parul Chakrabarti	*Tetrahedron*	**21,** 381, 1965
5. Isolation of Spergulagenic acid—a new sapogenin from Mollugo spergula.	Parul Chakrabarti, Deb Kumar Mukherjee, Ratneswar Chatterjee, A. K. Barua	*Ind. J. Chem.*	**3,** 283, 1965
6. Studies on the constitution of Spergulagenin A—A New Sapogenin from Mallugo spergula.	Parul Chakrabarti, A. K. Barua	*J. Ind. Chem. Soc.*	**42,** 137, 1965
7. On the constitution of Murrayanine, a carbazola derivative isolated from *Murraya koenigii* Spreng.	D. P. Chakraborty, B. K. Barman and P. K. Bose	Tetrahedron	**21,** 681, 1965
8. On the structure of Girinimbine, a pyrano-carbazole derivated, isolated from *Murraya koenigii* Spreng.	D. P. Chakraborty, B. K. Barman and P. K. Bose	Sci. & Cult.	**30,** 445, 1964
9. Chemical Taxonomy Part III: Isolation of mahoganin, a non-bitter constituent of *Swietenia mahogani*.	D. P. Chakraborty and B. K. Barman	*Sci. & Cult.*	**31,** 241, 1965
10. Separation of some natural coumarins by thin layer chromatography.	J. Dutta, M. Hoque, D. P. Chakraborty.	*Sci. & Cult.*	**31,** 241, 1965

	Title of papers	Authors	Where published	Reference: Vol. page and year
11.	The effect of psoralene on gonadal activity.	C. Deb, Amar Chatterjee, D. P. Chakraborty	Endokrinologie	**46,** 106, 1964
12.	Family Rutaceae: A bio-systematic view point.	D. P. Chakraborty	Proc. Sym. on Frontiers of Plant Sciences. Bull. Bot. Soc., Bengal.	In Press
13.	Antifungal activity of some constituents of *Murraya koenigii* Spreng.	K. C. Das, D. P. Chakraborty, and P. K. Bose	Experientia 1965	**21,** 390, 1965
14.	Spices of India.	H. L. Chakravarty and D. P. Chakraborty	Indian Agriculturist	**8,** 124, 1964 1965
15.	Genes and their expression —a review.	M. Chakravorty	J. Sc. Ind. Res.,	**24,** 30, 1965
16.	Purification and properties of dihydrolipoic dehydrogenase from mung bean seedling.	S. K. Mitra	Ind. J. Biochem.	**1,** 180, 1964
17.	Purification and properties of Glutamine Synthetase from *Azotobacter vinelandii.*	R. K. Mandal	Ind. J. Biochem.	In Press
18.	Conversion of shikimic acid to aromatic amino acids in cell-free extracts of mung bean seedling.	M. Bal and D. P. Burma	Trans. Bose Inst.	In Press
19.	Molecular Basis of gene expression.	D. P. Burma	Presented at the Symposium on 'Frontiers of plant Sciences', Bull. Bot. Soc. West Bengal.	In Press
20.	Biochemical studies on lysogenic host-virus relationship	M. Chakraborty, M. Bal, K. Chakraburtty and D. P. Burma	-do-	In Press
21.	Effect of polyribonucleotides on amino acid incorporation by chloroplast ribosomes.	S. Biswas and B. B. Biswas	Experientia	**21,** 251, 1965
22.	The effect of ultraviolet irradiation on protein and RNA synthesis by chloroplasts.	-do-	Expt. Cell Res.	In Press

	Title of papers	Authors	Where published	Reference: Vol., page and year
23.	Non-terminal incorporation of adenosine monophosphate from adenosine triphosphate by chloroplast extract.	S. Biswas and B. B. Biswas	Arch. Biochem. Biophys.	In Press
24.	Non-terminal incorporation of Guanosine monophosphate from Guanosine triphosphate by an enzyme system from Spinach chloroplasts.	A. Chakravorty and B. B. Biswas	J. Biol. Chem.	**240,** 1965 (In Press)
25.	Biosynthesis of Histones	A. Chakravorty and B. B. Biswas	Indian J. Biochem.	**2,** 62, 1965
26.	Construction of a Gas-Liquid Partition Chromatograph.	J. Dutta and M. Hoque	Trans. Bose Res. Inst.	**25,** 109, 1962
27.	Radio-active dust fall-out	J. Dutta and H. C. Chakrabortty	Sci. & Cult.	**28,** 371, 1962
28.	Radioactivity in rain-water	J. Dutta, H. C. Chakrabortty and A. M. Ghose.	Sci. & Cult.	**28,** 372, 1962
29.	A carrier-gas by pass system for multiple-column gas-liquid partition chromatography.	J. Dutta	Sci. & Cult.	**29,** 153, 1963
30.	Preliminary study on the up-take of radioactive elements by the plants grown on Monazite Area.	J. Dutta and H. C. Chakrabortty	Sci. & Cult.	**29,** 263, 1963
31.	Didecyl Phthalate as the liquid phase for the analysis of mixture of lower monohydric alcohols by gas liquid chromatography.	J. Dutta and M. Hoque	Trans. Bose Res. Inst.	In Press
32.	Measurement of coherent scattering cross-sections of Gamma Rays at small angles	A. Nath and A. M. Ghose	Nuclear Physics	**57,** 547, 1965
33.	Effect of Bremsstrahlung on the Measurement of large-Angle coherent scattering of Gamma Rays.	A. Nath	Sci. & Cult.	**30,** 502, 1964

	Title of papers	Authors	Where published	Reference: Vol., page and year
34.	Measurement of Absolute differential collision cross-sections of Co.^{60} photons for Compton Effect.	A. M. Ghose	Nuclear Physics	**65,** 145, 1965
35.	Uniform Spectral sensitivity Photon counter.	A. M. Ghose	Nuclear Instruments and Methods	**34,** 1, 1965
36.	Photoelectric cross-section of γ-rays for heavy elements.	A. M. Ghose	Proc. Nuclear Physics Symposium, Calcutta.	1965
37.	A 250 KV Cockcroft-Walton Accelerator with small selenium rectifiers and high-frequency input.	B. Mitra, H. N. Raha B. Ghose	Nuclear Instruments and Methods.	**32,** 342, 1965
38.	Cross-section of the Reac- 31 P(n, 2n)^{30}P with Neutrons of Energy 14.8 Mev.	B. Mitra, A. Chatterjee and A. M. Ghose	Proc. Nuclear Physics, Symposium, Calcutta.	1965
39.	Systematic Measurements of (n.p) cross-sections at 14.8 Mev.	B. Mitra and A. M. Ghose	Proc. Nuclear Physics, Symposium, Calcutta.	1965
40.	On the magneto-ionic components of Radio Waves.	S. K. Banerjee and S. R. Khastgir	Journal of Geophysical Research (U.S.A.)	**68,** No. 15, 3209, Aug. 1, 1964.
41.	Coupled Wave Equations in Magneto-ionic Theory.	S. K. Banerjee and S. R. Khastgir	Indian Journal of Physics.	**38,** No. 7, 347, July, 1964.
42.	On the 'hook'-field components superposed on the c-field change of a lightning discharge.	S. R. Khastgir and M. Rao	Proceedings of the World conference o on Radio-Meteorology, Boulder, Colorado, U.S.A.	Paper No. 2, Sept, 15, 1964.
43.	Lateral Corona Currents from the Return-stroke channel and the c-field change in a lightning discharge.	M. Rao, and H. Bhattacharya	Proceedings of the World conference on Radio Meteorology Boulder, Colorado, U.S.A.	Paper No. 3, Sept. 15, 1964.
44.	A study of the random motion of the Ionospheric Irregularities from auto-correlograms.	N. N. Sen, and S. R. Khastgir	Indian Journal of Pure and Applied Physics.	October, 1964
45.	On the Angle of Inclination of the Equivalent Lightning channel.	H. Bhattacharya, and M. Rao	Indian Journal of Physics.	**38,** No. 6, 283 June, 1964.

	Title of papers	Authors	Where published	Reference: Vol., page and year
46.	Effect of Ionospheric reflections on the nature of the waveforms of radio-atmospherics.	H. Bhattacharya, and M. Rao	Radio Science U.S.A.	(in press)
47.	Studies on the mechanism of auxin action: Auxin regulation of nucleic acid metabolism in pea internodes coconut milk nuclei.	R. Roychoudhury and S. P. Sen	Physiologia Plantarum	**17,** 352-362, 1964.
48.	Hormonal regulation of nucleic acid metabolism and its significance in the mechanism of hormone action.	R. Roychoudhury and S. P. Sen	Bill. Bot. Soc.	**18,** No. 1 (Suppl.) 45, 1 1964.
49.	Metabolic conversion of thymine-2-C^{14} and its incorporation into nuclear RNA of endosperm nuclei of Cocos nucifera. Linn.	R. Roychoudhury and S. P. Sen	Biochem. Biophys. Res. Comm.	**14,** No. 1 7-11, 1964.
50.	The mechanism of action of Plant Growth substances: The role of nuclear RNA in growth substance action.	R. Roychoudhury, Anima Dutta and S. P. Sen	Biochem. Biophys. Acta.	In Press, 1965.
51.	The mechanism of action of Plant Growth substances: Growth substance stimulation of amino acid incorporation into nuclear protein.	Anima Datta and S. P. Sen	Biochem. Biophys. Acta.	In press, 1965.
52.	Radiation induced morphological mutants of jute.	R. K. Basu	Journal of Genetics	Vol. 59, No. 2
53.	Morphological Aberrations produced by Ethyleneimine in Jute.	S. Bose, A. De	Science and Culture	Vol. 30, pp. 345-347 July, 1964.
54.	Morphological Aberrations produced by Ethylenimine in Jute.	S. Bose, A. De	Trans. Bose Res. Inst.	In Press.
55.	Studies on the development of improved strains of *Streptomyces citreus* through induced mutation.	P. M. Naha, P. Nandi.	Trans. Bose Res. Inst.	26(3), (1963).

Title of papers	Authors	Where published	Referenc: Vol., page and year
56. Induction of colonial para morph in *Sordaria bosensis.*	K. L. Chaudhuri.	Trans. Bose Res. Inst.	27(2), (1964).
57. Studies on the antibiotic producing *Streptomyces griseus* Ac_4 248.	A. K. Banerjee, P. Nandi.	Trans. Bose Res. Inst.	27(2), (1964).
58. Production of antibiotic by *Streptomyces citreus* in neutral soil and its stability.	P. M. Naha, P. Nandi.	Trans. Bose Res. Inst.	27(3), (1964).
59. Studies on the isolation and characterisation of a strain of *Pseudomonas* producing antibiotic pigment.	P. P. Mukherjee, P. Nandi.	Trans. Bose Res. Inst.	27(3), (1964).
60. Studies on the antimicrobial activity of *Pseudomonas aeruginosa* strain (Px).	P. P. Mukherjee, P. Nandi.	Trans. Bose Res. Inst.	27(4), (1964).

APPENDIX D

Fortysixth anniversary meeting of the Bose Institute 30th November, 1964

DIRECTOR'S REPORT

This evening the Bose Institute is celebrating the fortysixth anniversary of its foundation. We are very fortunate that in spite of the many pressing calls on his time, Shri Asoka Mehta, Deputy Chairman, Planning Commission, has accepted the invitation of the Bose Institute Council to deliver this evening the 26th Acharya Jagadish Chandra Bose Memorial address for which he has selected the topic "The Threshold of the Breakthrough; Technological and Social Transformation in India".

The present series of memorial addresses commenced at a time coinciding with the outbreak of the last World War; the British Government of the period learning from the experiences of the first World War, had instituted in this country on the British model, a D.S.I.R., whose first director was our esteemed friend, Dr. S. S. Bhatnagar; later till he passed away in 1955, Dr. Bhatnagar was responsible for the construction of many of the National Laboratories. His own laboratory was started in 1939 in the precincts of the Government Test House, Alipore. In 1940, Dr. Bhatnagar was invited to deliver the second lecture of the series, for which he selected the topic "Scientific Research and the Future of Indian Industry". The Council of the Bose Institute has since then invited distinguished authorities on the application of science to the problems of national welfare, to deliver some of the memorial addresses. I shall refer here to two of the important addresses, Prof. P. C. Mahalanobis on "Statistical Methods in National Development" and Sir J. J. Gandhy on "Technology and Economic Change in the Third Plan".

The incursion of the Chinese armed forces across some areas of the northern frontier of India caused disquiet amongst our people, whether our Government was giving serious consideration to the mobilisation of the resources of the country, including scientific potential, to the defence of the country. On the invitation of the Council of the Bose Institute Dr. S. Bhagavantam, Scientific Adviser to the Minister of Defence, chose for the 1963 Jagadis Chandra Memorial address "Place of Science on the Defence of the Country".

Dr. Bhagavantam gave a masterly discourse on the general problem of the place of science in the defence of the country, without indicating as some of us had hoped, how the principles discussed by him were being applied to the problem of our national defence. Probably our antagonists across the Indian frontier shared in our disappointment.

During the last two years another crisis, this time in our national economy has loomed large. One of the important causative factors is the increasing inability of our agricultural production to keep pace with the alarming rate of population growth—this has become a source of grave concern to all of us. Along with this problem another issue of our national policy is causing some of searching discussions amongst a large group of scientists—how soon with the present policy of depending so largely on foreign aid, loans, food supply, supply of capital goods and technical know-how from foreign countries, which though easing

the present economic situation, will enable the country ultimately to reach its goal of self-sufficiency in technology, industry and agriculture. We have so long been comforting ourselves with the belief that with such foreign aid the country will be spared the terrible struggle which some of the totalitarian countries had to undergo and some are still undergoing to enable them to build up out of their own resources viable capital industries and defence organisation. The question which many of us find disturbing is whether we are not getting too habituated to this dependence on foreign aid. We are aware of the many difficulties facing our people like population explosion, land fragmentation and soil impoverishment which hamper our self-sufficiency in food production. This problem is still facing many other countries, totalitarian as well as under-developed countries which have not been able to accumulate sufficient capital resources and technical know-how necessary for developing agriculture on modern technological basis as well as on equitable system of land utilisation.

We are very fortunate in having with us this evening Shri Asoka Mehta, Deputy Chairman, Planning Commission, to speak to us on some of these problems.

Shri Mehta before he was called to take up the difficult responsible post of Deputy Chairman, Planning Commission, was widely known and respected as an able exponent of democratic socialism. He with Shri Jayprakas Narayan, Acharya Narendra Dev were founders of the Socialist party of India. He had on several occasions been invited to participate in international conferences organised by the Congress for Cultural Freedom of which Shri Jayprakas Narayan is one of the Honorary Presidents.

Before requesting the speaker of the evening to deliver address, it is usual for the Director to review the work of the Institute during the past year.

I shall refer first to the loss the Institute has suffered by the passing away of one of its senior most members. I propose then to give a short review of the new trend of research we are taking up in the Bose Institute.

The plan for founding an institute like the Bose Institute occurred to Acharya Jagadis Chandra in 1897 when he was invited for the first time to lecture on his work in the Royal Institution, London. His many friends which included Rabindranath Tagore, Sister Nivedita, some American friends shared on his vision, and helped him to collect funds for founding a research Institute in India, where Indian could have the opportunity of doing independent research—and speak of their findings before gatherings of men of education and culture.

When in 1917 he founded the Bose Institute, Jagadis Chandra had with him a band of devoted friends and fellow workers, who served on the Governing Body of the Bose Institute and helped him to carry out his plans. Amongst them were honoured names like Rabindranath Tagore, Shrimati Abala Bose, Nilratan Sircar. There was another group which though not so distinguished but who since the foundation were intimately associated with the Bose Institute in the daily administration of the Institute. We mention the names of Sudhansumohan Bose, who acted as Secretary to the Governing Body since its foundation, Nagendra Chandra Nag, who left an important post in the Banaras Hindu University to become the Assistant Director and Abaninath Mitter who was Superintendent of the Bose

Institute since 1917 and who after his retirement in 1948, continued to be associated with the Bose Institute in many important capacities.

These were the people who helped Acharya Jagadis Chandra Bose during his life time; after his passing away they became the upholders of the tradition of Jagadis Chandra and their services were invaluable during the transition period following his passing away. To-night we have to mourn the death of last of this group of collaborators Abaninath Mitter who left us on the 11th November 1964, after a period of continued and valued association with the Bose Institute for over 48 years. Till his passing away, Abaninath was a member of the Council of the Bose Institute, Assistant Secretary to Governing Body, and Secretary of two Boards of Trusts, J. C. Bose had founded. Academically though not highly qualified, Shri Mitter was an unusually gifted craftsman who could employ his hands and brains in construction of machines of his own design. In 1916 while working as partner in a biscuit factory, he was called by his cousin Jagadis Chandra to assist him in carrying out his plan for the construction of the Bose Institute buildings with its many beautiful adaptations of Indian architectural and decorative motifs to the needs of a modern Institute. Abaninath had visited many museums, visited Banaras, Ajanta, making copies of classical Indian designs; later he instructed the craftsmen whom he recruited in Calcutta and in Banaras to carry out these designs. The Institute temple, the later laboratory buildings erected in the Institute premises, the premises on the bank of the river Hoogli built on an embankment encroaching on the foreshore of the river at Falta, were all constructed under his supervision. Shri Mitter's practical ability and strong commonsense were recognised by his friends who had got their initial training along with him in Japan; he was for a long time director of the Bengal Waterproof Works Co. and the Calcutta Chemical Co. The Bose Institute mourns the loss of a friend and trusted well-wisher.

I propose to give now a brief account of the new lines of activity which are being developed in the Bose Institute. The Institute was intended by its founder for the study of the nascent science which includes both life and non-life. As a biophysicist Jagadis Chandra was mainly interested in physical manifestations of the life phenomena, growth, electrical and mechanical responses to various forms of stimulation, gravity, light, mechanical and electrical stimuli. Other departments were opened in the Bose Institute by Jagadis Chandra but his main interest was in biophysical studies.

Since his time, the concept is being generally accepted that the life process is due to a sequence of biochemical activities through which an organism develops from the fertilized egg to the adult state, in course of which it passes through a reproductive stage. The Bose Institute is now being developed mainly for the study of biological sciences; the departments of Botany, Chemistry, Microbiology and Animal Physiology cooperate in these studies. Different types of living organisms in different stages of evolutionary development, single celled plants like bacteria, algae, yeast, molds, and multicelled organisms like higher plants and some of the higher animals are being studied from a comparative stand-point. While some genes which control the biochemical processes underlying cellular metabolism in different organisms show a large degree of similarity, the diversity in structure and degree of

specialisation in organ formation and their activities is found to be controlled by other genes in the same nucleus.

Physics is the basic science which provide the techniques for such studies, and also the ultimate discipline in terms of which all the life processes are to be interpreted. The border line which separates the interpretation of life in terms of physical concepts and something not definable in physical ones is continuously shifting. Probably in defining the separateness of life from the non-living we shall have to take refuge in some formulation of the complementarity principle of Bohr.

In the Bose Institute we are only interested in elucidating how the processes taking place in living organisms could be formulated in terms of the simplest physical concepts. During the last decade a revolutionary change has taken place in our understanding of life processes for which a new discipline with the same molecular biology has been proposed. It is based on the discovery that the genes in the cell nucleus which according to the geneticists are the carriers of all the hereditary information of an organism are made of long chain DNA molecules. The theory also gives a model of how this information contained in the DNA molecules are transferred to specific enzymes in the cell bodies which control all cellular activities and consequently the functions of the organism as a whole. The greatest success of molecular biology has been in the domain of single cell organisms bacteria, molds and fungi. Difficulties of interpretation begin to arise when it is found that in multicellular organisms, at a certain stage of development cells begin to differentiate leading to organ formation like roots, shoots, leaves, flowers in plants. New concepts have to be introduced to interpret why some portions of the genes in the nucleus become effective at later stages of the organism developments as in morphogenesis. These new conceptions are finding increasing applications in the interpretation of a host of existing observations where it is found that the introduction of a very minute quantity of foreign molecules including viruses either formed in the cell cytoplasm or introduced from outside can produce such large effects in the organism as a whole. I refer to the wholesale degeneration of normal tissues to neoplasmic cancer tissues, the formation of antibodies in response to introduction of antigens in the organism, the action of auxins and hormones in energising many kinds of cellular activities, the release of morphogenetic changes like flowering in plants, the transformation of a frog from its tadpole state to a full grown animal.

These are some of the problems which are being studied now in the Bose Institute.